Theor Appl Genet (2004) 108:1120–1130
DOI 10.1007/s00122-003-1526-3

ORIGINAL PAPER

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Genetic linkage maps of two apricot cultivars (*Prunus armeniaca* L.) compared with the almond Texas × peach Earlygold reference map for *Prunus*

Received: 14 July 2003 / Accepted: 29 October 2003 / Published online: 19 December 2003

Abstract Several genetic linkage maps have been published in recent years on different *Prunus* species suggesting a high level of resemblance among the genomes of these species. One of these maps (Joobeur et al., Theor Appl Genet 97:1034–1041 [(1998); Aranzana et al., Theor Appl Genet 106:819–825 (2002b)] constructed from interspecific almond Texas × peach Earlygold F_2 progeny (T×E) was considered to be saturated. We selected 142 F_1 apricot hybrids obtained from a cross between *P. armeniaca* cvs. Polonais and Stark Early Orange for mapping. Eighty-eight RFLP probes and 20 peach SSR primer pairs used for the 'reference map' were selected to cover the eight linkage groups. One *P. davidiana* and an additional 14 apricot simple sequence repeats (SSRs) were mapped for the F_1 progeny. Eighty-three amplified fragment length polymorphisms were added in order to increase the density of the maps. Separate maps were made for each parent according to the 'double pseudo-testcross' model of analysis. A total of 141 markers were placed on the map of Stark Early Orange, defining a total length of 699 cM, and 110 markers were placed on the map of Polonais, defining a total length of 538 cM. Twenty-one SSRs and 18 restriction placed in the T×E map were heterozygous in both parents (anchor loci), thereby enabling the alignment of the eight homologous linkage groups of each map. Except for 15 markers, most markers present in each linkage group in apricot were aligned with those in T×E map, indicating a high degree of colinearity between the apricot genome and the peach and almond genomes. These results suggest a strong homology of the genomes between these species and probably between *Prunophora* and *Amygdalus* sub-genera.

Communicated by C. Möllers

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Introduction

Apricot belongs to the *Rosaceae* family, subfamily *Prunoidese*, genus *Prunus* L., subgenus *Prunophora* (Neck.) *Focke*, section *Armeniaca* (Rehder 1949). The *Prunus* genus comprises other tree crops of high economical importance in temperate regions, including peach, cherry, almond and plum. In term of economics, apricot is the third most important species of the stone fruit crops with a worldwide production of approximately 2.74 millions tons (FAO 2002). Apricot is diploid ($2n$=16) and has a small genome (5.9×10^8 bp/$2n$) that is about twice the size of that of *Arabidopsis thaliana* and between that of peach (5.3×10^8 bp/$2n$) and cherry (6.8×10^8 bp/$2n$) (Arumuganathan and Earle 1991). Its level of heterozygosity is between that of peach (the lowest) and almond (Byrne and Littleton 1989). Our knowledge of the genetics of several of the *Prunus* species has greatly improved during the last decade with the use of molecular markers, but despite the number of breeding programs apricot is still one of the least genetically characterized species of the genus.

The construction of genetic maps for improving breeding selection in *Prunus* began only a few years ago with an emphasis on peach (Chaparro et al. 1994) due to the difficulties of mapping other outbreeding and heterozygous *Prunus* species. Preliminary results based on comparative mapping in the *Prunus* genus were obtained in an European *Prunus* mapping project (Arús et al. 1994). In recent years several linkage maps of *Prunus* fruit crops based on intra- or interspecific crosses have been developed using various kinds of markers. Among these maps are three in peach (Rajapakse et al. 1995; Dirlewanger et al. 1998; Lu et al. 1998), two in almond (Ballester et al. 1998; Joobeur et al. 1998), one in sour cherry (Wang et al. 1998), one in an interspecific

cross between almond and peach (Joobeur et al. 1998), one between peach and almond (Bliss et al. 2002), one between peach and *P. davidiana* (Dirlewanger et al. 1998) and one between peach and *P. ferganensis* (Dettori et al. 2001).

Apricot diversity has been studied using isozymes (Byrne and Littleton 1989), restriction fragment length polymorphisms (RFLPs) (de Vicente et al., 1998) and amplified fragment length polymorphisms (AFLPs) (Hagen et al. 2002). Two genetic linkage maps of apricot have been published (Hurtado et al. 2002; Vilanova et al. 2003). However these apricot maps, which were constructed mainly with dominant randomly amplified polymorphic DNA (RAPD) and AFLP markers, were not complete. Among the *Prunus* maps presently available, the map of Joobeur et al. (1998) is considered to be saturated. It was constructed in an interspecific almond Texas × peach Earlygold F_2 progeny, with isozymes and RFLP markers obtained with cDNA and genomic probes from five different *Prunus* species and apple (*Malus domestica)*. This map, named the TxE map, has been recently improved by adding 96 microsatellites markers developed in peach and cherry (Aranzana et al. 2002b). When comparing the maps of peach, almond, *P. ferganensis* and *P. davidiana*, a high level of synteny was observed. The alignment of markers common to all maps was nearly identical, suggesting a high level of resemblance among the genomes of these species. Similar results were obtained by Dirlewanger et al. (2002b) using simple sequence repeat (SSR) markers for comparison between sweet cherry and other *Prunus* species.

Taking these results into consideration, we hypothesized that the alignment of markers would also be conserved between apricot (*Prunus armeniaca*, sub-genus *Prunophora,* section *Armeniaca*) and the other *Prunus* species, especially almond (*P. dulcis*, sub-genus *Amygdalus*) and peach (*P. persica*, sub-genus *Amygdalus*), and we considered the map of Joobeur et al. (1998) as a standard for constructing both apricot maps. To test this hypothesis we selected a subset of polymorphic segregating markers among those used for constructing the T×E map of Joobeur et al. (1998) and those added by Aranzana et al. (2002b) to cover the whole genome of apricot at regular intervals. We paid special attention to SSRs, which are mostly multi-allelic co-dominant markers, to provide anchor loci useful for comparisons between both the apricot maps and between the apricot maps and almond × peach map. Additional AFLP markers were added to improve the density of the apricot maps. We report here the results and conclusions of this mapping strategy as applied to apricot.

