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Construction of a saturated linkage map for *Prunus* using an almond × peach F_2 progeny

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Abstract A map with 246 markers (11 isozymes and 235 RFLPs) was constructed using an interspecific F_2 population between almond (cv Texas) and peach (cv Earlygold). RFLPs were obtained using 213 probes from the genomic and cDNA libraries of different species (almond, peach, *P. ferganensis*, cherry, plum and apple), including 16 almond probes which correspond to known genes. All markers were distributed in eight linkage groups, the same as the basic chromosome number of the genus, covering a total distance of 491 cM. The average map density was 2.0 cM/marker and only four gaps of 10 cM or more were found; the two largest gaps were 12 cM each. This map was compared with one constructed previously with an intraspecific almond population sharing 67 anchor loci. Locus order was nearly identical and distances were not significantly different. A large proportion of the mapped loci (46%) had skewed segregations; in approximately half of them, the distortion was due to an excess of heterozygotes. One of the distorted regions could be associated with the position of the self-incompatibility gene of almond.

Key words Almond · Peach · *Prunus amygdalus* · *P. persica* · Isozymes · RFLPs · Mapping

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Introduction

The construction of linkage maps which cover the entire genome with markers at short intervals, called 'saturated' maps, is required for some of the applications of molecular markers in plant breeding (Tanksley et al. 1989). This is particularly true for the co-segregation analysis between markers and agronomic characters, often with polygenic inheritance, which allows the detection of quantitative trait loci (QTLs) responsible for the observed phenotypic variation. To obtain the maximum advantage from saturated maps, it is important that the markers chosen are highly reproducible and easily transferable to populations other than the one used for map construction. For this purpose, codominant markers like RFLPs, SSRs, or isozymes are among the best possible choices. A selected subset of markers from a saturated map, covering the genome at regular intervals and segregating in other breeding populations, can be used for linkage analysis with agronomically important characters segregating in these populations. Once the positions of genes or QTLs of interest are known, more markers within the target regions may be studied to find tighter linkages useful for marker-assisted selection or as a first step towards regional saturation for positional cloning (Tanksley et al. 1995).

Several marker maps of *Prunus* fruit crops have been published in the last 4 years. Three of them, using peach (Rajapakse et al. 1995), almond × peach (Foolad et al. 1995) and almond (Viruel et al. 1995) progenies, were constructed mainly with RFLP markers. Bošković

et al. (1997), using two cherry interspecific crosses, obtained linkage maps with only isozyme genes. Four more maps, obtained by Chaparro et al. (1994) and Dirlewanger et al. (1998 a) in peach, Stockinger et al. (1996) in cherry, and Dirlewanger et al. (1996) in a peach × *P. davidiana* progeny, were elaborated with RAPDs or AFLPs as the predominant marker type. However, none of the maps constructed with easily transferable markers (RFLPs and isozymes), ranging from the 127 markers mapped on the almond map by Viruel et al. (1995) to the 31 of the isozyme cherry map (Bošković et al. 1997), can be considered as sufficiently complete, since they often detect more than the expected eight linkage groups ($x = 8$ in *Prunus*) and have large regions (20 cM or more) without marker coverage.

One of the objectives of a project funded by the European Union, and involving six laboratories (Arús et al. 1994 a), was the construction of a saturated map for *Prunus* based on a common highly polymorphic interspecific almond × peach F_2. The map was elaborated with the collaboration of three partners from this project: IRTA in Cabrils and Mas Bové (Spain), INRA in Bordeaux (France), and ISF in Rome (Italy). Each group contributed a portion of the markers to the map presented in the present paper. This map is currently being used, by all the partners in the project, to select markers for the identification of useful linkages with major genes and QTLs for a wide range of characters of interest segregating in breeding populations of almond, cherry, peach and plum (Dirlewanger et al. 1996, 1998 b; Ballester et al. 1998).

