

# An expanded genetic linkage map of *Prunus* based on an interspecific cross between almond and peach

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**Abstract:** The genetic linkage map of *Prunus* constructed earlier and based on an interspecific F<sub>2</sub> population resulting from a cross between almond (*Prunus dulcis* D.A. Webb) and peach (*Prunus persica* L. Batsch) was extended to include 8 isozyme loci, 102 peach mesocarp cDNAs, 11 plum genomic clones, 19 almond genomic clones, 7 resistance gene analogs (RGAs), 1 RGA-related sequence marker, 4 morphological trait loci, 3 genes with known function, 4 simple sequence repeat (SSR) loci, 1 RAPD, and 1 cleaved amplified polymorphic sequence (CAP) marker. This map contains 161 markers placed in eight linkage groups that correspond to the basic chromosome number of the genus ( $x = n = 8$ ) with a map distance of 1144 centimorgans (cM) and an average marker density of 6.8 cM. Four more trait loci (*Y*, *Pcp*, *D*, and *SK*) and one isozyme locus (*Mdh1*) were assigned to linkage groups based on known associations with linked markers. The linkage group identification numbers correspond to those for maps published by the Arús group in Spain and the Dirlwanger group in France. Forty-five percent of the loci showed segregation distortion most likely owing to the interspecific nature of the cross and mating system differences between almond (obligate outcrosser) and peach (selfer). The *Cat1* locus, known to be linked to the *D* locus controlling fruit acidity, was mapped to linkage group 5. A gene or genes controlling polycarpel fruit development was placed on linkage group 3, and control of senesced leaf color (in late fall season) (LFCLR) was mapped to linkage group 1 at a putative location similar to where the *Y* locus has also been placed.

**Key words:** *Prunus*, molecular markers, RFLPs, resistance gene analogs (RGAs), polycarpel fruit, stone fruits.

**Résumé :** La carte génétique du genre *Prunus* produite précédemment à l'aide d'une population F<sub>2</sub> issue d'un croisement interspécifique entre l'amandier (*Prunus dulcis* D.A. Webb) et le pêcher (*Prunus persica* L. Batsch) a été bonifiée et compte maintenant : 8 locus isoenzymatiques, 102 ADNc du mésocarpe du pêcher, 11 clones génomiques du prunier, 19 clones génomiques de l'amandier, 7 analogues de gène de résistance (RGA), 1 marqueur correspondant à un gène ressemblant à un gène de résistance, 4 caractères morphologiques, 3 gènes de fonction connue, 4 microsatellites (SSR), 1 marqueur RAPD et 1 marqueur CAPS (« cleaved amplified polymorphic sequence »). Cette carte comprend 161 marqueurs formant huit groupes de liaison, ce qui correspond au nombre haploïde de chromosomes ( $x = n = 8$ ), et totalise 1144 centimorgans (cM), ce qui se traduit par une densité moyenne d'un marqueur à tous les 6,8 cM. Quatre autres caractères morphologiques (*Y*, *Pcp*, *D* et *SK*) et un locus isoenzymatique (*Mdh1*) ont été assignés à un groupe de liaison sur la base d'une liaison connue avec un marqueur. La numérotation des groupes de liaison est conforme à celle employée par les groupes de Arús en Espagne et de Dirlwanger en France. Quarante-cinq pour cent des locus montraient une distorsion de la ségrégation vraisemblablement en raison de la nature interspécifique du croisement et de différences quant au mode de reproduction des deux espèces, l'amandier étant une espèce à allofécondation obligatoire et le pêcher à autofécondation. Le locus *Cat1*, connu comme étant lié au locus *D* qui contrôle l'acidité des fruits, a été assigné au groupe de liaison 5. Un gène ou des gènes déterminant le nombre de carpelles au sein du fruit a été situé sur le groupe de liaison 3 et le contrôle de la couleur des feuilles senescentes à l'automne (LFCLR) a été placé sur le groupe de liaison 1 à un endroit semblable à celui où se trouve le locus *Y*.

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*Mots clés* : *Prunus*, marqueurs moléculaires, RFLP, analogues de gène de résistance (RGA), fruit à carpelles multiples, fruits à noyau.

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## Introduction

The genus *Prunus* (Rosaceae: Prunoideae) contains important fruit and nut crops (commonly called “stone fruits”) with different ploidy numbers, for example, sweet cherry, peach, and almond ( $2n = 2x = 16$ ); sour cherry ( $2n = 4x = 32$ ); and European plum ( $2n = 6x = 48$ ). Among the stone fruits, peach (*P. persica* L. Batsch) is genetically well characterized with at least 25 morphological traits described (Hesse 1975).

Genetic linkage maps with a high density of well-placed marker loci are useful for studying the organization of genes controlling expression of important traits, identifying linkages that are either fortuitous or undesirable, and facilitating marker-aided selection (MAS) to complement standard breeding procedures and improve selection efficiency for economic traits that are difficult to select by phenotype early in the life cycle (preferably before field planting). The availability of informative genetic maps will also enhance opportunities for identifying new genetic variability in underutilized and unadapted genetic resources.

