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A linkage map with RFLP and isozyme markers for almond

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Abstract Inheritance and linkage studies were conducted with seven isozyme genes and 120 RFLPs in the F_1 progeny of a cross between almond cultivars 'Ferragnes' and 'Tuono'. RFLPs were detected using 57 genomic and 43 cDNA almond clones. Eight of the cDNA probes corresponded to known genes (extensin, prunin (2), α-tubulin, endopolygalacturonase, oleosin, actin depolymerizing factor and phosphoglyceromutase). Single-copy clones were found more frequently in the cDNA (65%) than in the genomic libraries (26%). Two maps were elaborated, one with the 93 loci heterozygous in 'Ferragnes' and another with the 69 loci heterozygous in 'Tuono'. Thirty-five loci were heterozygous in both parents and were used as bridges between both maps. Most of the segregations (91%) were of the 1:1 or 1:1:1:1 types, and data were analyzed as if they derived from two backcross populations. Eight linkage groups covering 393 cM in 'Ferragnes' and 394 in 'Tuono' were found for each map. None of the loci examined in either map was found to be unlinked. Distorted segregation ratios were mainly concentrated in two linkage groups of the 'Ferragnes' map.

Key words Almond · *Prunus amygdalus* · Isozymes · RFLPs · Genetic maps

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Introduction

Long intergeneration periods, self-incompatibility and inbreeding depression have limited genetic studies in tree species. Almond (*P. amygdalus* Batsch, syn. *P. dulcis* (Miller) D. A. Webb), one of the first fruit crops domesticated by man (Spiegel-Roy 1986), is an example of this situation: only three genes of interest in breeding (self-compatibility, kernel taste and shell hardness) have so far been characterized (Kester and Asay 1975).

With the development of molecular markers, a new avenue for the study of plant genetics that overcomes most of the problems associated with woody species has been opened. A high number of polymorphic, codominant, Mendelian loci can be studied in a relatively short time using pre-existing segregating populations. These markers are useful for a broad range of applications in plant genetics and breeding (Tanksley et al. 1989).

Isozymes were the first kind of markers studied in almond. Fifteen isozyme genes have been reported, and most of the possible gene pairs tested for linkage (Hauagge et al. 1987; Arús et al. 1994a). Isozymes have provided important information on the level of genetic variation of almond and other related crops (Arulsekar et al. 1986; Byrne 1990) and allowed useful applications like cultivar fingerprinting (Cerezo et al. 1989), peach × almond F_1 hybrid identification (Chaparro et al. 1987) or the estimation of the rate of pollen migration under horticultural conditions (Jackson and Clarke 1991).

In addition to almond, the genus *Prunus* includes other important crops like peach, cherry, apricot and plum. All of them share the same basic chromosome number ($x = 8$) and one of the smallest DNA contents among cultivated species: 0.54–0.67 pg of DNA/2C (Dickson et al. 1992). This group of species is closely related as indicated by the fact that some of them are compatible or can be crossed through bridge species (Watkins 1976). Another important fruit crop, apple, is also related to *Prunus*. Apple is an amphydiploid spe-

cies, and Sax (1933) proposed that one of its component genomes belongs to a primitive form of the sub-family *Prunoideae*. Thus, information obtained on the genetics of one of these species is likely to be also useful for the rest.

Different kinds of molecular markers have been studied in all of the important species of *Prunus* and in apple, and isozymes have been reported to some extent in all of them (Torres 1990). Two maps, one in peach (Chaparro et al. 1994) and the other in apple (Hemmat et al. 1994), have been constructed using mainly random amplified polymorphic DNA (RAPD) markers. The level of variation for restriction fragment length polymorphisms (RFLPs) in peach was analyzed by Eldredge et al. (1992) and found to be sufficient for map construction in this species. However, RFLP maps of these species have not been reported yet. While RAPD markers have proven to be useful for map construction and as a source of a large amount of quickly screenable markers, it is difficult to establish homologies between RAPDs of different individuals of the same species or from different species (Grattapaglia and Sederoff 1994). In contrast, homologous RFLPs can be found across wide philogenetic ranges (Ahn and Tanksley 1993) and are more adequate for genome comparisons.