Materials and methods

Plant material

The mapping population was a controlled intraspecific cross between *Prunus armeniaca* (apricot) cv. Polonais, a French self-compatible cultivar (accession no. A1352), as the female parent, and cv. Stark Early Orange, a North American self-incompatible cultivar (accession no. A1145), as the source of pollen. In order to avoid accidental self-pollinations, Polonais was castrated previous to pollination, and progeny were checked for conformity during the mapping stage. Two hundred and fifty seedlings were obtained at INRA-Avignon. These were planted in the experimental fields of INRA-Domaine de Gotheron and maintained in the field under standard cultural conditions. This population segregates for resistance to 'plum pox virus' (PPV), *Xanthomonas prunii*, *Agrobacterium tumefaciens* as well as architectural, floral biology and fruit quality traits.

DNA isolation

Samples (20–30 g) of young expanded terminal leaves from both parents and the first 142 hybrids were collected in April 2000 and kept at –80°C until DNA isolation. About 15 g of leaf material was later ground in a mortar in the presence of liquid nitrogen. Genomic DNA isolation was then performed following the protocol of Bernatzky and Tanksley (1986b). DNA concentrations were measured using a spectrophotometer (UV-Visible, Beckman DU 530), checked on 1% TAE agarose gels and diluted to a final concentration of 250 ng μl^{-1}.

Genotyping

Three types of molecular markers (RFLPs, SSRs and AFLPs) were used to construct the linkage maps.

RFLP analysis

Aliquots (5 μg) of DNA from each cultivar and hybrid were digested separately with five restriction enzymes—*Bam*HI, *Dra*I, *Eco*RI, *Eco*RV, and *Hin*dIII—using 5 U of enzyme per microgram DNA. DNA fragments were then separated on 0.8% agarose gels run in NEB buffer (neutral electrophoresis buffer) (Bernatzky and Tanksley (1986a) for 17 h at 1 V cm^{-1}, vacuum blotted on positively charged nylon membrane Hybond N^+ (Amersham Pharmacia Biotech, Piscataway, N.J.) and kept dry. Two hundred and thirteen probes from genomic and cDNA libraries of different *Prunus* species were obtained from several laboratories involved in the European *Prunus* mapping project (Arús et al. 1994). The origin and terminology of the probes are given in Table 1. Following the screening of the selected DNA probes, those that revealed segregating RFLPs were labeled with α-[^{32}P]dCTP and the mapping blots hybridized at 55°C for a minimum of 18 h (Bernatzky and Tanksley 1986a). The membranes were then exposed to an X-ray film (Kodak X-Omat) for 1–4 days.

SSR analysis

Sixty-two peach [*Prunus persica* (L.)], seven sour cherry and one *P. davidiana* SSR primer combinations were tested for optimal annealing temperature using a Robocycler Gradient PCR (Stratagene, La Jolla, Calif.). Five genomic and nine EST (expressed sequence tag) apricot SSR primer pairs were added. (Tables 2, 3). PCR products were run on 1.5% agarose gels to verify amplification and select the best annealing temperature. Successfully amplified microsatellites were screened for both parents and a set of seven hybrids of the mapping progeny in order to establish segregation patterns. Segregating SSRs were then studied in the whole mapping population. They were PCR-amplified in a volume of 15 μl containing 10 m*M* Tris-HCl pH 9, 50 m*M* KCl, 0.1% of Triton X100, 1.5 m*M* MgCl2, 0.2 m*M* each dNTP, 0.25 U *Taq* DNA polymerase (Promega, Madison, Wis.), 30 ng of genomic DNA, 0.2 μM of the first primer and 34 p*M* of the second primer end-labeled with γ-[^{33}P]ATP using T4 polynucleotide kinase. PCRs

Table 1 Origin of the probes used for RFLP mapping in the apricot (*Prunus armeniaca*) population. The loci detected accumulated in apricot cvs. Polonais and Stark Early Orange

Probe origin	Terminology	Probes mapped	Loci detected	Anchor Loci	Source of probes
Almond genomic	AG	30	52	5	IRTA-Cabrils (Spain)
Almond cDNA	AC	11	14	4	IRTA-Cabrils (Spain)
Peach cDNA	PC	16	20	1	INRA-Bordeaux (France)
Prunus ferganensis genomic	FG	8	13	2	ISF-Rome (Italy)
Cherry cDNA	CC	17	24	4	HRI-East Malling (UK)
Almond—known genes	-	4	5	1	CSIC-Barcelona (Spain)
Apricot—known genes	-	2	3	1	INRA-Avignon (France)
Total		88	131	18	

Table 2 Origin of the microsatellites used for mapping in the apricot (*P. armeniaca*) population. Loci detected accumulated in cvs. Polonais and Stark Early Orange

Source species	Library	Terminology of primer	Number. of SSRs	Number of loci detected	Number of anchor loci	Source of SSRs
Peach	Genomic	pchgms	3	5	2	Sosinski et al. (2000)
Peach	cDNA	pchcms	1	1	-	Sosinski et al. (2000)
Peach	Genomic	UDP	4	6	2	Cipriani et al. (1999)
Peach	Genomic	UDP	4	7	3	Testolin et al. (2000)
Peach	Genomic	BPPCT	7	11	4	Dirlewanger et al. (2002a)
Peach	Genomic	CPPCT	1	2	1	Aranzana et al. (2002a
Apricot	cDNA	Pac	9	16	7	Decroocq et al. (2003)
Apricot	Genomic	AMPA	5	7	2	L.S. Hagen et al., in preparation
Prunus davidiana	cDNA	Pdav	1	2	-	V. Decroocq (personal communication)
Total			35	57	21	

Table 3 cDNA SSRs primer sequences (5′→3′) developed in apricot (*P. armeniaca*) and *Prunus davidiana*

