Randomly Amplified Polymorphic DNA-based Genetic Linkage Maps of Three Apple Cultivars

Patrick J. Conner¹, Susan K. Brown², and Norman F. Weeden

Department of Horticultural Sciences, New York State Agricultural Experiment Station, Cornell University, Geneva NY 14456

ADDITIONAL INDEX WORDS. *Malus × domestica*, 'Wijcik McIntosh', molecular markers, isozymes, apple genome, pseudo-testcross

ABSTRACT. Genetic linkage maps were created for three apple (*Malus × domestica* Borkh.) cultivars using data from two progenies ('Wijcik McIntosh' × NY 75441-67 and 'Wijcik McIntosh' × NY 75441-58). The maps consist primarily of randomly amplified polymorphic DNA (RAPD) markers, but also contain six isozyme loci and four morphological markers (*R_f*, fruit skin color; *V_f*, scab resistance; *Co*, columnar growth habit; *Ma*, malic acid). Maps were constructed using a double pseudo-testcross mapping format and JoinMap mapping software. An integrated 'Wijcik McIntosh' map was produced by combining marker data from both progenies into a single linkage map. Homologous linkage groups from paternal maps were paired with their counterparts in the 'Wijcik McIntosh' map using locus bridges composed of markers heterozygous in both parents of a progeny. The 'Wijcik McIntosh' map consists of 238 markers arranged in 19 linkage groups spanning 1206 cM. The NY 75441-67 map contains 110 markers in 16 linkage groups and the NY 75441-58 map consists of 183 markers in 18 linkage groups. The average distance between markers in the maps was ≈5.0 cM.

Molecular marker linkage maps are valuable tools for analyzing and selecting complex traits (Tanksley et al., 1989) and studying individual genes that control expression of polygenic traits (Stubber, 1992; Tanksley, 1993). Molecular markers linked to genes controlling complex tree and fruit quality characteristics in apple may allow breeders to define the inheritance of these traits (Weeden et al., 1994). Molecular marker-assisted selection (MAS) (Lande, 1991; Dudley, 1993) is potentially useful for apple breeding because seedlings may be preselected for traits of importance before years of costly field maintenance. Genetic maps will also be necessary for map-based cloning of genes that are not amenable to other cloning strategies (Wing et al., 1994).

Creating genetic maps traditionally involved evaluating many crosses to place relatively few markers on a map. The breeding constraints associated with woody perennials has precluded generating saturated classical genetic maps for these species. Protein (isozyme) and DNA-based markers made possible the creation of genetic maps for woody perennials (Weeden, 1994). Markers based on the polymerase chain reaction (PCR) method of DNA amplification (Saiki et al., 1988) have increased the speed with which genetic maps can be produced (Quiros et al., 1991; Weining and Langridge, 1991). Randomly amplified polymorphic DNA (RAPD) markers (Welsh and McClelland, 1990; Williams et al., 1990), which can quickly detect a large number of genetic polymorphisms, have led to the creation of genetic maps in a number of woody fruit crops including apple (*Malus × domestica*) (Hemmat et al., 1994); blueberry (*Vaccinium darrowi* and *V. elliotii*) (Rowland and Levi, 1994); *Citrus* (Cai et al., 1994); grape, *Vitis* (Lodhi et al., 1995); and peach (*Prunus persica*) (Chaparro et al., 1994).

Genetic maps for apple have been created using progeny of a cross between 'Rome Beauty' and the crab apple 'White Angel' (Hemmat et al., 1994). These maps were created using a double

pseudotestcross mapping format in which a separate map is created for each parent of a cross (Weeden, 1994). The two maps were composed of isozyme, restriction fragment-length polymorphism (RFLP), and RAPD markers. However, neither of the mapped apple cultivars is being actively used in the Cornell Univ. (Geneva, N.Y.) breeding program, and the progeny were not segregating for commercially important apple quality characters. We have now created RAPD-based linkage maps for three cultivars that are being used in the breeding program. These maps will be useful for examining the inheritance of traits affecting tree and fruit morphology and development.

Materials and Methods

PLANT MATERIAL. Two half-sib populations—NY 89117 ['Wijcik McIntosh' (WM) × NY 75441-67 (NY-67)] (114 trees), and NY 86229 [WM × NY 75441-58 (NY-58)] (172 trees)—have been used to complete genetic maps for the three parental cultivars. NY 89117 was produced in 1989 and NY 86229 in 1986 by S.K. Brown. NY-67 and NY-58 are scab-resistant seedlings derived from the cross 'Prima' × 'Spartan'. Both are heterozygous for the dominant *V_f* gene for resistance to apple scab (Dayton et al., 1970). WM is a scab-susceptible cultivar. Seed germination and seedling survival for the NY 89117 population was >90%, and seedlings were not selected, so this population represents a relatively unbiased sample. Seedlings from the NY 86229 population were screened for *V_f* resistance to apple scab. Susceptible plants (60%) were removed, making this a biased population.