Materials and methods

Plant material

Seedlings (1 1 1) from self-pollinating a single tree (MB 1-73 selected as a rootstock at IRTA-Mas Bové) of the cross between almond cv Texas, used as the female parent, and peach cv Earlygold, used as the source of pollen, were obtained during the Spring of 1992 and 1993 by IRTA. Graftwood or grafted plants on 'Garrigues' almond seedling rootstock of 75 of these individuals were distributed to INRA-Bordeaux and ISF-Rome during 1994 and 1995, where they were grown in their experimental fields. These 75 plants composed the 'Texas' × 'Earlygold' (T × E) population from which the map was constructed.

Isozymes

Ten enzyme systems, malate dehydrogenase (MDH), glucose-phosphate isomerase (GPI), phosphoglucomutase (PGM), leucine aminopeptidase (LAP), 6-phosphogluconate dehydrogenase (6PGD), malic enzyme (ME), aconitase (ACO), shikimate dehydrogenase (SDH), aspartate aminotransferase (AAT) and isocitrate dehydrogenase (IDH), were studied for polymorphism in the parents of T × E. Isozymes segregating in the progeny were employed as markers for map construction. Electrophoresis was performed in horizontal starch gels, using the methods of leaf extraction, gel preparation, and enzyme staining described in Arús et al. (1994 b).

DNA probes and RFLP methods

A total of 213 probes from different *Prunus* species and apple were used for RFLP analysis. Their origin and terminology are shown in Table 1. The sequence of 16 of the cDNA probes is known, so their homology with genes coding for known proteins in other species could be established. Six of them, coding for extensin (detecting locus *Ext1*), α-tubulin (*TubA2*, *TubA3*), actin depolymerizing factor (*Adf1*), phosphoglycerate mutase (*Pgl1*), oleosin (*Ole1*) and prunin (*Pru1*), were placed on the map constructed by Viruel et al. (1995). The rest, coding for lipid-transfer protein (*Ltp2*), jasmonic-induced protein (*Pij1*), pyruvate kinase (*Pyk1*), mandelonitryl lyase (*Mdl1*), tonoplast intrinsic protein (*Tpi1*), caffeic-o-methyl transferase (*Omt1*), prolin-rich protein (*Prp1*), and three tag sequences of *Arabidopsis* (*TSA2*, homologous to EST T22193, *TSA3* to EST T14021, and *TSA4* to EST T41929), were used for the first time. These probes were supplied by P. Puigdomènech, J. Garcia-Mas and M. Suelves from the Consejo Superior de Investigaciones Científicas (CSIC) of Barcelona, and are unpublished results of a joint CSIC-IRTA project.

DNA extraction followed the specifications of Viruel et al. (1995). ISF extractions were performed following the method of Doyle and Doyle (1987) with the modifications described by Quarta et al. (1994). Methods of DNA digestion, Southern blotting (5 µg of DNA per lane), probe hybridization, and labelling with ^{32}P-α-dCTP or with digoxygenin-11-UTP were as described by Viruel et al. (1995).

DNA was digested with five restriction enzymes, *Bam*HI, *Dra*I, *Eco*RI, *Hin*dIII and *Mva*I, and hybridized with DNA probes, first in the parents and later in individuals of the T × E population, for probes that detected RFLPs. ISF-Rome studied the FG probes from 1 to 119, INRA-Bordeaux all the PC probes, the AG probes from 101 to 116, and the FG probes from 201 to 230, while IRTA-Cabrils analyzed the probes AG1 to AG63, AC7 to AC55, CC2 to CC138, almond cDNAs corresponding to known genes, and all probes of other origin (plum, peach, and apple).

Inheritance and linkage analysis

Chi-square goodness-of-fit tests of 1 : 2 : 1 or 3 : 1 segregation ratios were done with LINKEM v.1.2 software (Vowden et al. 1995). An additional χ^2 test was performed on co-dominant markers to study the adjustment of the ratio of homozygotes vs heterozygotes to the expected 1 : 1 ratio. The map was constructed with MAPMAKER/EXP v. 3.0 (Lander et al. 1987). The Kosambi function was used to convert recombination units into genetic distances. Given the abundance of distorted segregations, the mapping procedure was adapted to minimize the risk of errors in the assignment of loci to linkage groups and in the estimation of their genetic distances. This procedure takes into account two characteristics of linkage estimation with distorted ratios (Lorieux et al. 1995): first, the maximum-likelihood estimation (MLE) of linkage between a non-distorted and a distorted locus is unbiased, and second, the bias of MLE distance between distorted loci decreases when the distance decreases and is lower for co-dominant than for dominant loci. Stringent conditions (LOD ≥ 7 and recombination fraction < 20) were set for establishing linkage groups using the 'group' command. An additional condition was that the assignment of any locus to a linkage group required a significant ($P \leq 0.001$)χ^2 test for independence using the contingency table with at least another locus of this group, as this test is robust against departures from Mendelian ratios (Bailey 1961). The χ^2 values were determined with LINKEM. Once groups were established, distorted or dominant loci were removed and, for linkage groups with nine or more markers remaining, we selected

