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Targeted mapping and linkage analysis of morphological isozyme, and RAPD markers in peach

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Abstract. Nine different F2 families of peach [*Prunus persica* (L.) Batsch] were analyzed for linkage relationships between 14 morphological and two isozyme loci. Linkage was detected between weeping (*We*) and white flower (*W*), 33 cM; double flower (*Dl*) and pillar (*Br*), 10 cM; and flesh color (*Y*) and malate dehydrogenase (*Mdh1*), 26 cM. A leaf variant phenotypically distinct from the previously reported wavy-leaf (*Wa*) mutant in peach was found in progeny of 'Davie II'. The new willow-leaf character (designated *Wa2*) was closely linked (0.4 cM) to a new dwarf phenotype (designated *Dw3*). Two families derived from the pollen-fertile cultivar 'White Glory' segregated for pollen sterility, but segregation did not follow a 3:1 ratio. Evidence is presented suggesting that 'White Glory' possesses a pollen-sterility gene (designated *Ps2*) that is non-allelic to the previously reported pollen-sterility gene (*Ps*) in peach. *Ps2* was linked to both weeping (*We–Ps2*, 15.5 cM) and white flower (*Ps2–W*, 25.3 cM). A genomic map of peach containing 83 RAPD, one isozyme, and four morphological markers was generated using an F2 family obtained by selfing an NC174RL × 'Pillar' F1. A total of 83 RAPD markers were assigned to 15 linkage groups. Various RAPD markers were linked to morphological traits. Bulked segregant analysis was used to identify RAPD markers flanking the red-leaf (*Gr*) and *Mdh1* loci in the NC174RL × 'Pillar' and 'Marsun' × 'White Glory' F2 families, respectively. Three markers flanking *Mdh1* and ten markers flanking *Gr* were identified. The combination of RAPD markers and bulked segregant analysis provides an efficient method of identifying markers flanking traits of interest. Markers linked to traits that can only be scored late in development are potentially useful for marker-aided selection in trees. Alternatives for obtaining additional map order information for repulsion-phase markers in large F2 populations are proposed.

Key words: RAPD – Bulked segregant analysis– Genomic mapping – Peach, *Prunus persica*

Introduction

Members of the genus *Prunus* are economically-important crops producing fruit [*Prunus armeniaca* L., *P. avium* L., *P. cerasus* L., *P. persica* (L.) Batsch, and P. salicina Lindl.], nuts (*P. dulcis* D. A. Webb) and timber (*P. serotina* Ehrh., *P. avium* L.). Few single gene markers have been identified in species of *Prunus* and little information on linkage relationships exists. Peach (*P. persica*) is the best genetically-characterized species in the genus with 40 morphological and isozyme markers (Hesse 1975; Yamazaki et al. 1987; Monet 1989). Peach is diploid with a low chromosome number (n = 8) and a genome size of 0.55 pg/2C, making it only twice the size of *Arabidopsis thaliana* L. (Arumuganathan and Earle 1991). Although numerous morphological and isozyme markers have been identified in peach, only four linkage groups have been described (Bailey and French 1949; Monet et al. 1985, 1988; Monet and Gibault 1991). Evidence for linkage

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between isozyme loci has been reported in *P. dulcis* (Hauagge et al. 1987) and *P. avium* (Santi and Lemoine 1990).

Genomic mapping studies of morphological traits in trees are rare. Trees are typically cross-pollinated and have large numbers of recessive lethals, making it difficult to obtain inbred progeny for mapping purposes. The inability to self has also precluded the identification of large numbers of morphological-trait loci in trees, because most allelic variants are recessive to wild-type alleles and must be homozygous for phenotypic expression. Genetic studies of morphological traits in trees are also hampered by the long juvenile period characteristic of many tree species. Markers closely linked to traits expressed late in development would be valuable for early selection in trees, even if the trait is highly heritable.

Isozymes and restriction fragment length polymorphisms (RFLPs) provide a source of selectively-neutral codominant markers for mapping. RFLPs have been used to develop extensive maps in many species (Gebhardt et al. 1991; Paran et al. 1991; Weeden et al. 1991). Random amplified polymorphic DNA markers (RAPDs) provide a new source of large numbers of molecular markers. Screening and mapping RAPD markers is relatively easy, but in contrast to RFLPs and isozymes, RAPDs are dominant markers. RAPDs have been used to develop extensive maps in loblolly pine (Grattapaglia et al. 1991) and apple (Weeden et al. 1991). Identification of markers flanking a trait of interest was thought to require mapping of the entire genome. However, whole-genome mapping is highly inefficient because only a small number of the polymorphisms identified will be closely linked to the trait of interest. Bulked segregant analysis (BSA) using RAPD markers has been shown to be a highly-efficient method for the targeted mapping of specific loci (Michelmore et al. 1991) or specific chromosomal intervals (Giovannoni et al. 1991).

The development of a peach linkage map combining molecular, isozyme, and morphological markers could be the basis for genomic maps in other species of *Prunus*. Comparison of the genomic maps of rodents and cattle has revealed a high degree of synteny (Womack et al. 1992). A high degree of synteny has also been reported between *Lycopersicon* and *Solanum*, two closely related genera (Gebhardt et al. 1991), and between maize and sorghum, two more distantly related species (Whitkus et al. 1992). In this paper we report new linkage relationships between morphological, isozyme, and RAPD markers in peach. Data is presented on the use of BSA to preferentially map specific loci. Additionally, methods for obtaining additional map-order information for repulsion-phase markers in F2 populations are proposed.

Materials and Methods

F2 populations

Nine different F2 families segregating for 14 morphological and two isozyme markers (Table 1) were obtained by selfing F1 trees derived from various parental crosses (Table 2). Selfing was accomplished by enclosing F1 trees in screened enclosures to

Table 1. Segregation and homogeneity chi-squares of morphological and isozyme markers used in the linkage studies

Locus	Phenotype	Test ratio	Segregation chi-square[c]	Homogeneity chi-square[c]
Br/br	Pillar/normal growth	3:1	15.51***	9.67** 1 *df*
Cat1-1/Cat1-2	Catalase	1:2:1	0.13 ns	na[d]
Dl/dl	Single/double flowers	3:1	0.85 ns	0.72 ns 1 *df*
Dw/dw	Normal/dwarf	3:1	0.63 ns	7.55 ns 3 *df*
Dw3/dw3[a]	Normal/dwarf	3:1	2.02 ns	na
G/g	Peach/nectarine	3:1	0.02 ns	1.18 ns 1 *df*
Gr/gr[b]	Red/green leaves	3:1	0.92 ns	10.97** 2 *df*
Mdh1-1/Mdh1-2	Malate dehydrogenase	1:2:1	0.81 ns	5.68* 1 *df*
We/we	Normal/weeping growth	3:1	2.84 ns	4.7 ns 3 *df*
Ps/ps	Pollen fertile/sterile	3:1	0.63 ns	na
Ps2/ps2[a]	Pollen fertile/sterile	3:1	26.57***	0.18 ns 1 *df*
Sh/sh	Nonshowy/showy flowers	3:1	4.39*	na
R/r	Pink/red flowers	3:1	2.00 ns	na
W/w	Pink/white flowers	3:1	2.30 ns	17.77*** 3 *df*
Wa2/wa2[a]	Normal/willow leaf	3:1	2.02 ns	na
Y/y	White/yellow flesh	3:1	36.83***	4.49* 1 *df*