MORPHOLOGICAL TRAITS. Skin color in apple is primarily controlled by a single gene, *R_f*, with red color dominant to yellow (Cheng et al., 1996). Skin color was evaluated in NY 86229. Trees producing fruit with 0% to 40% red coloration were classified as yellow fruited, and trees producing fruit with 41% to 100% red were classified as red fruited. Red-fruited trees were considered to have at least one copy of *R_f*. WM and NY-58 produced red fruit. WM is a columnar mutation of 'McIntosh' discovered in Canada (Fisher, 1969, 1970). It is heterozygous for the dominant columnar *Co* gene that greatly decreases branching, internode length, and plant height and increases stem diameter (Lapins, 1976). NY-67 and NY-58 lack *Co* and have a standard plant form. The presence of *Co* was assessed in progenies by visually categorizing trees as

Received for publication 2 Aug. 1996. Accepted for publication 14 Jan. 1997. This research was supported in part by USDA grant NRICRG 93-37300-8776. This article is a portion of a doctoral thesis submitted by Patrick Conner. Warren Lamboy is thanked for reviewing this paper. The cost of publishing this paper was defrayed in part by the payment of page charges. Under postal regulations, this paper therefore must be hereby marked *advertisement* solely to indicate this fact.
¹Current address: Dept. of Plant Biology, Plant Science Bldg., Cornell Univ., Ithaca NY 14853.

²To whom reprint requests should be addressed.

standard or columnar. Nybom (1959) demonstrated the occurrence of low-acid fruit (pH 3.8 and above), "sweets," to be due to a single gene, *ma*, in a homozygous recessive state. Fruit pH was determined for each fruiting tree in NY 86229 by juicing a slice from each sampled apple into a common mixture and then measuring the pH. Fruit with a pH ≥ 3.7 were scored sweet and those with a pH < 3.7 acid. Acid-fruited progeny were considered to have at least one copy of *Ma*. WM and NY-58 produce acid fruit and appear to be heterozygous *Ma ma*.

ISOZYME SYSTEMS. Isozyme analyses were conducted on extracts of young leaves before they had expanded fully. Analyses of polymorphism for aspartate aminotransferase (AAT; E.C.2.6.1.1), 6-phosphogluconate dehydrogenase (6PGD; EC 1.1.1.44), isocitrate dehydrogenase (IDH; EC 1.1.1.42), diaphorase (DIAP; EC 1.6.4.3), phosphoglucumutase (PGM; EC 2.7.5.1), glucose-phosphate isomerase (GPI; EC 5.3.1.9), peroxidase (PRX; EC 1.11.1.7), and triosephosphate isomerase (TPI; EC 5.3.1.1) were performed on horizontal starch gels as described by Weeden and Lamb (1985, 1987).

RAPD ASSAY. DNA was extracted using the procedure of Torres et al. (1993). DNA solutions were visually quantified using gel electrophoresis and comparison to known DNA concentrations. Amplifications were performed in 25 μ L of solution containing 13.2 μ L H₂O, 2.5 μ L 10 $\times$ buffer (500 mM KCl, 100 mM Tris-HCl pH 9.0, 1% Triton X-100), 1.5 μ L of 25 mM MgCl₂, 1.25 μ L of a 2.5 mM solution of each dNTP, 1.5 μ L of 10 μ M primer, 5 μ L of ≈ 10 ng μ L⁻¹ DNA solution, and 0.5 units *Taq* polymerase (Promega; Madison, Wis.). The reaction mixture was overlaid with two drops of mineral oil. Amplification was performed in an MJ PTC-100 programmable thermalcycler (MJ Corp., Watertown, Mass.) programmed for 40 cycles (94 °C for 1 min; 35 °C for 2 min; 72 °C for 2 min) followed by an 8-min extension at 72 °C, with maximum ramping speed between temperatures. Amplification products were resolved by electrophoresis on 2% agarose gels run at 100 V for 4 h in 1 $\times$ Tris-borate electrophoresis buffer (Sambrook et al., 1989). Gels were stained with ethidium bromide and photographed on a transilluminator using Polaroid Type 55 film (Polaroid Corp., Cambridge, Mass.). Segregation patterns were scored from the negatives.

Primers were synthesized at the Cornell Univ. New York State Center for Advanced Technology in Biotechnology, Genosys Biotech, Inc. (The Woodlands, Texas), Operon Tech. (Alameda, Calif.), and the Univ. of British Columbia Biotechnology Laboratory, (Vancouver, B.C., Canada). Primer sequences may be obtained from the authors and will be made available on the World Wide Web at <http://probe.nalusda.gov:8300/plant>.

LINKAGE ANALYSIS AND MAP CONSTRUCTION. Single-locus Mendelian segregation chi-square tests were conducted using the computer program LINKAGE-1 (Suiter et al., 1983). Linkage maps were constructed for each parent independently using the JoinMap computer program (Stam, 1993). A 4.0-linklod parameter and a maximum map distance of 30 cM were the minimum criteria used to define linkage groups. Map distances were computed using the Kosambi mapping function and the default maplod threshold of 0.05. A single integrated map was made for WM by combining data from both populations in JoinMap and using the same thresholds defined above. Linkage groups from NY-67 and NY-58 were paired with homologous linkage groups from WM using markers heterozygous in both parents. RAPD markers were considered homologous between the two populations if they were about the same size and occupied the same position in the overall banding pattern produced from the primer. Linkage groups were paired for comparison purposes only; no integration of the three cultivar maps was attempted. Independent estimates of recombination percentages between morphological and RAPD markers and their standard errors were included in the input data files as described in the JoinMap instruction manual. WM linkage groups were ordered from largest to smallest except for those found to be homologous to linkage groups from the maps of Hemmat et al. (1994), in which case the original designation was kept.

