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M. R. Foolad · S. Arulsekar · V. Becerra · F. A. Bliss

A genetic map of *Prunus* based on an interspecific cross between peach and almond

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Abstract A genetic linkage map of *Prunus* has been constructed using an interspecific F_2 population generated from self-pollinating a single F_1 plant from a cross between a dwarf peach selection (54P455) and an almond cultivar 'Padre'. Mendelian segregations were observed for 118 markers including 1 morphological (*dw*), 6 isozymes, 12 plum genomic, 14 almond genomic and 75 peach mesocarp specific cDNA markers. One hundred and seven markers were mapped to 9 different linkage groups covering about 800 cM map distance, and 11 markers remained unlinked. Three loci identified by three cDNA clones, PC8, PC5 and PC68.1, were tightly linked to the *dw* locus in linkage group 5. Segregation distortion was observed for approximately one-third of the markers, perhaps due to the interspecific nature and the reproductive (i.e. self-incompatibility) differences between peach and almond. This map will be used for adding other markers and genes controlling important traits, identifying the genomic locations and genetic characterizing of the economically important genes in the genus *Prunus*, as well as for marker-assisted selection in breeding populations. Of particular interest are the genes controlling tree growth and form, and fruit ripening and mesocarp development in peach and almond.

Key words Linkage map · RFLPs · *Prunus* spp. Molecular markers

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M. R. Foolad[1] · S. Arulsekar · V. Becerra · F. A. Bliss (✉)
Department of Pomology, University of California, Davis, CA 95616, USA

Permanent address:
[1] Department of Horticulture, The Pennsylvania State University, University Park, PA 16802, USA

Introduction

Genetic linkage analyses in the economically important fruit and nut crops of the genus *Prunus* (e.g. peach, nectarine, almond etc.) have been limited to a few genes controlling morphological differences and isozyme markers. Recently, with the increase in the availability of DNA-based molecular markers, linkage maps can be developed relatively easily using different types of segregating progenies (e.g. Chaparro et al. 1994; Belthoff et al. 1993). Molecular marker-based linkage maps have been useful for identifying and localizing important genes controlling both qualitatively and quantitatively inherited traits in a wide range of plant species (Tanksley et al. 1989). Also, these maps are promising tools for improving the efficiency of breeding and selection programs (Paterson et al. 1991) and should be especially useful for tree fruit and nut crops that have long juvenile periods and for which the large plant size necessitates substantial investments in time and land (Durham et al. 1992; Jarrell et al. 1992).

The procedure for constructing a genetic linkage map is straightforward: (1) identification of polymorphic marker loci, (2) verification of Mendelian segregation and (3) placement of loci in linear order relative to each other with the aggregation of linked markers into linkage groups. The small size of the peach [*P. persica (L.)* Batsch] genome, estimated at 0.55 pg/2C (Arumuganathan and Earle 1991) and 0.62 pg/2C (Belthoff et al. 1993), and of other diploid (2n=2x=16) members of *Prunus* should facilitate map-based cloning and the isolation of specific genes once highly saturated linkage maps are developed. Also, such linkage maps can be used to study genome organization and will greatly facilitate marker-assisted selection and breeding.

While several types of molecular markers are suitable, Restriction fragment length polymorphism (RFLP) markers have been the chosen tool by which to create linkage maps for many plants. "RFLP markers are virtually unlimited in numbers, the only restriction to the efficiency of the technique being the DNA sequence divergence between

the genotypes tested" (Ritter et al. 1990). Most molecular linkage maps are constructed around a core of polymorphic RFLPs, with the probes being either homologous or heterologous to the DNA sequences of plants in the mapping population. Homologous probes are derived from either total genomic DNA or developmentally- and/or tissue-specific cDNA from the same or closely-related species, while heterologous probes include conserved genes of known function that are available from various sources.

Belthoff et al. (1993) analyzed 35 RFLPs in segregating peach populations and placed them into five linkage groups, and 10 additional unlinked markers. That linkage map has since been expanded to include 57 markers [RFLP, randomly amplified polymorphic DNA (RAPD), morphological and isozyme] assigned to 9 linkage groups which cover 520 cM of the peach nuclear genome (Rajapakse et al. 1994). Chapparo et al. (1994) analyzed nine different F_2 peach populations for linkage relationships between 14 morphological loci, 2 isozyme loci and 83 RAPD markers, and assigned the markers to 15 different linkage groups. Ten isozyme loci were analyzed in segregating almond populations, with 6 being placed in 2 different linkage groups (Arus et al. 1994) A molecular marker-based genetic map for almond, *P. dulcis,* is being developed using both intraspecific and interspecific segregating populations (Messeguer et al. 1994).