In this paper we report our investigation of a number of RFLPs and several known isozyme genes that segregate in one intraspecific almond offspring. With this information we have expanded by 125 the number of known markers in almond and have constructed a map with them. Some of the RFLPs of this map are currently being used for the development of a densely populated map in *Prunus* in a cooperative effort with other European research groups (Arús et al. 1994b). Selected loci of the *Prunus* map will be analyzed in a set of breeding progenies of almond, peach, cherry and plum with the objectives of studying the cosegregation of markers with valuable agronomic characters and comparing the genome organization of different species.

Materials and methods

Plant material

A progeny of 60 F_1 plants derived from a cross between two almond cultivars, 'Ferragnes', which was used as the pistillated parent, and 'Tuono', served as the mapping population. These kind of populations, termed F_1 segregating progenies (Arús et al. 1994a) or pseudotestcrosses (Hemmat et al. 1994; Grattapaglia and Sederoff 1994), are commonly used in tree breeding and segregate for all loci heterozygous in each parent. 'Ferragnes' × 'Tuono' was obtained as part of the almond breeding program of IRTA's "Departament d'Arboricultura Mediterrània", and segregates for agronomic characters like blooming time, shelling percentage and presence of double kernels.

Isozymes

Enzyme extraction, electrophoresis and staining procedures, as well as genetic interpretation and linkage analysis for the seven isozyme genes used in this report can be found in Arús et al. (1994a). The isozyme genes studied were two of aspartate aminotransferase (*Aat-1*, and *Aat-2*) and one of each of phosphoglucomutase (*Pgm-2*), glucosephosphate isomerase (*Gpi-2*), isocitrate dehydrogenase (*Idh-2*), leucine aminopeptidase (*Lap-1*) and shikimate dehydrogenase (*Sdh-1*).

Sources of probes

A random genomic library was constructed with DNA extracted from young leaves of the almond cultivar 'Texas'. Genomic DNA was digested with *Pst*I, and fragments ranging in size from 0.5 to 2.0 kb were cloned into pBluescript plasmids (Stratagene). Recombinant clones transformed into *E.coli* DH5α cells according to the procedures of Sambrook et al. (1989) were selected on IPTG-X-Gal plates, and plasmids were isolated with the Wizard miniprep kit (Promega). Recombinant plasmids were digested with *Pst*I and subjected to electrophoresis on 1.2% agarose gels. The molecular weight of each insert was determined by comparison to molecular weight standards.

Clones from two cDNA libraries previously obtained, one from seedling root and the other from immature kernel (Garcia-Mas et al. 1992; Garcia-Mas et al. 1995), were also used as sources of RFLPs. Clones corresponding to eight known genes were also used. Three of them were obtained from the seedling root library, 4 more from the immature kernel library and 1 (endopolygalacturonase) was a heterologous probe from peach kindly provided by Dr. J. Speir (CSIRO, Australia). The main characteristics of these clones are given in Table 1.

RFLPs detected with genomic and cDNA probes were designated as AG and AC, respectively, followed by a number. Multiple loci detected by the same probe were labelled with a capital letter after this number (A, B, C, etc.). We considered that a clone detected a single locus when parents and progeny had a maximum of two major bands, or three bands when one of the alleles had a pattern of two bands.

RFLP methods

Genomic DNA was extracted from young leaves according to the method of Bernatzky and Tanksley (1986) with the modification that the DNA was resuspended in TE buffer after isopropanol precipitation and further purified through a CsCl gradient (Messeguer et al. 1994). DNA (5 μg) from the parents was digested with *Bam*HI, *Dra*I, *Eco*RI, *Hin*dIII and *Mva*I (3–4 U/μg DNA) according to the recommendations of the manufactures. Enzymes that produced polymorphism for a given probe were later used to digest the DNA of all individuals of the progeny. *Hpa*II was used with the genes actin

Table 1 Clones corresponding to known genes used as sources of RFLPs

Product	Gene abbreviation	Species	Reference
Prunin	*Pru1*	Almond	Garcia-Mas et al. (1995)
Prunin	*Pru2*	Almond	Garcia-Mas et al. (1995)
Oleosin	*Ole*	Almond	Garcia-Mas et al. (1995)
Extensin	*Ext*	Almond	Garcia-Mas et al. (1992)
α-Tubulin	*TubA*	Almond	Stöcker et al. (1993)
Phospho-glyceromutase	*Pgl*	Almond	Garcia-Mas et al. (1995)
Actin depolymerizing factor	*Adf*	Almond	Garcia-Mas et al. (1995)
Endo-polygalacturonase	*Pga*	Peach	Lee et al. (1990)

depolymerizing factor, α-tubulin and endopolygalacturonase, which are not polymorphic with these five restriction enzymes. Probes were labelled [^{32}P]-α-dATP using the random primer labelling method (Sambrook et al. 1989) or with digoxigenin-11-dUTP (Boehringer Mannheim) by insert amplification. Electrophoresis of plant DNA, Southern blotting, labelling of probes and hybridization were performed as described by Messeguer et al. (1994).