[a] New loci described in this manuscript
[b] Although the *Gr* allele is incompletely dominant, segregation at the *W* locus prevents the identification of all *GrGr* homozygotes and the data were analyzed as if *Gr* were completely dominant
[c] *, **, *** = significant at the 0.05, 0.01, and 0.001 level, respectively
[d] na = not applicable

Table 2. Origin, genotype, and progeny size of F2 populations used to perform cosegregation analysis

Cross number	Cross[a,b]	F1 genotype	F2[c] progeny size
1	WG × RR	*gr w we g ps2* / *Gr W We G Ps2*	771
2	MS × WG I	*W We G ps y Mdh1-2* / *w we g Ps Y Mdh1-1*	624
3	MS × WG II	*W We G ps Ps2 y Mdh1-2* / *w we g Ps ps2 Y Mdh1-1*	291
4	SM × WG I	*W We dw* / *w we Dw*	1356
5	SM × WG II	*W We dw Ps2* / *w we Dw ps2*	328
6	NC174RL × WG	*Gr W We* / *gr w we*	1751
7	NC174RL × PI	*Gr W Br g Dl Cat1-2* / *gr w br G dl Cat1-1*	1027
8	HG × PI	*Br g dw Dl* / *br G Dw dl*	574
9	MS × DIID	*ps R Dw Dl* / *Ps r dw dl*	864
10	DII selfed	*Wa2 Dw3* / *wa2 dw3*	554
11	GB selfed	*Mdh1-1 Y ps sh Cat1-2* / *Mdh1-2 y Ps Sh Cat1-1*	1595

[a] Peach clones used to generate the F1s were DII (Davie II), DIID (Davie II Dwarf), GB (Georgia Belle), HG (Honey Glo), Ms (Marsun), RR (Rutgers Redleaf 2n), SM (Sweet Melody), PI (Pillar), and WG (White Glory)
[b] Full-sib families not segregating for the new pollen-sterile phenotype are designated I and those segregating for the new pollen-sterile phenotype are designated II
[c] Linkage analysis for the *Cat1* locus in the NC174RL × 'Pillar' and 'Georgia Belle' F2 families was based on random samples of 160 and 135 individuals, respectively. Linkage analysis for RAPD markers was performed with a random sample of 96 individuals from the NC174RL × 'Pillar' and 'Marsun' × 'White Glory' F2 families

exclude pollinating insects. Seedlots belonging to different full-sib trees were kept separate. F2 seed of NC174RL × 'Pillar' and 'Georgia Belle' was planted directly in the field at a spacing of 0.15 m × 6 m. All other F2 seed was artificially startified at 4 °C. After stratification, seeds were planted in the greenhouse, grown to a height of about 10 cm, and transplanted to the field at a spacing of 0.23 m × 6 m. All F2 families were planted at the Sandhills Research Station, Jackson Springs, N.C. Characterization of progeny for the morphological traits *Br, G, Gr, Dl, Dw, We, W, Ps, Y, Sh*, and *R* (Table 1) was conducted in years 2 and 3 after initial establishment of trees in the field. Catalase (*Cat1*) and malate dehydrogenase (*Mdh1*) isozymes were characterized following Werner (1992) and Mowrey et al. (1990), respectively. Linkage analysis for all loci was based on the progeny sizes shown in Table 2, with the exception of *Cat1*, which was based on a random sample of 160 and 135 individuals from the NC174RL × 'Pillar' and 'Georgia Belle' F2 families, respectively.

RAPD

DNA was extracted from young expanding leaves using a CTAB procedure (Bousquet et al. 1990), or following Junghans and Metzlaff (1990). DNA was diluted to a working concentration of approximately 1 µg/µl by visual comparison to a lambda DNA standard on agarose gels. RAPD reactions were performed as described by Williams et al. (1990) using 15–20 ng of template, 20 ng of ten-base primers from Operon Technologies kits A–Z (Operon Technologies, Alameda, Calif.) or custom-made primers, and 1U of *Taq* DNA polymerase. Reactions were performed in 96-well microtitre plates using an MJ Research PTC 100 thermal cycler (MJ Research, Inc., Watertown, Mass.). RAPD fragment were size-fractionated on 1.5% agarose gels, stained with ethidium bromide and photographed.

Bulked segregant analysis

Bulked segregant analysis (BSA) was performed to identify RAPD markers linked to specific morphological traits following Michelmore et al. (1991). Composite DNA samples (bulks) for each phenotypic class were made by combining 2 µg of DNA from 8–12 F2 seedlings. Compsite DNA samples representing the two homozygote classes were made for the incompletely-dominant trait red leaf (*Gr*)/green leaf (*gr*) in the 174RL × 'Pillar' F2 family, and for the codominant trait malate dehydrogenase (*Mdh1-1*/*Mdh1-2*) in the 'Marsun' × 'White Glory' F2 family. Composite DNA samples were also made for contrasting phenotypes of the dominant traits, peach (*G*)/nectarine (*g*), and pollen-fertile (*Ps*)/pollen-sterile (*ps*) in the 174RL × 'Pillar' and 'Marsun' × 'White Glory' F2 families, respectively. Composite DNA samples were used to screen RAPD primers (Operon kits A-I) for banding polymorphisms. Polymorphisms were confirmed by comparison to parental banding phenotypes and by segregation analysis on 96 additional F2 individuals not comprising the original bulked samples.

Peach genomic map

A genomic map of peach was developed using the F2 family derived from selfing a NC174RL × 'Pillar' F1. NC174RL is a red-leafed, white-fleshed nectarine clone. 'Pillar' is an ornamental double-flowered peach clone demonstrating a unique upright growth habit. 'Pillar' has been cultivated in Japan for about 200 years (M. Yoshida, personal communication), and was introduced into the United States some 20 years ago. Both clones are of unknown pedigree. RAPD polymorphisms were revealed by screening both parents against 522 ten-base oligonucleotide primers (Operon kits A–Z and two custom-made primers). RAPD markers, the *Cat 1* isozyme marker, and five morphological markers (*Br*, *Dl*, *W*, *Gr*, and G), were tested for linkage using cosegregation analysis on a random sample of 96 NC174RL × 'Pillar' F2 progeny.

Data analysis

Segregation data for morphological, isozyme, and RAPD polymorphisms were tested for departures from the expected 3:1 or 1:2:1 Mendelian ratios using chi-square tests. RAPD polymorphisms demonstrating significant departure from the expected 3:1 ratio ($P \leq 0.05$) were not used in mapping. In some cases, more than one F1 tree from the same cross was used to generate F2 families. Data from each full-sib F2 family were tested for departure from homogeneity and combined if no significant departure ($P \leq 0.05$) was detected. Gene pairs segregating in two or more half-sib or unrelated families were tested for departure from homogeneity and also combined if no significant departure ($P \leq 0.05$) was revealed. The Macintosh version of MAPMAKER (Lander et al. 1987) was used for linkage analysis. Linkage groups were first identified using the 'group' command LOD > 4, $R_F = 0.4$. An initial map was generated for each group using the 'three-point' command, LOD > 3, $R_F = 0.4$. The 'ripples' command was used to verify linkage order. A framework map was constructed using only one marker from each set of closely-linked markers. Markers were retained with the framework only if LOD values for ripples was > 2. The Kosambi mapping function (Kosambi 1944) was used to convert recombination fraction into map distances.