Peach has been used as a parent in many of the linkage maps developed in *Prunus* because it is economically important, has a small genome size and is genetically the best characterized species in the genus. However, several authors have noted that peach cultivars have a low level of polymorphism based on isozyme studies (Arulsekar et al. 1986; Mowrey et al. 1990) and RAPD markers (Chapparo et al. 1994). Populations resulting from interspecific crosses provide greater diversity in segregating populations, which facilitates map making, but wide genomic differences may produce distorted segregation ratios. Almond and peach are closely related and easily intercrossable, and meiosis is regular in the F_1 plants which are fully fertile (Kester and Asay 1975). However, F_2 plants may be segregating for compatibility since peaches are self-compatible and most almonds are self-incompatible. The high degree of synteny reported among related plants, e.g. *Lycopersicon* and *Solanum* (Gebhardt et al. 1991); maize and sorghum (Whitkus et al. 1992), suggests that a map based on a peach×almond hybrid should also be useful for other stone fruit crops, i.e. plum, apricot, cherry and nectarine.

The peach and almond parents chosen for this study differ for many traits, some of which have been characterized genetically to a limited extent. The most obvious differences are for fruit type (fleshy vs. desiccated mesocarp) and tree size (brachytic dwarf vs. normal growth habit). The objective of this study was to construct a low-density genetic linkage map for *Prunus* using RFLP and isozyme markers and to place the dwarf (*dw*) locus in the initial linkage map. Additional loci controlling other traits will be added as the population is characterized more fully and when linkages from other populations can be integrated using genes common to both populations for anchors.

Materials and methods

Mapping population

An interspecific F_2 population generated by selfing a single F_1 plant of a cross of the dwarf peach selection 54P455 *(Prunus persica)*× almond cv 'Padre' (*P. dulcis*), was provided by Dr. T. Gradziel, Department of Pomology, UC, Davis. This population, hereafter denoted as Prunus mapping population 1 (PMP1), consists of 64 F_2 plants which are maintained in the field under standard cultural conditions for phenotypic evaluation, data collection and the leaf tissue used for isozyme analysis and DNA isolation.

Source of DNA probes

Three different sources of DNA probes were used in this study: (1) random genomic DNA clones from a diploid plum, cv 'Santa Rosa' (*P. salicina*), (2) selected random genomic DNA clones from almond (P. Arus, IRTA, Cabrills, Spain) and (3) mesocarp-specific cDNA clones from developing fruit of peach (cv 'Spring Lady').

Plum probes

A random genomic DNA clonal library was constructed using the diploid plum cv 'Santa Rosa'. The plum genomic DNA was double-digested with *Cla*I and *Pst*I restriction enzymes, and the resulting fragments were ligated into the multiple cloning site of p-Bluescript plasmid. The recombinant clones were transformed using *E. coli* strain DH5 alpha. A total of 98 clones were screened for polymorphism among the parents and F_1 plants using seven restriction enzymes (*Bam*HI, *Bgl*II, *Dra*I, *Eco*RI, *Eco*RV, *Hin*dIII and *Pst*I).

Almond probes

The *Pst*I almond genomic clones used in this mapping analysis were provided by Dr. P. Arus, IRTA, Cabrills, Spain. These were part of a set of 18 selected clones that represented specific linkage groups that are being developed by the Arus' group for almond genome mapping. They were screened for polymorphism among the parental and F_1 plants that produced the PMP1.

Peach cDNA probes

A Lambda cDNA library was made using mRNAs isolated from developing peach mesocarp tissue sampled 40–45 days after pollination from the early-maturing peach cv 'Spring Lady'. The mRNA isolation, cDNA synthesis and cloning were done using the "Lambda ZipLox System" from BRL-Life Technologies by Biogenetic Services Inc., Brookings, S.D. The primary library contained 4.5×10^6 plaque-forming units and was amplified and subcloned in pZL1 plasmid according to the protocols provided by BRL-Life Technologies, Gaithersberg, M. D. The cDNA insert was isolated by using a polymerase chain reaction (PCR) amplification procedure with the T7 and SP6 promoter primers. They were screened for polymorphism among the parental and F_1 plants using four restriction enzymes (*Eco*RI, *Eco*RV, *Dra*I and *Hin*dIII). Screening was done using Amersham's ECL (Enhanced Chemiluminescence) kit.

