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Mapping and Evaluation of *Malus ×domestica* Microsatellites in Apple and Pear

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ABSTRACT. We mapped DNA polymorphisms generated by 41 sets of Simple Sequence Repeat (SSR) primers, developed independently in four laboratories. All primer sets gave polymorphisms that could be located on our 'White Angel' x 'Rome Beauty' map for apple [*Malus sylvestris* (L.) Mill. Var. domestica (Borkh.) Mansf.]. The SSR primers were used to identify homologous linkage groups in 'Wijcik McIntosh', NY 75441-58, 'Golden Delicious', and 'Liberty' cultivars for which relatively complete linkage maps have been constructed from isozyme and Random Amplified Polymorphic DNA (RAPD) markers. In several instances, two or more SSRs were syntenic, and except for an apparent translocation involving linkage group (LG) 6, these linkages were conserved throughout the six maps. Twenty-four SSR primers were consistently polymorphic, and these are recommended as standard anchor markers for apple maps. Experiments on a pear (*Pyrus communis* L.) population indicated that many of the apple SSRs would be useful for mapping in pear. However some of the primers produced fragments in pear significantly different in size than those in apple.

Simple sequence repeats (SSRs), also called microsatellites, consist of different numbers of tandem repeats (two to six nucleotides in length) and have a total length of repeated units in the range of 10 to 60 bp. SSRs are highly informative genetic markers and have been used in saturation and construction of high-density maps of human and other mammalian systems. Based on properties such as co-dominant mode of inheritance and high degree of heterozygosity, SSRs have advantages over other types of markers. In plants, SSRs have been reported to be highly polymorphic and provide many alleles for each primer set in closely related species. Such markers are particularly useful in species with low genetic diversity (Cipriani et al., 1999; Katzir et al., 1996). The abundance and distribution of SSR markers throughout a genome was shown in rice (Wu and Tanksley, 1993), in soybean (Akkaya et al., 1995), and in grape (Thomas and Scott, 1993). SSR markers can serve as anchor markers between different genetic linkage maps within specific crops due to their high level of reproducibility and polymorphism (Beckmann and Soller, 1990). In many cases SSR markers can be transferred between genetic maps derived from different populations (Westman and Kresovich, 1998) and in comparative mapping (Byrne et al., 1996). Eighty percent transferability of microsatellites has been reported among species of Myrtaceae, Poaceae and Pinus, with 20% transferability across unrelated genera (Gupta and Varshney, 2000).

SSR markers have been developed in different laboratories for research on *Malus* species (Gianfranceschi et al., 1998; Guilford et al., 1997; Hokanson et al., 1998). *Malus* spp. genomes are abundant in microsatellites, and the frequency of occurrence of di- and tetra-nucleotides is comparable with other plant species (Guilford et al., 1997). In a 'Golden Delicious' genomic library, the most abundant di-nucleotide was $(GA)_{10}$ and in general tri- and tetra-nucleotides were less frequent (Hokanson et al., 1998). Gianfranceschi et al. (1998) reported 8.2 alleles per locus and on average 78% heterozygosity for 17 sets of SSR primers they developed in apple. Due to a high level of heterozygosity, as few as three selected SSR primers were sufficient to distinguish 21 apple cultivars (Guilford et al., 1997). Ciprani et al. (1999) reported that 88% of the microsatellites primers they developed for peach [*Prunus persica* (L.) Batsch] showed polymorphism and each had as many as two to four alleles. The same report indicated that 59% of the *Prunus* microsatellites gave correct amplification in a cross-species (almond, apricot, plum, sweet and sour cherry) survey. Yamamoto et al. (2001) reported 79 alleles from seven *Malus* SSR primers detected in *Pyrus*. The flanking regions for selected SSRs were highly conserved in different pear accessions. However, fragment sizes were different between apple and pear and among pear cultivars.

The purpose of this study was to map 41 SSRs developed from apple by different laboratories on the 'Rome Beauty ' x 'White Angel' linkage map (Hemmat et. al., 1994), and to identify corresponding linkage groups on four other apple maps. Flanking primers developed for apple were also evaluated on two pear cultivars.

Materials and Methods

PLANT MATRERIAL. Three apple populations: 'Rome Beauty' x 'White Angel' (RB x WA) (56 trees), 'Wijcik McIntosh' x NY 75441-58 (WM x NY-58) (60 trees), and 'Liberty' x 'Golden Delicious' (Li x GD) (55 trees) and one pear population, 'Bartlett' x NY 10353 (Ba x NY) were used in our study of genetic mapping of microsatellites. Plant material for the pear population was provided by Richard Bell, USDA, Kearneysville, W. Va.