Development of genetic linkage maps for fruit and nut crops has been slow because of inadequate sustained funding support and biological limitations, including long life cycles and poor genetic characterization. However, genetic maps have been developed in peach (Chaparro et al. 1994; Rajapakse et al. 1995; Dirlwanger et al. 1998), almond (Viruel et al. 1995), almond  $\times$  peach (Foolad et al. 1995; Joobeur et al. 1998), sweet cherry (Stockinger et al. 1996), and sour cherry (Wang et al. 1998). Furthermore, random amplified polymorphic DNA (RAPD) markers linked to several economic traits in peach were reported by Warburton et al. (1996). A genetic linkage map for *Prunus* based on an interspecific  $F_2$  population resulting from an almond (*P. dulcis*)  $\times$  peach (*P. persica*) hybrid using RFLP markers from cDNA clones of developing peach fruit mesocarp was described by Foolad et al. (1995). That map included 107 markers in nine linkage groups with a total genomic coverage of 800 cM. Here we describe an expanded *Prunus* linkage map with additional RFLP, isozyme, resistance gene analog (RGA), resistant gene analog-related sequence (RRS), RAPD, cleaved amplified polymorphic sequence (CAPs), and simple sequence repeat (SSR) markers along with tentative map locations of genes controlling fruit doubling (polycarpel), fruit acidity, sweet kernel, self-incompatibility (*Si*), and senesced leaf color and the nectarine (*G*) locus.

## Materials and methods

### Mapping populations

The interspecific mapping population (PMP1) is an  $F_2$  population ( $n = 64$  individuals) resulting from an almond (‘Padre’)  $\times$  dwarf peach selection (54P455) that was used to construct a linkage map described earlier (Foolad et al.

1995). In addition to PMP1, a segregating interspecific  $F_3$  population (PMP6), developed from a cross between an almond selection (6A11) and a dwarf peach (54P455), consisting of 113 individuals resulting from the self-fertilization of a single  $F_2$  plant was also used to detect markers linked to single vs. double (fused) fruit (polycarpel) expression. The peach parent 54P455 is common to both PMP1 and PMP6 mapping populations. Both populations were grown in the field under standard culture conditions for trait evaluations and data collection.

### DNA probes

Four sources of DNA probes were used for the RFLP markers. Random genomic clones from plum (PLG) and almond (AG) (*P. Arús*, IRTA, Cabrills, Spain, personal communication, as well as peach mesocarp cDNA clones (CPM) were previously described in Foolad et al. (1995). In addition, we also used DNA clones known to contain RGAs amplified from ‘Okinawa’ peach (described below). Two genes coding for polygalacturonase inhibitor protein (PGIP; cDNA clone from tomato provided by J.M. Labovitch, University of California at Davis) and another for dehydrins (DHN; cDNA clone from peach provided by T. Artlip, USDA-ARS, Keameysville, W.V.) and four SSR markers were also mapped.

The RFLP probes for locating RGAs were developed as follows: the peach rootstock cultivar ‘Okinawa’, which is resistant to root-knot nematodes (*Meloidogyne incognita* race1 and *M. javanica*), was used as the source of RGAs. PCR primers were designed based on low degeneracy within two conserved domains of the nucleotide binding site (NBS) of the *RPS-2* gene of *Arabidopsis thaliana* L. (Mindrinos et al. 1994 Shen et al. 1998). Primers S1 and AS1 (Leister et al. 1996) were also used. PCRs were conducted in a Perkin Elmer thermal cycler (Perkin Elmer, Norwalk, Conn.). The amplified products were cloned using the TA cloning Kit (Invitrogen, Carlsbad, Calif.) and cloned inserts sequenced using an ABI-377 sequencer (ABI, Foster City, Calif.) at the University of California at Davis Plant Genetics Facility. Sequences were analyzed using the ‘Sequencher’ software (Gene Codes Corporation, Ann Arbor, Mich.) and clones containing open reading frames and conserved regions were subjected to a BLAST search. Clones that showed high homology to cloned resistance genes and (or) RGAs identified in other plant species were considered putative RGAs and used as RFLP probes in the almond  $\times$  peach (PMP1) mapping population. Clones that did not show high homology were designated as RGA-related sequences (RRS) and also used as probes.