Results

Inheritance of morphological traits and isozymes

Chi-square tests were performed to test for departure of observed segregation ratios from expected Mendelian ratios and to verify that all traits investigated behaved as single-gene markers. The morphological and isozyme loci *Br*, *Dl*, *W*, *Gr*, *We*, *Ps*, *G*, *Y*, *Mdh1*, *Cat*, and *Dw*, had segregation ratios that generally did not depart significantly from the expected 3:1 or 1:2:1 ratio (see Table 1). However, all morphological loci segregating in the 'Georgia Belle' selfed family (*Sh*, *Y*, *Ps*) demonstrated a significant departure from the expected 3:1 segregation ratios. Therefore, linkage associations in the 'Georgia Belle' selfed family were tested using a chi-square test of independence and the data from this family were not used to calculate map-distance estimates. The *Br* locus demonstrated a significant departure from the 3:1 ratio in the 'Honey Glo' × 'Pillar' F2 family but segregated normally in NC174RL × 'Pillar'. The departure from homogeneity for the *W*-locus segregation data was due to a distorted ratio in a single family (NC174RL × 'White Glory') out of four derived from 'White Glory'. Elimination of the NC174RL × 'White Glory' family resulted in a non-significant homogeneity chi-square for *W*. The departure from homogeneity for the *Gr* locus was due to a distorted ratio in one family (NC174RL × 'Pillar') out of the three studied, and this family was not used in map-distance calculations involving *Gr*.

Pollen-sterile progeny were found in some families originating from 'White Glory', a pollen-fertile cultivar (Werner 1985). Pollen-fertile/-sterile phenotypic segregation ratios in the F2 progeny from 'White Glory' × 'Rutgers Redleaf 2n' and 'Sweet Melody' × 'White Glory'-I showed a significant departure from the expected 3 (fertile):1 (sterile) ratio (see Table 1). Only 16% of the progeny were pollen-sterile in each family. The observed frequency is not significantly different from the expected 13:3 ratio for a two-gene model with suppression-type epistasis (Suzuki et al. 1989). In the suppression-type epistasis model, pollen-sterile individuals would have the *ps2/ps2 Ps3/*-genotype. All other genotypes in this model would be pollen-fertile. An alternative explanation for the observed 16% pollen sterility is a single-locus model (*ps2*) with approximately 20% lethality of homozygous-recessive progeny (*ps2/ps2*).

A test for allelism of the new pollen-sterility locus originating from 'White Glory' with the previously reported pollen-sterility locus was possible in the 'Marsun' × 'White Glory'-II F2 family. Marsun is homozygous for the previously-reported pollen-sterility allele (*ps*). All three 'Marsun' × 'White Glory' F1 seedlings were pollen-fertile. However, 37% of the 291 seedlings in the 'Marsun' × 'White Glory'-II F2 family were pollen-sterile. The observed frequency of 37% pollen-sterile progeny is significantly different from 25% ($P \leq 0.001$) but is not significantly different from the 39% expected $[0.25 + 0.18 - [(0.25)(0.18)]]$ if *ps* and the new pollen-sterile phenotype are non-allelic and segregating independently. Data from the 'Marsun' × 'White Glory'-II F2 family and the phenotype of the three F1s suggests that the pollen-sterile gene(s) from 'White Glory' is not allelic to *Ps*. Cosegregation data from the 'Sweet Melody' × 'White Glory' and 'Rutgers Redleaf 2n' × 'White Glory' F2 families revealed that the new pollen-sterility locus (*Ps2*) maps to the *W* (25.3 cM) and *We* (15.5 cM) linkage group (see Fig. 3). The linkage associations were detected using both the two-gene suppression-type epistatic model and the single-gene partial-lethality model.

Selfed progeny of Davie II segregated for dwarf and 'willow-leaf' phenotypes (Figs. 1, 2). Chi-square analysis of the segregation data showed no significant departure from a 3:1 phenotypic segregation ratio for either

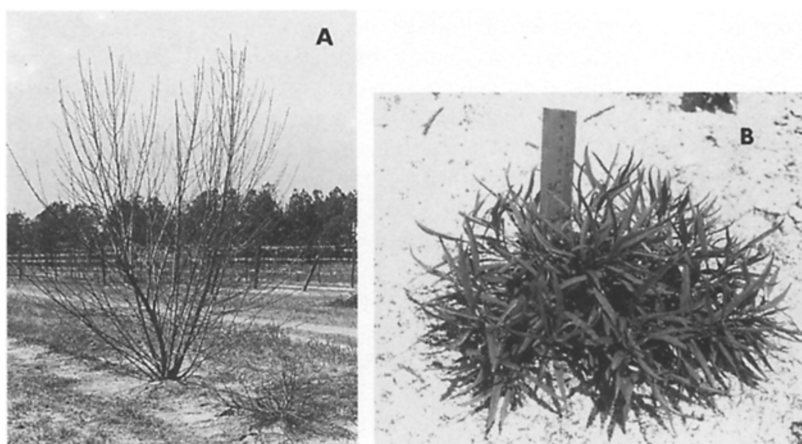

Fig. 1. Phenotype of the new dwarf (*dw3*) allele identified in selfed progeny of 'Davie II'. **A** Standard (left) and dwarf (right) segregants. **B** Two-year-old dwarf tree. The ruler shown is 38 cm tall

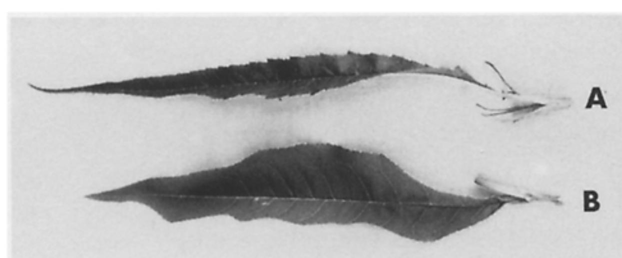

Fig. 2. Phenotype of the new willow-leaf allele identified in the clone 'Davie II'. **A** Willow leaf. **B** Wild-type leaf

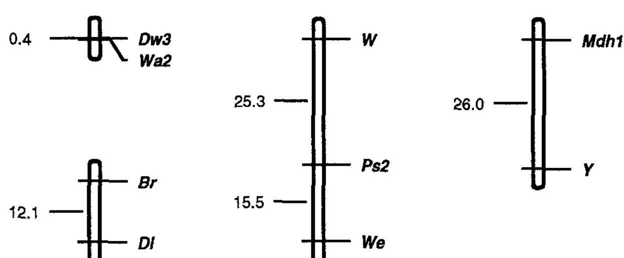

Fig. 3. Linkage groups of morphological and isozyme loci identified in this study and their map distances

the dwarf-stature or willow-leaf phenotypes (see Table 1), suggesting that each is controlled by a single locus. The willow-leaf phenotype was designated *Wa2* because of similarities to the previously-reported wavy-leaf (*Wa*) phenotype (Scott and Cullinan 1942). Both *Wa* and *Wa2* have serrated leaf edges and are associated with dwarfed growth. However, *Wa2* homozygotes do not have the wavy-leaf edge characteristic of *wa/wa* homozygotes. The new dwarf phenotype was designated *Dw3*. The *Dw3* phenotype differs from *Dw* and *Dw2*, two previously reported brachytic dwarf phenotypes (Hansche 1988), in having an extreme dwarf stature, willowy growth, and thin stems. Cosegregation analysis revealed that *Wa2* is closely linked to the *Dw3* locus (0.4 cM). It is possible that *Wa2* and *Dw3* represent different loci or new alleles of *Wa* and *Dw*, respectively.