Table 1 Description of the probes used for RFLP mapping in the T × E population

Probe origin	Terminology	No. of probes	Probes detecting >1 locus	No. of loci mapped	Source of probe
Almond genomic	AG	56	31	62	IRTA-Cabrils (Spain)
Almond cDNA	AC	27	11	29	IRTA-Cabrils (Spain)
Peach cDNA	PC	28	10	29	INRA-Bordeaux (France)
P. ferganensis genomic	FG	32	14	32	ISF-Rome (Italy)
Cherry cDNA	CC	25	7	30	HRI-East Malling (UK)
Peach genomic	B-	2	1	2	Clemson University (USA)
Plum genomic	PLG	6	2	11	UC Davis (USA)
Apple genomic	LY	5	2	6	Hort + Research (New Zealand)
Apple cDNA	MC	16	8	17	CPRO-DLO (The Netherlands)
Almond known genes	–	16	6	17	CSIC-Barcelona (Spain)
Total		213	92	235	

a first sequence of markers, using the 'order' command with 'multipoint criteria' with a strict threshold of LOD ≥ 4 for the first pass of ordering, and a LOD ≥ 3 for the second pass. Then, markers with co-dominant distorted segregations were added to this sequence one by one using the 'try' and 'ripple' commands and were retained only if the LOD for 'ripple' was higher than 3. These loci were considered as the framework of each linkage group. Using the 'try' and 'ripple' commands again, we first placed the remaining co-dominant markers, and then the dominant markers. For linkage groups where almost all loci studied had skewed ratios, a different approach was followed. All co-dominant loci were included in the analysis. The 'order' command was set with a strict threshold of LOD ≥ 5, an 'ordinary' threshold of LOD ≥ 4, and a distance ≤ 15 recombination units. The resulting sequence of markers was considered to provide the framework for these linkage groups. The remaining markers, first co-dominant and then dominant, were added later, with 'try' and 'ripple', to the framework sequence.

Each of the three research groups followed the same rules for data analysis. The data for each RFLP were scored independently by two members of each group. Conflicting results were re-examined and in case of disagreement the most conservative option was taken. After mapping, the 'error detection' command of MAPMAKER was used, and possible errors were re-examined. The final data analysis included all three datasets, and was performed at IRTA-Cabrils. Once the map was constructed, the banding patterns of the parents and the progeny of probes producing co-segregating RFLPs were compared. If the same pattern was observed, we retained the results of only one probe.

Loci order and genetic distances of this map were compared with those obtained in a map constructed previously by Viruel et al. (1995) in the almond F_1 segregating intraspecific population 'Ferragnès' × 'Tuono' (F × T), where four enzyme systems and 64 of the probes used for RFLP analysis were the same, resulting in 67 anchor loci. Markers produced by two of the probes (AG8 and AG32) which hybridized to more than three loci were discarded. Distances between the two most-separated anchor loci of each linkage group of three maps (two were constructed with the F × T population, one for each parent) were compared with a paired *t*-test.