DNA isolation

Total genomic DNA was extracted using the procedure described by Gepts and Clegg (1989), which had been modified from Saghai-Maroof et al. (1984). Ten grams of leaf tissue was ground to a fine powder in liquid nitrogen using a pestle and mortar. The pulverized tissue was incubated in 15 ml extraction buffer [50 m*M* TRIS-HCl pH 9.5, 10 m*M* EDTA 0.7 M NaCl, 1% SDS; and prior to use, 5%

polyvinylpolypyrrolidone (PVPP) and 2% 2-Mercapto-ethanol was added] at 60°C for 30 min with occasional gentle mixing. The mixture was extracted twice with an equal volume of chloroform:isoamyl alcohol (24:1). The DNA from the aqueous phase was precipitated with 2/3 volume of isopropyl alcohol. The DNA precipitate was dissolved in TE (10 m*M* TRIS-HCl pH 8.0; 1 m*M* EDTA) buffer and further purified by cesium chloride-ethidium bromide density gradient centrifugation. The yield of DNA after CsCl banding was approximately 15 µg/g of fresh leaf tissue.

Southern blotting, probe labelling and hybridization

From each individual 7 µg of total DNA was digested separately with the different restriction enzymes, including *Eco*RI, *Eco*RV, *Hin*dIII, *Bam*HI and *Dra*I, according to the manufacturer's instructions. The digested DNA from each individual was separated by electrophoresis in 0.8% agarose gel (25 V, 12 h). Lambda *Hin*dIII fragments were included in each gel for molecular weight determination. The DNA from the gel was transferred to a nylon (Zeta-Bind from AMF-CUNO, Conn.) membrane by the capillary transfer procedure (Southern 1975). The membrane was baked for 1 h at 80°C and stored until used for hybridization.

All of the plum clones were radiolabelled with [^{32}P] according to random hexamer primer method (Feinberg and Vogelstein 1983) to a specific activity of $0.5–1\times10^9$ cpm/mg DNA. Genomic blots were prehyridized (4–16 hr) and hybridized (12–24 hr) at 42 °C in a solution containing 5×SSPE, 5× Denhardt's (0.1% Ficoll, 0.1% polyvinylpyrrolidone, 0.1% BSA), 0.5% SDS, 200–400 mg/ml sheared, denatured salmon sperm DNA and 50% formamide. Hybridizations were carried out with a $4–5\times10^6$ cpm/ml solution. The membranes were washed three times, each for 20–30 min at 42°C, in a buffer containing 0.2×SSPE and 0.1% SDS. The membranes were exposed then to X-ray films (Kodak XAR-2) at –70°C for 8 h to 3 days with an intensifying screen.

All of the cDNA and almond genomic clones were labelled using the ECL (enhanced chemiluminescence) nonradioactive method (Amersham Corp, Arlington Heights, Ill.). New sets of Southern blots were prepared for the nonradioactive labelling. The labelling, prehybridization, hybridization, washing and detection solutions and conditions were as suggested by the manufacturer (Amersham).

Isozyme analysis

Vigorously growing leaves were sampled from the parental and F_2 individuals, and assayed for six isozyme genotypes using starch gel electrophoresis. These loci included Glucose phosphate isomerase (*Gpi-2*), 6-Phosphogluconate dehydrogenase (*6Pgd-2*), Phosphoglu-co mutase (*Pgm-1* and *Pgm-2*), Leucine aminopeptidase (*Lap-2*) and Isocitrate dehydrogenase (*Icd-2*). Gel electrophoresis, enzyme extraction and activity staining procedures were according to Arulsekar and Parfitt (1986).

Statistical and linkage analysis

The F_2 segregation data for the 110 codominant loci were subjected to the chi-square test for goodness-of-fit to an expected genotypic ratio of 1:2:1 and an allele frequency ratio of 1:1. Segregation data for the 8 dominant loci were examined for goodness-of-fit to the expected phenotypic ratio of 3:1.

Multipoint linkage analysis was performed by using the MAPMAKER computer program v. 3.0 (Lander et al. 1987; Lincoln et al. 1992). Initially the "group" command was used to assign marker loci into linkage groups using a minimum LOD score of 4.0. Subsequently, three-point linkage analysis was performed within each group to determine the maximum likelihood recombination fraction and the associated LOD score for each combination of loci. The commands "order", "try" and "compare" were then used to find the best order of the loci within each group. Haldane's mapping function (Haldane 1919) was used to convert recombination fraction into map distances.

Results

Identification of polymorphic DNA clones

In order to identify the DNA clones that reveal polymorphism between the peach (54P455) and almond ('Padre') parents each DNA clone was hybridized individually with the parental and F_1 genomic DNAs which were digested with different restriction enzymes electrophoresed and Southern blotted onto a nylon (Zeta Bind) membrane. The DNA clones that showed polymorphism between the parents along with a good hybridization signal were selected for segregation analysis in the F_2 progeny. Of the 98 plum genomic clones tested more than 50% had a very weak hybridization signal and were not useful. Twelve clones were clearly single to low-copy and showed polymorphism between the peach and almond parents with the restriction enzymes *Bam*H1 (2 clones), *Eco*RI (5 clones) *Eco*RV (1 clone), *Hin*dIII (3 clones) and *Dra*I (1 clone).