LOCI NOMENCLATURE. Marker names begin with either one or two capital letters indicating the primer origin, followed by a number specifying the exact primer, and then a small letter indicating the particular band on the gel corresponding to the marker. To distinguish between markers segregating in the two populations, markers segregating in NY 89117 were assigned letters a–h and markers segregating in NY 86229 were assigned letters u–z. Markers segregating in both populations kept their original NY 89117 designation of a–h, and were underlined in the WM map figures. Marker band size in base pairs is given immediately after the marker name. Fragment sizes were determined by comparison to a 100-bp DNA ladder (Gibco BRL, Gaithersburg, Md.). Fragment sizes for WM markers segregating in both populations are the average over both populations.

Results and Discussion

PRIMER SCREENING. Primers were screened using DNA from three progeny trees and the two parents of each cross. To complete the NY 89117 map, 548 primers were screened initially. One or more fragments were amplified by 448 (82%) of these primers. On

Table 1. Parental genotypes and linkage map position of assayed isozymes.

Enzyme system	Locus name	Parental genotype ^z			Linkage	
		WM	NY-67	NY-58	group	Maps
Aspartate aminotransferase	<i>Aat-1</i>	CD	AB	AB	1	WM, NY-67, NY-58
	<i>Aat-2</i>	CC	AB	---	7	NY-67
Diaphorase	<i>Diap-2</i>	AB	AB	AB	4	WM, NY-67
Isocitrate dehydrogenase	<i>Idh-1</i>	BB	AB	AB	1	NY-67, NY-58
Phosphoglucumutase	<i>Pgm-1</i>	CC	---	DD	---	---
	<i>Pgm-2</i>	BB	---	BB	---	---
	<i>Pgm-3</i>	nn	nn	nn	---	---
	<i>Pgm-5</i>	B null	AB	AA	4	NY-67
Peroxidase	<i>Prx-2</i>	AA	AA	AA	---	---
6-Phosphogluconate dehydrogenase	<i>Pgd-1</i>	AB	AD	AD	2	WM, NY-67, NY-58
Triosephosphate isomerase	<i>Tpi-2</i>	AA	AA	AA	---	---

^zWM = 'Wijcik McIntosh', NY-67 = NY 75441-67, NY-58 = NY 75441-58.

^yIsozyme loci were not assayed in the parent.

average, five bands were amplified per primer, with an average of one polymorphic band. The map was completed using 96 primers on 114 seedlings. Primers were selected based on scorable segregating bands, which produced 208 markers for an average of 2.2 RAPD markers per selected primer. Of 138 markers segregating in a 1 present : 1 absent ratio, 80 were heterozygous only in WM and 58 only in NY-67. Seventy additional markers were heterozygous in both parents (doubly heterozygous) and segregated in a 3:1 ratio.

Most primers used to complete the NY 89117 maps were also screened in NY 86229 to facilitate map integration. In this population, 87 primers were selected, which produced 266 markers with an average of 3.1 markers per selected primer. Of these markers, 76 were heterozygous only in WM, 97 only in NY-58, and 93 were doubly heterozygous.

ISOZYME LOCI. Seven enzyme systems tested revealed six different polymorphic loci in three cultivars that mapped to four linkage groups (Table 1). Four loci were polymorphic in WM and NY-58, and six loci were polymorphic in NY-67. The presence of progeny showing homodimeric A bands in both progenies provided evidence for a null allele for PGM-5 in WM. PGM-5, which mapped to linkage group 4 in the NY-67 map and remained unlinked in WM, has not been mapped previously in apple. DIAP-2 was only scored in NY 89117 and, therefore, could not be placed on the NY-58 map.

MONOGENIC SEGREGATION RATIOS. RAPD marker segregation ratios deviating significantly ($P < 0.05$) from the expected 1:1 or 3:1 ratios were found for 17% of NY 89117 markers (Table 2) and 24% of the NY 86229 markers (Table 3). Markers with skewed ratios were more than twice as likely to have been inherited from the male parent; 39 combined in NY-67 and NY-58 compared to 17 total in WM. An additional 44 markers with distorted ratios were heterozygous in both parents, and the source of the distortion was unclear. Markers with distorted segregation ratios were distributed throughout 14 linkage groups, but a large percentage was clustered on linkage group (LG) 5 in NY 89117 (31%) and LGs 5, 9, and 10 in NY 86229 (51%).

High degrees of segregation distortion for molecular markers have been detected within other annual and perennial species (Cai et al., 1994; Echt et al., 1994; Gebhardt et al., 1989). Pre- and postzygotic selection are the most common explanations for dis-

torted ratios. NY 89117 was an unselected population and seedling survival was >90%, so postzygotic selection is an unlikely explanation. NY 86229, however, represents a population of 173 trees from an original sowing of 430 seeds. Most seedlings were eliminated because they lacked the V_f gene, but other unintentional sources of seedling selection such as poor vigor cannot be ruled out. V_f has been mapped to LG 8 (Hemmat et al., 1995), and three markers in this region (S64x-1550, P198a-750, and S22y-410) show segregation distortion in NY 86229.