Linkage analysis

Chi-square goodness-of-fit values for the segregation of individual loci and for independent assortment between all pairs of loci, and the estimation of recombination frequencies between pairs of linked loci were performed with the LINKEM program (Vowden and Ridout 1994). This new software has the advantage over other well-known similar programs in that it allows the analysis of linkage between pairs of loci having all possible parental phase combinations (including coupling/repulsion cases), which is adapted to the analysis of F_1 segregating progenies like the one we have used for almond mapping.

Since the population analyzed was from a cross between two partly heterozygous genotypes, two maps were elaborated, one for loci heterozygous in 'Ferragnes' and one for loci heterozygous in 'Tuono'. Markers heterozygous in both parents, i.e. those segregating 1:2:1 or 1:1:1:1, were included in both maps and used as bridges between them. To simplify the analysis, loci segregating 1:1:1:1 were converted into two backcross ratios, as if they were 2 loci, 1 heterozygous in each parent. With this transformation, most loci segregated 1:1, and map construction was done with the backcross mode of MAPMAKER/EXP version 3.0 (Lander et al. 1987; Lincoln et al. 1992). Loci segregating 1:2:1 were added to these maps using the two-point linkage data obtained with the LINKEM program. Markers segregating 3:1 were excluded from linkage analysis since this is the least informative class of markers and to avoid possible problems of repulsion or mixed phases with other 3:1 or 1:2:1 segregating loci (Ritter et al. 1990; Arús et al. 1994a). Linkage groups were constructed using the 'group' command of MAPMAKER with the default linkage criteria of LOD = 3.0 and recombination fraction $\leqslant 0.3$. The Kosambi mapping function was used for the conversion of recombination fractions into genetic distances. An estimation of the total genetic distance using the information of all loci was obtained by elaborating an integrated map with JOINMAP (Stam 1993).

Results

One hundred probes revealed RFLPs in 'Ferragnes' and 'Tuono' for at least one of the restriction enzymes used. Fifty-seven of them were obtained from a genomic library and 43 were cDNA [20 from root and 22 from immature kernel libraries, and 1 heterologous probe (Pga) from peach]. These probes detected 125 RFLPs, 78 (1.5 RFLPs per probe) with genomic and 47 (1.1 RFLPs per probe) with cDNA probes. A high proportion (65%) of the cDNA clones identified single loci, whereas genomic clones hybridized to single loci in a significantly ($P < 0.05$) lower proportion (26%). Nineteen probes (15 genomic and 4 cDNA) revealed more than 1 RFLP. Four loci were found with genomic probes AG23 (A–D) and AG32 (A–D), 3 with clones AG24 (A–C) and AG25 (A–C), and 2 with each of 15 more probes.

Seven isozyme genes previously studied in this progeny (*Pgm-2*, *Lap-1*, *Aat-1*, *Aat-2*, *Sdh-1*, *Idh-2*, and *Gpi-2*) (Arús et al. 1994a) were used for mapping. Additional information was obtained for *Pgm-2*, where a new allele (*c*), which coded for an allozyme of slightly more cathodal mobility than the product of one of the already described alleles (*a*), was detected in some well-resolved gels. 'Ferragnes' had the *cb* genotype, 'Tuono' was *ab*, and the progeny segregated for the expected classes *ac*, *ab*, *cb* and *bb*.

Two maps were constructed with a total of 127 markers, one containing the 93 loci heterozygous in 'Ferragnes' and the other with the 69 loci heterozygous in 'Tuono' (Fig. 1). Thirty-five of them were heterozygous in both parents (1:1:1:1 and 1:2:1 segregations) and were used as anchor points between the two maps (Table 2). All loci heterozygous in 'Ferragnes' coalesced into eight linkage groups (G1F–G8F) for a total distance of 393 cM (average of 4.2 cM per marker). For 'Tuono', we found nine linkage groups. The two smallest of them had one anchor locus (AG10A and AG58A) mapped in linkage group 7 of 'Ferragnes' (G7F), and these were considered to belong to its homologous group in 'Tuono' (G7T). Moreover, tests of independence between two pairs of loci of these two small groups were significant (AG10A-AG8B and AC46-AG8B), and the distances and loci order between the 4 RFLPs included in the resulting linkage group could be estimated. The total distance covered by the eight groups of 'Tuono' (G1T–G8T) was 394 cM (average of 5.7 cM per locus). As in 'Ferragnes', no unlinked loci were found.