### Development of the CAP1 marker

Bulked segregant analysis using Operon oligonucleotide primer OPO6 (Operon Technologies, Alameda, Calif.) detected an RAPD fragment linked to the *Mi* gene in PMP2

(Gillen 2001). The RAPD fragment was cloned and converted to a more reliable sequence-characterized amplified region (SCAR) marker (Paran and Michelmore 1993). SCAR markers have been shown to be reliable and potentially codominant (Paran and Michelmore 1993). The SCAR primers developed from the sequence of the RAPD marker produced a 224-bp monomorphic fragment in PMP2 population and in the peach rootstock cultivars Nemaguard, Nemared, and Lovell (data not shown). Direct sequencing of the SCAR product of the PMP2 parents found differences for *AluI*, *HaeIII*, and *PvuII* restriction endonuclease recognition sites. A CAPs marker designated as CAP1 was created by digesting the SCAR product with *PvuII*, which gave fragments of 224 bp (no recognition sites) in 'Okinawa' F1, and 128 and 96 bp (one recognition site) in 'Harrow Blood'.

### Simple sequence repeats

Two SSR markers (GA34 = PceGA34; GA77 = PceGA77) derived from a sour cherry (*P. cerasus*) small-insert genomic DNA library (A. Iezzoni, Horticulture Dept., Michigan State University, East Lansing, Mich., personal communication) and one that was derived from a sweet cherry genomic DNA library (GK12A02; G. King, Horticultural Research Institute, Wellesbourne, U.K., personal communication) were screened for polymorphism in the PMP1 mapping population. The primer sequences for PceGA34 and PS12A02 are given in Downey and Iezzoni (2000). The primer sequences for GA77 were: 5'-CCTTACCCTGGCATCATCA-3' (forward) and 5'-CAGCTGAGCAGGCAACAAAA-3'. The PCRs were done in a 25- $\mu$ L volume and the reaction mixture contained 10 mM Tris-HCl (pH 8.2), 50 mM KCl, 0.0001% gelatin, 100  $\mu$ M of each of the dNTPs, 1.9 mM MgCl<sub>2</sub>, 0.125  $\mu$ M of each primer (forward and reverse), 1 U *Taq* DNA polymerase enzyme and 50 ng of genomic template DNA. Cycling parameters were as follows: 1 cycle of 95°C for 3 min; 35 cycles of 94°C for 1 min, 64°C for 1 min, 72°C for 2 min; followed by 10 min at 72°C. PCRs were carried out in a 96-well block Robocycler™ (Stratagene Inc., La Jolla, Calif.). A stop solution containing 95% formamide, 10 mM NaOH, 0.05% bromophenol blue, and 0.05% xylene cyanole was added to the PCR product. Three microlitres of the mixture was used in gel electrophoresis.

### Polyacrylamide gel electrophoresis and silver staining

Amplified PCR products were separated in a 6% denaturing polyacrylamide gel containing 8 M urea with 1 $\times$  Tris-borate as gel and running buffers. Electrophoresis was performed at 80 W constant power using a BIORAD Sequi-Gen™ (Biorad, Hercules, Calif.) sequencing gel system. A 25-bp ladder (Gibco BRL, Rockville, Md.) was used as a molecular size standard and a DNA sequencing reaction of the pGEM-3Z(+) plasmid was prepared (Promega Inc., Madison, Wis.) and loaded onto a gel to serve as a molecular size ladder (GATC). PCR products were visualized using a silver staining kit from Promega (Promega, Inc., Madison, Wis.) following the manufacturer's instructions. Stained gels were allowed to dry overnight and were scanned using an HP 400C (Hewlett Packard, Roseville, Calif.) scanner for documentation.

### Identification and screening for fruit doubling (polycarpel trait)

Single vs. fused carpels were scored in individual trees of the PMP6 population in late fall and early winter by collecting flower buds from different branches that were then examined under a stereodissecting microscope. Bulked segregant analysis (BSA) (Michelmore et al. 1991) was used to identify markers linked to this trait, and a tentative map location was postulated from the presence of common linked markers in the primary mapping population (PMP1).

### Statistical and linkage analyses

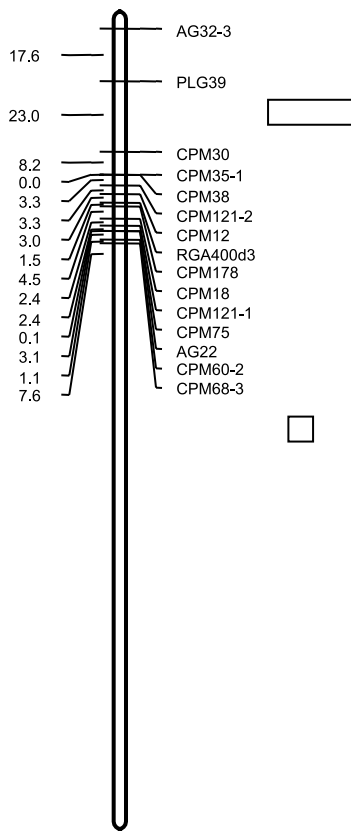
Our previous genetic linkage map was constructed using the MAPMAKER EXP version 3 software (Lander et al. 1987; Lincoln et al. 1992). Initially, the "group" command was used to assign marker loci to linkage groups using a minimum LOD score of 4.0. This was followed by three-point linkage analysis within each group to determine the maximum likelihood recombination fraction and associated LOD score for each combination of loci. The "order", "try", and "compare" commands were used to determine best order of loci within each group. After mapping, the "error detection" command was used and possible errors were re-examined. Kosambi's (Kosambi 1944) mapping function was used to convert recombination fractions into centimorgan map distances. The F<sub>2</sub> segregation data for all loci were subjected to a  $\chi^2$  test for goodness of fit to expected genotypic ratios.