Distorted segregation ratios were found for 13 of 16 markers on NY-58 LG 10, suggesting that selection has occurred against a gene on this linkage group. The columnar gene, *Co*, has been mapped to WMLG 10. Because *Co* has a pronounced effect on tree growth, we investigated the possibility that the cause of the segregation distortion was a gene from NY-58 LG 10 interacting with *Co* to produce a lethal genetic combination. However, markers with distorted segregation from NY-58 LG 10 randomly assort with *Co*, indicating that *Co* is not associated with the segregation distortion occurring on this linkage group.

A source of prezygotic selection is self-incompatibility. Most apple cultivars are self-incompatible, with incompatibility being determined by a multiallelic *S* gene having a gametophytic effect (Modlibowska, 1945). Linkage between the *S* incompatibility locus and an isozyme locus of aspartate aminotransferase (AAT) has been reported, described as glutamate oxaloacetate transaminase (GOT) by Manganaris and Alston (1987) and Batlle et al. (1995). If two cultivars share *S* alleles, segregation at *Aat-1* will be distorted. Neither progeny differed significantly from the 1:1:1:1 *Aat-1* ratio expected from a cross between two heterozygous parents; therefore, self-incompatibility does not appear to be responsible for any selection in these crosses. Segregation distortion was much more common in the male than the female parent, suggesting some form of selection on the male gamete.

LINKAGE ANALYSIS. Researchers have often discarded markers whose segregation ratios were aberrant. However, those who have included these markers found them to map with the same proficiency as markers with standard ratios (Bradshaw et al., 1994). We also found that markers with skewed ratios mapped with about the same efficiency (95%) as markers displaying expected ratios

Table 2. The linkage status, number, and parental origin of randomly amplified polymorphic DNA markers showing distorted segregation in NY 89117.

Linkage group	No. of markers	Heterozygous parents ²		
		WM	Both	NY-67
1	3	2	1	0
2	2	0	1	1
3	2	0	1	1
5	11	1	4	6
7	3	1	1	1
8	4	1	3	0
9	2	0	0	2
10	1	1	0	0
11	2	1	1	0
13	2	0	2	0
17	1	0	1	0
Unlinked	2	1	1	0
Total	35	8	16	11

²Indicates parent that is heterozygous for the marker; WM = 'Wijcik McIntosh', NY-67 = NY 75441-67, both indicates the marker is heterozygous in both parents.

Table 3. The linkage status, number, and parental origin of randomly amplified polymorphic DNA markers showing distorted segregation in NY 86229.

Linkage group	No. of markers	Heterozygous parents ²		
		WM	Both	NY-58
1	1	0	1	0
2	4	0	2	2
4	1	0	0	1
5	10	4	3	3
6	5	0	4	1
7	6	2	1	3
8	4	1	1	2
9	9	0	6	3
10	14	1	5	8
11	3	0	3	0
12	4	1	1	2
13	2	0	1	1
Unlinked	2	0	0	2
Total	65	9	28	28

²Indicates parent that is heterozygous for the marker; WM = 'Wijcik McIntosh', NY-58 = NY 75441-58, both indicates the marker is heterozygous in both parents.

Table 4. Linkage map data for 'Wijcik McIntosh', NY 75441-67, and NY 75441-58 maps.

Cultivar map	No. of markers	No. of linkage groups	Total map length (cM)	Avg. distance ² (cM)
Wijcik McIntosh				
NY 89117	130	20	789	6.1
NY 86229	181	18	858	4.7
Integrated ³	238	19	1206	5.1
NY 75441-67	110	16	692	6.3
NY 75441-58	183	18	898	4.9

²Average distance between markers on the map.³Integrated map of 'Wijcik McIntosh' based on markers segregating in NY 86229, NY 89117, or both.

(93%). We decided to include markers with aberrant segregation ratios because deleting them may have prevented us from mapping genes in these regions.

The production of inbred mapping populations is not feasible in apple due to self-incompatibility and severe inbreeding depression. A substitute mapping scheme is the double-pseudotestcross method (Weeden, 1994). To create a segregating mapping population, two heterozygous cultivars are crossed. Three types of segregation can be observed for RAPD markers in the progeny: 1) markers heterozygous in parent A and homozygous absent in parent B; 2) markers heterozygous in parent B and homozygous absent in parent A; and 3) markers heterozygous in both parents. Marker types 1 and 2 segregate in a 1 present : 1 absent ratio, while type 3 markers segregate in a 3 present : 1 absent ratio. Because the parents of this cross are genetically distinct, a single map cannot be produced from this data, rather two parental maps are created, each comprised of segregating markers heterozygous in that parent. Thus, the map of parent A will consist of marker types 1 and 3, and the map of parent B will consist of marker types 2 and 3.