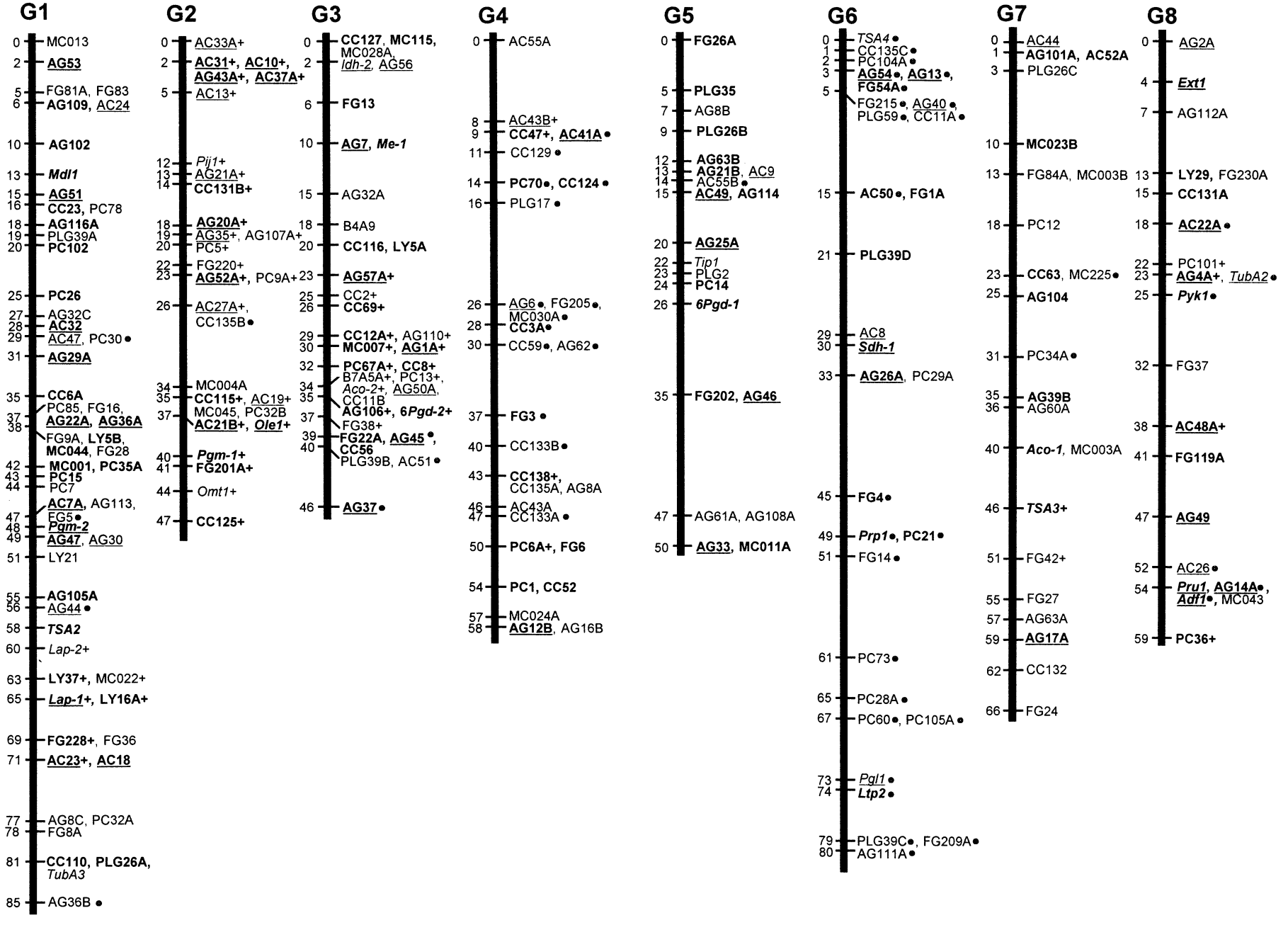
Results

From the ten enzyme systems studied, three (GPI, MDH and AAT) were monomorphic in the T × E population. The remaining seven segregated and 11 isozyme genes could be scored: two for PGM (*Pgm-1*, and *Pgm-2*), 6PGD (*6Pgd-1* and *6Pgd-2*), ACO (*Aco-1* and *Aco-2*) and LAP (*Lap-1* and *Lap-2*), and one for ME (*Me-1*), IDH (*Idh-2*) and SDH (*Sdh-1*). For two of them, *Aco-2* and *Me-1*, this is the first time they have been studied in peach and almond.