## Results and discussion

### The genetic linkage map

The expanded genetic linkage map from the *Prunus* mapping population, PMP1, includes 161 markers placed into eight linkage groups (Fig. 1), which corresponds to the basic chromosome number of the genus *Prunus* ( $x = n = 8$ ). Among these markers, 143 are RFLPs, 8 are isozyme loci, 4 are SSRs, 1 is a CAPs marker, 1 is a RAPD marker, and 4 are morphological trait loci. The number and distribution of the various markers among the eight linkage groups is shown in Table 1. The linkage map covers a total distance of 1144 cM. Although all of the markers mapped in this study coalesced into the eight linkage groups, there are still large gaps present, particularly in groups 1, 2, 3, and 4. This could in part explain the longer map distance covered in this map compared with the 491-cM distance reported by Joobeur et al. (1998). This difference could also be due to variations in the rate of recombination in the two sets of parents used in these two studies. The eight linkage groups were identified by assigning numbers from one to eight that correspond to the eight linkage groups reported by Joobeur et al. (1998). This was done by locating common isozyme and RFLP loci (AG and PLG clones) as anchors between the two maps. Linkage groups 1, 3, and 6 were comparatively larger than the other groups and this is in agreement with the results reported by Joobeur et al. (1998).

Segregation distortion was observed for 45% of all the markers mapped in this study. Skewed segregation ratios have been reported frequently in interspecific crosses of different plant species and for all types of genetic markers



(e.g., morphological, isozyme, RFLPs, and AFLPs). The percentage of loci exhibiting skewed segregation was highly variable depending on the species (e.g., 69% in *Cryptomeria japonica* (Nikaido et al. 1999), 36% in *Oryza sativa* L. (Xu et al. 1997), 23% in a *Helianthus argophyllus* T.&G. × *H. annuus* L. cross (Quillet et al. 1995), and 46% in almond, peach, *P. ferganensis*, cherry, plum, and apple (Joobeur et al. 1998). It is important to note that almond and peach spe-

cies are distinct in their mating system, in that almond is self incompatible (obligate out crossing species) and peach is predominantly self pollinating, with the exception of some sterile male cultivars.

Among the RFLP markers, seven were dominant and the remainder displayed codominant inheritance; 12% of the RFLP markers (17 of the 143) detected two or more loci and all others were segregating for a single locus. Sixty-five of

**Table 1.** Distribution of genetic markers in eight linkage groups.

Marker type	Linkage groups								Total
	1	2	3	4	5	6	7	8	
Isozymes	3	1	3	0	1	0	0	0	8
Plum genomic (PLG)	2	1	1	2	2	2	1	0	11
Almond genomic (AG)	4	1	6	1	2	3	2	0	19
Peach cDNA (CPM)	18	4	19	8	11	24	11	7	102
RGAs	3	1	0	0	0	0	0	3	7
RRS	1	0	0	0	0	0	0	0	1
CAP1	0	0	1	0	0	0	0	0	1
SSRs	0	2	0	1	0	0	0	1	4
RAPD	0	0	1	0	0	0	0	0	1
Known function genes	0	0	0	0	0	0	3	0	3
Trait loci	1	0	0	0	1	2	0	0	4
Total	32	10	31	12	17	31	17	11	161

the 143 RFLP markers exhibited skewed segregation (16 showed deficiency for peach, 39 showed deficiency for almond and 10 showed an excess of heterozygotes).

A peach microsatellite marker previously designated pchgms1 (Sosinski et al. 2000; Lu et al. 1998) that was found distantly linked to *Mi* (49.2 cM) in linkage group-2 of a peach × peach (PMP2) mapping population (Gillen 2001) was also found to be segregating in the PMP1 mapping population and was mapped in linkage group 2 (Fig. 1). The two GA dinucleotide SSR markers (GA34 and GA77) derived previously from a tetraploid sour cherry genomic library were mapped in linkage groups 2 and 4, respectively (Fig. 1). Another SSR marker, GK12A02, derived from a diploid sweet cherry (*P. avium*), was mapped in LG 8 (Fig. 1). This suggests that some SSRs can be useful for comparing linkage relationships and studying synteny among different *Prunus* species.