Of the 130 cDNA clones from peach mesocarp that were examined, 63 were clearly single to low copy and polymorphic with the restriction enzymes *Eco*RI (18 clones), *Eco*RV (18 clones), *Dra*I (16 clones) and *Hin*dIII (11 clones). Similarly 14 almond genomic clones were polymorphic for *Eco*RI (2 clones), *Dra*I (5 clones), *Bam*HI (4 clones) and *Hin*dIII (3 clones).

The enhanced chemiluminescence procedure (Amersham) used in this study utilizes the light-emitting reaction mediated by the horseradish peroxidase catalyzed oxidation of luminol, with chemical enhancers to increase the light output. The light emission is then captured on blue light-sensitive autoradiography film. The procedures were found to be simple, fast and as sensitive as [^{32}P] for probes used in this study. Similar banding patterns were observed for [^{32}P] labelling and non-radioactive, chemiluminescent labelling procedures. In our laboratory, we have routinely reprobed our blots at least 12 times and a maximum of 15 times without losing resolutions. Southern blots hybridized with a plum probe (PLG 35a) labelled with ^{32}P and with the chemiluminescent label are shown in Fig. 1.

Segregation of marker loci

Genotypic segregation data and the chi-square values for goodness-of-fit to 1:2:1 and 3:1 Mendelian ratios in the F_2 population for the 118 markers scored are shown in Table 1. Each of the 12 plum clones identified a single codominant locus, and of these markers, 6 showed segregation distortion. Of the 63 cDNA clones, 14 revealed multiple loci (2–3), and the other 49 revealed a single locus each. A cDNA clone (PC-6) labelled with the chemiluminescent procedure and segregating in the F_2 population is shown in Fig. 2. In total, 80 loci were scored for the cDNA clones, 2 of which were inherited as dominant markers (presence vs. absence). Of the 80 markers, 33 showed skewed segregation at either the 5% or 1% level of probability (Table 1). The 14 almond genomic clones tested revealed 19 loci

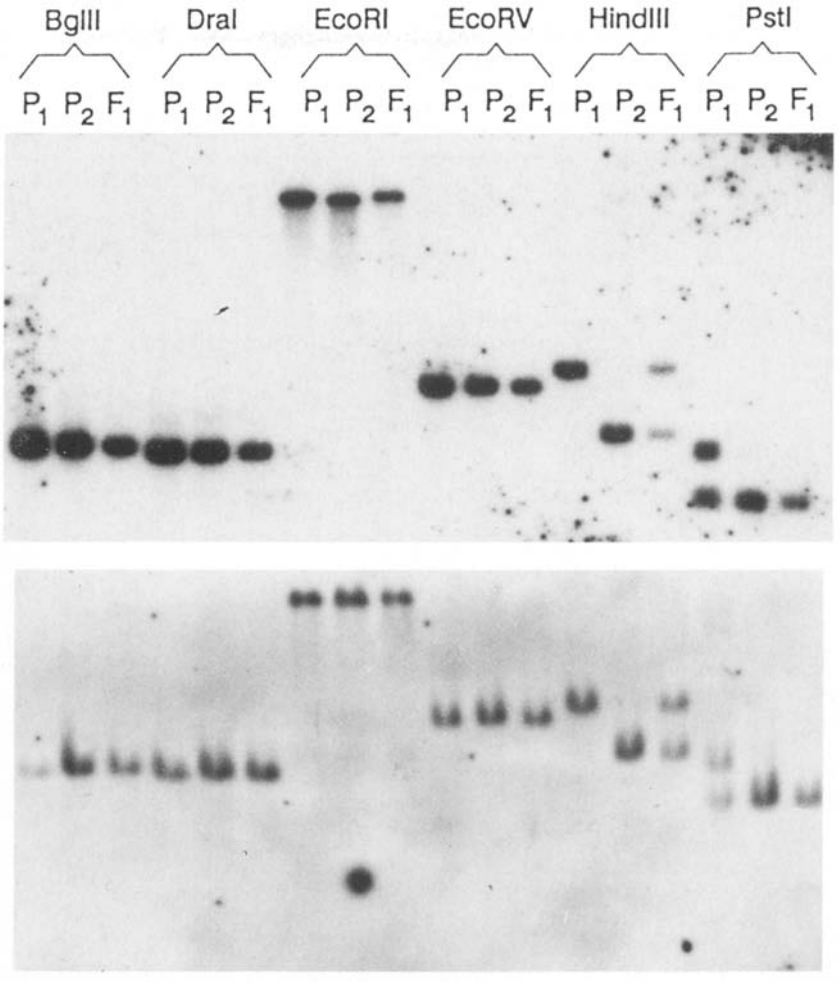

Fig. 1 Genomic Southern blots showing the peach parent (P_1), almond parent (P_2) and F_1 hybrid (F_1) digested with six restriction enzymes and hybridized with the plum genomic-clone PLG35 labelled with [^{32}P] (*above*) and non-radioactive chemiluminescent label (*below*). The plum clone reveals polymorphism for the *Hin*dIII restriction enzyme