RFLPs were detected with 213 probes. One-hundred-and-one of them were genomic clones and the remaining 112 were cDNAs (including known genes). Using these clones we found 235 RFLPs (an average of 1.1 markers per probe). A single locus was detected by most (191) of the clones. Fourteen clones detected two loci each, three (AG8, CC135 and PLG26) had three loci, and one (PLG39) gave four loci. Seventy four of the probes detected monomorphic bands in addition to the segregating RFLP, indicating that they hybridized to more than one locus. Thus, we estimate that a total of 92 probes (43%) detected more than one locus, and this was more frequent in genomic probes (50%; 50 probes), than in cDNA clones (38%; 42 probes).

All markers coalesced into eight linkage groups, named G1 to G8 (Fig. 1). G1, G3, G5, G7, and G8 were constructed using an initial set of loci with non-distorted ratios. For G2, G4, and G6 we followed the mapping procedure for groups having most of their loci with skewed segregations. The distribution of markers

Fig. 1 Molecular map obtained with the T × E almond × peach F_2 progeny. Linkage groups have been labelled as G1 to G8. Probes hybridizing to more bands than explained by a single locus have been designated with an *A* after the locus name if only one locus was mapped, and with a *B, C or D* when more than one locus were studied. *Loci in bold characters* compose the framework of each linkage group. *Loci following commas* were placed at the same position. *Loci marked with a black dot* after their name had distorted segregations. *Loci with a +* after the locus name were distorted and had a significant excess of heterozygotes. *Underlined loci* are anchor points with the almond F × T map


G1
0 MC013
2 AG53
5 FG81A, FG83
6 AG109, AC24
10 AG102
13 Mdl1
15 AG51
16 CC23, PC78
18 AG116A
19 PLG39A
20 PC102
25 PC26
27 AG32C
28 AC32
29 AC47, PC30
31 AG29A
35 CC6A
37 PC85, FG16,
38 AG22A, AG36A
FG9A, LY5B,
MC044, FG28
42 MC001, PC35A
43 PC15
44 PC7
47 AC7A, AG113,
FG5
48 Pgm-2
49 AG47, AG30
51 LY21
55 AG105A
56 AG44
58 TSA2
60 Lap-2+
63 LY37+, MC022+
65 Lap-1+, LY16A+
69 FG228+, FG36
71 AC23+, AC18
77 AG8C, PC32A
78 FG8A
81 CC110, PLG26A,
TubA3
85 AG36B
G2
0 AC33A+
2 AC31+, AC10+,
AG43A+, AC37A+
5 AC13+
12 Pij1+
13 AG21A+
14 CC131B+
18 AG20A+
19 AG35+, AG107A+
20 PC5+
22 FG220+
23 AG52A+, PC9A+
26 AC27A+,
CC135B
34 MC004A
35 CC115+, AC19+
37 MC045, PC32B
AC21B+, Ole1+
40 Pgm-1+
41 FG201A+
44 Omt1+
47 CC125+
G3
0 CC127, MC115,
MC028A,
2 Idh-2, AG56
6 FG13
10 AG7, Me-1
15 AG32A
18 B4A9
20 CC116, LY5A
23 AG57A+
25 CC2+
26 CC69+
29 CC12A+, AG110+
30 MC007+, AG1A+
32 PC67A+, CC8+
34 B7A5A+, PC13+,
Aco-2+, AG50A,
35 CC11B
AG106+, 6Pgd-2+
37 FG38+
39 FG22A, AG45,
40 CC56
PLG39B, AC51
46 AG37
G4
0 AC55A
8 AC43B+
9 CC47+, AC41A
11 CC129
14 PC70, CC124
16 PLG17
26 AG6, FG205,
MC030A
28 CC3A
30 CC59, AG62
37 FG3
40 CC133B
43 CC138+,
CC135A, AG8A
46 AC43A
47 CC133A
50 PC6A+, FG6
54 PC1, CC52
57 MC024A
58 AG12B, AG16B
G5
0 FG26A
5 PLG35
7 AG8B
9 PLG26B
12 AG63B
13 AG21B, AC9
14 AC55B
15 AC49, AG114
20 AG25A
22 Tip1
23 PLG2
24 PC14
26 6Pgd-1
35 FG202, AG46
47 AG61A, AG108A
50 AG33, MC011A
G6
0 TSA4
1 CC135C
2 PC104A
3 AG54, AG13,
FG54A
5 FG215, AG40,
PLG59, CC11A
15 AC50, FG1A
21 PLG39D
29 AC8
30 Sdh-1
33 AG26A, PC29A
45 FG4
49 Prp1, PC21
51 FG14
61 PC73
65 PC28A
67 PC60, PC105A
73 Pgl1
74 Ltp2
79 PLG39C, FG209A
80 AG111A
G7
0 AC44
1 AG101A, AC52A
3 PLG26C
10 MC023B
13 FG84A, MC003B
18 PC12
23 CC63, MC225
25 AG104
31 PC34A
35 AG39B
36 AG60A
40 Aco-1, MC003A
46 TSA3+
51 FG42+
55 FG27
57 AG63A
59 AG17A
62 CC132
66 FG24
G8
0 AG2A
4 Ext1
7 AG112A
13 LY29, FG230A
15 CC131A
18 AC22A
22 PC101+
23 AG4A+, TubA2
25 Pyk1
32 FG37
38 AC48A+
41 FG119A
47 AG49
52 AC26
54 Pru1, AG14A,
Adf1, MC043
59 PC36+