The PCR-based marker designated CAP1 showed dominant expression (Table 2) and mapped to linkage group 3 in PMP1 (Fig. 1). However, in PMP2, an intraspecific peach mapping population, Gillen (2001) found it to be linked 9.5 cM from *Mi* and placed it in linkage group 2. This is consistent with other studies where markers later shown to be linked to *Mi* were also in linkage group 2 (Joobeur et al. 1998; Pere Arús, IRTA, Carretera de Cabrils, Cabrils, Spain, personal communication). These disparities may be due to the differences in mapping populations, i.e., intraspecific (PMP2) vs. interspecific (PMP1), and to the fact that the CAP1 marker was scored differently. In PMP1, the susceptible phenotype (associated with three bands from the peach parent) was scored as dominant, whereas in PMP2, the resistant phenotype (associated with one uncut band) was dominant. The dominant expression of CAP1 may be explained by the fact that the CAP1 primers appear to amplify more than one sequence in the peach genome (Gillen 2001). Therefore, it is possible that there are two different loci in PMP1 and PMP2. These inconsistencies among map placements remain, but overall the consensus is that *Mi* is most likely located in linkage group 2.

Eight isozyme loci, including aspartate amino transferase (AAT), catalase (CAT), glucose phosphate isomerase (GPI), isocitrate dehydrogenase (ICD), leucine amino peptidase (LAP), malate dehydrogenase (MDH), phosphoglucomutase (PGM), and 6-phosphogluconate dehydrogenase (6-PGD),

were placed in this linkage map based on segregation in the PMP1 population. The *Mdh1* locus, previously reported by Werner and Moxley (1991) to be associated with low vigor in peach, was segregating in the PMP6 population and we were able to assign a tentative map position for it in linkage group 1 based on common markers segregating in both the PMP6 and PMP1 populations (Warburton et al. 1996). In their intraspecific peach × peach F<sub>2</sub> population, Dirlwanger et al. (1998) also placed the *Mdh1* locus in linkage group 1 in nearly in the same position. That supports our tentative placement of this isozyme locus. Of the nine isozyme loci, four (*Lap2*, *Gpi2*, *Pgm2* and *Mdh1*) were placed in linkage group 1 (Fig. 1). *Pgm1* was placed in linkage group 2, *Icd2*, *Aat1*, and *6-Pgd2* were placed in group 3, and the *Cat1* locus was placed in group 5. The isozyme marker order and location in the respective linkage groups were in agreement with the configurations published by Joobeur et al. (1998).

#### Fruit acidity trait linked to the *Cat1* locus

The location of the *Cat1* isozyme locus is reported here for the first time. It was mapped to linkage group 5 (Fig. 1). Catalase, a tetrameric enzyme in plants, has been reported to be polymorphic in peach. Plant introductions of Chinese origin are predominantly homozygous for the fast allele, *Cat1-1*, but most American cultivars are homozygous for the slow allele (*Cat1-2*) (Werner 1992). The peach and almond parents of our mapping population, PMP1, were polymorphic for *Cat1* and segregation in the F<sub>2</sub> population supported codominant expression at a single locus, but with a deficiency of plants homozygous for the peach allele. Monet et al. (1996) reported that *Cat1* and the *D* locus controlling fruit acidity were tightly linked, thus offering a possible explanation for the intriguing observation that different groups of cultivars show high frequencies of either one or the other *Cat1* allele. Historically, peach cultivars from the U.S.A. have been selected for high acidity, whereas in Asia there is a preference for low-acid fruit. Depending on the linkage configuration of the *Cat1* and *D* loci in founder parents, selection for either low or high acid likely led to observed high or low frequencies of either the slow or fast allele of the *Cat1* locus. Although segregation for fruit acidity is usually expressed quantitatively, the *D* locus likely has a major ef-

**Table 2.** Segregation of marker genotypes,  $\chi^2$  values, and the linkage group in which the marker is mapped.

Co-dominant markers	No. of F <sub>2</sub> plants in each genotypic class				$\chi^2$	Linkage group
	Almond	Het	Peach	Total		
CPM178	11	34	12	57	2.15	1
CPM205	18	39	3	60	12.90**	1
LFCLR	11	28	10	49	1.04	1
RGA400C1	10	40	10	60	6.6	1
CPM159	9	38	8	55	8.05*	2
GA34	8	44	11	63	10.20**	2
PCHGMS1	15	41	7	63	7.76*	2
RGA100	10	33	10	53	3.18	2
CPM165	15	29	11	55	0.74	3
CPM188	16	30	16	62	0.064	3
CPM154	13	32	10	55	1.79	3
CPM207	18	34	11	63	1.95	3
CPM206	7	37	11	55	7.14*	4
CPM202	13	31	12	55	0.67	4
GA77	8	42	14	64	7.37*	4
CPM217	17	43	2	62	16.54**	5
CPM214	14	42	6	62	9.87**	5
CPM189	15	35	12	62	1.32	5
CPM133	13	43	4	60	13.95**	5
CPM204	14	40	7	61	7.52*	5
Cat1	15	32	2	49	11.48**	5
CPM140	10	29	18	57	2.26	6
SI locus	0	36	27	63	24.42**	6
PGIP1	4	34	24	62	13.48**	7
PGIP2	4	34	24	62	13.48**	7
DHN1	2	46	14	62	19.16**	7
CPM179	3	30	23	56	14.57**	7
CPM158	10	32	12	54	2	7
CPM199	8	37	12	57	5.63*	7
CPM196	10	41	11	62	6.48*	7
CPM146	7	35	21	63	7.01*	7
CPM150	11	36	3	50	12.23**	8
CPM198	8	38	12	58	6.13*	8
CPM148	9	38	14	61	4.5	8
GK12A02	12	36	12	60	2.39	8
RGA16	12	21	23	56	7.80*	8
RGA15C1	8	35	13	56	4.19	8
Dominant markers						
RRS51d1		44	8	52	2.56	1
RGA400d3		48	12	60	0.8	1
RGA52d1	17	36		53	1.41	1
CAP1	16	45		61	0.049	3
G LOCUS		42	14	56	0	5
RGA15d4		41	4	45	6.22*	8
Unlinked markers						
Gland	16	47		63	0.005	Unlinked
CPM169	13	38	4	55	10.96**	Unlinked
S6-Pdh	13	36	11	60	2.59	Unlinked
GK08E08	6	44	12	62	12.06**	Unlinked

