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I. Verde · M. Lauria · M. T. Dettori · E. Vendramin
C. Balconi · S. Micali · Y. Wang · M. T. Marrazzo
G. Cipriani · H. Hartings · R. Testolin · A. G. Abbott
M. Motto · R. Quarta

Microsatellite and AFLP markers in the *Prunus persica* [L. (Batsch)]×*P. ferganensis* BC_1 linkage map: saturation and coverage improvement

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Abstract A set of 146 single sequence repeats (SSRs) and 14 amplified fragment length polymorphism (AFLP) primer combinations were used to enrich a previously developed linkage map obtained from a (*Prunus persica*×*P. ferganensis*)×*P. persica* BC_1 progeny. Forty-one SSR primer pairs gave polymorphic patterns detecting 42 loci. The restriction/selective primer AFLP combinations produced a total of 79 segregating fragments. The resulting map is composed of 216 loci covering 665 cM with an average distance of 3.1 cM. Novel regions were covered by the newly mapped loci for a total of 159 cM. Eight linkage groups were assembled instead of the earlier 10 as two small groups (G1a and G8b), previously independent, were joined to their respective major groups (G1b and G8a). Several gaps were also reduced resulting in an improved saturation of the map. Twelve gaps ≥10 cm are still present. A comparative analysis against the *Prunus* reference map (71 anchor loci) pointed out an almost complete synteny and colinearity. Six loci were not syntenic and only two were not colinear. Genetic distances were significantly longer in our map than in the reference one.

Keywords Peach · Linkage map · SSR · Synteny · Comparative mapping

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I. Verde (✉) · M. T. Dettori · E. Vendramin
S. Micali · R. Quarta
Istituto Sperimentale per la Frutticoltura (CRA),
Via di Fioranello 52, Ciampino Aeroporto,
00040 Rome, Italy
E-mail: ignazio.verde@entecra.it
Tel.: +39-06-79348150
Fax: +39-06-79340158

M. Lauria · C. Balconi · H. Hartings · M. Motto
Istituto Sperimentale per la Cerealicoltura (CRA),
Via Stezzano 24, 24100, Bergamo, Italy

Y. Wang · A. G. Abbott
Department of Genetics and Biochemistry,
Clemson University, Clemson, SC 29634, USA

M. T. Marrazzo · G. Cipriani · R. Testolin
Dipartimento di Scienze agrarie e ambientali,
Università di Udine, Via delle Scienze 208,
33100, Udine, Italy

Present address: Y. Wang
Department of Plant Breeding, Cornell University,
Ithaca, NY 14853, USA

Introduction

Peach [*Prunus persica* (L.) Batsch] is a temperate fruit species of economic importance. It ranks second in fruit production in Europe after apple. It is a self-fertile diploid species ($2n = 16$), autogamous in nature. Such a reproductive behavior, together with the narrow genetic base of commercial varieties (Scorza et al. 1985), has led to a low variability among the peach germplasm. However, its small genome, almost twice larger than *Arabidopsis* (Baird et al. 1994), self-compatibility and short intergeneration period make the peach a model species for genetic studies.

Several linkage maps, obtained by using molecular markers, are available today in peach (Chaparro et al. 1994; Rajapakse et al. 1995; Dirlewanger et al. 1996, 1998; Lu et al. 1998; Dettori et al. 2001; Foulongne et al. 2003). Linkage maps also have been developed from peach×almond F_2 progenies (Foolad et al. 1995; Joobeur et al. 1998; Bliss et al. 2002). In particular, the 'Texas' (almond)×'Earlygold' (peach) linkage map (T×E), earlier issued by Joobeur et al. (1998) and recently updated by Aranzana et al. (2003) and Dirlewanger et al. (2004a), is currently considered to be the reference map of the *Prunus* genus.

A combination of several molecular markers such as restriction fragment length polymorphisms (RFLPs), random amplified polymorphic DNAs (RAPDs), (AFLPs) and SSRs was used in the above mapping works. Though codominant and highly transferable in different populations and related species, the RFLPs are hampered

by the complex procedure and the extensive labor required. Moreover, the low level of variability (Rajapakse et al. 1995; Dirlewanger et al. 1998; Dettori et al. 2001) has greatly limited their use for assisted breeding in peach. Conversely, RAPDs can be easily obtained through PCR technology, yet their dominant pattern of inheritance and their low degree of reproducibility make them of little use for genetic studies. In this regard AFLPs, which are dominant but more robust as markers and with a higher level of polymorphism (many loci usually detected in a single experiment), appear more attractive. AFLP fragments can be also cloned to obtain codominant STS markers (Lu et al. 1999; Wang et al. 2002a).