(5 dominant and 14 co-dominant). Seven markers showed deviations from the expected Mendelian ratio (Table 1).

The six isozyme markers (*Gpi-2*, *6Pgd-2*, *Pgm-1*, *Pgm-2*, *Lap-2* and *Icd-2*) that were polymorphic between peach and almond were scored for segregation in the F_2 population. Segregation ratios for *Gpi-2* and *Lap-2* showed significant deviation from the expected 1:2:1 ratio. In addition to the molecular markers, the F_2 plants were scored for normal versus brachytic dwarf growth habit, which segregated as a monogenic trait (3 normal: 1 dwarf), as expected.

The analyses of the 118 loci showed that 48 deviated significantly from expected Mendelian ratios (Table 1). Eleven markers showed a deficiency for the peach genotype, 33 showed a deficiency of the almond genotype and 4 loci had an excess of the heterozygote class.

Linkage groups

The 118 markers that included 111 RFLPs, 6 isozymes and the *dw* locus were analyzed using "MAPMAKER" to detect linkage groups. One hundred and seven markers were linked to at least 1 other marker, and a map consisting of 9 linkage groups was generated using a highly significant LOD score of 4.0 (Fig. 3). Eleven markers remained unlinked. The MAPMAKER analysis was done both with and without the error detection facilities turned on. While MAPMAKER did point out some "candidate errors", upon rechecking our scoring and entering of data in the computer, we did not find the reported errors. Therefore, the results reported here are without the error detection facilities turned on. The size of the linkage groups ranged from 3.9 cM (Group 7) to 194.6 cM (Group 5), with a total genome coverage of approximately 800 cM and an average distance of 7.5 cM between pairs of loci.

Among these linkage groups, the markers in groups 2, 6, 7 and 9 were without any segregation distortion, whereas a number of markers in the other groups showed different levels of skewness. Of the 48 markers that showed significant deviation from the expected ratios, 25 were located in linkage group 5. The *dw* locus, which controls growth habit, was mapped in linkage group 5 at the same location as the cDNA marker PC 8. In addition, 2 other cDNA markers, PC 5 and PC 68.1, were found to be flanking the *dw* locus at 3.5 and 3.3 cM distance. However, all 3 cDNA markers exhibited segregation distortion, with a deficiency of the almond allele.

Table 1 Monogenic segregation of genetic markers in an F_2 population of a cross between peach (54P455) and almond ('Padre')

