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A set of simple-sequence repeat (SSR) markers covering the *Prunus* genome

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Abstract A set of 109 microsatellite primer pairs recently developed for peach and cherry have been studied in the almond × peach F_2 progeny previously used to construct a saturated *Prunus* map containing mainly restriction fragment length polymorphism markers. All but one gave amplification products, and 87 (80%) segregated in the progeny and detected 96 loci. The resulting *Prunus* map contains a total of 342 markers covering a total distance of 522 cM. The approximate position of nine additional simple sequence repeats (SSRs) was established by comparison with other almond and peach maps. SSRs were placed in all the eight linkage groups of this map, and their distribution was relatively even, providing a genome-wide coverage with an average density of 5.4 cM/SSR. Twenty-four single-locus SSRs, highly polymorphic in peach, and each falling within 24 evenly spaced approximately 25-cM regions covering the whole *Prunus* genome, are proposed as a 'genotyping set' useful as a reference for fingerprinting, pedigree and genetic analysis of this species.

Keywords Microsatellites · Reference map · Map comparison · Marker-assisted selection · Genotyping set

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Introduction

The genus *Prunus* encompasses some of the most important temperate fruit species, including peach (*P. persica*), apricot (*P. armeniaca*), cherry (*P.avium* and *P. cerassus*), plum (*P. japonica* and *P. domestica*) and almond (*P. amygdalus*). A saturated linkage map with restriction fragment length polymorphism (RFLP) and isozyme markers was constructed for the genus using an F_2 population of a cross between almond and peach (Joobeur et al. 1998). The construction of this map was with a view of its use as a reference for map comparisons between different species and also for the construction of framework or reduced maps, i.e. maps with markers evenly spaced at distances of 15–25 cM, in different populations. These maps would allow the location of major genes or QTLs (quantitative trait loci) segregating in these progenies. For that reason, RFLP markers were chosen because of their high quality and transferability between different populations of the same species and between different species of the genus.

Selected markers of the reference *Prunus* map have been used for map construction and agronomic trait genetic analysis in several almond (Joobeur et al. 2000; Ballester et al. 2001), almond × peach (Jáuregui et al. 2001) and peach (Dirlewanger et al. 1997, 1998; Dettori et al. 2001) populations. However, two circumstances limit their widespread use in *Prunus*. First, RFLPs require relatively laborious and complex methods that are

not always available to all laboratories. Second, the low level of variability of certain species such as peach and, to a lesser extent, apricot (Byrne 1990), results in fewer informative markers in an intraspecific context. Dirlewanger et al. (1998) and Dettori et al. (2001) found, respectively, 24% and 28% polymorphism in the RFLP probes they tested in peach progenies. As a consequence, the maps obtained only with these markers were incomplete, and the addition of other markers like amplified fragment length polymorphisms (AFLPs) or random amplified polymorphic DNAs (RAPDs) was necessary for sufficient genome coverage.

Given their high polymorphism, codominant inheritance and the simplicity of the methods required for their development, microsatellite or simple-sequence repeat (SSR) markers seem to be the appropriate marker system to solve these problems. Many SSRs have been recently developed in peach (Testolin et al. 2000; Sosinski et al. 2000; Aranzana et al. 2002; Dirlewanger et al. 2002) and cherry (Downey and Iezzoni 2000; Cantini et al. 2001). With these markers, which have a high level of observed heterozygosity (0.37–0.41) in peach cultivars (Aranzana et al. 2002; Dirlewanger et al. 2002), it seems probable that enough polymorphism for map construction will be found. In this paper we have placed the majority of the available SSRs on the *Prunus* saturated map, allowing us to develop a resource useful for map comparison or marker-assisted selection in fruit crops. A set of single-locus and highly polymorphic peach SSRs that cover the whole *Prunus* genome has been chosen, and it is proposed as a reference set for cultivar identification and variability or pedigree analysis in this species.