SSRs are mostly codominant markers, frequently showing multiple alleles and a high rate of polymorphism. They are mainly single locus markers easily obtainable via standard PCR technology. These features make SSRs the markers of choice for various purposes in plant genetics. Several SSRs have been developed worldwide in *Prunus* species (Cipriani et al. 1999; Sosinski et al. 2000; Testolin et al. 2000, 2004; Aranzana et al. 2002; Dirlewanger et al. 2002; Wang et al. 2002a; Georgi et al. 2003; Verde et al. 2004). The high level of polymorphism reported for some of them in peach segregant progenies (Dettori et al. 2001; Foulongne et al. 2003) can help in saturating genomic regions yet uncovered. Moreover, SSRs being mainly single locus markers, targeted map saturation can be achieved based on microsatellites placed in anchored maps.

In the present work many of the available *Prunus* SSRs as well as AFLP markers were placed on the (*Prunus persica*×*P. ferganensis*)×*P. persica* map (P×F) developed by Dettori et al. (2001) in which 10 QTLs had been already identified (Verde et al. 2002). The addition of new markers allowed to increase map density and to localize previously uncovered regions. A better coverage of the genome can be helpful both for QTL detection and for comparative analyses within the genus.

Materials and methods

Plant materials and DNA extraction

A BC_1 progeny (70 individuals randomly chosen in a population of 297) derived by crossing an accession of *Prunus ferganensis*, as male donor parent, with the peach selection IF7310828, as female recurrent parent, was used as mapping population. The accession of *P. ferganensis*, collected in a natural Chinese population, carried a source of resistance to powdery mildew. Genomic DNA was extracted following the Doyle and Doyle (1990) procedure with the modifications described in Dettori et al. (2001).

Genotyping

Simple sequence repeats

Microsatellites developed by several authors were employed (Table 1). Designations are according to those adopted in the original papers with the exception of the CPPCT series (Aranzana et al. 2002) for which Aranzana et al. (2003) was followed and the UDP series for which the year reference was omitted (e.g., UDP96-001 is now UDP-001). Four SSRs have never been described before (Table 2). They were obtained by Wang et al.

Table 1 SSRs: designation, origin and polymorphism in P×F progeny

SSR name	Species	No. of SSR tested	No. ofSSR segregating	Rate of polymorphism (%)	Reference
BPPCT	Peach	41	18	43.9	Dirlewanger et al. (2002)
CPPCT	Peach	35	7	20.0	Aranzana et al. (2002)
pchgms	Peach	26	7	26.9	Sosinski et al. (2000) Georgi et al. (2003) Wang et al. (2002b) Verde et al. (2004)
UDA	Almond	44	9	20.4	Testolin et al. (2004)
Total		146	41	28.1	

Table 2 SSR markers developed from the Clemson BAC library

SSR name		Primer sequences (5′ → 3′)	Repeat motif	Length (bp)[a]	Ta (°C)
pchgms27	F	GGCTTTGTGTGGTTGAGGTT	$(TTA)_7$	204	55
	R	GCCCAAGTCAACTCGTAAGG			
pchgms28	F	GCGCCATTGTCACAAAATC	$(GA)_{24}$	194	58
	R	CGAGCCATCTGTCAGGTACA			
pchgms29	F	CCTGAAGAAGGTGGACCAGA	$(GA)_{21}$	128	58
	R	CCTCCCAATTCAAATTCCCT			
pchgms41	F	GGAAATTCCCTGTCCTTCCT	$(AG)_9$	217	57
	R	CCTCGAACTAGTTGCCTTTGA			

[a]The length was determined from the sequenced results of the isolated 'Nemared' subclones

(2002b) from the BAC library of 'Nemared' available at Clemson University. PCR reactions were performed according to the procedures described in Dettori et al. (2001). PCR products were separated by electrophoresis in a 3% MetaPhor (Cambrex) agarose gel in 1×TBE buffer (~5 V/cm) and then stained with ethidium bromide. Each microsatellite was tested in the parents and in the F_1 hybrid and only those showing useful polymorphic patterns were analyzed further in the whole BC_1 progeny.

Amplified fragment length polymorphisms

AFLP markers were obtained following the protocol of Vos et al. (1995) with minor modifications. DNA was digested with an *Eco*RI/ *Mse*I enzyme combination. Preamplifications were carried out using standard AFLP *Eco*RI and *Mse*I primers (Vos et al. 1995) containing one selective nucleotide (*Eco*RI + A/ *Mse*I + C or *Eco*RI + C/ *Mse*I + A). Selective amplifications were performed using various combinations of *Eco*RI primers with two or three selective nucleotides and *Mse*I primers with three selective nucleotides (Table 3).