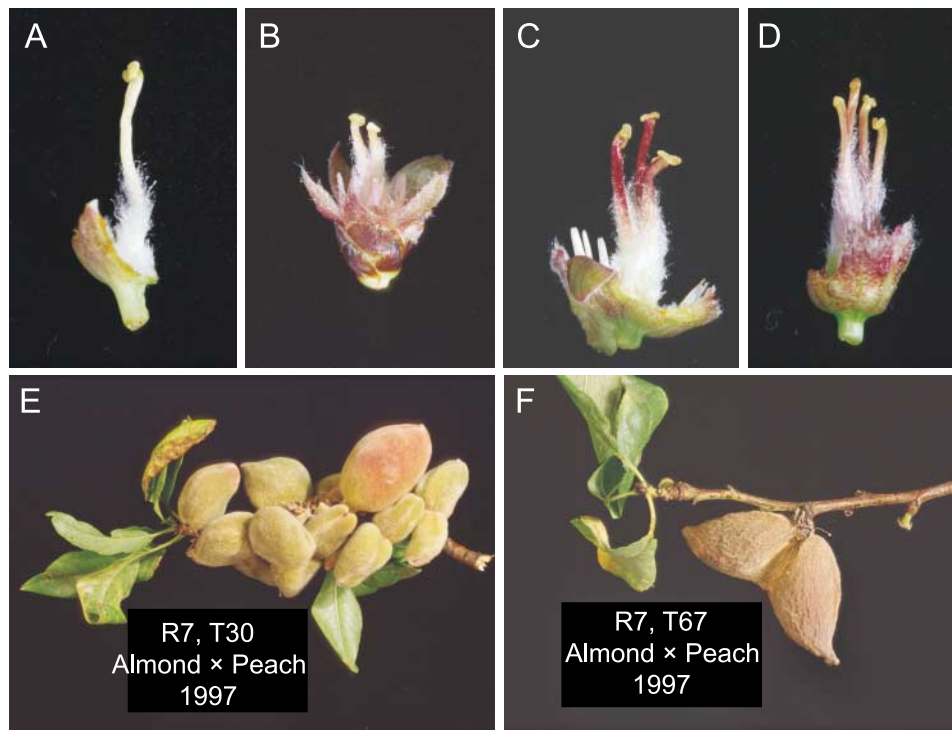
**Note:** \*, Significant at the 5% probability level; \*\*, significant at the 1% probability level.

fect on total fruit acidity. Using a peach intraspecific F<sub>2</sub> population, Dirlewanger et al. (1998) also mapped the fruit acidity locus *D* to the distal end (top) of group 5, similar in placement to that of the *Cat1* locus in this study, i.e., linkage group-5.

#### **Peach vs. nectarine trait**

Individual trees in the PMP1 population produced fruits that were scored phenotypically as either glabrous (*G*<sub>-</sub>) or pubescent (*gg*). The *G* (nectarine) locus was placed at the proximal end (bottom) of group-5 in our map and in the

**Fig. 2.** Polycarpel formation in population PMP6. A, B, C, D, showing single, two, three, and four carpels, respectively. E and F show fully formed fused fruits.



**Table 3.** Distribution of trees in the F<sub>3</sub> (6A11 × 54P455) population (PMP6) with flowers having different number of carpels.

Generation	Percentage of flowers with single carpel									
	0–10	11–20	21–30	31–40	41–50	51–60	61–70	71–80	81–90	91–100
	(No. of trees)									
Parent 1 (54P455)										1
Parent 2 (6A11)										1
F <sub>1</sub> parent										1
F <sub>2</sub> parent										1
F <sub>3</sub> population	13	5	2	2	6	8	3	4	4	52

same group by Dirlewanger et al. (1998). Because Werner and Creller (1997) determined that the *G* and sweet kernel (*sk*) loci were linked with an estimated map distance of 12 cm, *sk* can also be assigned to linkage group 5 (Fig. 1).