Linkages between morphological traits and isozyme loci

A total of 51 pairwise combinations involving 14 morphological and two isozyme loci were tested for linkage (Table 3). Linkage was accepted for gene pairs having a linkage chi-square value greater than 10.8 ($P < 0.001$, 1 *df*). Four linkage groups were identified (Fig. 3). Most linkage associations were tested in at least two F2 families and were confirmed across families. In addition to the *W-Ps2-We* linkage group previously discussed, three other gene linkages were detected: *Br–Dl* (10 cM), *Wa2–Dw3* (0.4 cM), and *Mdh1–Y* (26 cM).

Linkage between *Mdh1* and *Y* is noteworthy. The *Mdh 1-1* allele is very rare in commercial North American peach germplasm (Arulsekar et al. 1986). This is surprising, because 'J. H. Hale' and 'Elberta', the principal progenitors of commercial North American germplasm, are both heterozygous at the *Mdh1* locus. Previous investigators have proposed that linkage of *Mdh1-1* to an undesirable trait could explain the low frequency of the *Mdh1-1* allele in commercial peach germplasm (Arulsekar et al. 1986). 'J. H. Hale' is pollen-sterile (*ps/ps*) and 'Elberta' carries the pollen-sterility allele (*Ps/ps*) (Myers et al. 1989). We hypothesized that the *Mdh1* and *Ps* loci were linked, and that selection against pollen sterility in offspring of 'J. H. Hale' and 'Elberta' would have co-eliminated the *Mdh1-1* allele. The 'Marsun' × 'White Glory'-1 and 'Georgia Belle' F2 families were used to test the hy-

Table 3. Marker combinations, F2 populations analyzed, and chisquare values obtained for the isozyme and morphological marker loci tested for linkage

Loci tested	F2 populations examined[a]	Linkage chi-square[b]
Br-Cat1	7	0.9
Br-Dl	7, 8	462.4*
Br-Dw	8	0.1
Br-G	7, 8	0.2
Br-Gr	7	0.1
Br-W	7	3.8
Cat-Dl	7	0.5
Cat1-G	7	2.4
Cat1-Gr	7	0.1
Cat1-Mdh1	11	8.7
Cat1-Ps	11	7.1
Cat1-Sh	11	4.7
Cat1-W	7	0.1
Cat1-Y	11	11.0
Dl-Dw	8, 9	6.5
Dl-G	7, 8	0.1
Dl-Gr	7	2.2
Dl-Ps	9	1.1
Dl-R	9	4.9
Dl-W	7	2.4
Dw-G	8	0.6
Dw-We	4, 5	1.4
Dw-Ps	9	0.5
Dw-Ps2	5	0.8
Dw-R	9	1.3
Dw-W	4, 5	0.5
Dw3-Wa2	10	469.5*
G-Gr	1, 7	7.3
G-Mdh1	3	0.8
G-We	1, 2, 3	0.9
G-Ps	2, 3	0.1
G-Ps2	1, 5	1.1
G-W	1, 2, 3	1.5
Gr-We	1, 6	0.9
Gr-Ps2	1	0.4
Gr-W	1, 6	0.9
Mdh1-We	2	1.6
Mdh1-Ps	11	2.7
Mdh1-Sh	11	0.8
Mdh1-Y	2, 3, 11[c]	31.5*
Mdh1-W	2, 3	10.2
We-Ps	2	0.0
We-Ps2	1, 5	201.2*
We-W	1, 2, 3, 4, 5	118.2*
Ps-Ps2	3	0.2
Ps-R	9	5.2
Ps-Sh	11	0.4
Ps-W	2	0.1
Ps-Y	11	0.3
Ps2-W	1, 5	19.6*
Sh-Y	11	0.3

[a] Population numbers correspond to the cross numbers described in Table 2

[b] * = linkages significant at the 0.001 level

[c] Due to segregation distortion at the Y locus in F2 population number 11 (Georgia Belle selfed), only populations 2 and 3 were used to calculate the map distance between *Mdh1* and *Y*

pothesis of linkage between the *Mdh1* and *Ps* loci. Cosegregation data revealed that the *Mdh1* locus is not linked to pollen sterility (*ps*). However, evidence for linkage between the *Y* and *Mdh1* loci was obtained (Table 3). The existence of linkage between *Y* and *Mdh1* cannot explain the rarity of the *Mdh1-1* allele in commercial peach germplasm because 'J. H. Hale' and 'Elberta' are both homozygous for yellow flesh (Myers et al. 1989). Since both cultivars were homozygous for yellow-flesh color (*y*/*y*) and heterozygous *Mdh1-1*/*Mdh1-2*, selection for yellow-flesh color in offspring of these cultivars would have had no impact on the frequency of either *Mdh1* allele. The low frequency of the *Mdh1-1* allele could have resulted from drift because often only a few seedlings from a cross are ever used as parents. A second alternative is the known association of the *Mdh1-1* allele with a low-vigor phenotype (Werner and Moxley 1991). Poor seedling competition could have resulted in early death or selection against *Mdh1-1* homozygotes.

Inheritance of RAPD polymorphisms

Approximately 16% of the 522 RAPD primers tested yielded polymorphic fragments between 'Pillar' and NC174RL. Most polymorphic primers yielded only one polymorphic band between 0.5 kb to 2.0 kb in size. Most polymorphic bands were inherited in a Mendelian fashion (Table 4). Polymorphic bands demonstrating a significant departure from a 3:1 ratio ($P \leq 0.05, 1\ df$) in the sample of 96 progeny were not used in mapping.

Targeted RAPD mapping of morphological and isozyme loci

Four traits were selected for targeted mapping using bulked segregant analysis: *Gr* and *G* in NC174RL × 'Pillar' and *Mdh1* and *Ps* in 'Marsun' × 'White Glory'-I. The *Gr* allele is incompletely dominant and the *Mdh1* allele is codominant, hence contrasting phenotypic bulks for these loci were composed of alternative homozygotes, whereas the contrasting phenotypic bulks for *Ps* and *G* contained both homozygous-dominant and heterozygous individuals in the dominant allele bulk. Bulked segregant analysis identifies RAPD markers specific to each of the alternative codominant or incompletely-dominant alleles. Only markers specific to the dominant allele are identified when heterozygotes are included in the dominant allele bulk.

Gr locus. Bulked segregant analysis screening of 174 primers revealed 11 polymorphisms (from nine primers) between the red-leaf (*Gr*/*Gr*) versus green-leaf (*gr*/*gr*) bulks (Fig. 4). The polymorphisms were confirmed by testing the parents NC174RL (*GrGr*) and 'Pillar' (*grgr*).