Linkage analysis

Data were analyzed with the JoinMap 3.0 software (Van Ooijen and Voorrips 2001) by using the CP population type. Linkage groups were established at LOD score ≥3 and recombination fraction ≤ 0.4. For the mapping procedure the Rec and LOD thresholds of 0.4 and 0.5 were used, respectively. Markers that increased by three the goodness-of-fit (χ^2) of the map (Jump threshold ≥3) were removed and later added one by one. These loci were forced in the map and tentatively placed only if they did not give rise to major rearrangements. Otherwise, they were not assigned to a specific position within their group, but only listed as belonging to it. Departures from the Mendelian ratio were tested by using the chi-square goodness-of-fit test available in JoinMap 3.0. The Kosambi mapping function was used to convert recombination units into genetic distances.

By using the F_1 hybrid 1:1 segregating markers, a robust framework for each linkage group was obtained with MAPMAKER EXP 3.0 following the same procedure described in Dettori et al. (2001). To minimize the risk of unreliable orders, when JoinMap produced arrangements in conflict with the MAPMAKER framework or with other published anchored maps, the alternative orders were tested as "fixed" in JoinMap and the map with the best goodness-of-fit (χ^2) was accepted. After mapping, the "error detection" command of MAPMAKER and the "genotype probabilities" option of JoinMap were employed and possible errors were rechecked.

Results

Markers

Simple sequence repeats

Screening of 146 primer pairs was performed in the parents of the BC_1 progeny. All but four (UDA-009, UDA-022, UDA-037, UDA-041) gave amplification products. Forty-one were polymorphic (28.1%) and detected 42 loci segregating in the offspring (Table 1). Only one detected two loci while four displayed null alleles (see electronic supplementary Table S1). Remarkable differences in the rate of polymorphism were observed among the different sets of SSRs. The BPPCT set gave the highest rate of polymorphism (43.9%) while the CPPCT, pchgms and UDA sets gave 20.0, 26.9 and 20.4%, respectively (Table 1). Twenty-five loci out of 42 (59.5%) segregated in the F_1 hybrid

Table 3 AFLPs: primer combinations, number of fragments and polymorphism

*Eco*RI/*Mse*I selective nucleotides	No. of total fragments	No. of polymorphic fragments	No. of polymorphic fragments/No. of combinations
CA/AAC	104	7	
CA/AAG	130	14	
CA/AAT	146	11	
CA/ACT	102	10	
CA/ACA	109	13	
CG/ACC	24	4	
Total EcoRI + 2/MseI + 3	615	59	9.8
AAC/CAA	56	5	
ACT/CCA	41	5	
ACT/CCG	15	1	
AAC/CTA	34	1	
ACC/CGA	32	1	
ACC/CCT	33	1	
AAC/CAT	32	4	
ACC/CGC	26	2	
Total EcoRI + 3/MseI + 3	269	20	2.5
Total	884	79	5.6

(Table see electronic supplementary S2), 10 (23.8%) in the recurrent parent, and seven (16.7%) in both parents (six as codominant and one as dominant). Thirty-five loci were surely homozygous in the *P. ferganensis* accession (Table S1). Twenty-five loci were homozygous in the recurrent parent. Considering all the microsatellite primers tested, the rate of homozygosis should be about 97% for the *P. ferganensis* accession and 88% for the peach selection IF7310828. Three (7.1%) microsatellites had skewed segregations ($P < 0.05$).

Twenty-one of the polymorphic microsatellites employed had already been mapped in the T×E reference map (Dirlewanger et al. 2004a) while nine almond SSRs (UDA) were mapped for the first time in *Prunus*.

Amplified fragment length polymorphisms

Fourteen primer combinations were assayed and 79 AFLP markers were detected (5.6 markers per combination ranging from 1 to 14; Table 3). A total of 884 fragments were scored with an average of 63.1 fragments per combination, ranging from 15 (*Eco*RI + ACT/ *Mse*I + CCG) to 146 (*Eco*RI + CA/ *Mse*I + AAT). Remarkable differences in the number of useful polymorphic bands were observed among the combinations. The highest number of polymorphisms (59 bands) was obtained by the *Eco*RI + 2/ *Mse*I + 3 primer combination type (9.8 markers per combination), while the use of three selective nucleotides in both *Eco*RI and *Mse*I primers produced only 20 polymorphic bands (2.5 markers per combination).