Marker	Genotype[a]			χ^2 (1:2:1)	q[b]	χ^2 (1:1)[c]
	p/p	p/d	d/d			
Group 1						
PLG2	6	42	15	9.57**	0.57	2.57
PLG35	3	40	21	14.13**	0.64	10.13**
AG33	15	35	13	0.90	0.48	0.13
PC2	13	34	13	1.07	0.50	0.00
PC20.2	3	43	17	14.62**	0.61	6.22*
PC21	13	36	12	2.02	0.49	0.03
PC43	11	34	16	1.62	0.54	0.82
PC84	4	43	15	13.19**	0.59	3.9
AG19.3	44		18	0.52 (3:1)[d]	–	
Group 2						
PLG5	8	33	11	4.12	0.53	0.35
AG37	15	30	17	0.19	0.52	0.13
AG32.2	15	32	14	0.18	0.49	0.03
AG7	10	38	13	3.98	0.52	0.30
PC3	20	30	13	1.70	0.44	1.56
PC6	11	37	15	2.43	0.53	0.51
PC19.1	10	38	15	3.48	0.54	0.79
PC19.2	13	37	13	1.92	0.50	0.00
PC22.2	8	36	16	4.53	0.57	2.13
PC35.2	13	31	17	0.54	0.53	0.52
PC40	10	35	17	2.61	0.56	1.58
PC57	15	30	18	0.43	0.52	0.29
PC60.1	15	29	18	0.55	0.52	0.29
PC66.1	16	29	16	0.15	0.50	0.00
PC68.2	11	36	14	2.28	0.52	0.30
PC69	18	29	16	0.52	0.48	0.13
PC83	11	36	14	2.28	0.52	0.30
PC107	15	29	17	0.28	0.52	0.13
PC119	23	24	15	5.23	0.44	2.06
AG2.2	17	45		0.99 (1:3)	–	
AG3	18	45		0.64 (1:3)	–	
Group 3						
PLG6	20	25	12	3.11	0.43	2.25
PLG26	8	35	20	5.35	0.60	4.57*
AG11	6	36	21	8.43*	0.62	7.14**
AG32.1	10	37	14	3.30	0.53	0.52
PC11	8	35	18	4.61	0.58	3.28
PC12	12	39	11	4.16	0.49	0.03
PC18	11	37	14	2.61	0.52	0.29
PC30	12	36	12	2.40	0.50	0.00
PC35.1	12	33	14	0.97	0.52	0.14
PC38	12	37	14	2.05	0.52	0.13
PC46	6	39	17	8.03*	0.59	3.90
PC60.2	12	33	12	1.42	0.50	0.00
PC62	9	32	20	4.11	0.59	3.97
PC68.3	12	35	13	1.70	0.51	0.03
PC65	6	40	16	8.45*	0.58	3.23
PC70	7	38	15	6.40*	0.57	2.13
PC75	10	36	16	2.77	0.55	1.16
PC85	9	34	20	4.24	0.59	3.84*
PC121.1	11	39	13	3.70	0.52	0.13
AG22	11	50		6.38 (1:3)	–	
PC121.2	52		11	7.14* (3:1)	–	
Group 4						
PLG 10	20	43	0	21.10**	0.34	12.70**
PLG17	17	33	13	0.65	0.47	0.51
AG12	24	36	2	17.23**	0.32	15.61**
PC34	10	34	16	2.27	0.55	1.2
PC41	17	37	7	6.05*	0.42	3.28
PC53	18	37	7	6.23*	0.41	3.90*
PC58	11	34	18	0.95	0.56	1.56
PC66.2	13	26	19	1.86	0.55	1.24
PC78	23	36	1	18.53**	0.32	16.13**
Group 5						
PLG35a	14	42	8	7.38*	0.45	1.13
PLG59	11	41	12	5.09	0.51	0.03
AG16	25	36	0	22.48**	0.30	20.49**
AG17	11	42	9	7.94*	0.48	0.13
AG18	20	39	4	11.70**	0.37	8.13
AG19.1	12	42	7	9.49**	0.46	0.82
AG19.2	12	46	4	16.58**	0.44	2.06
PC5	23	39	0	21.19**	0.31	17.06**
PC8	24	38	0	21.74**	0.31	18.58**
PC7.1	8	42	13	7.79*	0.54	0.79**
PC7.2	23	40	0	21.38**	0.32	16.79**
PC9.2	21	35	7	7.00*	0.39	6.22
PC13	10	38	15	3.48	0.54	0.79
PC15.1	9	37	16	3.90	0.56	1.58
PC16.1	23	37	0	20.90**	0.31	17.63**
PC16.2	15	40	5	10.00**	0.42	3.33
PC17.2	12	46	5	14.90**	0.44	1.56
PC20.1	15	43	5	11.57**	0.42	3.17
PC22.2	19	35	6	7.30*	0.39	5.63*
PC22.3	20	38	5	9.83*	0.38	7.14**
PC23	14	41	5	10.77**	0.43	2.70
PC28.1	26	35	1	21.19**	0.30	20.16**
PC39	10	43	9	9.32**	0.49	0.03
PC49	15	41	6	9.06*	0.43	2.61
PC52	11	38	12	3.72	0.51	0.03
PC61	19	39	4	11.39**	0.38	7.26**
PC64	10	40	10	6.67*	0.50	0.00
PC68.1	23	38	0	21.03**	0.31	17.34**
PC76	10	33	16	2.05	0.55	1.22
PC116	24	35	4	13.48**	0.34	12.70**
PC28.2	22	38		0.30 (1:3)	–	
dw	25	38		1.14 (1:3)	–	
Group 6						
PC9.1	11	36	16	2.08	0.54	0.79
PC15.2	10	33	19	2.87	0.57	2.61
Group 7						
PC45	13	36	12	2.02	0.49	0.03
PC104	9	35	13	3.53	0.54	0.56
Group 8						
PLG36	22	36	6	9.00*	0.38	8.00**
PC17.1	23	35	5	11.06**	0.36	10.29**
PC48	22	36	4	12.06**	0.35	10.45**
PC67	22	37	3	13.97**	0.35	11.65**
PC113	19	36	4	10.49**	0.37	7.63**
Group 9						
PLG86	10	37	17	3.09	0.55	1.53
AG32.3	8	35	14	4.23	0.55	1.26
PC59	10	33	19	2.87	0.57	2.61
PC71	9	37	15	3.95	0.55	1.18
PC90	9	37	15	3.95	0.55	1.18
Pgm-1	13	35	14	1.06	0.51	0.03
Unlinked						
PLG39	11	43	10	7.59*	0.49	0.03
AG35	8	38	15	5.30	0.56	1.61
PC20.3	2	44	17	17.06**	0.62	7.14**
PC81	16	40	3	13.20**	0.39	5.73*
PC109	23	28	10	5.95	0.39	5.54*
Gpi-2	8	43	10	10.38**	0.52	0.13
6Pgd-2	18	27	17	1.06	0.49	0.03
Pgm-2	12	37	13	2.35	0.51	0.03
Lap-2	5	38	19	9.48**	0.61	6.32*
Icd-2	12	35	15	1.32	0.52	0.29
AG2.1	44		18	0.52 (3:1)	–	