SSR	Forward primer	Reverse primer
PacA10	tgagcataattggggcag	gccagagaagccatttcagt
PacA18	tccaaacctaccgtttctcat	caacagcacaaacagaaccac
PacA33	tcagtctcatcctgcatacg	catgtggctcaaggatcaaa
PacB22	aaccagttgcctctagattttg	agctgaaagtcaattcagagtagtt
PacB26	ccaatcatgaaatcataaagcaa	tgggatgtcctattgttttca
PacB35	attgcgatttcggtctgtt	ccatcccaaattgcttactt
PacC3	tgacttgatcagactcgaca	ttgcatttgcatttacaataga
PacC25	gtgttttgacaagaaatgaattg	tccattcgcagtaaaattaaac
PacA58	gacattgcgatttcggtc	tccatcccaaattgcttact
PdavW3	gagggctggatcatgacg	aacccagtggcacaatcgta

Table 4 Primer combinations used in AFLP analysis: digest and selective nucleotides

*Eco*RI	*Mse*I	Designation
AAC	CAC	E32M48
AAG	CGA	E33M55
ATA	CCA	E43M51
ATG	CCC	E45M52
ATA	CCC	E43M52
AGC	CGA	E40M55
AAA	AA	E31M11
AAC	AC	E32M12
ACG	AG	E37M13

were performed in an MJR PTC-100 thermal cycler (MJ Research, Waltham, Mass.) under the following conditions: an initial denaturation for 3 min 30 s at 95°C, followed by 30 cycles of 1 min at 94°C, 1 min at 49–57°C, 30 s at 72°C and a final extension of 5 min at 72°C. The PCR products were first denaturated by the addition of 1 volume of tracking dye solution (98% formamide, 10 m*M* EDTA, 0.005% bromophenol blue and xylene cyanol) and heated for 3 min at 95°C and then visualized by electrophoresis on a 6% polyacrylamide, 7.5 *M* urea sequencing gel (Sequagel-National diagnostics) for 2.5 h at 70 W with 1× TBE after a pre-run for 20 min at 80 W.

Following electrophoresis, the gel was dried and exposed to an X-ray film (Kodak X-Omat) for 1–4 days.

AFLP analysis

AFLP analysis was performed as described by Vos et al. (1995). Two different pre-selective PCR amplifications were performed: one using *Eco*RI+A (E01) and *Mse*I+C (M02) primers; the second one using *Eco*RI+A (E01) and *Mse*I+A (M01) primers.

Selective amplification reactions were performed with two primers having the same sequences as the couple E01/M01 or the couple E01/M02, respectively, but with one to two additional selective nucleotide(s) at the 3′ end of the *Mse*I primer and two additional ones for *Eco*RI (Table 4). AFLP markers were designated by the name of the two primers (e.g. E33M55) used to amplify the DNA, followed by the molecular size (the number of nucleotides of the amplification product as estimated from the mobility in the gel compared to a size standard).

Scoring of markers

Mapping data were obtained by visual scoring of the banding pattern twice independently. Only unambiguous, distinct bands were scored. Conflicting readings were re-examined and eliminated in the case of disagreement. Markers heterozygous in one parent and those monomorphic heterozygous in both parents (dominant

markers) were scored for presence or absence of the band considered. Polymorphic markers heterozygous in both parents (bridge-markers) were scored according to the standard coding system described in Maliepaard et al. (1997) using different characters to denote different alleles of a marker, including 'A', 'B', 'C', 'D', and '0' for a null allele.

Preparation of data files

Markers obtained with the RFLP, SSR and AFLP procedures were separated into four types: (1) those heterozygous segregating for the female parent; (2) those heterozygous segregating for the male parent; (3) those polymorphic segregating in the progeny for both parents (bridge-markers); (4) those monomorphic in both parents but showing segregation in the progeny. Only markers (1) and (2) that segregated in a 1:1 (present:absent) ratio and bridge-markers (3) that segregated in a 1:1:1:1 ratio were kept for being included into the mapping files. Markers (3) were beforehand converted into 1:1 markers according to the parental origin of the alleles. All of the markers used for mapping were then maintained as two separate datasets: one consisting of those markers only segregating in cv. Polonais; the other consisting of only those segregating in cv. Stark Early Orange. Bridge-markers were added to each parental data file, and a mirror of the scored markers was added into the datasets for internal phase determination at each locus during mapping analysis.

Chi-square tests of goodness-of-fit were performed on segregation data for all markers to determine their agreement with the expected ratios.

Linkage analysis and map construction

Separate genetic linkage maps were constructed for each parent following the "double pseudo-testcross" model of analysis (Grattapaglia and Sederoff 1994). Linkage analysis was performed using MAPMAKER/EXP 3.0 software (Lincoln et al. 1992) for the construction of both parental maps. Markers were first divided into linkage groups using a critical LOD score threshold of 5.0 and an arbitrary maximum recombination fraction of 0.35. A subset of high-confidence core markers was then selected at LOD=10.0. Markers remaining unlinked at this threshold and markers departing from the Mendelian ratio at the α=0.05 level of confidence were checked and added one by one into the framework map. Marker distances were calculated using the Kosambi mapping function (Kosambi 1944). Loci polymorphic heterozygous in both parents were used as anchor-loci for the alignment of the maps. After mapping, the original scorings for single loci flanked by double crossovers were re-examined.

Results

RFLP analysis

A total of 180 *Prunus* DNA probes selected out of 213 probes mapped in T×E were screened for polymorphism and segregation on the sorting blots: 144 were polymorphic (80%) with at least one of the five restriction enzymes. Sixty among these (42%) gave a simple pattern consisting of two to four bands. An average of six bands was obtained. One hundred and ten probes (61% of the total) generated RFLPs segregating in cvs. Polonais or Stark Early Orange or in both parents, of which 88 were mapped on the whole population after considering their distribution in the 'reference map' to obtain an optimal coverage of the genome. They revealed a total of 131 loci.