Forty-three (54.4%) AFLP fragments in the F_1 hybrid and 15 (19.0%) in the recurrent parent segregated in a 1:1 ratio, while 21 (26.6%) segregated in both parents in a 3:1 ratio (Table S2). Twenty-five loci (31.6%) significantly deviated ($P < 0.05$) from the expected Mendelian ratio.

Linkage map

One hundred and twenty-one newly scored markers were analyzed together with the previous set of 118 loci (Dettori et al. 2001). All but six loci (one AFLP, one SSR, two RFLPs and two RAPDs) were assigned to eight linkage groups. Four RFLPs, previously unmapped, were also placed. Seventeen markers (15 AFLPs, one SSR, one RFLP) were assigned but not ordered. One hundred and seven loci (63 AFLPs, 40 SSRs, four RFLPs) were added to the previous map; they were evenly distributed along the genome.

The resulting map (Fig. 1) comprises 216 loci (78 RFLPs, 63 AFLPs, 57 SSRs, 16 RAPDs and two morphological markers) linked in eight linkage groups. The nomenclature and orientation of each group is given according to previously published *Prunus* linkage maps (Viruel et al. 1995; Joobeur et al. 1998; Dettori et al. 2001). The number of loci per linkage group ranges from 48 in G2 to 16 in G5, with an average of 27. The map covers 665 cM of the peach genome against 521 cM of the previous one. The length of each linkage group ranges from 136 cM of G1 to 65 cM of G4. The average distance between adjacent loci decreases from 4.8 cM to 3.1 cM. The group with the highest density is G2 with an average distance of 1.7 cM per marker, while the less saturated is G5 with an average of 4.3 cM. The map shows a total of 12 gaps from 10 cM to 21 cM in length.

Novel regions of the genome (gray bars in Fig. 1), for a total of 159 cM, were covered by the new set of markers. Thirty-three loci, for a total of 131 cM, were localized in distal genome portions and distributed in all groups but G3. Fifty-five centimorgans were found in G5, which had been previously composed of only four markers covering 16 cM. Two AFLP markers (CA/ ACA7 and AAC/CAA1), adding 28 cM to G1, permitted the joining of G1a and G1b. Seventy-four loci were placed inside the previous frame and allowed saturating the map and merging G8a and G8b.

Rearrangements with respect to the loci mapped in the previous P×F map were observed in few regions of the genome. The more evident was found in G2 where two SSRs, UDP-406 and UDP-410, were relocated at 80 and 83 cM respectively, instead of 38 and 39 cM. Small inversions arose in five more regions (G2, G3, G5, G6 and G7) and involved closely linked loci. The "fixed order" of JoinMap was used in these cases to test the alternative order solutions, which were, however, discarded because of their worst goodness-of-fit. In G1 (between UDP-022 and UDA-031) and in G6 (between PC73 and PC28), JoinMap produced orders in conflict with other maps; however, the map with "fixed order" was retained owing to better chi-square values.

Twenty-eight skewed loci (23.1%) were found within the new set of markers. Seven clustered in G3 in the same distorted region detected in the previous map. All the markers of this group segregated in a 1:1 ratio informative for the F_1 hybrid, and in the skewed cluster the selection was always in favor of the heterozygous individuals and against the homozygotes for the peach allele. Nine skewed loci could not be ordered and the remaining twelve were distributed in G2 (three loci), G4 (four loci), G6 (two loci), G7 (two loci) and G8 (one locus).

Comparative analysis with the *Prunus* reference map

Seventy-one RFLP and SSR markers were in common with the *Prunus* reference map (Joobeur et al. 1998; Aranzana et al. 2003; Dirlewanger et al. 2004a) allowing a comparative analysis. Among them six (AG2, AG8, AG47, AG60, MC011 and CPPCT002) were not syntenic. The loci order was conserved with two exceptions. One was found in G1 and involved the position of locus PC35 with respect to loci PC85, AG36a and pchgms3. Since all these loci segregated in the F_1 hybrid, the log-likelihood could be calculated for the two alternative