Genetic linkage maps of 'Rome Beauty' x 'White Angel', (Hemmat, et al., 1994) and 'Wijcik McIntosh' x NY 75441-58, (Conner et al., 1997) were used for linkage analysis and mapping of the SSRs. A Microsoft Excel macro, QUIKMAP, designed by Weeden, and Barnard (1994) to calculate the frequency of the matches between

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Table 1. SSR primers[z] used for mapping on genetic linkage map of three apple populations and one pear population.

Primer name	Primer forward sequence	Primer reverse sequence
*SSRCO	CGC TTC GAA AAC CTC AAG AGA TCA TC	GAC GCC ATA CCC ATT GAC GA
CH01B12	CGC ATG CTG ACA TGT TGA AT	CGG TGA GCC CTC TTA TGT GA
CH01E12	AAA CTG AAG CCA TGA GGG C	TC AAT TCA CAT GAG GCT G
CH01 G12	CCC ACC AAT CAA AAA TCA CC	TGA AGT ATG GTG GTG CGT TC
CH01H01	GAA AGA CTT GCA GTG GGA GC	GGA GTG GGT TTG AGA AGG TT
CH01H10	TGC AAA GAT AGG TAG ATA TAT GCC A	AGG AGG GAT TGT TTG TGC AC
CH02B10	CAA GCA AAT CAT CAA AGA TTC AAG	CAA GTG GCT TCG GAT AGT TG
CH02B12	GGC AGG CTT TAC GAT TAT GC	CCC ACT AAA AGT TCA CAG GC
CH02C06	TGA CGA AAT CCA CTA CTA ATG CA	GAT TGC GCG CTT TTT AAC AT
CH02D12	AAC CAG ATT TGC TTG CCA TC	GCT GGT GGT AAA CGT GGT G
CH01F02	ACC CAC ATT AGA GCA GTT GAG G	CTG GTT TGT TTT CCT CCA GC
*GD-6	ATT TTG GAA CAC ACA CTC CCT CAG A	GAC GCC ATA CCC ATT GAC GAT T
*GD-12	TTG AGG TGT TTC TCC CAT TGG A	CTA ACG AAG CCG CCA TTT CTT T
*GD-96	CGG CGG AAA GCA ATC ACC T	GCC AGC CCT CTA TGG TTC TCA GA
GD-100	ACA GCA AGG TGT TGG GTA AGA AGG T	TGC GGA CAA AGG AAA AAA AAA AGT G
*GD-103	CGG CGA GAA AAA AAA ACA ATG	GGA TAA CCG TCC CCC TCT TC
*GD-112	GCT GCG AGC GAA CCT GAA	ACC TGA ATC CGA CCC CTT TTC
*GD-126	ATT TCG TTG CTG TGC TCG TC	AAC CTC CTT CAT TAC ATT CCA CTC T
*GD-127	CTT TCG GAT GCC TTT TGC T	GCT TTC GGA TTC TTC GTC TTC
*GD-136	CGG CGA GAA AAA AAA ACA ATG	GTG GAT AAC CGT CCC CTC TTC
GD-142	GGC ACC CAA GCC CCT AA	GGA ACC TAC GAC AGC AAA GTT ACA
*GD-144	GCA AAC ATA AAA CCC ATC ATT ACC	GTA GCC CTC AAA GAA CTC CTT GT
GD-147	TCC CGC CAT TTC TCT GC	AAA CCG CTG CTG CTG AAC
*GD-153	AAG CCC TAT CTA TTC TTC ACA CAC A	CCT TTC CCC CTC ACA CAA C
*GD-154	CGT GGA GGA AGA GAA AGA GGC	GCAGAG TGA CCC TGA AGA CAA
*GD-158	CAA CCA CTC AGA ATG ACA CCA AC	GAG AAA GAC GAT GGC TAC GAG ATT
*GD-161	TTT GGG TCC CTG AGA GTC CAG TG	GTC GTT TGA AAT CCA TCC CTC CAT
GD-162	GAG GCA AGT GAC AAA GAA AGA YG	AAA ATG TAA CAA CCC GTC CAA GTG
*NZ-02b1	CCG TGA TGA CAA AGT GCA TGA	ATG AGT TTG ATG CCC TTG GA
*NZ-03c1	GCT CTC ATC TTC ACA GAT AA	AGA CCC GGA AAA TTC TAT
*NZ-04h11	CTT CCA TCG AGATTG CATC ATA	CGA ATT GAG AGG TCG TCG TT
*NZ-05g8	CGG CCA TCG ATT ATC TTA CTC TT	GGA TCA ATG CAC TGA AAT AAA CG
*NZ-17e6	AAC ACG CCA TCA CAC ATC	CTG TTT GCT AGA AGA GAA GTC
NZ26c6	GAC GAA GAA CTC GCC GGC GC	CGA GGA CCA ACC CAC ACA CAA
*NZ-28f4	TGC CTC CCT TAT ATA GCT AC	TGA GGA CGG TGA GAT TTG
*UK-MS11H08	TAT TAG TGC TTG ACA GAC	CAG CAT GAT TAG TAG TTT AG
*UK-MS14B04	CCT TAA GAA TCA TGT GAT	ACT AAT GGC ACA AAG ATT GT
*UK-MS14H03	CGC TCA CCT CGT AGA CGT	ATG CAA TGG CTA AGC ATA
*UK-MS02A01	CTC CTA CAT TGA CAT TGC AT	TAG ACA TTT GAT GAG ACT G
*UK-MS01A03	AGC AGT ATA GGT CTT CAG	TGC GTA GAT AAC ACT CGA T
*UK-MS01A05	GGA AGG AAC ATG CAG ACT	TGA TGT TTC ATC TTC ACA
*UK-MS02H06	AGA CGA ACA CAA GTA GTA	CTA ATA CAG TCA TAC AG