Table 5 Probes mapping at a different locus in almond Texan (T) × peach Earlygold (E) than in apricot cvs. Polonais (P) and/or Stark Early Orange (S). These loci correspond to duplicated regions of the genome or small gene families

Probe[a]	Linkage group		
	P	S	T×E
AC33	1, 2	2	2
CC12	-	3, 6	3
CC2	1, 3	-	3
PC6	8	4	4
AG25	1	5	5
CC132	6	7	7
AG2	1	8	8
PC29	6	8	6
FG42*	6	6	7
CC131*	1	-	2, 8
CC52*	2	2	4
PC60*	2	-	6
PC21*	-	8	6

[a] Probes mapping in apricot at a locus with no counterpart in T×E are denoted with an asterisk

A single locus was detected for most. Thirty-eight were heterozygous in Polonais, and 45 heterozygous in Stark Early Orange, with a segregation ratio of 1:1. Forty-eight loci (37%) were heterozygous for both parents, 18 segregated 1:1:1:1, three segregated 1:2:1 and 27 segregated 3:1. Among these 131 loci, only those segregating 1:1:1:1 and 1:1 were placed on the maps making a total of 101 loci (119 accumulated in both maps). Twenty-nine probes (33%), generated markers detecting two or more loci in apricot, including those segregating 1:2:1 and 3:1. Among these, eight probes generated duplicate markers mapping two loci in apricot instead of one in the reference map (Table 5). Five RFLPs were not syntenic, revealing a locus in a different linkage group than in the T×E map (Table 5).

SSR analysis

Eighty-five primer combinations from different sources were tested for polymorphism. Eight primer pairs gave no amplification product in apricot (9%), four (5%) produced only weak bands and 63 were polymorphic (74%). Segregation was demonstrated for 56 of them (66%), among which 23 were heterozygous for a single parent, segregating 1:1, and 33 heterozygous for both parents, segregating 1:1:1:1. Two loci were detected with four of the primer combinations (7%) including *P. davidiana*.

Twenty peach primer pairs were selected for mapping using the same criteria as for RFLP. One *P. davidiana* and 14 apricot primer combinations were kept for mapping. In total, 36 SSR loci were included in the mapping data (Table 2): nine were heterozygous in Stark Early Orange, six heterozygous in Polonais and 21 heterozygous for both parents (60%). A total of 30 SSR markers were mapped in Stark Early Orange and 27 in Polonais, among which 21 allowed both parental maps to be anchored to

each other and to the T×E map. The *P. davidiana* primer pair (PdavW3) detected two loci placed onto different linkage groups in Polonais (LG1) and Stark Early Orange (LG8). One peach SSR locus (CPPCT004C) heterozygous in both parents was not syntenic and placed at a single locus in LG6 in apricot. Another peach SSR locus (UDP98-406) was syntenic compared to the map of Dettori et al. (2001) but was not placed in the T×E map. E. Dirlewanger (personal communication) reported a similar position in LG2 of *Prunus cerasifera*.

AFLP analysis

The optimum number of additional primer bases was first investigated on the two parents and a subset of ten hybrids before being applied to the entire family in order to obtain the maximum number of clearly scoreable bands. Only highly reproducible markers with a strong intensity were kept for mapping. The AFLP markers were developed using two types of selective primer combinations for the reactions. Using Eco adaptator+3/Mse adaptator+3, an average of 29 distinct bands out of 59 were amplified, and 2–13 polymorphic segregating AFLP markers were detected per primer combination. Ten primer combinations were tested and six combinations kept for mapping. Using Eco adaptator+3/Mse adaptator+2 nucleotides, an average of 79 legible bands out of 110 were amplified, and 7–24 polymorphic segregating AFLP markers identified per reaction. Five primer combinations were tested and three kept for mapping (Table 4). A total of 411 bands were scored: 112 (27.3%) were polymorphic, among which ten were not segregating; 102 loci (24.8%) were heterozygous in Stark Early Orange and 77 (18.8%) in Polonais, among which 38 markers were monomorphic segregating 3:1 in both parents. Fourteen AFLPs were dropped from the dataset because of ambiguities during the scoring step or because they were revealed to be alleles of markers already scored. A total of 88 AFLP loci segregating 1:1 were included in the dataset (34 in Polonais and 54 in Stark Early Orange).

Inheritance and map construction

Linkage analysis was established at a LOD score of 10.0 to select a sub-set of high-confidence markers corresponding to the core map of each parent. Eight linkage groups were obtained for each parent. Only six markers were not linked in Stark Early Orange and four in Polonais. At a LOD score of 5.0 for Stark Early Orange and 6.0 for Polonais all of the markers were linked. Five AFLPs were discarded during the mapping stage because discrepancies were found between the original distances using RFLPs and SSRs and the final distances following integration of the AFLPs. Twenty-one SSRs and 18 RFLPs were mapped in both apricot maps, thereby enabling the alignment of the eight homologous linkage groups (anchor-loci). A strict correspondence between the eight linkage groups in each apricot map and T×E could be established, which allowed us to use the same terminology for linkage group numbers as Joobeur et al. (1998). A total of 251 markers corresponding to 203 different loci were distributed in both maps. One hundred and forty-one markers (131 loci) were placed in the map of Stark Early Orange, defining a total distance of 699 cM. The average number of loci per linkage group was 16, with a minimum of 12 in LGs 6 and 8 and a maximum of 22 in LG2. The average distance between adjacent loci was 5.3 cM. The maximum distance between two adjacent markers was 31 cM in LG1. Only six intervals were longer than 15 cM—three were in LG1. One hundred ten markers (102 loci) were placed in the map of Polonais, defining a length of 538 cM. The average number of loci per linkage group was 13, with a minimum of nine for LG8, and a maximum of 22 for LG1. The average distance between adjacent loci was also 5.3 cM, with a maximum of 33 cM in LG7. We observed only six intervals longer than 15 cM—these were distributed in six of the eight linkage groups. However, the density of markers was irregular among linkage groups since it ranged from 3.6 cM in LG2 to 8.6 cM in LG1 of Stark Early Orange, and from 4.1 cM in LG4 to 6.8 cM in LG3 of Polonais. Sixteen markers (11.3%) deviated significantly from the expected segregation ratio in Stark Early Orange: ten markers at a threshold of 1% and six markers at a threshold of 0.1%; nine of these were located in LG6 (Fig. 1). No segregation distortion was observed at the 5% threshold of confidence in the map of Polonais.