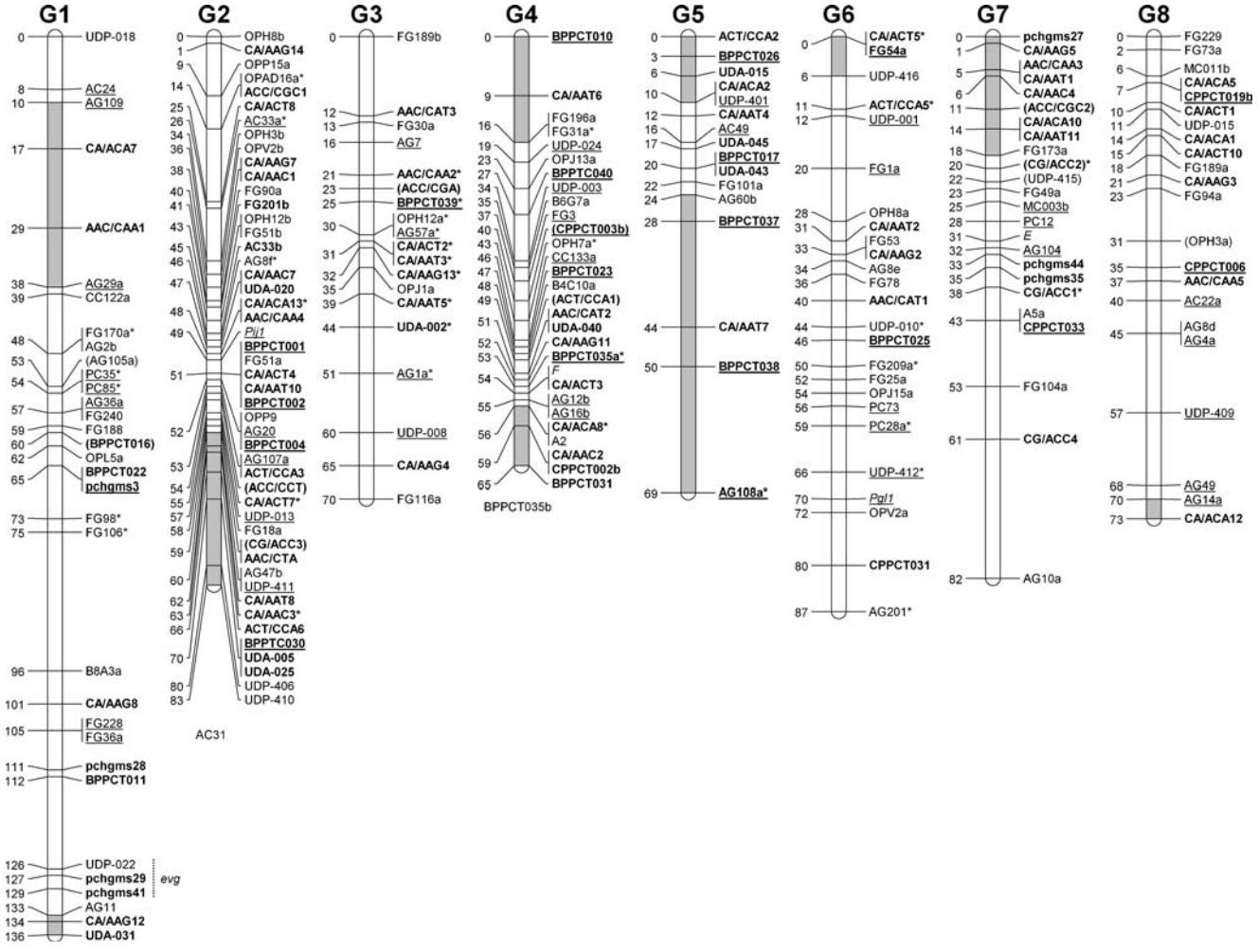

Fig. 1 P×F linkage map. Probes detecting loci others than those found in *Prunus* maps were named with different alphabetical letters (b, c, d, etc.) after the locus name. Loci with an *asterisk* after the locus name had distorted segregations ($P < 0.05$). Loci added in the present work are in *bold* and novel regions are in gray. *Underlined* loci are anchor points with the *Prunus* reference map. Loci in parenthesis were tentatively placed. The approximate position of the evergrowing gene (*evg*) is indicated by a *dotted vertical line*

arrangements using the "try" command of MAPMAKER. The loci order finally established was 1.34×10^8-fold more likely than the T×E alternative (log-likelihood = 8.13). Another exception was found in G6 where in FG209a was located next to BPPCT025, while in T×E it was next to *Pgl1*. Three markers, BPPCT016 and AG105a in G1 and CPPCT003b in G4, are in distinct positions in the two maps. These markers, when added to the framework, caused a "jump" higher than 3; therefore they were tentatively placed on the map but were not considered in the comparative analysis.