Linkage analysis was performed using the JoinMap mapping program, which does not restrict data sets to a single segregation type (Stam, 1993). Using MAPMAKER software and a population of 58 plants, Hemmat et al. (1994) were able to map only ~60% of the doubly heterozygous markers compared to >90% of the markers heterozygous in a single parent. By increasing the size of our mapping populations (114 and 172 trees) and using JoinMap software, we were able to map doubly heterozygous markers with the same efficiency as markers heterozygous in a single parent (~93%).

Two maps originally were produced for WM: one from NY 89117 data and the other from NY 86229 data. The NY 89117 map consisted of 130 markers arranged into 20 linkage groups spanning 789 cM, and the NY 86229 map consisted of 181 markers in 18 linkage groups spanning 858 cM (Table 4). Once two independent maps of WM were made using the marker data from each progeny independently, all marker data were combined to produce a single integrated map. Integration involves using markers for which segregation data is available in both progenies. If two markers are segregating in both progenies, JoinMap computes the weighted average of the recombination frequency between the two markers. This causes the shorter interval in one parent to be lengthened and the longer interval in the other parent to be compressed (Jacobs et al., 1995). Depending on the number of markers in common and the differences between maps, marker rearrangements between the single progeny maps and the integrated maps may occur. However, the increase in the number of markers and the number of progeny used to estimate map distances should result in a more accurate map.

Many of the same primers were used in both populations to facilitate integration of the WM maps. The maps shared 62

markers, 26% of the total markers. The large number of markers in common between the WM maps enhanced integration. Only two regions (LG 14 and the lower section of LG 8) could not be combined, because only one marker was common to both populations; thus, the orientation of the linkage groups relative to each other could not be determined.

Rearrangements of markers and changes in map distances occur between individual WM maps and the integrated map, but in general, the agreement between maps is good. Hauge et al. (1993) developed an integrated *Arabidopsis thaliana* map and noted a reordering of markers between the independent maps and the integrated map, mostly in regions that have few shared markers. Order differences also occurred more often between the NY 89117 map and the integrated map because NY 89117 is based on a smaller progeny size; thus, the recombination estimates have a lower weight than those from NY 86229.

The integrated WM map consists of 238 markers comprising 19 linkage groups with a total map distance of 1206 cM (Fig. 1). The NY-67 map has 110 markers in 16 linkage groups spanning 692 cM. The NY-58 map consists of 183 markers comprising 18 linkage groups with a total distance of 898 cM. NY 89117 has 28 unlinked markers and NY 86229 has 6 unlinked markers. Linkage distances between markers varied from 0 to 26.7 cM, with an average distance of 5 to 6 cM between markers in each map (Table 4). RAPD markers are distributed throughout the maps, although small clusters of markers occur. The maps have almost the same number of linkage groups as the chromosome number of *Malus* ($n = 17$), with the WM and NY-58 maps having 2 and 1 extra linkage group(s) respectively, and NY-67 having only 16 linkage groups. More markers are needed to resolve the data into 17 linkage groups.

MAJOR GENE MAP INTEGRATION. Four major genes— R_f , V_f , Ma , and Co —have been located on the maps using data from these mapping progenies and independent estimates of linkage distances from other progenies (Table 5). Anthocyanin production in apple fruit skin appears to be dominant and controlled by a single gene (R_f) (Alston and Watkins, 1974; Crane and Lawrence, 1930). Cheng et al. (1996) have used bulked segregant analysis (BSA) to identify a 10-base oligomer producing fragments associated with red and yellow skin color. Marker B226y-1175 is identical to the fragment A¹ that was found to be tightly linked to a dominant allele for red fruit color. WM and NY-58 appear to be heterozygous for R_f , and tight linkage between R_f and B226y-1175 has been confirmed in NY 86229 (Table 5), placing R_f on the end of LG 3 on the NY-58 and WM maps. Although NY-67 has been shown to be heterozygous for R_f (Cheng et al., 1996), R_f can not be placed on the NY-67 map because NY 89117 has just begun to produce fruit, and segregation data for fruit color is not sufficient to compute linkage.

Several RAPD markers have been mapped to the region surrounding the V_f gene for resistance to apple scab (Hemmat et al.,

Fig. 1. Genetic linkage maps of 'Wijcik McIntosh' (WM), NY 75441-67 (NY-67), and NY 75441-58 (NY-58) apple cultivars. Solid black bars represent WM linkage groups (center), light shaded bars represent NY-67 (right), and open bars represent NY-58 (left). The number of each linkage group is circled at the top. The WM linkage groups are based on an integrated linkage map consisting of markers from NY 89117 and NY 86229, except for the bottom of linkage group 8 and all of LG 14 where there was insufficient data to integrate the maps. In these two cases, the NY 89117 linkage group is on the right and the NY 86229 linkage group is on the left. Markers mapped in both populations and used to integrate the WM maps are underlined. Doubly heterozygous markers used to pair homologous linkage groups from the male parent with their counterparts on the WM map have lines drawn between their positions on each map. Linkage distances between markers (cM) are indicated by the numbers placed in the corresponding interval on the map. Markers falling within 1 cM of each other were placed at the same position on the map, but are listed in the correct order.