The distribution of markers along the linkage groups was far from uniform with clusters of tightly linked loci and regions with a low density of markers. The maps of 'Ferragnes' and 'Tuono' differed from each other in the position of the clusters, which were more common in the more populated 'Ferragnes' map (G2F, G3F, G4F, G6F and G8F) and less so in the 'Tuono' map (G2T). The three largest gaps occurred in G7T between markers AG8B and AG10A (35 cM), which corresponds to the situation described in the previous paragraph; in G4F, between markers AC34 and AG28B (28 cM), and in G3T, between isozyme genes *Idh-2* and *Aat-1* (28 cM). Additional evidence on the linkage between the two latter genes was found in one more almond progeny by Arús et al. (1994a).

RFLPs detected by the same probe were generally located in different linkage groups with four exceptions: AG25A and AG25B were tightly linked (3 cM) in G5T, and three more pairs of loci, AG23B and AG23C, AG8A and AG8B, and AC33A and AC33B, were placed at

Table 2 Distribution of segregation types produced by different sources of markers

	n^a	1:1	1:1:1:1	1:2:1	3:1	Total
Isozymes	6	4	1	2	–	7
RFLPs (genomic)	51	55	15	4	4	78
RFLPs (cDNA)	43	33	12	1	1	47
Total	–	92	28	7	5	132