Map length

The map length of Polonais was 538 cM and that of Stark Early Orange, 699 cM (Fig. 1). Fifty-eight loci were common between Polonais and T×E, 57 between Stark Early Orange and T×E and 39 between Polonais and Stark Early Orange, which enabled us to compare the maps. Distances between markers were generally longer in the apricot maps than those between the homologous markers in T×E (Table 6). The intervals in Polonais were, on average, 12% longer than those in T×E, and the intervals in Stark Early Orange were, on average, 33% longer in LGs 2–8 and 80% longer in LG1 than the homologous intervals in T×E. The ratios were calculated by comparing the loci placed in the apricot maps with the homologous ones in T×E. The length of Stark Early Orange and Polonais corresponded to 93% and 98% of the map length estimated for T×E, respectively. The number of loci distributed onto the map of Stark Early Orange was 30% greater than that on Polonais, with a similar average density for most of the groups except LG1. LG1 was about 50% longer in Stark Early Orange than in Polonais, with a density of markers twofold lower than that in Polonais (8.6 cM vs. 4.7 cM, respectively). This density could be correlated to the level of heterozygosity observed; of all the linkage groups the level of hetero-

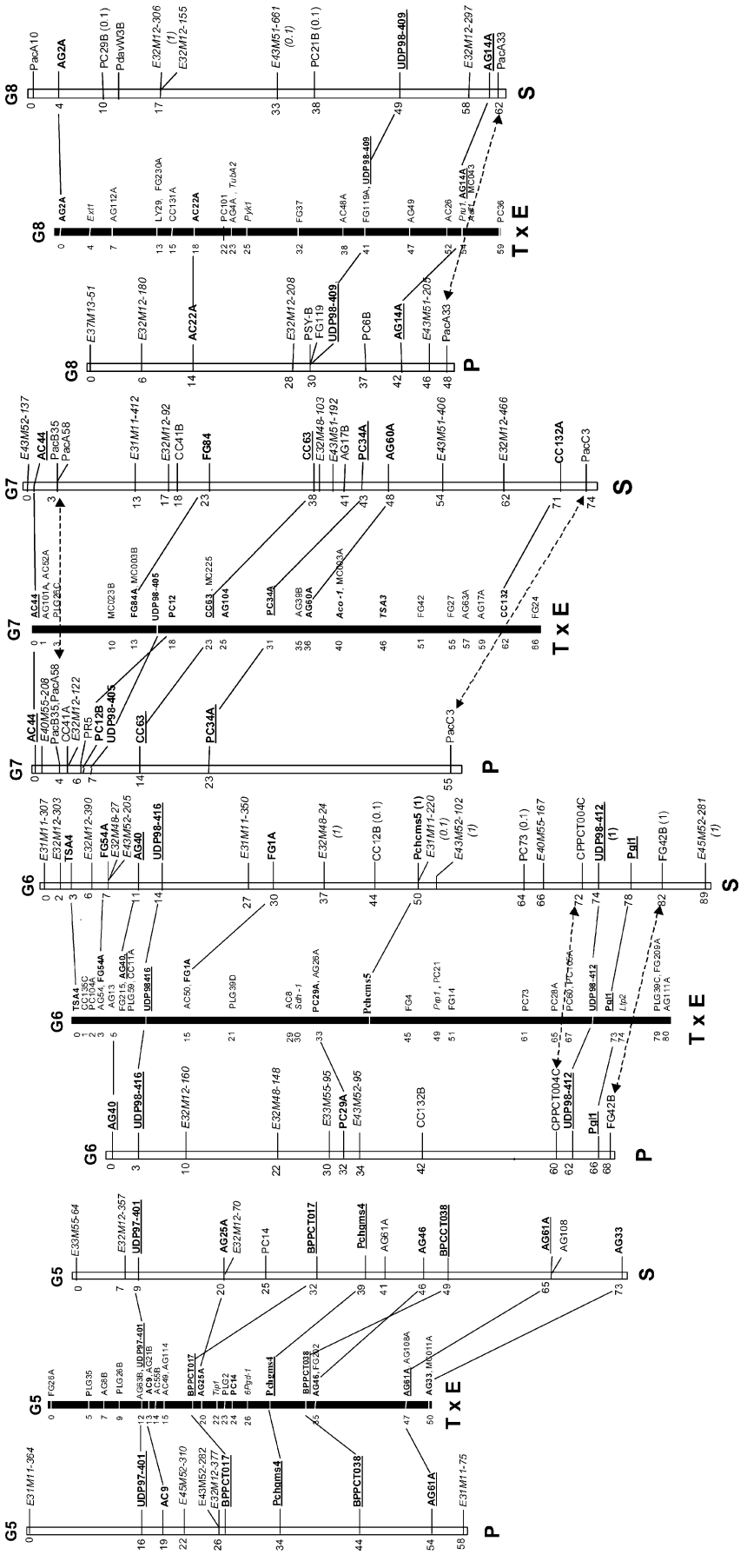

Fig. 1 Genetic maps obtained with the Polonais (*P*) × Stark Early Orange (*S*) (P×S) progeny compared to that of Texas × Earlygold (*T*×*E*) almond × peach. Linkage groups (*G*) have been labeled using the same terminology as that used for the T×E map. Probes or SSRs generating markers that could be mapped at more than one locus are designated with an *A*, *B* or *C* *following* the locus name, even if only one has been mapped in P×S. Loci designated *bold type* are present in T×E and Polonais or T×E and Stark Early Orange. Anchor-loci between T×E and both apricot maps are *underlined*. Anchor-loci between Polonais and Stark Early Orange without any corresponding loci in the T×E map are connected by a *dotted line*. AFLPs are in *italics*. Distorted loci at the level of 1% are denoted with a *1* following the name, and those at the 0.1% level by *0.1*