Materials and methods

Plant material and DNA extraction

The F_2 progeny (n = 82) obtained from selfing a single individual (MB 1-73) of the cross between "Texas" almond and "Earlygold" peach was used as the mapping population. This population (T × E) was used by Joobeur et al. (1998) for map construction with RFLP and isozyme markers. Genomic DNA was extracted from young leaves following the method described by Bernatzky and Tanksley (1986) with further $CsCl_2$ purification (Viruel et al. 1995).

Microsatellite analysis

Details of the microsatellites analysed and their origin are given in Table 1. As a parameter of variability for the selection of some of these microsatellites we have used the power of discrimination ($PD = 1 - \Sigma g_i^2$, where g_i is the frequency of the ith genotype at this locus) (Kloosterman et al. 1993), using data provided in the papers described in Table 1. One of the microsatellites used, pchgms6, has not been described earlier. It was obtained from the peach "Bicentennial" genomic library described by Sosinski et al. (2000), and the primers used were: 5′-CATTGTTCATGGGAGGAATT-3′ (forward) and 5′-AGAACATTCCTAAAGGAGCA-3′ (reverse). The same terminology is used as in the original description with the exception of peach CPPCT SSRs (Aranzana et al. 2002), where a three digit code number has been used (i.e., CPPCT6 or CPPCT33 in the former paper are now CPPCT006 and CPPCT033, respectively).

Amplification was conducted in a total volume of 15 µl with 60 ng DNA, 0.8 µ*M* of both specific primers, 166 µ*M* of dATP, dGTP and dTTP, 2 µ*M* of dCTP, 1 U *Taq* polymerase, 1.5 m*M* $MgCl_2$, and 1 µCi α-[^{33}P]-dCTP in 1 × PCR buffer (75 m*M* Tris HCl, pH 8.8, 20 m*M* $(NH_4)_2SO_4$, 0.01% Tween-20). The amplification program consisted of 1 min at 94 °C, 34 cycles of 30 s at 94 °C, 30 s at the appropriate annealing temperature and 1 min at 72 °C, followed by a 5-min extension at 72 °C. Polymerase chain reaction (PCR) products were denatured by adding 10 µl of 95% formamide/dye solution and heating for 10 min at 100 °C. Electrophoresis was carried out in 35 × 50-cm^2 gels with 6% denaturing polyacrylamide containing 7.5 *M* urea and run at a constant power of 80 W in 1 × TBE buffer. After 2 h of electrophoresis, the gel was transferred onto a Whatman 3 MM chromatography paper, vacuum-dried and exposed for 1 day to X-ray film.

Table 1 Summary of characteristics of the microsatellite primer pairs used in this study

Species	Repeat	Origin	Designation	Number described	Number tested	Number segregating	Reference
Peach	CT and GT	Two enriched genomic libraries of "Redhaven"	UDP	26	25	15	Cipriani et al. (1999); Testolin et al. (2000)
Peach	CT	Enriched genomic library of "O'Henry"	CPPCT	35	28	25	Aranzana et al. (2002)
Peach	CT	Enriched genomic library of "O'Henry"	BPPCT	41	31	27	Dirlewanger et al. (2002)
Peach	CT and CA	Genomic ("Bicentennial") and cDNA ("Suncrest") libraries	pchgms/pchcms	11	11	9	Sosinski et al. (2000)
Sweet cherry	CT, CA and GA	Genomic library from "Valerij Tschakhalov"	PMS	6	5	3	Cantini et al. (2001)
Sweet cherry	GA, GT and GTT	Enriched genomic library from "Napoleon"	PS	6	6	5	Joobeur et al. (2000); Cantini et al. (2001)
Sour cherry	GA	Genomic library of "Erdi Botermo"	Pce	6	3	3	Cantini et al. (2001); Downey and Iezzoni (2000)
Total				131	109	87	

SSRs were first studied in the parents, the hybrid and one plant of the progeny to detect polymorphism. Polymorphic and well-amplified markers were later run with the whole population.