Genetic distances spanned by the colinear loci were compared. They amounted to 457 cM (69% of the total length) in the P×F map and to 340.8 cM (66%) in the T×E map. On average, P×F distances were 1.34-fold longer than the T×E distances. Among the linkage groups, this rate ranged from 0.83 for G7 to 1.92 for G3 (Table 4). The paired *t* test was performed considering the two most distant loci in each linkage group. The same test was also applied taking into account all the 46 common segments bounded by two successive colinear anchor loci (data not shown). The results ($t = 3.10$, $P = 1.72 \times 10^{-2}$, $df = 7$; $t = 3.09$, $P = 3.44 \times 10^{-3}$, $df = 45$) revealed significant differences between the two maps. The differences may be underestimated since the two maps were elaborated with different softwares (JoinMap for P×F and MAPMAKER for T×E). In fact, van Ooijen et al. (1994) and Qi et al. (1996) report that JoinMap produces shorter maps than those of MAPMAKER. The framework map elaborated with MAPMAKER (not shown here) contained a subset of 128 loci segregating in a 1:1 ratio in the F_1 hybrid and was used to perform a homogenous comparison. This reduced map covered 822 cM arranged in eight linkage groups corresponding to 86% of the P×F total length. Thirty-six loci of the F_1 hybrid map were in common with the *Prunus* reference map. These anchor loci covered 472 cM (57% of the F_1 hybrid map) against 243.2 cM of the T×E map (47% of the total T×E length).

Table 4 Comparison between distances covered by the most distant common loci in each linkage group in P×F maps, obtained by using JoinMap and MAPMAKER, and in T×E

Group	Segments	P×F distances (cM) JoinMap	T×E distances (cM) MAPMAKER	Differences (cM)	Ratio P×F/T×E
G1	AC24-FG36a	97	60.8	36.2	1.60
G2	AC33a-BPPCT030	44	30.1	13.9	1.46
G3	AG7-UDP-008	44	22.9	21.1	1.92
G4	BPPCT010-AG12b	55	51.7	3.3	1.06
G5	BPPCT026-AG108a	66	41.1	24.9	1.61
G6	FG54a-Pgl1	70	67.3	2.7	1.04
G7	MC003b-CPPCT033	18	21.6	−3.6	0.83
G8	CPPCT019-AG14a	63	45.3	17.7	1.39
Total		457 (69%) MAPMAKER	340.8 (66%) MAPMAKER	116.2	1.34
G1	AG29a-FG36a	84	37.2	46.8	2.26
G2	Pij1-UDP-013	15	8.6	6.4	1.74
G3	AG7-UDP-008	95	22.9	72.1	4.15
G4	BPPCT010-AG12b	77	51.7	25.3	1.49
G5	BPPCT026-AC49	11	10.0	1.0	1.10
G6	UDP-001-UDP-412	95	54.5	40.5	1.74
G7	MC003b-CPPCT033	26	21.6	4.4	1.20
G8	CPPCT019b-UDP-409	69	36.7	32.3	1.88
Total		472 (57%)	243.2 (47%)	228.8	1.94

Thus, genetic distances were 1.94-fold longer in the peach×*P. ferganensis* than in the peach×almond map. As expected, the paired *t* tests, performed considering both eight linkage groups and 23 common segments, revealed significant differences between the two maps ($t = 3.29$, $P = 1.33\times10^{-2}$, $df = 7$, $t = 4.2$, $P = 3.66\times10^{-4}$, $df = 22$).

Discussion

Markers

In agreement with the results observed in other *Prunus* species on the transportability of microsatellite markers across the genus (Dirlewanger et al. 2002; Cipriani et al. 1999; Lambert et al. 2004), as much as 40 (91%) almond SSRs provided amplification in peach. We found a 28.1% rate of polymorphism, a value lower than that observed with the UDP set of SSRs previously tested in the same progeny (Dettori et al. 2001), which amounted to 65.4%. The almond SSRs and the CPPCT set displayed the lowest rate of polymorphism (20.4 and 20.0%, respectively). This result was expected for the UDA primer set since it had been developed from almond. In fact, Ellegren et al. (1997) and Huang et al. (1998) report a lower degree of polymorphism in species different from the one employed for SSR isolation. The CPPCT, like the BPPCT series, had been obtained from a CT enriched library of the peach cv 'O'Henry'. Surprisingly, the rate of polymorphism of the BPPCT markers was twice as much as that of the CPPCT markers. Considering all the 172 SSRs tested in our progeny, we observed a rate of 33.1% polymorphism, a value noticeably lower than that reported in other *Prunus* progenies (Foulongne et al. 2003; Aranzana et al. 2003; Dirlewanger et al. 2004b; Lambert et al. 2004). The lower resolution of agarose in comparison with acrylamide could partly explain this discrepancy. Only four SSR primer pairs displayed null alleles and only two singled out more than one locus in our progeny. These results are consistent with those of Aranzana et al. (2003) and Lambert et al. (2004), confirming the high level of codominance among microsatellites and their nature of mostly single locus markers.