[a] Number of enzyme systems for isozymes or probes for RFLPs

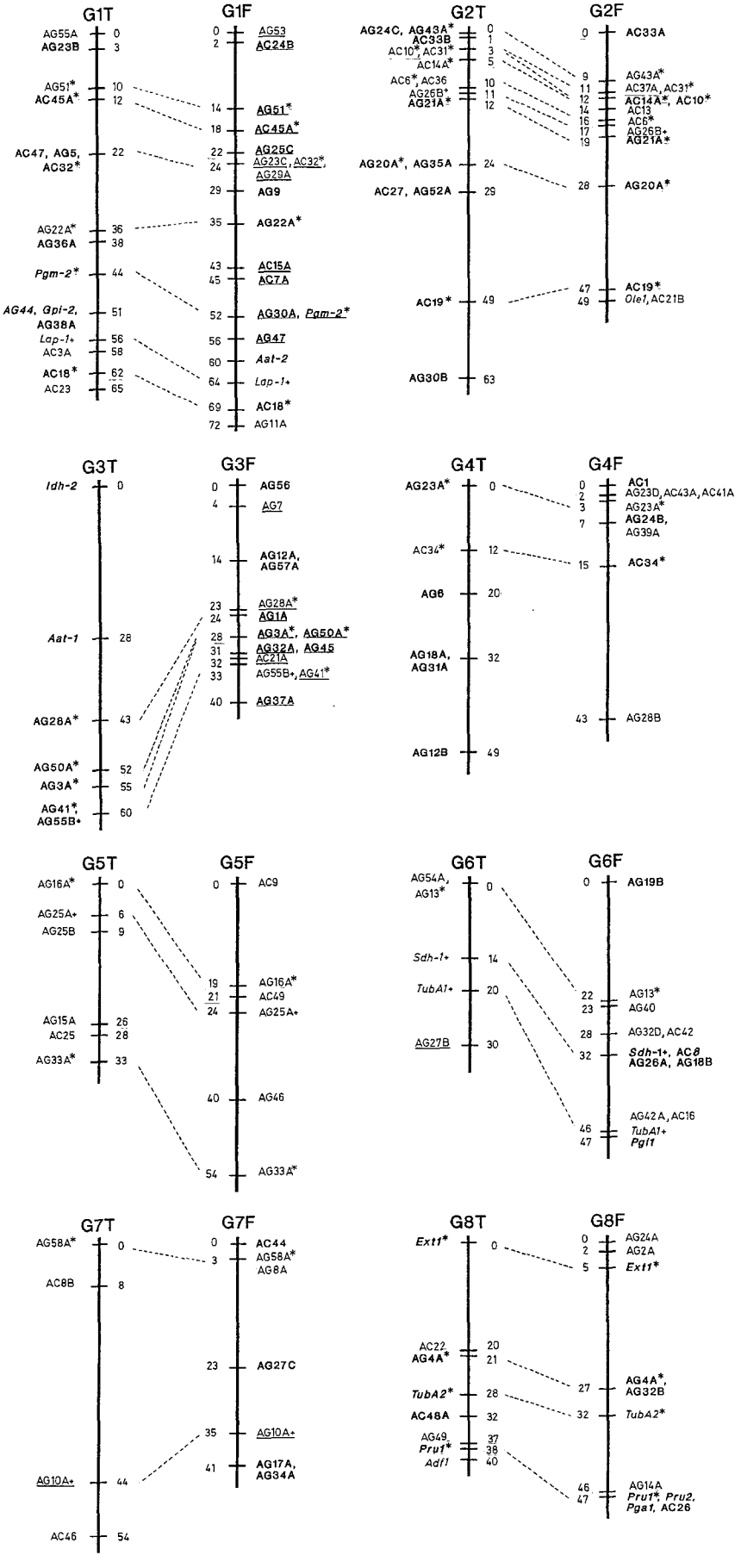

Fig. 1 Molecular linkage maps of almond and a comparison between the linkage groups of 'Ferragnes' (*G1F–G8F*) and 'Tuono' (*G1T–G8T*). Loci in *bold characters* have been ordered with a LOD score of 3.0 or higher. Loci segregating 1:1:1:1, analyzed as two backcrosses, are marked with an *asterisk* following the locus name; F_2 segregating loci, with a +. *Underlined loci* had distorted segregations. Correspondences between anchor loci of homologous linkage groups are shown by *dashed lines*

similar positions in homologous groups 1, 7 and 2, respectively. The three latter cases have been considered here as pairs of tightly linked loci, but we cannot discard the possibility that they correspond to individual loci heterozygous in both parents.

Skewed segregations were observed in 24 (26%) loci of 'Ferragnes', whereas only 2 (3%) deviated from the expected Mendelian ratios in 'Tuono' (Table 3). Most aberrant segregations occurred in G1F, in 13 out of 19 loci, and in G3F, in 10 out of 14 loci. Eight loci with 1:1:1:1 ratios present in these linkage groups, segregated normally in 'Tuono' and were distorted in 'Ferragnes' when converted into two 1:1 ratios. The rest of skewed segregations corresponded to AG27B, located in a distal position of G6T, and to AG10A, which segregated 1:2:1; it was not possible to assign the distorted ratio of the latter to any of the two parents' maps.

Using the 35 anchor loci we could assign each linkage group of 'Ferragnes' to its homolog of 'Tuono' (Fig. 1). Anchor loci ranged from a maximum of 9 in group 2 to a minimum of 2 in groups 4 and 7, with an average of 4.5 bridges per linkage group. The order of the anchor loci when more than 2 occurred at a given linkage group was conserved. The genetic distance between the most separated anchor points of all linkage groups was compared with a paired t-test with the objective of finding possible differences in recombination rates between both parents (Table 4). The sum of the distances of the common fragments was slightly larger (17 cM) in 'Tuono' than in 'Ferragnes', and the t-test was not statistically significant ($t = -0.89$; $P = 0.41$). In an attempt to establish a comparison between the homologous linkage groups of the 'Ferragnes' and 'Tuono' maps we plotted the number of loci of each linkage group and also their total map distance. The correlation was significant ($r = 0.76$; $P < 0.05$) between numbers of loci but not significant between map distances ($r = 0.21$; $P = 0.62$).