Linkage analysis

Markers were scored as codominant (1:2:1) or dominant (3:1) segregations. Segregation ratios were analyzed with a Chi-squared test. The SSR scores were included in the RFLP and isozyme dataset of the previous map and analysed for linkage using MAPMAKER v. 3.0 software (Lander et al. 1987). The Kosambi mapping function was used for converting recombination frequencies into genetic distances. Linkage groups were established with a LOD = 5.0. A first framework of the map was constructed with the 'order' command using a LOD ≥ 3 only with the codominant loci. Dominant loci and codominant markers placed with lower probability were added to this framework with the 'try' and 'ripple' commands. Map drawing was with the FITMAPS v. 1.0 program (Graziano and Arús 2002).

Results

One hundred and nine microsatellite primer pairs were assayed in DNA samples of "Texas", "Earlygold" and the hybrid plant (MB 1-73) used for the production of the F_2 progeny. All but one (pchcms3) yielded amplification products and amplified fragment sizes in the range expected for each microsatellite. Eighty-seven of them (80%) were polymorphic between the parents and segregated in the progeny. These primer pairs yielded a total of 96 scorable loci; 81 were single-locus and of the six remaining, CPPCT024 detected four loci, CPPCT003 three loci and BPPCT009, BPPCT021, CPPCT004 and CPPCT019, two loci each. Codominant inheritance occurred in 70 loci, and 26 (27%) were scored as presence versus absence of amplified fragment, which corresponds to a dominant model of inheritance. For these loci, the amplified allele originated from almond in three cases and peach in the other 23. Thirteen of the latter loci produced an additional amplified DNA fragment in the almond parent and F_2 individuals homozygous for the almond allele.

All of the markers studied were integrated in the eight linkage groups (G1–G8) of the T × E map (Fig. 1). Forty-one (43%) markers had segregation ratios significantly different from the expected ($P \leq 0.05$). In all cases, these loci were placed in regions of the map that contain markers with distorted segregations as detected by Joobeur et al. (1998). The total size of the map after the addition of these 96 SSRs (39% more markers) was 522 cM, 6% longer than the previous T × E map. The average marker density increased from 2.0 to 1.5 cM per marker considering all markers and from 2.7 to 2.2 cM per marker if only one marker was taken into account in loci with two or more markers. The map density considering only SSRs was of 5.4 cM per marker.

SSRs were distributed throughout all linkage groups of the T × E map (Table 2). The number of SSRs per linkage group ranged from 21 in G1 to 7 in G3. The pattern of distribution of the SSRs in the map appeared to be random. Only nine pairs of the SSRs mapped cosegregated, and a few clusters of four or more SSRs separated by less than 5 cM occurred on G5 and G7. The observed number of SSRs mapped on each linkage group was not significantly different from the expected ($\chi^2 = 7.09$; 7 *df*), assuming that it was proportional to the number of markers already mapped. A few SSRs cosegregated with the most distal markers of the linkage group, e.g. CPPCT016 on G1, BPPCT010 on G4 or CPPCT030 on G6, or were located at the end of the linkage groups, e.g. CPPCT021 on G6. Three of the four gaps that were greater or equal to 10 cM of the original map were filled with SSRs, reducing the size of the longest gap to 11 cM on G6, between markers FG14 and PC73, followed by one gap of 9 cM also on G6, five gaps of 8 cM (two on G2 and one on G5, G6 and G7) and two gaps of 7 cM (both on G7).

Some of the SSRs mapped are anchor points on published maps. Five of the six SSRs mapped by Joobeur et al. (2000) in almond and 9 of the 17 SSRs located in a peach map constructed by Dettori et al. (2001) were located in the expected homologous linkage groups of the T × E map. Given the colinearity between these maps and the fact that many anchor loci, mainly RFLPs, exist between them, it was possible to establish the approximate positions of nine additional SSRs (one from the almond and eight from the peach maps) in T × E. Some of these markers lie on regions with low SSR density or on some of the major gaps, like UDP98-406 and UDP98-410 on G2, UDP98-416 on G6 or UDP98-415 on G7. With the inclusion of these markers, the number of SSRs placed on the *Prunus* map increases to 105.