Table 4. Origin and segregation behavior or RAPD markers segregating in NC174RL × 'Pillar' F_2 progeny. Each RAPD marker is designated by primer/polymorphic fragment size (kb)

RAPD[a] marker	Primer[b] sequence	Marker[c] origin	Segregation[d] chi-square
JXC-04/1.60	GGCACTACAA	'Pillar'	1.6
JXC-04/0.28	GGCACTACAA	NC174RL	0.9
OPA-01/0.42	CAGGCCCTTC	NC174RL	0.5
OPA-04/0.75	AATCGGGCTG	'Pillar'	0.0
OPA-04/1.29	AATCGGGCTG	NC174RL	0.1
OPA-13/0.93	CAGCACCCAC	NC174RL	0.4
OPB-18/0.79	CCACAGCAGT	'Pillar'	0.9
OPB-18/0.28	CCACAGCAGT	NC174RL	0.5
OPC-05/0.96	GATGACCGCC	'Pillar'	1.4
OPC-10/0.78	TGTCTGGGTG	NC174RL	0.0
OPC-11/1.10	AAAGCTGCGG	'Pillar'	0.9
OPC-17/1.27	TTCCCCCCAG	'Pillar'	0.0
OPC-17/0.48	TTCCCCCCAG	NC174RL	1.4
OPD-01/0.84	ACCGCGAAGG	NC174RL	1.3
OPD-03/1.10	GTCGCCGTCA	'Pillar'	3.0
OPD-18/0.67	GAGAGCCAAC	NC174RL	0.1
OPD-20/1.75	ACCCGGTCAC	NC174RL	0.0
OPD-20/1.19	ACCCGGTCAC	NC174RL	0.2
OPE-15/0.75	ACGCACAACC	'Pillar'	3.0
OPE-17/0.98	CTACTGCCGT	'Pillar'	4.0*
OPE-19/0.42	ACGGCGTATG	'Pillar'	1.3
OPE-20/2.15	AACGGTGACC	NC174RL	0.5
OPF-02/0.73	GAGGATCCCT	NC174RL	0.0
OPF-02/0.53	GAGGATCCCT	'Pillar'	0.9
OPF-04/2.00	GGTGATCAGG	'Pillar'	2.0
OPF-04/0.53	GGTGATCAGG	NC174RL	2.4
OPF-10/1.77	GGAAGCTTGG	NC174RL	0.3
OPF-11/1.26	TTGGTACCCC	'Pillar'	1.9
OPF-15/0.65	CCAGTACTCC	NC174RL	0.4
OPF-15/0.61	CCAGTACTCC	'Pillar'	2.2
OPF-17/0.93	AACCCGGGAA	NC174RL	0.4
OPG-05/0.88	CTGAGACGGA	NC174RL	9.1**
OPG-13/1.18	CTCTCCGCCA	'Pillar'	0.1
OPG-13/0.78	CTCTCCGCCA	NC174RL	0.0
OPH-05/0.48	AGTCGTCCCC	'Pillar'	0.4
OPI-01/1.15	ACCTGGACAC	NC174RL	0.8
OPI-02/0.88	GGAGGAGAGG	NC174RL	0.1
OPI-02/1.20	GGAGGAGAGG	'Pillar'	1.9
OPI-07/2.02	CAGCGACAAG	'Pillar'	0.1
OPI-07/1.43	CAGCGACAAG	'Pillar'	1.0
OPI-15/1.13	TCATCCGAGG	NC174RL	0.9
OPI-16/1.09	TCTCCGCCCT	'Pillar'	0.2
OPJ-06/0.74	TCGTTCCGCA	'Pillar'	5.1*
OPJ-11/1.23	ACTCCTGCGA	NC174RL	2.9
OPJ-19/1.44	GGACACCACT	NC174RL	3.4
OPJ-19/0.29	GGACACCACT	NC174RL	0.1
OPJ-20/0.55	AAGCGGCCTC	NC174RL	4.3*
OPK-01/0.86	CATTCGAGCC	NC174RL	11.3**
OPK-06/0.81	CACCTTTCCC	NC174RL	0.5
OPK-11/1.02	AATGCCCCAG	NC174RL	96.9**
OPK-12/1.54	TGGCCCTCAC	'Pillar'	0.1
OPL-01/1.55	GGCATGACCT	NC174RL	0.3
OPL-05/0.31	ACGCAGGCAC	'Pillar'	2.5
OPL-18/0.96	ACCACCCACC	NC174RL	0.5
OPM-02/0.42	ACAACGCCTC	NC174RL	2.5
OPM-06/0.85	CTGGGCAACT	'Pillar'	2.9
OPM-11/1.24	GTCCACTGTG	NC174RL	5.7*
OPM-15/1.02	GACCTACCAC	'Pillar'	0.2
OPN-07/1.58	CAGCCCAGAG	NC174RL	0.1
OPN-08/2.03	ACCTCAGCTC	'Pillar'	0.5
OPN-09/1.32	TGCCGGCTTG	'Pillar'	0.6
OPN-14/0.77	TCGTGCGGGT	NC174RL	1.0
OPN-14/0.35	TCGTGCGGGT	NC174RL	0.2
OPO-20/0.29	ACACACGCTG	NC174RL	0.9
OPO-20/0.31	ACACACGCTG	'Pillar'	0.9
OPP-08/1.18	ACATCGCCCA	NC174RL	1.9
OPP-13/0.99	GGAGTGCCTC	NC174RL	3.4
OPP-14/0.64	CCAGCCGAAC	NC174RL	2.6
OPP-14/0.58	CCAGCCGAAC	NC174RL	0.1
OPQ-06/0.95	GAGCGCCTTG	NC174RL	0.9
OPQ-14/0.81	GGACGCTTCA	'Pillar'	5.1*
OPQ-14/0.77	GGACGCTTCA	NC174RL	5.1*
OPQ-18/0.76	AGGCTGGGTG	NC174RL	0.2
OPR-08/1.10	CCCGTTGCCT	'Pillar'	1.0
OPR-08/0.43	CCCGTTGCCT	'Pillar'	0.1
OPS-11/1.00	AGTCGGGTGG	'Pillar'	2.1
OPS-19/1.34	GAGTCAGCAG	'Pillar'	5.6*
OPT-07/1.27	GGCAGGCTGT	'Pillar'	0.9
OPT-07/0.54	GGCAGGCTGT	'Pillar'	8.6**
OPU-06/2.22	ACCTTTGCGG	NC174RL	4.6
OPU-07/2.30	CCTGCTCATC	'Pillar'	1.5
OPU-07/2.10	CCTGCTCATC	NC174RL	0.2
OPU-12/1.25	TCACCAGCCA	NC174RL	1.2
OPU-19/1.45	GTCAGTGCGG	'Pillar'	0.7
OPV-02/0.84	AGTCACTCCC	NC174RL	0.0
OPV-08/1.05	GGACGGCGTT	NC174RL	5.5*
OPV-20/1.06	CAGCATGGTC	NC174RL	0.9
OPW-07/1.46	CTGGACGTCA	'Pillar'	0.9
OPW-12/1.54	TGGGCAGAAG	'Pillar'	1.9
OPW-12/1.31	TGGGCAGAAG	NC174RL	1.3
OPX-02/0.72	TTCCGCCACC	NC174RL	0.2
OPX-08/0.99	CAGGGGTGGA	'Pillar'	1.3
OPX-08/0.50	CAGGGGTGGA	NC174RL	1.3
OPX-11/0.75	GGAGCCTCAG	NC174RL	0.1
OPX-15/1.43	CAGACAAGCC	'Pillar'	0.4
OPY-03/0.68	ACAGCCTGCT	'Pillar'	3.1
OPY-07/1.19	AGAGCCGTCA	'Pillar'	0.2
OPY-07/0.59	AGAGCCGTCA	'Pillar'	2.4
OPY-14/0.83	GGTCGATCTG	'Pillar'	3.2
OPY-16/0.94	GGGCCAATGT	NC174RL	0.0
OPZ-03/0.69	CAGCACCGCA	'Pillar'	0.4
OPZ-12/0.47	TCAACGGGAC	NC174RL	93.4**
OPZ-19/1.10	CACCCCAGTC	NC174RL	0.1