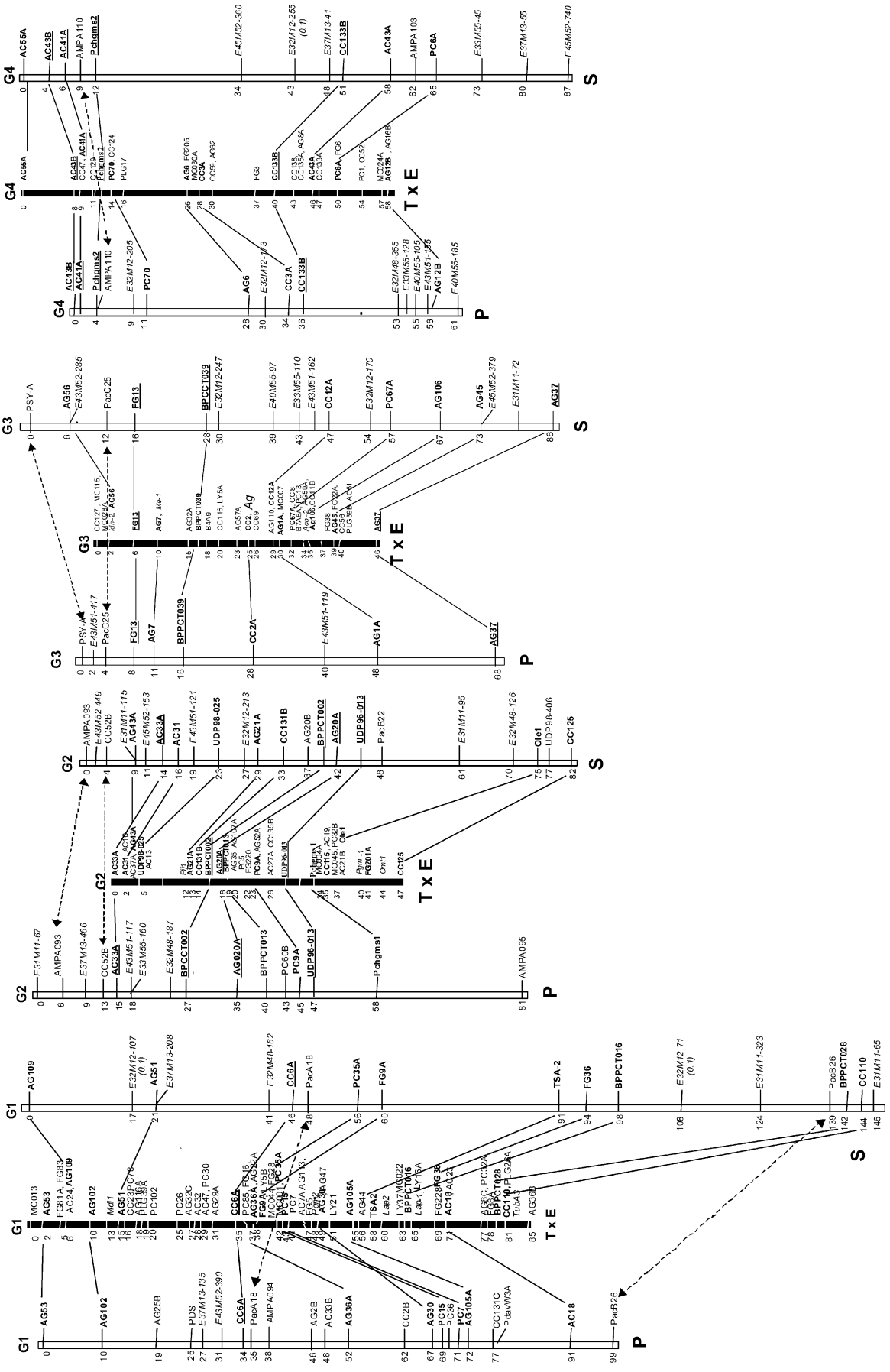

Fig. 1 continued

Table 6 Distance comparison between homologous fragments of the linkage groups of the intra-specific apricot maps of Polonais (P) and Stark Early Orange (S) and the almond × peach map of T×E

Linkage group	P/T×E		S/T×E		P/S	
	Interval	Difference	Interval	Difference	Interval	Difference
LG1	AG53-AC18	22	AG109-CC110	69	CC6-PacB26	−29
LG2	AC33B-Pchgms1	10	AG43A-CC125	28	UDP96–013-ParVG1	−5
LG3	FG13-AG37	20	AG56-AG37	36	PsyB-AG37C	−18
LG4	AC43-AG12	7	AC55A-PC6A	15	AC43B-CC133B	−11
LG5	UDP97-401-AG61A	3	UDP97-401-AG33	26	UDP97-401-AG61A	−18
LG6	AG40-Pgl1	−1	Tsa4-Pgl1	2	AG40-FG42	−7
LG7	AC44-PC34A	−8	AC44-CC132	8	AC44-PacC3	−17
LG8	AC22A-AG14A	−8	AG2A-AG14A	3	UDP98-409-PacA33	5
Total		45		187		−100

zygosity was lowest in LG1 of Stark Early Orange. Distances in Polonais and Stark Early Orange were compared for all linkage groups with those in T×E. The comparisons involved most of the distance of each map: 75% of Polonais (P) and 73% of T×E for P/T×E, 89% of Stark Early Orange (S) and 89% of T×E for S/T×E, 71% of Polonais and 70% of Stark Early Orange for P/S (Table 6). A paired *t*-test did not detect significant difference between the distance of Polonais and that of T×E (t=0.21), but the difference was significant between Stark Early Orange and T×E and between Polonais and Stark Early Orange (t=0.02 and t=0.01, respectively).

Discussion

Markers

Our mapping strategy was mainly based on RFLP markers from the reference *Prunus* map. The heterologous probes tested represented the three *Prunus* subgenera (*Prunophora*, *Amygdalus* and *Cerasus*). They gave very satisfactory results in term of hybridization response, indicating a high degree of homology with apricot DNA. Of the probes mapped, 33% detected two loci or more in apricot. This was observed with cDNA probes as well as genomic probes, suggesting duplicated regions of the genome or multiple loci. A portion of the additional monomorphic bands revealed by the probes might be heterozygous in one parent and not segregating due to the features of the F_1 progeny. Consequently, additional loci could potentially be detected in other apricot progeny. By adding the results obtained when screening the probes on five additional apricot cultivars (results not shown), we estimated that 42% of the probes would detect two loci or more. These results agree with those of Joobeur et al. (1998). These researchers found that about 90% of the probes detected a single polymorphic locus (69% in apricot), but that, if the additional monomorphic bands were considered, then 43% of the total probes detected more than one locus in peach and almond and that genomic probes detected the additional loci more frequently than cDNA clones (50% of the probes vs. 38%, respectively). We found a similar difference between genomic and cDNA probes in apricot (50.3% and 33%, respectively).