was far from uniform, with marker clusters and zones of low marker density. Genomic and cDNA probes were distributed evenly across linkage groups. The highest number of markers was concentrated in G1. This linkage group had 59 markers, while the second largest group was G3 with 35 markers. The number of markers contained in the rest of the groups ranged from 21 in G5 and G8 to 30 in G6. The total distance covered by the map was 491 cM, representing an average density of 2.0 cM/marker considering all the markers, and 2.7 cM/marker if only one marker was taken into account in regions with co-segregating markers. Only in G1, did the distance exceed 80 cM, whereas the shortest, G2, G3 and G5, were 50 cM or less. The density of markers was irregular among linkage groups, ranging from 1.3 cM/marker in G3 to 2.9 cM/marker in G7. Loci separated by a distance of 6 cM or less covered most of the map (77%). The two largest gaps were 12 cM, one located in G6 between AG26A and FG4 with the other in G5 between AG46 and AG61A. Only two more large gaps of 10 cM each were found, both in G6.

For most RFLPs (199) and all isozymes both parental alleles were detected and they were scored as co-dominant. The remaining 36 RFLPs were studied for the presence or absence of a DNA fragment and were scored as dominant markers. This generally occurred (25 RFLPs) in multiple-copy clones, because the presence of complex banding patterns made it difficult to efficiently score all the segregating loci.

Goodness-of-fit tests of 1:2:1 or 3:1 segregations revealed that 112 markers (46%) had distorted ratios. In 57 (56%) of the co-dominant distorted loci, the test of goodness-of-fit for homozygotes vs heterozygotes was significant and the heterozygous class was over-represented in all cases. Loci with non-Mendelian ratios were generally clustered (Fig. 1) and the direction of the distortion for markers placed in a given cluster was the same. Loci with biased segregations in G3, G4, and G8 had an excess of almond alleles whereas, in G1, peach alleles were favored. In G6, we found an excess of almond alleles in loci at one extreme of the linkage group (*TSA4*) and an excess of peach alleles in loci mapped at the other extreme (AG111A). All but three markers of G2 were distorted with a significant excess of heterozygotes in most of them. A proportion of heterozygotes significantly higher than expected occurred also in most skewed loci of G1 and G3, and in some loci of G8. In G5, all but one locus showed Mendelian segregation, and similarly in G7 all but four of the markers followed Mendelian ratios.