[a] The first three letters of the marker designation indicate the primer source. OPA-OPZ = Operon technology oligonucleotide kits A–Z; JXC = custom made oligonucleotide
[b] Primer sequences shown 5′–3′
[c] Marker origin was determined by genotyping NC174RL and 'Pillar' for RAPD markers
[d] *, ** = significant deviations from a 3:1 segregation ratio at the 0.05 and 0.01 level, respectively

Segregation analysis on 96 additional F2 individuals not comprising the original bulks showed four markers linked to the incompletely-dominant *Gr* allele (Fig. 5a) and six linked to the recessive *gr* allele (Fig. 5b). Only one polymorphic band revealed in the bulk comparison was not linked to either of the *Gr* alleles. Placement

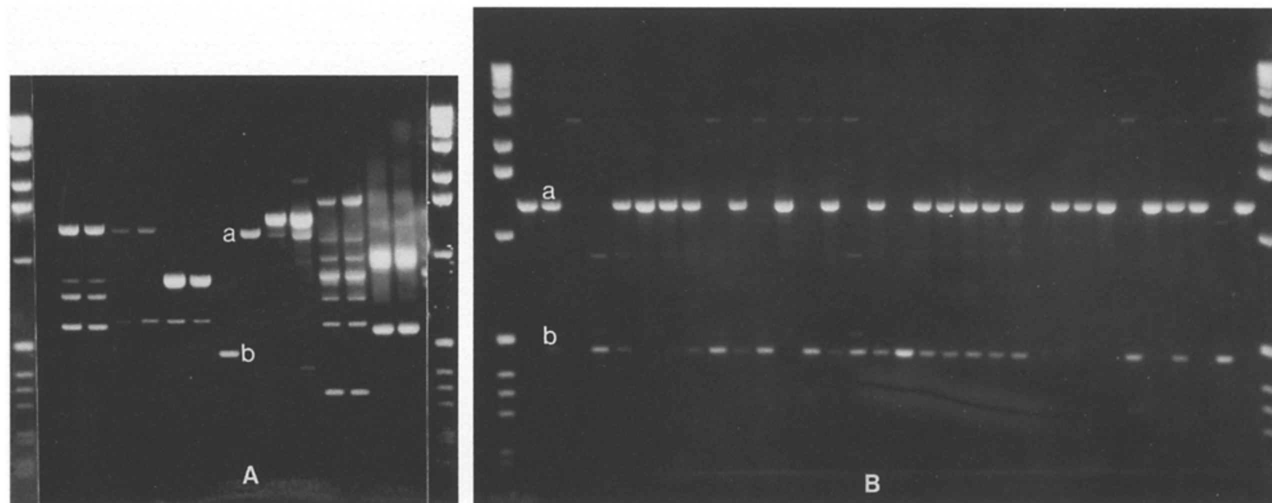

Fig. 4. **A** RAPD polymorphisms observed using bulk segregant analysis for the *Gr* (*left lane*) vs *gr* (*right lane*) alleles. Primers OPC-14 through OPC-20 are shown left to right in pairs. Polymorphisms revealed with OPC-17 at 1.27 kb (a) and 0.48 kb (*b*). 1-kb ladder shown in first and last lanes. **B** Segregation for markers OPC-17/1.27 (*a*) and OPC-17/0.48 (*b*) in a sample of 32 NC174RL × 'Pillar' F_2 progeny. 1-kb ladder shown in first and last lanes. *Lane 2 through 33* were scored from left to right as +/−, +/+, −/−, −/+, +/+, +/−, +/+, +/+, −/+, +/+, −/+, +/−, −/+, +/+, −/+, +/+, −/+, +/+, +/+, +/+, +/+, +/+, NR, +/−, +/−, +/−, −/+, +/−, +/+, +/−, −/+, +/−, for OPC-17/1.27\OPC-17/0.48, respectively

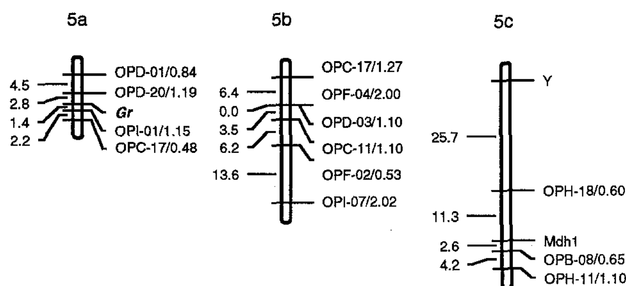

Fig. 5. Genetic maps of polymorphic markers detected using bulked segregant analysis flanking the *Gr* (*5a*), *gr* (*5b*) and *Mdh1-1* (*5c*) alleles. The *Gr* and *gr* alleles were mapped in the NC174RL × 'Pillar' F2 population and the *Mdh1-1* allele in the 'Marsun' × 'White Glory' F2 population

of the recessive *gr* allele relative to repulsion-phase RAPD markers was not possible because of several factors. Epistatic interaction between *Gr* and *W* (white-flower locus) causes suppression of the *Gr* phenotype and precludes characterization of *Gr* in *w/w* homozygotes (Chaparro et al., manuscript in preparation). Additionally, segregation at the *W* locus made it difficult to phenotypically differentiate the *GrGrWw* and *GrgrWW* genotypes. Therefore, segregation data for the *Gr* locus was analyzed as if *Gr* were completely dominant. Hence, the sample size of 96 was not large enough to determine linkage order for closely-linked repulsion-phase RAPD markers.

Mdh1 Locus. Bulked segregant analysis screening of 170 primers (Operon kits A-I) on bulked samples of *Mdh1-1/Mdh1-1* vs *Mdh1-2/Mdh1-2* homozygotes revealed three polymorphisms. Segregation analysis on 96 additional F2 individuals not comprising the original bulked samples demonstrated that the three markers were linked to the *Mdh1-1* allele (Fig. 5c). No markers were found flanking *Mdh1-2*.

G and Ps Loci. Bulked segregant analysis screening of 170 primers (Operon kits A–I) did not identify any markers linked to either the *G* or *Ps* loci. In contrast to *Gr* and *Mdh1*, the alleles for *G* and *Ps* are dominant. The dominant phenotypic bulk for these two loci is composed of both homozygous-dominant and heterozygous individuals. Only RAPD markers linked to the dominant allele will be observed as polymorphic. Our results confirm that bulked segregant analysis identifies fewer RAPD polymorphisms when heterozygotes are included in the bulk sample for the dominant allele (Giovannoni et al. 1991).