### Polycarpel (*Pcp*) trait expression

Fruit doubling, or fused multiple fruits, occur in stone fruits when plants are subjected to environmental stress during critical stages of floral bud development where two or more carpels develop in each flower (Johnson et al. 1992; see also Sharock 1993 for review). However, phenotypic expression varies among varieties, such as between sweet cherry (Micke et al. 1983) and peach (S. Johnson, personal communication), suggesting that a plant's genotype also influences expression.

We observed the F<sub>3</sub> almond × peach population (PMP6) for variation in the number of developing carpels per flower, which we described as the polycarpel trait (Fig. 2). The al-

mond and peach parents of the original hybrid and the F<sub>2</sub> parent that was selfed to produce the segregating F<sub>3</sub> population were each observed during three seasons to produce only flowers having one carpel (single fruits) (Table 3). However, among the F<sub>3</sub> plants in the PMP6 population grown during the same years and at the same location as the parents, 39 plants had flowers in which there were only single carpels and 13 plants had 91% or more flowers with single carpels and an occasional double carpel, but no higher multiples. On 28 plants, less than 50% of the flowers a single carpel, with the majority of flowers having multiple carpels. Flowers having as many as six fused carpels were observed and five plants had only polycarpel flowers (i.e., no single-carpel flowers were observed). Based on the single-carpel phenotypes of the two original parents and the selfed F<sub>2</sub> parent, as well as on the phenotypic distribution of F<sub>3</sub> trees classified for carpel number per flower, along with percentage of flowers per tree with varying number of carpels, we concluded the following: (i) single carpel expres-

sion is dominant to polycarpel expression, (ii) segregation at either one or two genes can account for the underlying genetic control of expression in PMP6, and (iii) expression of this trait may also be influenced by non-genetic factors.

In the same segregating population (PMP6), polycarpel expression was observed to be associated with specific alleles of the isozyme loci *Aat1* and *6-Pgd2*. Knowing that *Aat1* was linked to RAPD marker OPD-7 (520 bp) (Warburton et al. 1996), we performed BSA for polycarpel vs. single-carpel expression. Results indicated that the gene(s) controlling single vs. polycarpel expression were putatively located 16.8 and 20.2 cM from *Aat1* and *6-Pgd2*, respectively, and OPD-7 was 4.9cM from *Aat1*. Because these three markers were also segregating in PMP-1, the carpel development locus (*Pcp*) was estimated to be between *Aat1* and *6Pgd2* in linkage group 3 (Fig. 1).

Information regarding genetic control of polycarpel expression can be used to facilitate breeding for this trait. Assuming that single-carpel is dominant to polycarpel expression, a tree with all polycarpel fruit (recessive) can be used as a tester to cross with experimental trees of unknown genotype. In the progeny, frequency (percentage of flowers) and intensity (number of carpels per flower) of polycarpel expression for each tree would indicate the genotypic potential for fruit doubling of the test tree. Trees producing test-cross progeny with all single-carpel flowers would likely be free of double or multiple fused fruit in commercial production fields. A second approach would be to identify molecular markers linked to allele(s) responsible for polycarpel development, so that screening can be done in the seedling stage without waiting for sexual reproduction and occurrence of suitable environmental stress to predispose fruit doubling expression.

### Self-incompatibility (*SI*) locus

Several of the alleles controlling the self-incompatibility trait were cloned from almond, sequenced, and PCR primers were designed by Tamura et al. (2000). These primers amplified PCR products from the almond and peach parents, and the  $F_1$  and the  $F_2$  progenies of the PMP1 population. Segregation in  $F_2$  progenies showed complete deficiency for the almond genotype. Because of the gametic incompatibility system known to operate in almond species, one of the almond alleles present in the  $F_1$  hybrid does not allow pollen containing the same allele to travel through the pistil, thereby causing complete deficiency of the almond genotype for *SI* locus occurring in the  $F_2$  progeny. It is interesting to note that most of the markers surrounding the *SI* locus were also distorted towards deficiency for almond genotypes. Previously, Foolad et al. (1995) suggested that the *SI* locus might be located in this chromosomal region based on the severity of segregation distortion that was observed. Ballester et al. (1998), using the segregation of allozymes of stelar RNAses in an almond  $\times$  almond population, mapped the self-incompatibility locus at the bottom end of linkage group 6, which corresponds to our map location.