Map coverage with SSRs was of 430 cM (82%) of the total 522 cM. Most chromosomes were well covered leaving only a few large gaps, the largest of 23 cM on the central part of G6 and two more of 18 cM on the distal part of G8. The remaining gaps were shorter than 12 cM. To provide a more precise picture of the SSR dis-

Table 2 Distribution of the SSRs studied here and the rest of the markers (RFLPs and isozymes) in the linkage groups (G1–G8) of the Texas × Earlygold map

	G1	G2	G3	G4	G5	G6	G7	G8	Total
SSRs	21	10	7	15	11	12	12	8	96
Other markers	59	29	35	28	21	30	23	21	246
Total	80	39	42	43	32	42	35	29	342
Distance (cM)	85	50	51	61	49	88	80	60	522
Percentage increase over previous map	0	6	9	5	–2	10	21	1	6
Percentage coverage with SSRs	94	80	61	98	82	97	70	63	82

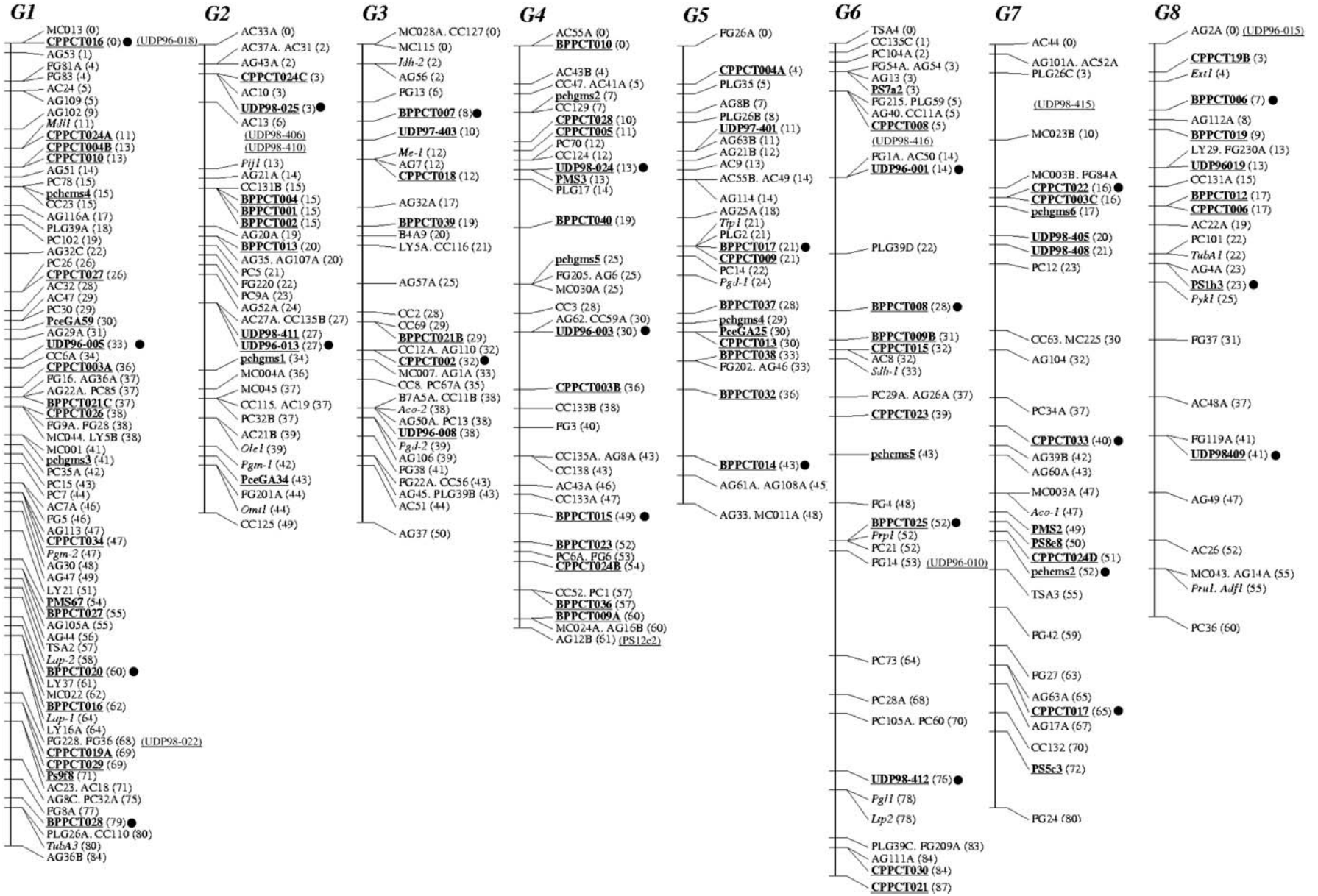
G1
MC013 (0)
CPPCT016 (0) ● (UDP96-018)
AG53 (1)
PG81A (4)
PG83 (4)
AC24 (5)
AG109 (5)
AG102 (9)
Mill (11)
CPPCT024A (11)
CPPCT004B (13)
CPPCT010 (13)
AG51 (14)
PC78 (15)
pchems4 (15)
CC23 (15)
AG116A (17)
PLG39A (18)
PC102 (19)
AG32C (22)
PC26 (26)
CPPCT027 (26)
AC32 (28)
AC47 (29)
PC30 (29)
PceGA59 (30)
AG29A (31)
UDP96-005 (33) ●
CC6A (34)
CPPCT003A (36)
PG16. AG36A (37)
AG22A. PC85 (37)
BPPCT021C (37)
CPPCT026 (38)
PG9A. PG28 (38)
MC044. LY5B (38)
MC001 (41)
pchgms3 (41)
PC35A (42)
PC15 (43)
PC7 (44)
AC7A (46)
PG5 (46)
AG113 (47)
CPPCT034 (47)
Pgm-2 (47)
AG30 (48)
AG47 (49)
LY21 (51)
PMS67 (54)
BPPCT027 (55)
AG105A (55)
AG44 (56)
TSA2 (57)
Lup-2 (58)
BPPCT020 (60) ●
LY37 (61)
MC022 (62)
BPPCT016 (62)
Lup-1 (64)
LY16A (64)
PG228. PG36 (68) (UDP98-022)