Table 3 Segregations and goodness-of-fit tests for loci showing skewed segregations in the cross 'Ferragnes' × 'Tuono'. For 1:1 segregations, A and C are phenotypes with the two alternative alleles of its heterozygous parent. The alleles present in A (or C) individuals of different loci were in the same phase in its heterozygous parent. For the 1:2:1 segregation of locus AG10A, A and C are the two homozygotes and H the heterozygote

Linkage group	Locus[a]	Expected segregation	A	C	H	χ^2	P
G1F	AG53	1:1	19	40		7.47	0.006
G1F	AC24B	1:1	20	38		5.59	0.02
G1F	AG51*	1:1	21	37		4.41	0.04
G1F	AC45A*	1:1	18	36		6.00	0.01
G1F	AG25C	1:1	20	36		4.57	0.03
G1F	AG29A	1:1	21	38		4.90	0.03
G1F	AC32*	1:1	21	38		4.90	0.03
G1F	AG23C	1:1	20	36		4.57	0.03
G1F	AC15A	1:1	17	40		9.28	0.002
G1F	AC7A	1:1	19	39		6.90	0.009
G1F	*Pgm-2**	1:1	17	35		6.23	0.01
G1F	AG30A	1:1	19	40		7.47	0.006
G1F	AG47	1:1	21	37		4.41	0.04
G3F	AG7	1:1	36	19		5.25	0.02
G3F	AG28A*	1:1	39	20		6.12	0.01
G3F	AG1A	1:1	39	19		6.90	0.009
G3F	AG3A*	1:1	42	17		10.59	0.001
G3F	AG50A*	1:1	42	16		11.66	<0.001
G3F	AG32A	1:1	40	19		7.47	0.006
G3F	AG45	1:1	38	19		6.33	0.01
G3F	AC21A	1:1	35	18		5.45	0.02
G3F	AG41*	1:1	39	20		6.12	0.01
G3F	AG37A	1:1	38	20		5.59	0.02
G6T	AG27B	1:1	45	10		22.7	<0.001
7	AG10A	1:2:1	23	9	22	9.11	0.01

[a] Loci order is the same as in the linkage groups. Loci marked with an asterisk following the locus name segregated 1:1:1:1 and were transformed into two backcross segregations

Discussion

Polymorphism in the parents

Two almond cultivars 'Ferragnes' and 'Tuono' were used as parents of the segregating offspring used in the investigation described herein. Polymorphisms for isozymes and RFLPs were abundant, confirming previous observations on the high level of heterozygosity in almond (Byrne 1990). Thus, the task of finding sufficient variability for the construction of the map was simplified. From the seven isozyme genes and 125 RFLPs studied, 74% were heterozygous in 'Ferragnes' and a significantly lower proportion (56%) were heterozygous in 'Tuono'. 'Ferragnes' was recently selected from the offspring of the cross between two distant almond cultivars: 'Ai' from the South-Eastern French region of Provence and 'Cristomorto' from the Puglia region of Southern Italy. 'Tuono' is a traditional cultivar that also comes from Puglia. From these observations a higher level of heterozygosity would be expected in 'Ferragnes' than in 'Tuono', as confirmed with our data.

DNA clones

RFLPs were obtained from relatively similar numbers of clones from a *Pst*I almond genomic and two cDNA

Table 4 Comparison of map lengths (given in Kosambi units) between homologous fragments of the linkage groups of 'Ferragnes' and 'Tuono'

Linkage group	Loci	Distance in 'Ferragnes'	Distance in 'Tuono'	F–T[a]
1	AG51-AC18	55	52	3
2	AG43A-AC19	38	49	−11
3	AG28A-AG55B	10	17	−7
4	AG23A-AC34	12	12	0
5	AG16A-AG33A	35	33	2
6	AG13-*TubA 1*	24	20	4
7	AG58-AG10A	32	44	−12
8	*Ext 1-Pru 1*	42	38	4
Total		248	165	−17

[a] F–T = Distance in 'Ferragnes' minus distance in 'Tuono'

libraries. Probes apparently detecting more than 1 locus were frequent (57%), indicating that duplicated sequences occur relatively often in the almond genome. RFLPs produced by the same probe were generally located in different linkage groups, and the pattern of distribution of RFLPs obtained from different probes did not appear to detect sets of loci representing duplications of entire chromosomal pieces.

A significantly higher proportion of multiple loci was found with genomic probes (74%) than with cDNA probes (35%). In contrast to our observations, *Pst*I genomic libraries have resulted in a majority of single-copy clones in other species like tomato (Tanksley et al. 1987) or maize (Burr et al. 1988). *Pst*I is a methylation-sensitive enzyme that is likely to yield DNA probes mainly from coding regions of the genome. McCouch et al. (1988) also found a considerable proportion of *Pst*I clones detecting multiple-copy RFLPs in rice. These results were attributed to the lower degree of methylation of rice as compared to other species. Unpublished results from our group also reveal a low level of methylation in almond suggesting that, as in rice, this may be the cause of the lower proportion of single-copy *Pst*I probes.