1995). V_f indirectly was placed on LG 8 of NY-67 using linkage data from a separate screened progeny (Table 5). No data were available for V_f in the mapping populations because susceptible seedlings were removed from NY 86229 before the mapping analysis and no reliable screening data were available for NY 89117. However, linkage distances between the surrounding markers—P198a-750, S5a-2200, and OU1a-400—are very similar to those reported by Hemmat et al. (1995). V_f could not be placed on the NY-58 map due to a lack of polymorphism in markers in close proximity to V_f due to selection against V_f .

The malic acid locus, *Ma*, was placed at one end of LG 8 on the NY 86229 WM map through linkage to two RAPD markers—S65z-600 and B180a-700 (Table 5). Linkage between *Ma* and S65z-600 was confirmed in an additional progeny (Table 5). *Ma* could not be placed on the NY-58 map because no homologous counterpart exists for this region of LG 8. NY 89117 did not segregate for malic acid, preventing *Ma* from being placed on the NY-67 or the NY 89117 WM map.

The columnar gene, *Co*, was mapped to LG 10 primarily using data from NY 86229. However, linkage data for P459z-800 and *Co* from an additional WM progeny also was included (Table 5). Columnar form data from NY 89117 were not included due to the presence of a large number of intermediate growth forms in this progeny, making it difficult to assess trees for the presence of *Co* (Conner et al., 1994).

HOMOLOGY BETWEEN MAPS. Once the integrated WM map was produced, homologous linkage groups from NY-67 and NY-58 could be paired with their WM counterparts. Homologous linkage groups were aligned using markers heterozygous in both parents that serve as locus bridges between maps (Echt et al., 1994). Counterparts were found for 13 linkage groups in all three maps, two linkage groups (6 and 14) only had counterparts in WM and NY-58, two (16 and 17) had counterparts only in WM and NY-67, and one (18) only in NY-67 and NY-58. In addition, two linkage groups (19 and 20) were present only in WM or NY-58 (21 and 22). Often small linkage groups in one map were joined using an homologous linkage group on the reciprocal map, decreasing the total number of linkage groups in each parental map. The number of locus bridges between linkage groups in the separate maps varied from ten between WM and NY-58 LGs 5 and 6, to only one in several instances. Pairing linkage groups with only one bridge in common should be viewed with caution until more markers are found to confirm homology.

The relative orientation of homologous linkage groups can be deduced for those groups sharing two or more markers. In general, the order of markers on the separate maps were in agreement; however, some rearrangements were evident. Most of these were simple reversals of tightly linked marker pairs, although larger rearrangements did occur. The largest rearrangement was evident with B474y-710, which is located on nearly opposite ends of LG 11 in the WM and NY-58 maps. Differences in map order usually result from the effect of neighboring markers that differ between the two maps or the decreased informativeness of doubly heterozygous markers. Other possibilities for error include misscoring markers or misidentifying homologous bands. Underlying genetic causes such as translocations or inversions also cannot be ruled out.

Where possible, our linkage groups were compared with those maps created by Hemmat et al. (1994). Homology between linkage groups was deduced using morphological and isozyme markers. R_f was mapped to the end of LG 3 in the 'Rome Beauty' x 'White Angel' (RB x WA) map (Weeden et al., 1994), and this was true in the WM and NY-58 maps. Homology in LG 8 was determined by the placement of the V_f gene for resistance to scab that was mapped to LG 8 by Hemmat et al. (1995).

Isozyme markers can be ideal anchor points for comparing separate maps. In our crosses, we were able to map *Aat-1*, *Aat-2*, *Idh-1*, *Pgd-1*, *Pgm-5*, and *Diap-2*. However, since *Aat-1* and *Idh-1* are linked and *Pgm-5* has not been mapped previously, we were only able to confirm homology between four linkage groups using these systems. Homology in LG 1 was determined by the isozymes *Aat-1* and *Idh-1* and in LG 2 by *Pgd-1*. Homology in LG 7 was determined by *Aat-2*, and *Diap-2* determined the homology of LG 4 in each map.

Sixteen RAPD primers used by Hemmat et al. (1994) to create the RB x WA map were also scored in at least one of our mapping progenies. These primers produced 14 bands that were about the same size as the reported band sizes in the RB x WA progeny (± 25 cM). However, in only one case could homology be confirmed between RAPD markers. Marker S34c-540 is linked with *Aat-1* on both maps (LG 1), making it likely that they are homologous markers. Eight bands occurred singly in linkage groups so that homology could not be confirmed, and five bands occurred in pairs that were contradictory between maps. RAPD markers were, thus, less useful for determining homologous linkage groups from the unrelated maps than they were for determining homologous linkage groups between maps developed from half-sib progenies.

Table 5. Estimates of recombination frequencies (RFs) used to map morphological markers and their sources.