In the preliminary experiments, 64% of the probes used for screening revealed a banding pattern made up of six bands or more. Considering that 61% of the probes mapped in apricot gave analog results, with banding patterns containing monomorphous markers along with those segregating, this observation was compatible with the detection of small gene families. This may be a consequence of ancient polyploidy in *Prunus*. By combining the mapping data obtained with different progenies, it would be possible to detect the position of all loci identified by a given probe. This information would make it possible to identify duplicate regions in apricot and other *Prunus* species. Single-copy polymorphic RFLPs would thus be preferable markers for comparative mapping and aligning maps.

A total of 88.6% of the *Prunus* SSR primer pairs mapped in T×E by Aranzana et al. (2002b) gave an amplification product. This rate increased to 90.3% for peach primer pairs. These results are in agreement with those observed on apricot (92.7%) by Dirlewanger et al. (2002a) when testing peach microsatellite primer combinations in different *Prunus* species, including apricot. Conservation of the flanking region on both sides of the repeats is very high in *Prunus*, particularly between *Amygdalus* and *Prunophora* sub-genera demonstrating a high transportability of SSR loci. Cipriani et al. (1999) reported a similar transportability from peach to different species of *Prunus*. Nevertheless, in our study, although 70% of the peach primer pairs generated polymorphic loci, only 53.2% of them were segregating, despite the higher level of heterozygosity in apricot than in peach. This rate is lower than that attained with RFLPs (61%). Our results may be the consequence of short microsatellites. Aranzana et al. (2002b) reported a higher level of segregating polymorphism (80%) with SSR markers in T×E. Their results demonstrate that the cross-species transportability criterion is not sufficient to validate the discrimination capability of a given SSR and does not provide much information on the level of polymorphism that could be obtained in a given species. In addition, one SSR locus (CPPCT004C) was not syntenic (Fig. 1). This primer combination revealed two loci in T×E (Aranzana

et al. 2002b): one in LG5 (CPPCT004A) and the other in LG1 (CPPCT004B). The pattern of the bands observed in apricot was clearly compatible with the presence of two loci, one monomorphic and the other polymorphic heterozygous for both parents. Another possibility is the presence of one or more null alleles, suggesting the possibility of three loci in *Prunus* species. Aranzana et al. (2002b) observed that about 7% of the SSR pairs detected more than one locus. We found similar results in apricot (7%). However, it is important to note that the annealing temperature used for the apricot PCR amplifications was lower than that used in peach and almond and that the fragments amplified may not be those anticipated. This could be a major drawback in comparative mapping across sub-genera.

One major advantage of SSRs is that they generate double heterozygous markers that allow homologous linkage groups to be aligned through the use of 'anchor-loci'. Sixty percent of the polymorphic SSRs (59.5% for peach primer pairs) corresponded to this type of loci. They were fully informative, detecting three or four alleles segregating 1:1:1:1 for both parents at a given locus. This proportion increased to 64.3% when only the SSRs developed in apricot were taken into consideration, and 78% for those developed only from an apricot cDNA library. However, Decroocq et al. (2003) found that the optimal utility of the apricot EST-SSRs was attained for closely related species belonging to the same subgenus, *Prunophora*. These results suggest that SSR primer pairs, particularly EST-SSRs issued from a given species, have a better discrimination capability inside the same species than those elaborated in another species, even of the same genus. However, the differences observed in apricot between genomic and cDNA-SSRs need to be demonstrated on a larger dataset. When compared to the results obtained in apricot with RFLPs, where 20.5% of the probes gave fully informative anchor-loci (23.9% including those 1:2:1), SSR markers, preferably single-locus SSR markers, appeared to be the best available choice for merging maps and linking homologous linkage groups in maps constructed in different progenies.

In our study, the AFLP technique was chosen as the means to easily increase the density of markers. AFLP markers have been shown to be reproducible, reliable, locus-specific and efficient markers for rapidly increasing the coverage of genetic maps (Waugh et al. 1997). The proportion of distorted ratio fragments (10.2%) was higher than that observed with the other techniques (5% for RFLPs and 5.7% for SSRs) but lower than that observed by Hurtado et al. (2002) in apricot (13%). Several publications on genetic linkage maps have reported that AFLPs using the *Eco*RI/*Mse*I restriction enzyme combination tend to cluster around centromeric regions (see Castiglioni et al. 1999). In agreement with the results of Vilanova et al. (2003), we did not observe this clustering in our study and AFLP distribution was rather uniform. This observation could suggest that centromeric regions of apricot have relatively fewer repeats compared to both the rest of the genome and to other crops. Nevertheless, by adding AFLPs to the core map obtained using RFLP and SSR markers, we generated maps that are comparatively longer than maps generated with SSRs and RFLPs alone. This increase in the total length was mainly caused by the addition of markers to regions near the edge of some groups (See Fig. 1: LG2 in Polonais, LGs 4 and 5 in Polonais and Stark Early Orange, LG6 in Stark Early Orange) where RFLPs were poorly represented. This location could thus allow the mapping of regions generally poor in coding regions.

Heterozygosity and origin of cultivars

The number of markers positioned in Stark Early Orange was 30% greater than in Polonais. This was correlated to the level of heterozygosity detected in Stark Early Orange and Polonais with AFLPs (24.6% and 18.9%, respectively) and RFLPs (27% and 21.1%, respectively). The difference in heterozygosity observed with both techniques is in agreement with the known geographical origin of the cultivars. As reported by Hagen et al. (2002), Stark Early Orange belongs to the Diversification group that includes cultivars from former USSR and Central Asia. These are characterized by a large variability for most agronomical traits, heavy chill requirements and late flowering. Polonais belongs to the Mediterranean Basin group, which includes cultivars from Turkey, southern Europe and northern Africa. Their genetic base is reported to be much narrower than that of the Diversification group. An additional cause of inbreeding and reduced heterozygosity in Polonais is its self-compatibility in contrary to Stark Early Orange. A similar result was obtained in the almond map Ferragnès × Tuono (Viruel et al. 1995). In addition to the observed heterozygosity, the polymorphism detected was higher than that obtained by Dirlewanger et al. 1998 (27.3% vs. 17% with AFLP) and Rajapakse et al. 1995 (33.2% vs. 18% with RFLP) in peach. This is in agreement with a broader genetic base in apricot than peach and is also linked to the different origin of the cultivars.