Known genes

Clones of known genes produced RFLPs with different patterns of inheritance. Oleosin, endopolygalacturonase and actin depolymerization factor were encoded each by a single locus (*Ole 1*, *Pga 1* and *Adf 1*, respectively). One polymorphic gene (*Pgl 1*) was detected for phosphoglyceromutase, but the presence of one monomorphic band suggested the existence of at least one more gene coding for this enzyme. Extensin produced a complex banding pattern. The bands with the strongest signal segregated 1:1:1:1 and were attributed to the *Ext 1* gene. One to four more bands that produced a lower signal did not segregate or gave unclear patterns of variation and were not scored. A probe for α-tubulin revealed 2 polymorphic loci, *TubA 1* and *TubA 2*, the latter giving a stronger signal than the former, located in groups 6 and 8, respectively, of both maps. Two cDNA clones corresponding to two legumin-like storage proteins (prunins), which were very abundant in the immature kernel cDNA library (Garcia-Mas et al. 1994), were also tested for polymorphism. The sequence of their mature peptide is similar (63% of identity), and their cDNA clones produced autoradiograms in which the bands with the stronger signal segregated as a single gene (*Pru 1* or *Pru 2*). Bands with a weaker signal were also detected for each probe, corresponding to the stronger bands of the other probe, indicating that hybridization to the homeologous prunin gene occurred to some extent, as was expected from their sequence similarity. *Pru 1* and *Pru 2* were heterozygous in 'Ferragnes' and mapped to the same position close to the distal part of G8T, suggesting their possible origin as tandemly duplicated genes.

Distribution of segregation types

Most of the loci studied segregated according to a 1:1 ratio, whereas the number of loci segregating as F_2s (1:2:1 and 3:1 ratios) was very small (Table 2). When F_1 segregating progenies were used for map construction in potato (Gebhardt et al. 1991) or apple (Hemmat et al. 1994), the proportions of backcross segregations largely exceeded those of F_2 segregations. This situation can be explained on the basis of both theoretical and practical reasoning. If two individuals are taken at random from a population in Hardy-Weinberg equilibrium and they are crossed, the probability that their offspring will segregate as a backcross for a locus with two alleles of frequencies p and q ($p+q=1$) is $4pq(p^2+q^2)$. This probability is always larger than the chance of the same offspring to segregate like an F_2 ($4p^2q^2$) provided that p and $q \neq 0$. The ratio $4pq(p^2+q^2):4p^2q^2$ is minimal when $p=q=0.5$ and has a value of 2:1. On average, and with the assumption that the distribution of allele frequencies is uniform, the proportion between backcross and F_2 progenies will be 3:1 (which corresponds to the ratio between the surfaces limited by the two curves of the probability function and the q axis). The hypothesis of genotype frequencies close to a Hardy-Weinberg equilibrium in a sample of almond cultivars seems to be acceptable on the basis of the existing isozyme evidence (Arulsekar et al. 1986), and it may be true also for other cross-pollinating species that are vegetatively propagated as crops. In 'Ferragnes' × 'Tuono' we found that over the 104 loci that segregated for two alleles, 92 were backcrosses and only 12 segregated 1:2:1 or 3:1. The proportion of backcrosses (88%) exceeded the expected 75%, with the difference being significant ($\chi^2=10.05$, $P<0.01$). The bias towards 1:1 segregations can be explained by the way in which we selected the polymorphic probes. Since the parents of 1:2:1 or 3:1 segregations have the same RFLP genotype, they are often discriminated against by other segregation types (1:1 or 1:1:1:1) that show polymorphism between the parents. In highly polymorphic species like almond, there is still another factor that decreases the proportion of crosses that will segregate as F_2s. Loci segregating for three or four alleles will be recovered at considerable frequencies – in 'Ferragnes' × 'Tuono' we found 28 (21% of the total 132 loci scored) – allowing their study as two backcross progenies. The final outcome will be the predominance of 1:1 segregations, as exemplified by the fact that 120 of the 132 (91%) polymorphic markers detected in 'Ferragnes' × 'Tuono' could be analyzed as backcrosses.