Genomic map of peach

A linkage map with 15 linkage groups containing 83 RAPD, one isozyme, and four morphological markers was developed for the NC174RL × 'Pillar' F2 family (Fig. 6). Of the 522 oligonucleotide primers screened in this F2 family, 83 revealed distinct polymorphisms (Table 4). Because some oligonucleotide primers re-

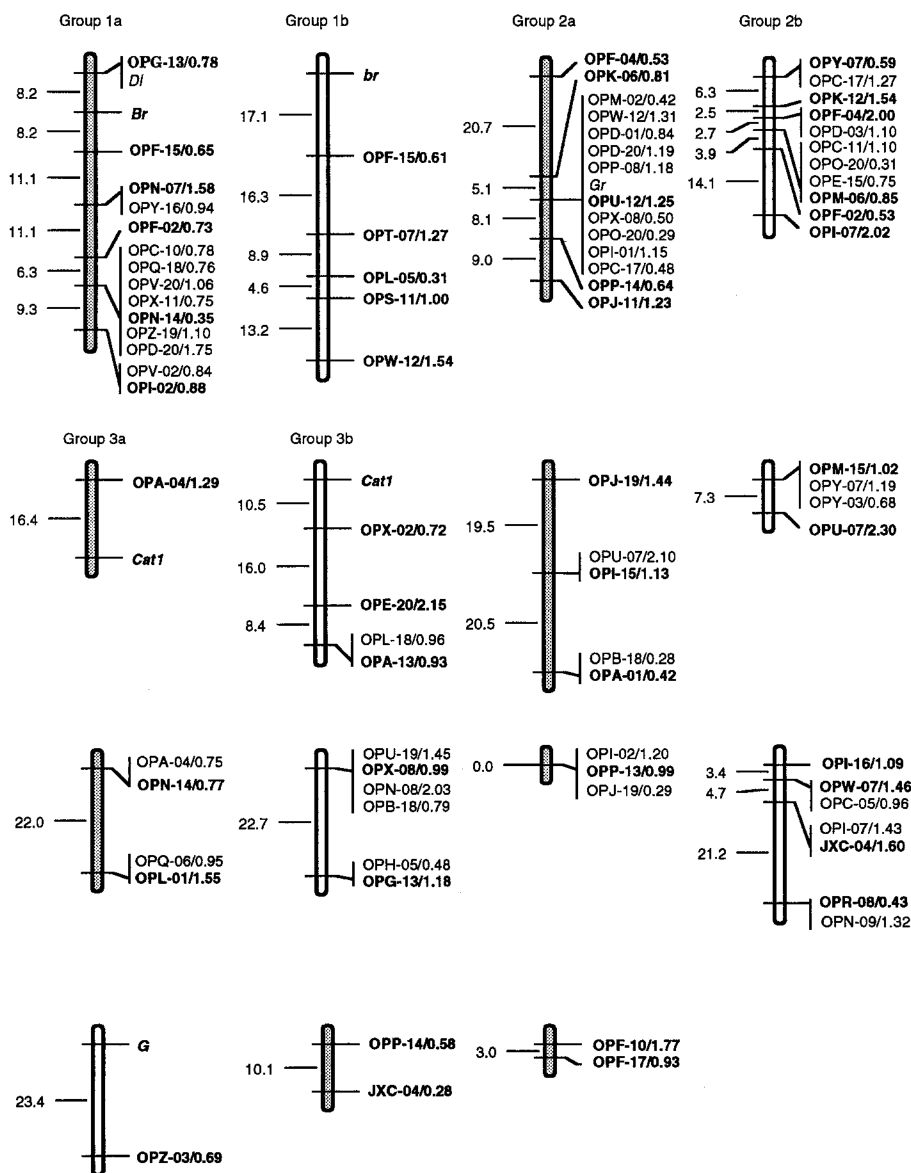

Fig. 6. Peach genomic map incorporating morphological, isozyme and RAPD markers segregating in the NC174RL × 'Pillar' F2 family. *Shaded and non-shaded chromosomes* represent linkage groups contributed by 174RL and 'Pillar', respectively. Markers in *bold* represent framework map. See text for details

vealed more than one polymorphic band, a total of 103 polymorphic bands was detected with these 83 primers. Of these 103 polymorphisms, 13 showed segregation distortion in the F2 and were not used in mapping. Of the remaining 90 polymorphisms, seven did not demonstrate linkage with any other RAPD, isozyme, or morphological marker. The linkage groups cover approximately 396 cM with an average density of 4.8 cM/marker. A total of 16 linkage groups is expected, two for each chromosome representing each of the homologs. The homology of linkage groups 1a-1b, 2a-2b, and 3a-3b was established. RAPD markers flanking the *Cat*, *Br*, *Dl*, and *G* loci were identified. An additional linkage group comprised of three RAPD markers and the *Y* and *Mdh1* loci was identified using bulked segregant analysis in the 'Marsun' × 'White Glory' F2 family (Fig. 5).

Discussion

A combined morphological, isozyme, and RAPD linkage map consisting of 15 linkage groups was developed for peach. An additional linkage group of three RAPD markers flanking *Mdh1-1* was also discovered. Examination of nine different F2 families revealed four new linkage groups involving isozyme and morphological traits. The previous genetic map of peach consisted of

four linkage groups identified in four different crosses (Bailey and French 1949; Monet et al. 1985, 1988; Monet and Gibault 1991). Further characterization of two families with RAPD markers resulted in the establishment of 14 additional linkage groups.

RAPD markers in peach are inherited in simple Mendelian fashion, as demonstrated in other species (Williams et al. 1990; Giovannoni et al. 1991; Michelmore et al. 1991). The level of RAPD polymorphism in peach is low. Only 16% of the 522 RAPD primers tested yielded polymorphisms in a cross between two very diverse peach clones, NC174RL and 'Pillar'. This level of polymorphism is much lower than that observed in two other tree species, *Pinus taeda* L. and *Eucalyptus grandis* (Hill) Maiden (Grattapaglia, personal communication) where most primers yield more than one polymorphism. The low level of RAPD polymorphism parallels the low level of isozyme polymorphism in peach (Mowrey et al. 1990). Although genetic variation in peach is low, a large number of single-gene phenotypic variants have been identified, probably due to peach being a self-pollinated species. This selfing ability has been an advantage in our mapping studies because large F2 families can be obtained with little effort from a single heterozygous F1 individual.

We have identified RAPD markers flanking the codominant loci *Gr* and *Mdh1* by BSA. BSA screening of 170 RAPD primers did not identify any markers linked to the dominant traits peach/nectarine and pollen-fertile/pollen-sterile. However, screening of additional oligonucleotide primers against the parents and subsequent cosegregation analysis revealed that OPZ-03/0.69 was linked to the peach/nectarine locus. When dominant traits are analysed by BSA and heterozygotes are present in the bulk representing the dominant phenotype, RAPD markers linked to recessive alleles are not detected, thereby reducing the efficiency of BSA by half.

Peach breeders generate large F2 populations to recombine traits from different varieties. Plant breeding is based upon phenotypic selection. Markers that would allow seedling-stage selection of traits expressed late in development would be particularly useful in trees because of their long generation times. Markers flanking recessive alleles such as nectarine would allow the identification and selection of heterozygous individuals not expressing the trait. Although the RAPD marker OPZ-03/0.69 is linked to the peach/nectarine locus, its distance from the morphological locus limits its potential utility. This ability to genotype individuals can be used to reduce the number of backcross generations necessary for introgression of genes between populations or species (Tanksley et al. 1989).