### Orange vs. yellow leaf color

Connors (1920) was the first to study flesh (mesocarp) color of peach fruit, and he and others concluded that white

flesh is dominant to yellow and controlled by a single gene, *Y* (Bailey and French 1949). However, the intensity of yellow varies, with some cultivars having fruit that is yellow or light orange, whereas others have fruit that is dark orange. Morrison (1990) reported that yellow fruit contained about 289 mg beta-carotene/100 g fruit (based on fresh-fruit weight), orange fruit had 445 mg beta-carotene/100 g fruit, and white fruit contained no beta-carotene. She reported that leaves of the white-fruited genotypes averaged 6309 mg beta-carotene/100 g leaves (dry weight), yellow contained 18 624 mg/100 g, and orange contained 23 860 mg/100 g. She found a strong positive correlation between beta-carotene content of the leaves and of the fruit. Furthermore, she suggested that breeders might be able to use this relationship to select for orange-fruited phenotypes at the seedling stage rather than waiting until fruits are produced on two-year-old trees.

We observed that the underlying color owing to beta-carotene becomes visible in the fall, when leaves senesce and chlorophyll becomes degraded. Trees in the PMP1 mapping population were scored for orange vs. yellow leaf color. The segregation ratio fit a single gene model with orange being dominant to yellow, and the locus was subsequently mapped to linkage group-1. We were unable to score this population for fruit mesocarp color because many trees failed to set fruit, presumably because of infertility owing to the interspecific nature of the hybrid population. Warburton et al. (1996) identified molecular markers linked to the *Y* locus in an unrelated intraspecific  $F_2$  peach population, and used markers common to both that population and PMP1 to place the locus in the linkage group now referred to as LG 1. Morrison (1990) found no beta-carotene in white-fleshed fruit, but leaves on those same trees (genotypes) contained about one-third the amount of beta-carotene as leaves on trees with yellow fruit. Because both traits, white vs. yellow flesh color and yellow vs. orange senescent leaf color, map to a similar location in the same linkage group, it is possible that a single gene in linkage group 1 controls beta-carotene expression of leaves and fruit or that more complex control of expression of beta-carotene is involved.

### RGA probes

PCR amplifications of 'Okinawa' DNA using primers S1 and AS1 (Leister et al. 1996) resulted in one fragment of the expected size of approximately 500 bp. This fragment was cloned and produced two distinct nucleotide sequences. The first sequence, RRS 51, contained 401 nucleotides and BLAST analysis did not detect significant similarity to known resistance genes or their homologs. The second clone, RGA52, was 519 bp long. BLAST search indicated high similarity to several resistance genes and resistance gene homologs. Both clones detected multicopy sequences, with some being polymorphic in mapping population PMP1, and RRS51 and RGA52 were mapped to linkage group 1. The presence of numerous other monomorphic bands suggests the possibility of a multigene family.

PCR primers designed based on low degeneracy within two conserved domains (leucine-rich repeats and P-loops) of the nucleotide binding site (NBS) of the *RPS-2* gene of *Arabidopsis thaliana* were used in several combinations re-

sulting in single or very low number of bands with the expected molecular size of about 500 bp. Sequence analysis of the products of each band resulted in more than one nucleotide sequence, some containing open reading frames. BLAST search resulted in significant matches with published sequences of cloned genes, e.g., TMV ( $1.5 \times 10^{-27}$ ) and L6 ( $1 \times 10^{-26}$ ) (Whitham et al. 1994 and Lawrence et al. 1995, respectively) as well as resistance gene homologs found in soybean and potato (Leister et al. 1996; Kanazin et al. 1996). Other primer combinations amplified two bands of size 700 and 500 bp and sequence analysis of the cloned fragments resulted in no open reading frames and no internal domains. When clones containing these sequences were used as RFLP probes, a large number of bands were observed suggesting they might represent a multicopy gene family. They were mapped into linkage groups-1,-2 and-8 (Fig. 1).

### Genes with known function

The DHN gene mapped to linkage group 7. Two loci coding for polygalacturonase inhibitor proteins, PGIP-1 and PGIP2, were segregating and mapped to the same location on linkage group 7, indicating that this may be a tandem duplication of this gene. PGIP is known to confer resistance to several fungal pathogens including *Botrytis* fruit rot (Sharrock and Labavitch 1994).

The dehydrins occur as a multigene family in plants and are known to be involved in freeze and drought tolerance (Close 1997). Seasonal variation and expression patterns of dehydrins between deciduous and evergreen peaches were also reported by Artlip et al. (1997). We also observed that dehydrins occur as a multigene family in *Prunus*; however, only a single gene was segregating in PMP1 that was able to be mapped in linkage group 7 of the present map.

### Conclusions and perspectives

Our current linkage map includes genes that control economically and physiologically important traits. The tentative map location for expression of the polycarpel trait (i.e., multiple fused fruit) further substantiates that this trait is genetically controlled, as well as influenced by environmental stress. It also gives an insight into the evolution of fruit structure in the family Rosaceae. It should be noted that several species in this family produce fused fruits, such as the berry-type fruits.

The majority of the RFLP markers used in this study are derived from developing peach mesocarp tissue and can be easily converted to ESTs after end sequencing of the cDNA clones and their functions could be ascertained by searching GenBank. Additional saturation of markers is certainly needed to fully utilize this map in marker assisted breeding of these important fruit and nut crops.

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