[z] Primers identified by CH were developed at the Institute of Plant Sciences, Swiss Federal Institute of Technology, Universitätstrasse 2, CH-8092 Zürich, Switzerland, GD primers were developed at the USDA/ARS-Plant Genetic Resources Unit, Cornell University, Geneva, NY. Primers identified as NZ, were developed at the Horticulture and Food Research Institute of New Zealand. Primers designated as UK, were developed at the Plant Genetics and Development, Horticulture Research International, Wellesbourne in the United Kingdom and sequences generously supplied by Dr. G. King.
*Primers used on pear population.

the marker loci and the SSR loci, was used to identify the position of the SSR loci on the linkage maps. The identified SSRs for each linkage group served as anchor points to find corresponding linkage groups in other populations.

DNA isolation and PCR amplification of SSR loci. Genomic DNA was extracted from very young leaf tissue using CTAB buffer according to Saghai-Maroof et al. (1984) and a modification of the method by Doyle and Doyle (1990). A total volume of 25 μL reaction mixture containing 2.5 μL 10× buffer, 1.5 μL of 2.5 mM of each dNTP, 10 μM of each primer and 0.5 U *Taq* polymerase (Promega Corp., Madison, Wis.) and 200 ng DNA was subjected to PCR amplification in a thermocycler (PTC-100; MJ Corp., Watertown, Mass.). Cycling parameters for amplification of SSR included an initial cycle of 60 s at 94 °C followed by 40 cycles of 60 s at 94 °C, 120 s at the annealing temperature (between 49 to 59 °C), 120 s at 72 °C and a final cycle of 8 min at 72 °C.

Sources of primer sequences. In total, 41 SSR primer sets were used on apple and 27 sets on pear (Table 1). Primers identified as GD were developed at the USDA–ARS, Plant Genetic Resources Unit, Cornell University, Geneva, N.Y. (Hokanson et al., 1998). Primers designated CH were developed at the Institute of Plant Sciences, Swiss Federal Institute of Technology, Zurich, Switzerland

(Gianfranceschi et al., 1998). Primers identified as NZ were developed at the Horticulture and Food Research Institute of New Zealand, Auckland (Guilford et al., 1997). Seven primer sets, designated as UK, were developed in the United Kingdom and generously supplied by G. King (Plant Genetics and Development, Horticulture Research International, Wellesbourne).

Electrophoresis of the amplification products. PCR products were separated on NuSieve GTG agarose gel (FMC Bioproducts, Rockland, Maine) using 1× TBE buffer, at ≈2.6 V·cm–1. The gels were run ≈1.5 to 2 h. The concentration of agarose used in the gels varied between 3.5% to 4.25% depending on the size of the amplified fragment to be resolved. Gels were stained with ethidium bromide and photographed under UV (λ-302 nm) light using Polaroid type 55 film.

Results

Polymorphism. An initial comparison of agarose (2% to 4% NuSieve or equivalent) and denaturing sequencing gels indicated that most polymorphisms resolved on the sequencing gels also could be resolved on agarose gels. Thus the present analysis was performed solely on agarose gels. All primer sets except UK11H08 gave clear polymorphisms in at least one of the apple populations. As expected, 'White Angel' produced the most segregating bands (84%). NY 75441-58 produced the second most (72%), and the remaining four cultivars all displayed about 60% segregating bands for the SSR loci (data not presented).