Population	Source	Marker pair	RF (%)	SE
Rome Beauty x White Angel	Cheng et al., 1996	B226a-1160, R_f	1.4	12
Wijcik McIntosh x NY 75441-23	Cheng et al., 1996	B226a-1160, R_f	0.0	---
Esopus Spitzenburg x NY 75441-67	Cheng et al., 1996	B226a-1160, R_f	3.9	20
NY 489 x NY 61343-1	Cheng et al., 1996	B226a-1160, R_f	4.6	4.4
Wijcik McIntosh x NY 75441-58	P. Conner ^{3,x}	B226y-1175, R_f	2.3	3.6
Golden Delicious x Prima	M. Hemmat ^w	OU1-400, V_f	5.6	2.7
Golden Delicious x Prima	M. Hemmat ^w	P198-750, V_f	23.9	5.1
Golden Delicious x Prima	M. Hemmat ^w	S5-2200, V_f	0.0	1.4
Wijcik McIntosh x White Angel	P. Conner ^x	P459z-800, <i>Co</i>	14.0	6.2
Wijcik McIntosh x NY 75441-58	P. Conner ^{3,x}	B180a-700, <i>Ma</i>	22.1	8.2
Wijcik McIntosh x NY 75441-58	P. Conner ^{3,x}	S65z-600, <i>Ma</i>	5.0	4.0
Wijcik McIntosh x White Angel	P. Conner ^x	S65z-600, <i>Ma</i>	9.9	3.0

³SE not given in source.

³Included as raw data in JOINMAP.

^xUnpublished data.

^wPersonal communication.

Using a combination of methods, linkage groups 1, 2, 3, 4, 7, and 8 have been matched between the WM progeny maps and the WA x RB maps. To find homology between the other linkage groups, many primers need to be screened to find homologous RAPD markers, or additional isozyme and RFLP markers need to be mapped, since they are more useful in determining homology.