CPPCT019A (69)
CPPCT029 (69)
Ps9t8 (71)
AC23. AC18 (71)
AG8C. PC32A (75)
PG8A (77)
BPPCT028 (79) ●
PLG26A. CC110 (80)
TubA3 (80)
AG36B (84)
G2
AC33A (0)
AC37A. AC31 (2)
AG43A (2)
CPPCT024C (3)
AC10 (3)
UDP98-025 (3) ●
AC13 (6)
(UDP98-406)
(UDP98-410)
Pij1 (13)
AG21A (14)
CC131B (15)
BPPCT004 (15)
BPPCT001 (15)
BPPCT002 (15)
AG20A (19)
BPPCT013 (20)
AG35. AG107A (20)
PC5 (21)
PG220 (22)
PC9A (23)
AG52A (24)
AC27A. CC135B (27)
UDP98-411 (27)
UDP96-013 (27) ●
pchgms1 (34)
MC004A (36)
MC045 (37)
CC115. AC19 (37)
PC32B (37)
AC21B (39)
Ole1 (39)
Pgm-1 (42)
PceGA34 (43)
PG201A (44)
Omt1 (44)
CC125 (49)
G3
MC028A. CC127 (0)
MC115 (0)
Idh-2 (2)
AG56 (2)
PG13 (6)
BPPCT007 (8) ●
UDP97-403 (10)
Me-1 (12)
AG7 (12)
CPPCT018 (12)
AG32A (17)
BPPCT039 (19)
B4A9 (20)
LY5A. CC116 (21)
AG57A (25)
CC2 (28)
CC69 (29)
BPPCT021B (29)
CC12A. AG110 (32)
CPPCT002 (32) ●
MC007. AG1A (33)
CC8. PC67A (35)
B7A5A. CC11B (38)
Aco-2 (38)
AG50A. PC13 (38)
UDP96-008 (38)
Pgd-2 (39)
AG106 (39)
PG38 (41)
PG22A. CC56 (43)
AG45. PLG39B (43)
AC51 (44)
AG37 (50)
G4
AC55A (0)
BPPCT010 (0)
AC43B (4)
CC47. AC41A (5)
pchgms2 (7)
CC129 (7)
CPPCT028 (10)
CPPCT005 (11)
PC70 (12)
CC124 (12)
UDP98-024 (13) ●
PMS3 (13)
PLG17 (14)
BPPCT040 (19)
pchgms5 (25)
PG205. AG6 (25)
MC030A (25)
CC3 (28)
AG62. CC59A (30)
UDP96-003 (30) ●
CPPCT003B (36)
CC133B (38)
PG3 (40)
CC135A. AG8A (43)
CC138 (43)
AC43A (46)
CC133A (47)
BPPCT015 (49) ●
BPPCT023 (52)
PC6A. PG6 (53)
CPPCT024B (54)
CC52. PC1 (57)
BPPCT036 (57)
BPPCT009A (60)
MC024A. AG16B (60)
AG12B (61) (PS12e2)
G5
PG26A (0)
CPPCT004A (4)