Using the subset of 67 markers (63 RFLPs and four isozymes) homologous between this map and the intraspecific almond map constructed by Viruel et al. (1995), we were able to compare the T × E map with those of each almond parent: the 'Ferragnès' (F) and the 'Tuono' (T) maps having 52 and 37 anchor loci, respectively. Twenty two loci were common to all three maps. The following results were obtained: (1) a one-to-one correspondence between the eight linkage groups found in each map could be established, which allowed us to use the same terminology for linkage-group number as Viruel et al. (1995), (2) on comparing the locus order for all linkage groups, except G7 where only two anchor loci existed, all but two loci had the same order; these loci were AC26 and AG14A at the same distance (2 cM) in G8 of the T × E and F maps, but in an inverted order; (3) distances in T × E were compared with those in F for all linkage groups and with those in T for all groups except G7 because there were no markers in common (Table 2). The comparisons involved most of the distance of each map: 76% of T × E and 82% of F for T × E/F, and 53% of T × E and 68% of T for T × E/T. The intervals of the T × E map averaged 15% longer than those of the F map, and 2% shorter than those of the T map. The paired *t*-test did not detect significant differences between the distances of T × E compared to the almond maps: $t = 0.89$ in T × E/F and $t = 0.12$ in T × E/T. A similar test performed by Viruel et al. (1995) for the comparison between T and F homologous distances was also not significant. Important differences were apparent in the individual behavior of some of the linkage groups having most loci with distorted ratios. For G2, which could be studied in detail due to the presence of 14 anchor loci and a good coverage of the homologous regions, distances were longer in the almond maps (24% in F and 29% in T) than in the interspecific map. The opposite occurred in G6, with seven anchor loci, where the compared interval of T × E was about two and three times as long as those of T and F, respectively.

Table 2 Distance comparison between homologous fragments of the linkage groups of the almond × peach map T × E and the intraspecific almond maps of 'Tuono' (T) and 'Ferragnès' (F)

Linkage group	T × E/F		T × E/T	
	Interval	Difference	Interval	Difference
G1	AG53–AC18	0	AG51–AC23	1
G2	AC33–*Ole1*	−12	AC33B–AC19	−14
G3	AG56–AG37A	4	*Idh-2*–AG50A	−20
G4	AC41A–AC43A	1	AG6–AG12B	3
G5	AC9–AG33A	−17	AG25A–AG33A	3
G6	AG13–*Plg1*	45	AG45A–*Sdh-1*	13
G7	AC44–AG17	18	–	–
G8	AG2–*Pru1*	9	*Ext1*–*Adf1*	10
Total		48		−4

Discussion

A map with 246 markers was constructed in an interspecific almond × peach F_2 progeny. Linkage analysis identified eight linkage groups that included all the

markers analyzed. Eight linkage groups were also found in the almond map constructed by Viruel et al. (1995), and using the 67 anchor loci it was shown that the linkage groups of both maps were homologous. Given that the basic chromosome number of *Prunus* is $x = 8$, these results suggest that each linkage group corresponds to one of the chromosomes of the genus.

Probes from genomic and cDNA libraries of five *Prunus* species and apple were used for RFLP detection. Cherry, *P. ferganensis*, plum and apple probes gave good results when hybridized with the DNA of the T × E population, indicating their high degree of homology with almond and peach DNA. For 16 of the cDNA probes we knew the homology of their sequences with that of genes of known function in other species. Their 18 RFLPs may be of special relevance since knowledge of their function may be useful for establishing cause and effect relationships with morphological or physiological variability. Other probes were obtained from research groups involved in fruit-tree mapping. They detect RFLPs that may be used as landmarks for the establishment of consensus maps for the Rosaceae and can lead to a broader use of results obtained independently by different groups worldwide.

A high proportion of loci with a non-Mendelian segregation was found in our almond × peach cross (46%). Linkage analysis with such data may result in a poor estimation of genetic distances and in an erroneous assignment of marker order (Lorieux et al. 1995). These risks were limited by the introduction of special precautions in the mapping procedure and with the comparison of the locus order and distances obtained in the T × E map and the almond F × T map. Given that marker order was essentially conserved and that the genetic distance covered by the almond and T × E maps was not significantly different, we conclude that the presence of distorted segregations did not have a major effect on the map presented in this paper. On the other hand, the similarity between map distances obtained between inter- and intra-specific populations does not support the hypothesis that the size of the T × E map would be more compressed due to a non-random reduction of recombination rates as may have occurred in other species (Gebhardt et al. 1991; Causse et al. 1994).