Literature Cited

- Alston, F.H. and R. Watkins. 1974. Apple breeding at East Malling. Proc. Eucarpia Fruit Breeding Symp. 1973:14-29.
- Battle, I., F.H. Alston, and K.M. Evans. 1995. The use of the isoenzymic marker gene *GOT-1* in the recognition of the incompatibility *S* alleles in apple. Theor. Appl. Genet. 90:303-306.
- Bradshaw, Jr., H.D., M. Villar, B.D. Watson, K.G. Otto, S. Stewart, and R.F. Stettler. 1994. Molecular genetics of growth and development in *Populus*. III. A genetic linkage map of a hybrid poplar composed of RFLP, STS, and RAPD markers. Theor. Appl. Genet. 89:167-178.
- Cai, Q., C.L. Guy, and G.A. Moore. 1994. Extension of the linkage map in *Citrus* using random amplified polymorphic DNA (RAPD) markers and RFLP mapping of cold-acclimation-responsive loci. Theor. Appl. Genet. 89:606-614.
- Chaparro, J.X., D.J. Werner, D. O'Malley, and R.R. Sederoff. 1994. Targeted mapping and linkage analysis of morphological, isozyme, and RAPD markers in peach. Theor. Appl. Genet. 87:805-815.
- Cheng, F., N.F. Weeden, and S.K. Brown. 1996. Identification of codominant RAPD markers tightly linked to fruit skin color in apple. Theor. Appl. Genet. 93:222-227.
- Conner, P.J., M.H. Hemmat, S.K. Brown, and N.F. Weeden. 1994. Examination of quantitative trait loci in apple (*Malus x domestica*) using molecular markers. HortScience 29:529.
- Crane, M.B. and W.J.C. Lawrence. 1930. Fertility and vigor of apples in relation to chromosome number. J. Genet. 22:153-163.
- Dayton, D.F., J.B. Mowry, L.F. Hough, C.H. Bailey, E.B. Williams, J. Janick, and F.H. Emerson. 1970. Prima-an early fall red apple with resistance to apple scab. Fruit Var. Hort. Dig. 24:20-22.
- Dudley, J.W. 1993. Molecular markers in plant improvement: Manipulation of genes affecting quantitative traits. Crop Sci. 33:660-668.
- Echt, C.S., K.K. Kidwell, S.J. Knapp, T.C. Osborn, and T.J. McCoy. 1994. Linkage mapping in diploid alfalfa (*Medicago sativa*). Genome 37:61-71.
- Fisher, D.V. 1969. Spur-type strains of 'McIntosh' for high density planting. B.C. Fruit Growers' Assn. Quart. Rpt. 14:3-10.
- Fisher, D.V. 1970. Spur strains of 'McIntosh' discovered in British Columbia, Canada. Fruit Var. Hort. Dig. 24:27-32.
- Gebhardt, C., E. Ritter, T. Debener, V. Schachtschabel, B. Wakemeier, H. Uhrig, and F. Salamini. 1989. RFLP analysis and linkage mapping in *Solanum tuberosum*. Theor. Appl. Genet. 78:65-75.
- Hauge, B.M., S.M. Hanley, S. Cartinhour, J.M. Cherry, H.M. Goodman, M. Koorneef, P. Stam, C. Chang, S. Kempin, L. Medrano, and E.M. Meyerowitz. 1993. An integrated genetic/RFLP map of the *Arabidopsis thaliana* genome. Plant J. 3:745-754.
- Hemmat, M., N.F. Weeden, A.G. Manganaris, and D.M. Lawson. 1994. Molecular marker linkage map for apple. J. Hered. 85:4-11.
- Hemmat, M., N.F. Weeden, H.S. Aldwinckle, and S.K. Brown. 1995. Molecular markers for the scab resistance (*V_s*) region in apple. HortScience 30:855.
- Jacobs, J.M.E., H.J. Van Eck, P. Arens, B. Verkerk-Bakker, B. te Lintel Hekkert, H.J.M. Bastiaanssen, A. El-Kharbotly, A. Pereira, E. Jacobsen, and W.J. Stiekema. 1995. A genetic map of potato (*Solanum tuberosum*) integrating molecular markers, including transposons, and classical markers. Theor. Appl. Genet. 91:289-300.
- Lande, R. 1991. Marker-assisted selection in relation to traditional methods of plant breeding, p. 437-451. In: H.T. Stalker and J.P. Murphy (eds.). Plant breeding in the 1990s. CAB Intl., Wallingford, U.K.
- Lapins, K.O. 1976. Inheritance of compact growth type in apple. J. Amer. Soc. Hort. Sci. 101:133-135.
- Lodhi, M.A., M.J. Daly, G.-N. Ye, N.F. Weeden, and B.I. Reisch. 1995. A molecular marker based linkage map of *Vitis*. Genome 38:786-794.
- Manganaris, A.G. and F.H. Alston. 1987. Inheritance and linkage relationships of glutamate oxaloacetate transaminase isoenzymes in apple. Theor. Appl. Genet. 74:154-161.
- Modlibowska, I. 1945. Pollen tube growth and embryo sac development in apples and pears. J. Pomol. 21:57-89.
- Nybohm, N. 1959. On the inheritance of acidity in cultivated apples. Hereditas 45:332-350.
- Quiros, C.F., J. Hu, P. This, A.M. Chevre, and M. Delseny. 1991. Development and chromosomal localization of genome-specific markers by polymerase chain reaction in *Brassica*. Theor. Appl. Genet. 82:627-632.
- Rowland, L.J. and A. Levi. 1994. RAPD-based genetic map of blueberry derived from a cross between diploid species (*Vaccinium darrowi* and *V. elliotii*). Theor. Appl. Genet. 87:863-868.
- Saiki, R.K., D.H. Gelfand, S. Stoffels, S. Scharf, R.H. Higuchi, G.T. Horn, K.B. Mullis, and H.A. Erlich. 1988. Primer-directed enzymatic amplification of DNA with thermostable DNA polymerase. Science 239:487-491.
- Sambrook J., E.F. Fritsch, and T. Maniatis. 1989. Synthesis of uniformly labeled DNA probes using random oligonucleotide primers, p. 10-13. In: Molecular cloning. 2nd ed. Cold Spring Harbor Lab., Cold Spring Harbor, N.Y.
- Stam, P. 1993. Construction of integrated genetic linkage maps by means of a new computer package: JoinMap. Plant J. 3:739-744.
- Stuber, C.W. 1992. Biochemical and molecular markers in plant breeding. Plant Breeding Rev. 9:37-61.
- Suiter, K.A., J.J.F. Wendel, and J.S. Case. 1983. Linkage-1: A PASCAL computer program for the detection and analysis of genetic linkage. J. Hered. 74:203-204.
- Tanksley, S.D., N.D. Young, A.H. Paterson, and M.W. Bonierbale. 1989. RFLP mapping in plant breeding: New tools for an old science. Bio/Technology 7:257-263.
- Tanksley, S.D. 1993. Mapping polygenes. Annu. Rev. Genet. 27:205-233.
- Torres, A.M., N.F. Weeden, and A. Martin. 1993. Linkage between isozyme, RFLP, and RAPD markers in *Vicia faba*. Theor. Appl. Genet. 85:937-945.
- Weeden, N.F. and R.C. Lamb. 1985. Identification of apple cultivars by isozyme phenotypes. J. Amer. Soc. Hort. Sci. 110:509-515.
- Weeden, N.F. and R.C. Lamb. 1987. Genetics and linkage analysis of isozyme loci in apple. J. Amer. Soc. Hort. Sci. 112:865-87.
- Weeden, N.F. 1994. Approaches to mapping in horticultural crops, p. 57-68. In: P.M. Gresshoff (ed.). Plant genome analysis. CRC Press, Boca Raton, Fla.
- Weeden, N.F., M. Hemmat, D.M. Lawson, M. Lodhi, R.L. Bell, A.G. Manganaris, B.I. Reisch, S.K. Brown, and G.-N. Ye. 1994. Development and application of molecular marker linkage maps in woody fruit crops. Euphytica 77:71-75.
- Welsh, J. and M. McClelland. 1990. Fingerprinting genomes using PCR with arbitrary primers. Nucleic Acids Res. 18:7213-7218.
- Weining, S. and P. Langridge. 1991. Identification and mapping of polymorphisms in cereals based on the polymerase chain reaction. Theor. Appl. Genet. 82:209-216.
- Williams, J.G.K., A.R. Kubelik, K.J. Livak, J.A. Rafalski, and S.V. Tingey. 1990. DNA polymorphisms amplified by arbitrary primers are useful as genetic markers. Nucleic Acids Res. 18:6531-6535.
- Wing, R.A., H. Zhang, and S.D. Tanksley. 1994. Map-based cloning in crop plants. Tomato as a model system: I. Genetic and physical mapping of *jointless*. Mol. Gen. Genet. 242:681-688.