Map comparison

Eight linkage groups strictly homologous to the eight groups in T×E were obtained with a marker distribution and coverage satisfactory in most groups (Fig. 1). Only two linkage groups presented a gap longer than 25 cM: LG1 in Stark Early Orange and LG7 in Polonais (Fig. 1). The distribution of loci in both maps was complementary, thereby allowing a potential density of markers of less than 3.5 cM after merging both maps. The most striking result of our comparative mapping of apricot with the 'reference map' was the high similarity in marker order. It was nearly identical with that in T×E with a few exceptions. Five RFLP loci were not syntenic with T×E (Table 5). However, Joobeur et al. (1998) used the "A",

"B", "C" etc. terminology to identify probes that detected more than one locus even if only one was mapped in T×E, and this was the case in most of the loci of Table 5. Our conclusion is that these probes detected more than one locus and that the loci placed in P×S were different from that ones in T×E because the markers associated to these loci were not segregating in the respective progenies. Six order inversions of RFLP markers mapped with LOD=2.0 were observed in apricot maps compared to T×E: two in Polonais in LGs 1 (Fig. 1) and 7; four in Stark Early Orange in LGs 1, 2 and 5. Two order inversions of SSR markers mapped with LOD=2.0 were observed in LG5 of Stark Early Orange. We concluded that the most probable cause of these discrepancies were artifacts due to the difference of precision when mapping the markers in apricot and T×E progenies. The map of Joobeur et al. (1998), as improved by Aranzana et al. (2002b), was constructed using an interspecific F_2 population of 75 individuals, while the apricot maps were constructed using a F_1 intraspecific population of 142 individuals. In addition, a high proportion of loci presenting skewed segregations was found in T×E (46%) and, consequently, linkage analysis might result in a poor estimation of genetic distances and an erroneous assignment of marker order (Lorieux et al. 1995). We found a very low proportion of distorted loci in apricot, as did Hurtado et al. (2002), probably related to the use of a F_1 intraspecific progeny for mapping. All of the distorted loci were found in the male parent and mainly in LG6 between the FG1A and Pgl1 markers (Fig. 1). The self-incompatibility allele was mapped in this group in almond in the neighborhood of Pgl1 (Ballester et al. 1998), and the presence of distorted loci in LG6 was attributed to the self-incompatibility allele in almond × peach (Joobeur et al. 1998; Bliss et al. 2002) and apricot (Vilanova et al. 2003). Our results are in agreement with these observations.

The map length of Polonais was 538 cM and that of Stark Early Orange, 699 cM. The difference was of particular importance for LG 1 (Table 6) and was probably due to local differences in the recombination rate; it was comparatively more important in LG1 of Stark Early Orange than in LG1 of other Prunus species. However, if we compare the length of the apricot maps to the map lengths reported in other diploid *Prunus* species, and particularly to those in the *Amygdalus* sub-genus, we observe similar differences, which mainly depend upon the cross used for mapping: 396 cM and 712 cM for peach (Chaparro et al. 1994; Dirlewanger et al. 1998), 393 cM for almond (Viruel et al. 1995), 521 cM for peach × (peach × *P. ferganensis*) (Dettori et al. 2001), 491 cM and 522 cM for peach × almond (Joobeur et al. 1998; Aranzana et al. 2002b) and 800 cM and 1,144 cM (Foolad et al. 1995; Bliss et al. 2002) for two maps issued from a cross between almond and peach. In this latter case, 45% of the loci showed segregation distortion. In the *Cerasus* sub-genus the maps available are still incomplete (Wang et al. 1998). In the *Prunophora* sub-genus, Hurtado et al. (2002) obtained a length of 467 cM for the Valenciano apricot cultivar and 511 cM for the Goldrich apricot cultivar and, more recently, Vilanova et al. (2003) obtained a length of 602 cM with a map constructed in an apricot F_2. However, the number of linkage groups in these maps was different than expected, and they remain incomplete. Consequently, our results agree with these previous results in apricot and with those obtained in *Amygdalus* sub-genus.

The results obtained in this study using a comparative approach support the conclusions drawn from investigations with other *Prunus* species of a high conservation of the genomes in the *Prunus* genus. The conserved structural organization of the apricot, peach and almond genome could indicate the existence of a possible common ancestor to the *Prunophora* and *Amygdalus* sub-genera. The construction of our apricot maps was clearly facilitated by the information provided by the map of Joobeur et al. (1998), which enabled the selection of well-distributed markers, validating the original mapping strategy chosen at the outset of this project. The use of comparative mapping between different *Prunus* species appears to be a powerful tool for speeding up the construction of genetic maps. By choosing a set of transferable single-locus markers evenly distributed at intervals of 10–15 cM along the linkage groups of a pre-existing map, it should be possible to construct reduced anchored maps in other progenies more easily saturated. It could also be possible to focus on a particular region of a linkage group by selecting targeted polymorphic markers. The additional information given by the apricot SSRs placed on both apricot maps could improve the saturation of the "*Prunus* reference map" by adding a few codominant loci. The use of the reference map, including the data obtained in all maps available in *Prunus* species, could make it possible to map QTLs or genes useful for agronomic improvement and to transfer markers closely linked to these traits from one species to another.

Acknowledgements The authors wish to thank Jocelyne Kervella and Mireille Faurobert (GAFL-INRA Avignon) for their critical reading of this manuscript and all of the groups involved in the European *Prunus* mapping project (AIR3-CT93-1585) for kindly providing the DNA probes and SSR primers that we used for mapping—Stephane and Véronique Decroocq (UREFV-INRA-Bordeaux) for providing the apricot cDNA-SSR clones; Isabelle Marty and Gaia Sarkissian (SQPOV-INRA-Avignon) for the apricot probes; Jamila Chaib, Guillaume Gerez and Lamia Krichen for their participation. They also wish to thank Guy Clauzel, INRA-Domaine de Gotheron for maintaining the hybrid population. This work and that of L.S. Hagen were funded by the European project ABRIGEN-FAIR6 CT98–4345 "Optimization of apricot improvement by a joint conventional and molecular approach applied to the main agronomic traits".

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