Most of the populations currently available for map construction and cosegregation analysis between markers and agronomic characters in fruit trees and in many tree crop species are F_1 segregating progenies. Linkage analysis in these progenies presents specific problems because they segregate for a mixture of backcross and F_2 type segregations and by the fact that the phases of the alleles of each parent are unknown. Link-

age between pairs of loci segregating 1:2:1 (or 3:1)/ 1:1, or 3:1/3:1 in repulsion or coupling/repulsion, are poorly informative when compared to the typical F_2 or backcross progenies (Ritter et al. 1990; Arús et al. 1994a). Moreover, simple software programs allowing multipoint analysis of F_1 segregating populations have not been released yet. Our results suggest that these populations behave essentially as two backcross progenies, one for each parent, and can be analyzed as such with well-established programs like MAPMAKER. In highly polymorphic species, some of the analyzed loci will segregate 1:1:1:1, allowing the detection of anchor points between the two maps. Additional information can be obtained by F_2-type segregations that can be added to the backcross map as anchor points, often with less precision that the remaining loci.

Linkage maps

Eight linkage groups were constructed with the 93 loci heterozygous in 'Ferragnes', and the same number was constructed with the 69 loci heterozygous in 'Tuono'. Unlinked loci were not found in either map. For each linkage group of one map its homolog could be established in the other map by the presence of a minimum of 2 and a maximum of 9 anchor loci. These results suggest that the eight linkage groups found in both maps correspond to the eight chromosomes of the haploid complement of *P. amygdalus*. This hypothesis should be valiated by additional experiments ('in situ' hybridization or other cytogenetic studies) that would allow a correspondence to be established between molecular marker and physical data.

The characteristics of the 'Ferragnes' and 'Tuono' maps are very similar. The order of anchor loci in linkage groups was identical. Distances of homologous fragments of each linkage group were also similar, suggesting that no differences in recombination rates occurred on the basis of sex or genotype. The significant correlation found between the number of loci of homologous linkage groups indicates a proportional distribution of loci between both maps. On the other hand, the lack of significance of the regression between map distances of homologous linkage groups implies a certain degree of heterogeneity that can be attributable to a poor estimation of the total distance in the groups with a low number of loci.

The resemblance between both maps can be extended to its total map distance: 393 cM for 'Ferragnes' and 394 cM for 'Tuono'. A map elaborated with JOINMAP using the data of all markers yielded a total estimated distance of 455 cM. A distance comparable to ours (396 cM) was found by Chaparro et al. (1994) with 90 markers, mostly RAPDs, in the closely related peach. Maps of other species with low DNA content are in general larger than that of *Prunus:* Arabidopsis, with a size of 630 cM (312 markers) (Reiter et al. 1992) and a C value approximately half of that of *Prunus*, or rice, with a size of 1400 cM (135 markers) and a C value about 1.5 times that of *Prunus* (McCouch et al. 1988). Although comparisons of maps between different species have to be treated cautiously due to important sources of heterogeneity, these observations would be in agreement with the relatively small genome of *P. amygdalus* and would also indicate a low recombination rate in almond or in the almond cultivars used in this research.

Skewed segregations

A total of 25 loci showed departures from Mendelian ratios. The fact that we developed one map for each parent allowed us to determine for most of the loci just which parent was responsible for such distortions. Most (23) loci with skewed segregations were located in two linkage groups of 'Ferragnes': 13 in G1F and 10 in G3F. Alleles in excess of both distorted groups were always located on one of the two homologous 'Ferragnes' chromosomes. Pooled frequencies of the remaining non-distorted loci segregating 1:1 in G1F (5) and G3F (3) also had a significant distortion towards the alleles of the same homologous chromosome of 'Ferragnes' ($\chi^2 = 5.45$, $P < 0.05$ for G1F; $\chi^2 = 6.44$, $P < 0.05$ for G3F), suggesting that to some extent all of the loci of these linkage groups were affected by the distortion. Taking the values of χ^2 as a measure of the selection intensity (Table 3), we observed that the highest of them were in the distal (AG53) and central (AC15A) part of G1F and in the central part (loci AG3A and AG50A) of G3F, suggesting that at least in the longest G1F group (72 cM) more than 1 locus of the same chromosome may have been selected. Distorted segregations occurred to a much more limited extent in 'Tuono' (only in AG27B), indicating that if selection acted at the gametophytic level, most of it took place on the female side. The lack of regions with distorted segregations common to both maps implies that if selection affected individual genes, they would segregate as backcrosses (as opposed to F_2s) if post-zygotic or they would occur at the gametophytic level.

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