Five of the tested SSRs (pchgms43, pchgms44, pchgms45, pchgms46 and pchgms47) had been isolated previously for covering specific regions of the genome (Verde et al. 2004), by using the BAC library available at Clemson University with the targeted approach described in Wang et al. (2002a). Among them, only pchgms44 was polymorphic and was placed in the targeted region of G7 wherein a major QTL for powdery mildew resistance had been located (Verde et al. 2002). Three more SSRs (pchgms28, pchgms29 and pchgms41), previously isolated from the same BAC library and linked to the evergrowing (*evg*) gene (Wang et al. 2002b), were placed in our map at one end of G1 allowing the localization of this important trait (Fig. 1).

The level of heterozygosis in the donor and recurrent parent was extremely low. The selection IF7310828 was obtained from a cross between 'J.H. Hale'×'Bonanza', the latter variety being a complex hybrid with four different ancestors in its pedigree. In spite of this complex origin, the level of heterozygosis was low (12%), confirming the high inbreeding level of the cultivated peach germplasm (Scorza et al. 1985). *Prunus ferganensis* was homozygous for at least 97% of the SSR loci analyzed.

This result is consistent with the low rate of cross-pollination (5%) reported in peach (Hesse 1975), with the clone of *P. ferganensis* being an accession collected in a natural environment. This high level of homozygosis makes the F_1 progenies of little use for mapping the peach genome: had we used the F_1 progeny as mapping population, only five SSR loci out of 143 in *P. ferganensis* and 17 in the peach selection IF7310828 would have surely segregated.

AFLPs were applied to increase markers density. *Eco*RI + 2/ *Mse*I + 3 combinations gave on average the highest number of polymorphic fragments. This result is in accordance with Dirlewanger et al. (1998). AFLP markers were evenly distributed across the genome, as found in other *Prunus* progenies (Vilanova et al. 2003; Lambert et al. 2004); only one cluster of seven loci was observed at one end of G7. In other plant species, several authors (Castiglioni et al. 1999 and references therein) report that the *Eco*RI/ *Mse*I combinations often cluster around the centromeric regions. Unlike Lambert et al. (2004), we found only two AFLP loci, extending the total map length of 6 cM, at the end of P×F linkage groups.

AFLP markers displayed a proportion of distorted loci four times as much as SSRs. Considering the 239 markers employed, 50 skewed loci (20.9%) were found in our progeny. A cluster of distorted markers was detected only in the central region of G3. No inference about the type of selection (gametic or zygotic) could be done for a BC_1 model since only the homozygotes carrying the peach alleles could be scored. The proportion of distorted loci was low compared to the data report in the literature for interspecific crosses (Jenczewski et al. 1997; Fishman et al. 2001; Schwarz-Sommer et al. 2003). Within *Prunus*, Bliss et al. (2002) and Aranzana et al. (2003) using peach×almond F_2 progenies reported a proportion twice as much as that in our work. In a *P. persica*×*P. davidiana* F_2 progeny, Foulongne et al. (2003) detect 30% of distorted loci. Several genetic mechanisms, always involving selection at the gamete or zygote stage, have been reported for segregation distortions (Fishman et al. 2001). To a certain extent, skewed segregation may be related to the level of divergence between parental genotypes. Grandillo and Tanksley (1996), reviewing the results of several interspecific crosses of *Lycopersicon* spp. reported in the literature, suggest that the rate of distorted segregations increases with the distance between species that are crossed. In this regard, our results suggest the cultivated peach to be more closely related to *P. ferganensis* than to *P. davidiana* and almond.

Linkage map

One hundred and seven loci were added to the former framework. The addition of new markers allowed the reduction of the number of linkage groups to eight, as expected in peach. In particular, G8a and G8b had not been linked previously, even if they have a 12 cM overlapping region (between AG4a and UDP-409), because most of the markers of each group were uniparentally informative. Distal portions of the genome were localized by the new set of loci. The average loci distance decreased in all linkage groups, with the only exception of G5, and the number of average gaps per Morgan was reduced from 2.9 to 1.8. The addition of 74 loci inside the previous frame produced an expansion of only 2 cM. These results give a positive feedback about the reliability of the present map. Owing to its marker density (3.1 cM/locus and 1.8 gaps/M), its genome coverage and the high number of codominant markers (78 RFLPs and 57 SSRs) it can be considered to be one of the most saturated and complete maps in *Prunus* after the T×E one.