*, ** Significant at the 5% and 1% probability levels, respectively

[a] p/p, Homozygous for *Prunus persica* alleles; p/d, heterozygous; d/d, homozygous for *P. dulcis* alleles

[b] Allele frequency for P. dulcis

[c] Expected allele frequency ratio

[d] Expected genotypic frequency for a dominant locus

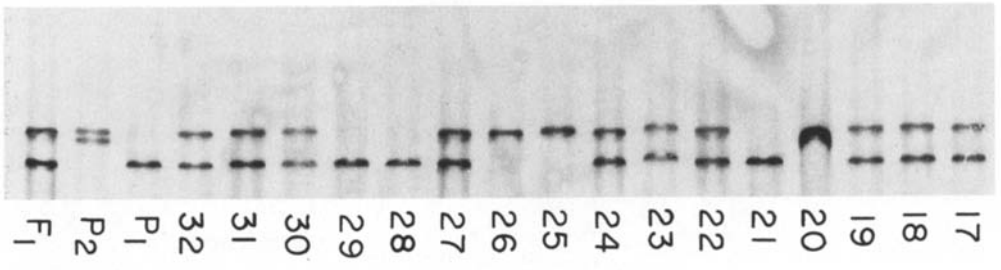

Fig. 2 The F_2 progeny segregating for the peach cDNA clone PC6 labelled with chemiluminescent label. P_1 peach parent, P_2 almond parent, F_1 interspecific F_1 hybrid between peach and almond