A high number of distorted segregations (37%) was also found in the almond × peach progeny used by Foolad et al. (1995). As already pointed out by these authors, part of these distortions may be attributed to selection in the field favoring the most vigorous individuals. The T × E population has 111 individuals but only 75 of them could be studied; the rest were discarded due to slow growth or poor compatibility with the rootstock used for grafting. This source of selection possibly occurred in addition to others which may have taken place at the pre- or post-zygotic level before seed germination.

One of the sources of pre-zygotic selection could be tested with our data. The F_1 between 'Texas' and 'Earlygold' is putatively heterozygous at the gametophytic self-incompatibility (SI) locus, having a self-compatibility (*Sc*) allele from the peach parent and a self-incompatibility (*Si*) allele from the almond parent. Selfing of an individual of this progeny would produce a semi-compatible reaction, where the *Si* pollen would be unable to fertilize the ovule, and only *SiSc* and *ScSc* individuals, in the same proportions, would be produced. Thus, a strong selection against the almond alleles is expected at the region around the SI locus. In a recent study, Ballester et al. (1998) located the SI gene of almond in the neighborhood of *Pgl1* (5 cM apart) on G6. As predicted, a gross segregation distortion favoring the peach alleles was found in *Pgl1* and in the markers flanking this gene over a distance of more than 30 cM.

Approximately half of the distorted loci, most of them located on G1, G2, G3 and G8, had a significant departure towards an excess of heterozygotes. Post-zygotic selection against one of the homozygotes may generate a proportion of heterozygotes higher than expected. This may be the case for homozygotes for the almond allele in G1, and for the peach allele in G3 and G8, which were all clearly under-represented. For G2, selection favoring heterozygotes at the sporophytic level for one or more genes may have been the cause of a lower than expected number of both homozygotes. An alternative explanation would be that two alleles in repulsion for two linked genes would have been selected against at the post-zygotic level. These two hypotheses correspond to a situation of overdominance in the former case and pseudo-overdominance in the latter. Dominance and additivity appear as the most general effects of genes involved in the inheritance of agronomic characters (de Vicente and Tanksley 1992; Tanksley 1993). Overdominance was proposed to explain heterosis in maize F_1 hybrids (Stuber et al. 1992), but in an experiment designed to analyze overdominance vs dominance, Xiao et al. (1995) found that dominance was the major factor contributing to hybrid vigor in rice. In the case of G2, testing for these hypotheses would require a much larger population and a very detailed genetic map of the region under selection.

The average density of markers was high (between 2.0 and 2.7 cM/marker), and only four gaps of 10–12 cM were found. This represents an average of 0.8 gaps of ≥ 10 cM per Morgan, lower than that calculated for three of the most complete plant maps published; that of tomato (Tanksley et al. 1992) with 1.2 cM/ marker and 1.8 gaps /M, potato (Tanksley et al. 1992) with 0.7 cM/marker and 1.8 gaps/M, and rice (Causse et al. 1994) with 2.0 cM/ marker and 1.9 gaps/M. These data suggest that the T × E map has a level of saturation similar to these maps, and therefore that it covers most of the distance of the *Prunus*

genome and has a sufficient marker density for use in plant breeding. However, its total distance (491 cM) is clearly shorter when compared to the potato (684 cM), tomato (1276 cM) and rice (1491 cM) maps. This difference may be due either to the small nuclear DNA content of the *Prunus* genome, about two and four times smaller than the rice and tomato genomes respectively (Arumuganathan and Earle 1992), or to a lower recombination rate in *Prunus* than in these species, or both.

The long inter-generation period and the large size of tree species are responsible for the slow progress of fruit breeding and make it more difficult to gather data on the mapping or co-segregation of markers with agronomic characters than in other species. Thus, research leading to the development of marker maps, and their use in fruit improvement, may have a practical impact larger than in herbaceous species. The map presented in this paper was constructed with RFLP and isozyme markers, which can be easily transferred to other populations of peach and almond or even to other species of the Rosaceae. A cooperative effort is needed to establish consensus maps with other groups working in *Prunus*, *Malus*, *Fragaria*, or *Rosa*. This would allow the mapping of useful genes or QTLs, likely to be homologous among different species, and to find markers sufficiently linked to them for their immediate use in breeding programs as tools for early and efficient selection.

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