Rearrangements of a few loci were observed with respect to the previous map. Two SSRs, UDP-406 and UDP-410, were relocated at the end of G2. These two markers in the previous map had only two useful linkages with a pair of closely linked SSRs (UDP-013 and UDP-411). The new loci provided several linkages helping in a better definition of the location of the above SSRs. Evidences of their current position are given by other *Prunus* maps recently published (Hurtado et al. 2002; Foulongne et al. 2003; Dirlewanger et al. 2004b; Lambert et al. 2004). Other rearrangements of minor entities involved closely linked loci. The low number of seedlings in our progeny, the close linkages among the loci involved and the poor informativeness (i.e. 3:1 segregation ratio) of some of them, may explain these discrepancies.

The "fixed order" option of JoinMap was used to obtain the current order in G1 and G6; these results point out to the fact that JoinMap does not always guarantee the best order solution as reported in literature (Qi et al. 1996):

Comparative analysis

Sixty-one syntenic loci with the *Prunus* reference map allowed a comparative analysis. The distribution of the distal anchor loci in P×F and T×E maps, the latter considered to be complete and saturated (Aranzana et al. 2003; Dirlewanger et al. 2004a), suggests that our map approximately covers the whole *Prunus* genome.

The loci order was almost identical in both maps with two exceptions. One was in G1 and involved the RFLP marker PC35. The same P×F order was found in an apricot map (Lambert et al. 2004). This inversion involved closely linked loci and is likely a mapping artifact due to the low number of individuals in both maps (T×E $n = 82$, P×F $n = 70$) rather than a real chromosome rearrangement. In addition, the severe reduction of recombination observed in the *Prunus* reference map in comparison with P×F in that region (Table 4) may also lead to misestimating the order of closely linked loci (Causse et al. 1994; Lorieux et al. 1995). The second

exception deserves a separate discussion. It involves the marker FG209, which was placed about 20 cM apart its expected position. It seems unlikely that it is a real inversion, as only one marker was relocated. So far, there is no evidence regarding whether it is a mapping artifact or a duplicated locus.

Genetic distances were significantly longer in the P×F map than in the T×E map, up to 1.94-fold in the homogenous comparison. Foulongne et al. (2003) reported a similar result comparing the recombination frequencies of peach×*P. davidiana* and T×E (1.78-fold). Reductions in recombination frequencies are often observed in interspecific mapping populations (Gebhardt et al. 1991; Causse et al. 1994; Chetelat et al. 2000). This is generally related to a decrease in the frequency of chiasmata due to a reduced homology between the DNA strands (Brots and Haber 1987; Dooner and Martínez-Férez 1997). Although both P×F and T×E maps were obtained by using interspecific crosses, the differences observed in the genetic distances and in the proportion of skewed loci confirmed that *P. persica* is more closely related to *P. ferganensis* than to *P. amygdalus*. The same result was achieved by Quarta et al. (2001) using a clustering approach. On the basis of its morphological traits, *P. ferganensis* is considered a close relative of the cultivated peach (Hesse 1975). It offers the best fruit quality among the wild peach species and it is currently cultivated in the Fergana Valley (Uzbekistan) and in Western China (Okie and Rieger 2003). Foulongne et al. (2003) also suggest that peach is more related to *P. davidiana* than to almond and report that Miller (1768) distinguished the subgenus *Amygdalus* and the subgenus *Persica*. Our data would fit better with this classification than with the one currently adopted in which all the mentioned species are grouped in the *Amygdalus* subgenus (Hesse 1975).

A practical consequence of recombination shrinkage in interspecific crosses is the difficulty in breaking linkages when favorable traits are linked to undesirable ones. This could hamper the use of related species for introgressing important genes into the cultivated species. Our results showed that recombination frequencies between peach and *P. ferganensis* are sufficiently high to suggest the potential use of *P. ferganensis* as a source of useful genes in peach breeding programs.

In conclusion, the current linkage map represents a valuable resource for plant breeders working on *Prunus*. Fifty-seven microsatellites are scattered in this map, out of which 27 were not included in the *Prunus* reference map. Nine almond SSRs were mapped for the first time. Owing to the high level of colinearity and synteny among the *Prunus* species (Dirlewanger et al. 2004a), the present map can help in covering the *Prunus* genome with microsatellite markers, as proposed by Aranzana et al. (2003).

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