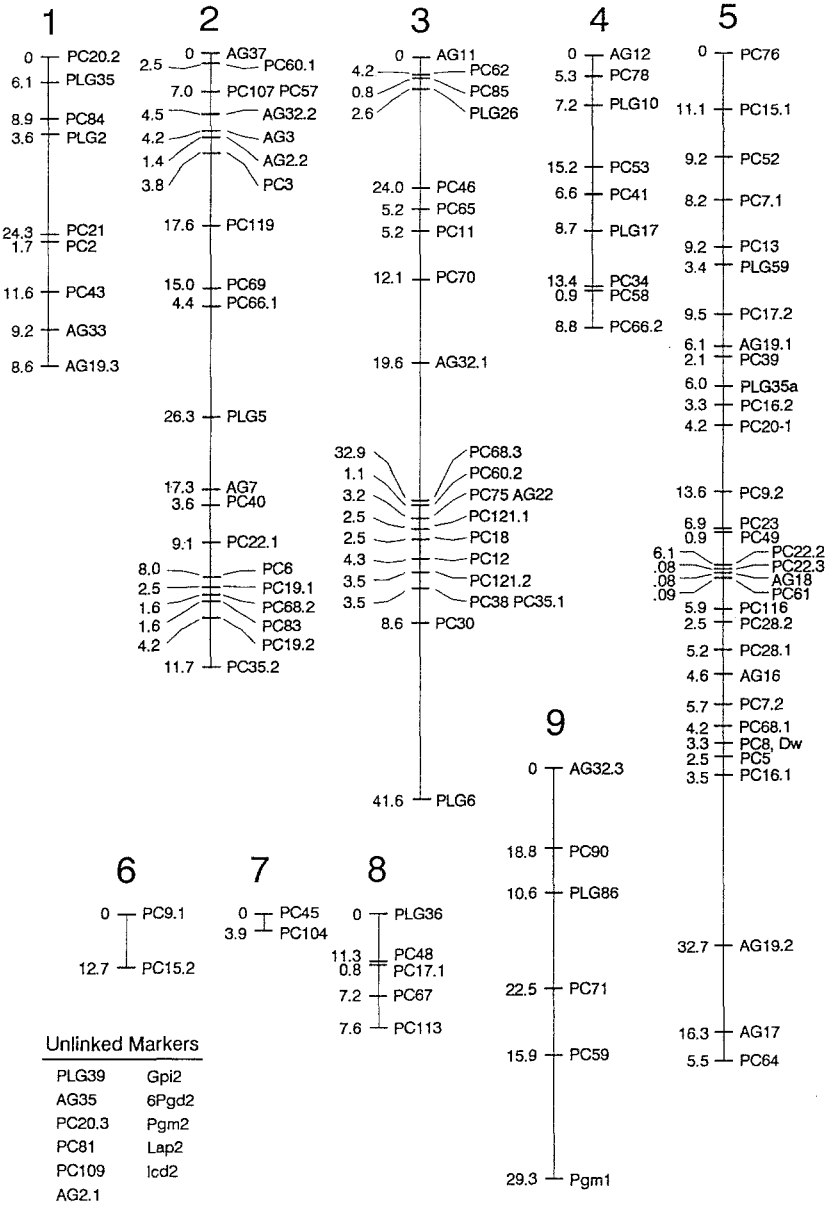

Fig. 3 Linkage map generated from the F_2 population resulting from the interspecific cross of peach×almond. The *number* identifying the linkage group is shown at the *top* of each group. The names of the loci are shown on the *right* (*PLG* plum genomic clones, *AG* almond genomic clones, *PC* peach cDNA clones), and the centi-Morgan distances are shown on the *left*

Discussion

Genetic linkage maps within *Prunus* have been constructed for peach (*P. persica*) (Chaparro et al. 1994; Belthoff et al. 1993) and almond (*P. dulcis*) (Messeguer et al. 1994). In the current study we have presented a genetic linkage map generated from an interspecific peach×almond F_2 population. Peach is predominantly self-fertilizing, while almond shows obligate out-crossing due to a gametophytic self-incompatibility system (Hesse 1975; Kester and Asay 1975). Because it is predominantly self-pollinating and relatively few parents have bean used for breeding, genetic polymorphism among US peach cultivars is quite limited (Arul-

sekar et al. 1986; Scorza et al. 1985) compared to that in almond cultivars. We chose almond and peach as parents for our mapping population because the species are genetically diverse, can be hybridized easily, and the F_1 is quite fertile. There were a sufficient number of segregating markers to generate a low- to medium-density linkage map having 6 major and 3 minor linkage groups.

The existence of large gaps in the map which are without marker coverage is evident, and there are more linkage groups than the haploid number of chromosomes (x=8), including groups with only 2–5 marker loci (i.e. groups 6, 7 and 8). Additional markers must be mapped in order to achieve a complete and uniform coverage of the entire genome.

Segregation distortion was observed for approximately 37% of the marker loci scored. This distortion may be due to the interspecific nature of the cross between peach and almond. Peach is a self-fertilizing species, whereas almond is an obligate outcrosser with a gametophytic incompatibility mating system. Peach and almond can hybridized readily, and the F_1 hybrid is quite vigorous and self-fertile. However, the F_2 population may exhibit a low to moderate amount of hybrid breakdown (DE Kester, personal communication; personal observation). In our F_2 population, out of the total of approximately 90 individuals, the other seedlings were too small and weak to harvest any leaves. The occurence of hybrid breakdown and hybrid weakness indicates that there is a moderate amount of genetic differentiation between peach and almond, which might contribute to the segregation distortion that was observed. It is important to note that peach and almond differ substantially in their physiology and developmental processes, and many recombinations of the genetic factors affecting these processes will most likely be selected against. It is also possible that linkage group 5 may include loci controlling compatibility since that group contains more than one-half of the distorted loci and distortion is due primarily to a deficiency of almond alleles. Segregation distortion has been commonly observed in interspecific mapping populations of several crops, e.g. tomato (Bernatzky and Tanksley 1986; Nienhuis et al. 1987) and in wide crosses (Weeden 1989). Aberrant segregation ratios may also result from the expression of linked lethal genes either at the gametic or zygotic stages of plant development (Zamir and Tadmor 1986).

This and other *Prunus* linkage maps can be used to locate genes of economic and biological interest, for map-based cloning of important genes, to aid in analyzing quantitatively inherited traits and for tagging genes to increase efficiency of selection and breeding, particularly in the perennial tree fruit and nut crops with a long generation time where selection at the seedling stage will save substantial time, land and labor. Regions of the *Prunus* genome where the order of genes and markers is conserved across several species can be used as anchors for high-resolution mapping. Future maps constructed from different interspecific crosses may also give insight into chromosomal rearrangements that are important factors in the evolution of the various *Prunus* species which are phenotypically quite different.

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