PLG35 (5)
AG8B (7)
PLG26B (8)
UDP97-401 (11)
AG63B (11)
AG21B (12)
AC9 (13)
AC55B. AC49 (14)
AG114 (14)
AG25A (18)
Tip1 (21)
PLG2 (21)
BPPCT017 (21) ●
CPPCT009 (21)
PC14 (22)
Pgd-1 (24)
BPPCT037 (28)
pchgms4 (29)
PceGA25 (30)
CPPCT013 (30)
BPPCT038 (33)
PG202. AG46 (33)
BPPCT032 (36)
BPPCT014 (43) ●
AG61A. AG108A (45
AG33. MC011A (48)
G6
TSA4 (0)
CC135C (1)
PC104A (2)
PG54A. AG54 (3)
AG13 (3)
PS7a2 (3)
PG215. PLG59 (5)
AG40. CC11A (5)
CPPCT008 (5)
(UDP98-416)
PG1A. AC50 (14)
UDP96-001 (14) ●
PLG39D (22)
BPPCT008 (28) ●
BPPCT009B (31)
CPPCT015 (32)
AC8 (32)
Sdh-1 (33)
PC29A. AG26A (37)
CPPCT023 (39)
pchems5 (43)
PG4 (48)
BPPCT025 (52) ●
Prp1 (52)
PC21 (52)
PG14 (53) (UDP96-010)
PC73 (64)
PC28A (68)
PC105A. PC60 (70)
UDP98-412 (76) ●
Pgi1 (78)
Lp2 (78)
PLG39C. PG209A (83)
AG111A (84)
CPPCT030 (84)
CPPCT021 (87)
G7
AC44 (0)
AG101A. AC52A
PLG26C (3)
(UDP98-415)
MC023B (10)
MC003B. PG84A
CPPCT022 (16) ●
CPPCT003C (16)
pchgms6 (17)
UDP98-405 (20)
UDP98-408 (21)
PC12 (23)
CC63. MC225 (30
AG104 (32)
PC34A (37)
CPPCT033 (40) ●
AG39B (42)
AG60A (43)
MC003A (47)
Aco-1 (47)
PMS2 (49)
PS8e8 (50)
CPPCT024D (51)
pchems2 (52) ●
TSA3 (55)
PG42 (59)
PG27 (63)
AG63A (65)
CPPCT017 (65) ●
AG17A (67)
CC132 (70)
PS5c3 (72)
PG24 (80)
G8
AG2A (0) (UDP96-015)
CPPCT19B (3)
Ext1 (4)
BPPCT006 (7) ●
AG112A (8)
BPPCT019 (9)
LY29. PG230A (13)
UDP96019 (13)
CC131A (15)
BPPCT012 (17)
CPPCT006 (17)
AC22A (19)
PC101 (22)
TubA1 (22)
AG4A (23)
PS1h3 (23) ●
Pyk1 (25)
PG37 (31)
AC48A (37)
PG119A (41)
UDP98409 (41) ●
AG49 (47)
AC26 (52)
MC043. AG14A (55)
Frul. Adf1 (55)
PC36 (60)