Mapping studies in out-crossing tree species can use existing full-sib crosses containing markers segregating 3:1 and 1:1. However, peach is inbred and full-sib (F1) families have few markers segregating 1:1 or 3:1. Segregating populations in peach are generated by selfing F1s of wide crosses. The peach F2 progeny therefore contain a mixture of dominant markers in coupling- and repulsion-phases. Linkage detection in F2 populations in highly inefficient for dominant markers in repulsion (Mather 1936; Allard 1956; Robinson 1971) and results in homolog-specific maps (two maps per chromosome). In order for RAPD markers to be useful in marker-aided selection of quantitative and qualitative traits in F2 populations, these homolog-specific maps must be aligned. The test cross is the most efficient inheritance model for detecting linkage between dominant markers in both coupling and repulsion (Mather 1936; Allard 1956; Robinson 1971). Accordingly, an efficient way to align homolog-specific maps would be to generate a small test-cross population in conjuction with the F2 of interest. The test-cross parent should be an unrelated clone representing a different germplasm, or a different related species, to maximize the number of RAPD marker segregating 1:1. It is unlikely that a tester recessive for most morphological and RAPD loci could be found in available peach germplasm. However, almond (*P. dulcis* L.) is sexually compatible with peach and high levels of RAPD polymorphism should be present between the species. Morphological loci could be placed on the integrated RAPD linkage map generated in the test cross using F2 segregation data for coupling-phase markers.

Several other alternatives are possible for the alignment of repulsion-phase linkage groups in existing F2 populations where generating a test cross may not be possible. Incorporation of a small number of codominant markers by using single-copy RAPD bands as RFLP probes would facilitate the alignment of pairs of homologous maps following the concept of "allelic bridges" (Ritter et al. 1990). Alternatively, F2 progeny used to generate the map could be genotyped by performing F3 progeny tests. This would require the generation and genotyping of an F3 family from each F2 individual used to generate the original map. Such an effort would be extremely laborious.

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References

Allard R (1956) Formulae and tables to facilitate the calculation of recombination values in heredity. Hilgardia 24:235–278

Arulsekar S, Parfitt D, Beres W, Hansche P (1986) Genetics of malate dehydrogenase isozymes in peach. J Hered 77:49–51

Arumuganathan K, Earle E (1991) Nuclear DNA content of some important plant species. Plant Mol Biol Rep 9: 208–218

Bailey J, French A (1949) The inheritance of certain fruit and foliage characters in the peach. Mass Agr Exp Sta Bul 452

Bousquet J., Simon L, LaLonde M (1990) DNA amplification from vegetative and sexual tissues of trees using polymerase chain reaction. Can J For Res 20:254–257

Gebhardt C, Ritter E, Barone A, Debener T, Walkemeier B, Schachtschabel U, Kaufmann H, Thompson R, Bonierbale M, Ganal M, Tanksley S, Salamini F (1991) RFLP maps of potato and their alignment with the homoeologous tomato genome. Theor Appl Genet 83:49–57

Giovannoni J, Wing R, Ganal M, Tanksley S (1991) Isolation of molecular markers from specific chromosomal intervals using DNA pools from existing mapping populations. Nucleic Acids Res 19:6553–6558

Grattapaglia D, Wilcox P, Chaparro J, O'Malley D, McCord S, Whetten R, McIntyre L, Sederoff R (1991) A RAPD map in 60 days. Proc 3rd Int Congress of Plant Mol Biol 2224

Hansche P (1988) Two genes that induce brachytic dwarfism in peach. HortScience 23:604–606

Hauagge R, Kester D, Asay R (1987) Isozyme variation among California almond cultivars: I. Inheritance. J Am Soc Hort Sci 112:687–693

Hesse C (1975) Peaches 285–335 In: Janick J, Moore J (eds) Advances in fruit breeding. Purdue University Press West Lafayette, Indiana, pp 623

Junghans H, Metzlaff M (1990) A simple and rapid method for the preparation of total plant DNA. BioTechniques 8:176

Kosambi D (1944) The estimation of map distances from recombination values. Ann Eugen 12:172–175

Lander E, Green P, Abrahamson J, Barlow A, Daly M, Lincoln S, Newberg L (1987) MAPMAKER: An interactive computer program for constructing primary linkage maps of experimental and natural populations. Genomics 1:174–181

Mather K (1936) Types of linkage data and their value. Ann Eugen 7:251–264

Michelmore R, Paran I, Kesseli RV (1991) Identification of markers linked to disease-resistance genes by bulked segregant analysis: a rapid method to detect markers in specific genomic regions by using segregating populations. Proc Natl Acad Sci USA 88:8928–9832

Monet R (1989) Peach genetics: past present and future. In: Coston D (ed) 2nd Int peach symposium. Acta Hort 254: 49–57

Monet R, Gibault B (1991) Polymorphisme de l'a amylase chez le pecher. Etude genetique. Agronomie 11:353–358

Monet R, Bastard Y, Gibault B (1985) Etude genetique et amelioration des peches plates. Agronomie 5:727–731

Monet R, Bastard Y, Gibault B (1988) Etude genetique du caractere "port pleureur" chez le pecher. Agronomie 8:127–132

Mowrey B, Werner D, Byrne D (1990) Inheritance of isocitrate dehydrogenase, malate dehydrogenase, and shikimate dehydrogenase in peach and peach × almond hybrids. J Am Soc Hort Sci 115:312–319

Myers S, Okie W, Lightner G (1989) The 'Elberta' peach. Fruit Var J 43:130–138

Paran I, Kesseli R, Michelmore R (1991) Identification of restriction fragment length polymorphic DNA markers linked to downy mildew resistance genes in lettuce using near-isogenic lines. Genome 34:1021–1027

Ritter E, Gebhardt C, Salamini F (1990) Estimation of recombination frequencies and construction of RFLP linkage maps in plants from crosses between heterozygous parents. Genetics 125:645–654

Robinson R (1971) Gene mapping in laboratory mammals. Part A. Plenum Publishing Co., London

Santi F, Lemoine M (1990) Genetic markers for *Prunus avium* L: inheritance and linkage of isozyme loci. Ann Sci For 47:131–139

Scott D, Cullinan F (1942) The inheritance of wavy-leaf character in the peach. J Hered 33:293–295

Suzuki D, Griffiths A, Miller J, Lewontin R (1989) An introduction to genetic analysis. W.H. Freeman and Co, New York

Tanksley S, Young N, Paterson A, Bonierbale M (1989) RFLP mapping in plant breeding: new tools for an old science. Bio/technol 7:257–264

Weeden N, Timmerman G, Hemmat M, Loaiza-Figueroa F, Lu J (1991) Evaluation and characterization of RAPD markers for genome mapping. Proc 3rd Int Congress of Plant Mol Biol 1721

Werner D (1985) 'White Glory' weeping nectarine. HortScience 20:308–309

Werner D (1992) Catalase polymorphism and inheritance in peach. HortScience 27:41–43

Werner D, Moxley D (1991) Relationship between malate dehydrogenase isozyme genotype and plant vigor in peach. J Am Soc Hort Sci 116:327–329

Whitkus R, Doebley J, Lee M (1992) Comparative genome mapping of sorghum and maize. Genetics 132:1119–1130

Williams JGK, Kubelik AR, Livak KJ, Rafalski JA, Tingey SV (1990) DNA polymorphisms amplified by arbitrary primers are useful as genetic markers. Nucleic Acids Res 18:6531–6535

Womack J, Dietz A, Gallagher D, Li L, Zhang N, Neibergs H, Moll Y, Ryan A (1992) Addition of 43 informative comparative anchor loci to the bovine synteny map. Proc 1992 Miami Bio/Technology Winter Symposium 2:8

Yamazaki K, Okabe M, Takahashi E (1987) Inheritance of some characteristics and breeding of new hybrids in flowering peaches. Bull Kanagawa Hort Exp Sta 34:46–53