Table 3 Intervals in which the eight linkage groups (G1–G8) of the Texas × Earlygold map have been divided, number of SSRs of each interval and the marker selected in each interval for the "genotyping set"

	G1	G2	G3	G4	G5	G6	G7	G8
Mean interval size (cM)	21.3	25.0	25.5	20.3	24.5	22.0	20.0	20.0
Interval 1								
No. SSRs	5	6	4	7	4	3	5	6
Marker for genotyping set[a]	CPPCT016 (0.46)	UDP98-025 (0.81)	BPPCT007 (0.79)	UDP98-024 (0.82)	BPPCT017 (0.76)	UDP96-001 (0.57)	CPPCT022 (0.88)	BPPCT006 (0.66)
Interval 2								
No. SSRs	7	4	3	3	8	5	1	1
Marker for genotyping set[a]	UDP96-005 (0.53)	UDP96-013 (0.73)	CPPCT002 (0.63)	UDP96-003 (0.83)	BPPCT014 (0.67)	BPPCT008 (0.69)	CPPCT033 (0.60)	PS1h3* (0.34)
Interval 3								
No. SSRs	5	–	–	5	–	1	4	1
Marker for genotyping set[a]	BPPCT020 (0.78)	–	–	BPPCT015 (0.89)	–	BPPCT025 (0.70)	pchcms2* (0.49)	UDP98-409 (0.50)
Interval 4								
No. SSRs	4	–	–	–	–	3	2	–
Marker for genotyping set[a]	BPPCT028 (0.58)	–	–	–	–	UDP98-412 (0.84)	CPPCT017 (0.69)	–

[a] In parenthesis, value of the discrimination power for the marker as calculated in the papers in which these markers were described or from unpublished results by the authors (*)

tribution, we divided the distance of each linkage group into intervals of equal size between 20 and 25.5 cM and counted the number of markers within each of them (Table 3). Most of the 24 intervals obtained in this manner (19) had three or more SSRs, and those with only one or two SSRs accounted for 19% of the total distance of the T × E map. All intervals contained at least one single-locus microsatellite that was polymorphic in peach, and we chose one of them among those having a high power of discrimination value to define a minimal set of markers that could be used for fingerprinting or other variability studies in this species.

Discussion

A set of 109 SSRs recently developed by various research groups in peach and cherry produced 96 markers that were added to the reference *Prunus* map. Most of these markers (70) behaved as codominant and, from the 26 that were scored as dominant, the amplified fragment was inherited from the peach parent in 23 cases and from the almond parent in the other three. The absence of amplified fragments (null alleles) can be attributed to low conservation of the primer regions of these SSRs between peach and almond, as found in the comparison of SSRs between closely related genomes in other crops like sugarbeet (Rae et al. 2000). The high frequency of null alleles of almond compared to that of peach is a logical consequence of the fact that most of the SSRs studied were developed in peach. More than half of the almond null alleles (13) produced amplification residues in the almond parent or in the homozygous individuals for the allele inherited from it in the F_2 progeny, suggesting that the almond allele competed disadvantageously with the peach allele in the PCR reaction. The behavior of almond alleles as nulls in the presence of the corresponding peach allele is an important observation for pedigree analysis of interspecific *Prunus* hybrids using SSRs, because the patterns observed in the progenies may not conform exactly to the predictions due to the absence of the allele of one of the parents. When all of the peach SSRs that gave an amplification product in the almond parent were taken into consideration, their percentage of transportability was high (89%), in agreement with their high level of genetic resemblance (peach and almond belong to the *Amygdalus* subgenus). Lower values have been found for peach SSRs in *Prunus* species belonging to other subgenera, like apricot with an 84% of amplification (Hormaza 2002) or cherry with an 81% (Dirlewanger et al. 2002).

The addition of 96 SSRs has improved the *Prunus* map, filling some existing gaps and increasing the density. Groups with two or more cosegregating markers were frequent (71 map positions), which can be attributed in most cases to the size of the T × E population (n = 82). The analysis of additional individuals would probably increase the resolution of this map. The results obtained provide additional evidence of the completeness of the

◀ **Fig. 1** Map of the "Texas" × "Earlygold" F_2 population with the markers located by Joobeur et al. (1998) plus the SSRs mapped in this paper (in *bold* and *underlined*). In *parenthesis following* marker names is the genetic distance from the top of each linkage group in centiMorgans. SSRs from other maps are placed on the *right* of the map in their approximate position, *underlined* and in *parenthesis*. *Black dots* to the *right* of SSR names indicate that they belong to the peach genotyping set

present map and its value as a resource for genetic analysis in this group of species. The pattern of distribution of the SSRs placed on the T × E map was apparently random, as also found when used in other species such as rice (Temnykh et al. 2000), wheat (Röder et al. 1998), soybean (Cregan et al. 1999b) or sugarbeet (Rae et al. 2000). Map coverage was high (82%), suggesting that a selected subset of these SSRs would be useful for establishing framework maps anchored in the T × E map in different populations of the same or different *Prunus* species. These maps would facilitate genome comparisons among species or to establish the position of genes or QTLs obtained in one population on a detailed integrated map of the genus, making it simpler to find markers useful for their selection. SSR-based map comparisons are already possible with the current information, and in this paper it has been possible to determine the approximate position of nine additional SSRs mapped in other populations (Joobeur et al. 2000; Dettori et al. 2001).

The existence of a set of single-locus, codominant and highly polymorphic markers that are distributed along the whole *Prunus* genome and quickly and easily detectable would be a powerful tool for variability analysis and fingerprinting. SSRs are the only molecular markers currently available that fit all these requirements. The use of such a set by different research groups would allow direct comparison of results, which is hardly feasible with the most widely used DNA markers of the recent past, like RAPDs or AFLPs. Macaulay et al. (2001) proposed the same approach for barley and chose a set of 48 SSRs as the 'genotyping set' for this species. We propose an analogue development for peach: the *Prunus* genome has been divided into 24 intervals 20.0–25.5 cM in length, and one peach single-locus highly polymorphic SSR has been selected for each one. Fingerprints of different peach cultivars for this set of SSRs may be useful to establish pedigree relationships, to evaluate rates of mutation of different SSRs and to detect conserved linkage blocks that include interesting alleles of major genes and QTLs. It would be worthwhile expanding this genotyping set to other *Prunus* species, but for that it is necessary to determine whether the SSRs selected for peach also produce amplified and polymorphic DNA fragments in the other species.

One of the critical points that will allow the study in depth of the quantitative characters of interest for peach and other fruit trees, such as fruit size, time of maturity, sweetness or skin color, is the possibility of constructing reduced maps quickly and cheaply for several segregating populations that are anchored with common markers in a general map of the genus. The SSR map presented here is a first step towards this goal. With the SSRs of known map position, and considering the 37% of observed heterozygosity estimated for peach by Aranzana et al. (2002), a set of 35–40 heterozygous markers should be found in the average parental genotype which would segregate in its progeny. If these markers were uniformly distributed in the peach genome, one would expect to find a segregating marker every 13–15 cM, enough for acceptable genome coverage. Uniform distribution of the polymorphic markers is, however, very unlikely and, in practice, these results indicate that the number of SSRs should be increased to have a high probability of finding at least one segregating marker in each region of the map. Considering the 24 intervals in which we have divided the *Prunus* genome, at least six markers should be in each of them to ensure that one is heterozygous in the tested genotype with a probability of 0.95. Only nine of the intervals, representing 29% of the genome, include six or more SSRs. A more desirable situation would be to have at least nine markers per interval, which would require adding a minimum of 110 SSRs appropriately distributed in the *Prunus* map but would give a high probability ($P = 0.95$) of having all, or all but one, intervals with at least one heterozygous SSR. For this purpose, targeted strategies using bacterial artificial chromosome clones detected with RFLP probes located in the regions of interest (Cregan et al. 1999a) would be an efficient approach.

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