Of the 41 primer sets producing amplified product, all generated fragments near the predicted size except for GD-127 and GD-103. The GD-127 primer set did not amplify a 120 bp fragment in the RB x WA cross, but produced the expected fragment in the other two apple populations as well as in the pear population. For GD-103 an 80 bp fragment was polymorphic in the RB x WA progeny rather than the expected 108 bp fragment. Several SSR primers, including GD-6, GD-103, GD-153, and GD-158 generated additional fragments, some of which were polymorphic and assorted independently of the primary polymorphism. Increasing the annealing temperature in these cases often led to the loss of all amplification products rather than loss of just the unexpected fragments. Finally, even after multiple attempts, certain SSR primers sets failed to generate fragments in specific crosses: UK2H06 and NZ5g8 in WM x NY-58, and GD-161 in Li x GD.

Distribution of SSR markers on the standad

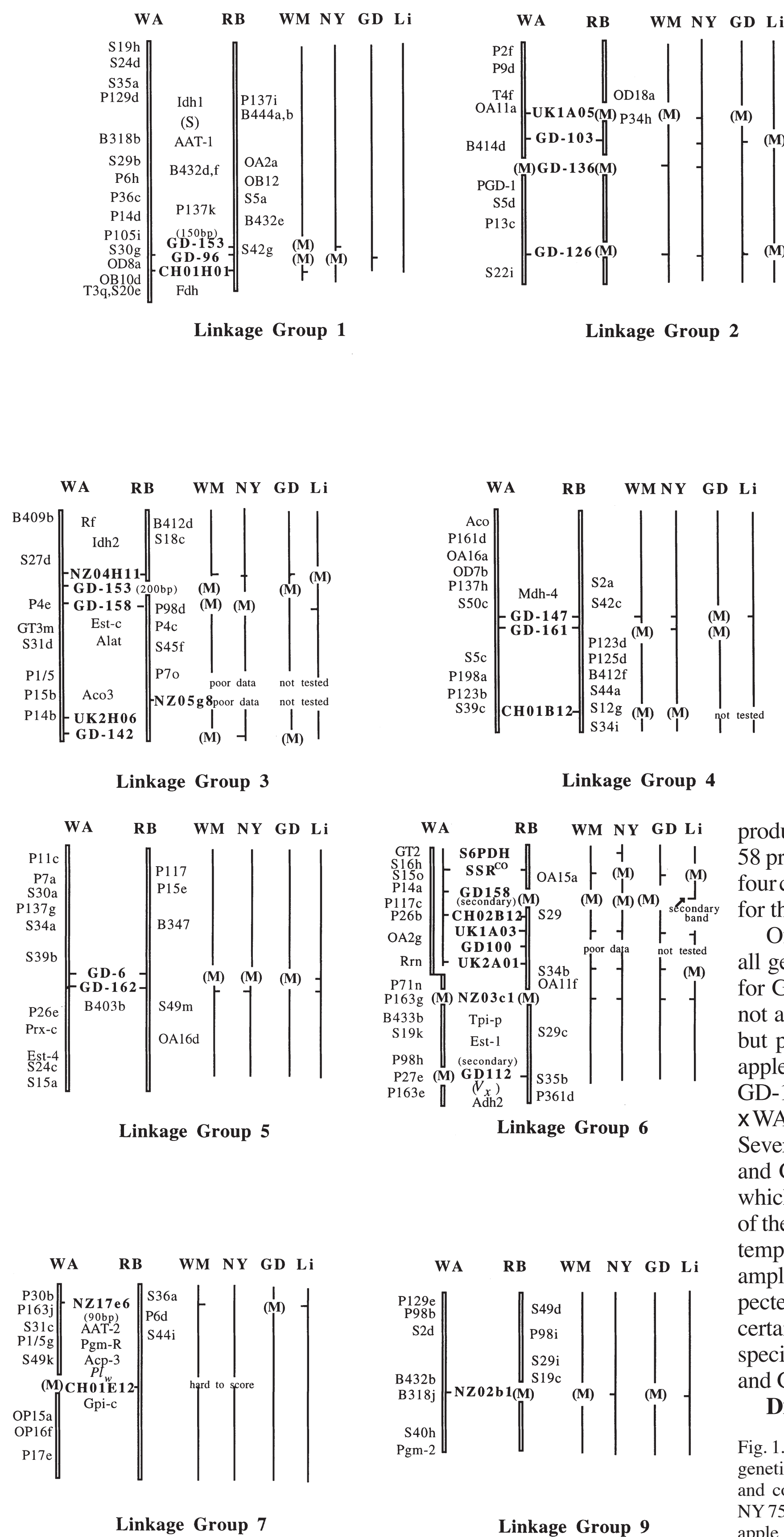

Fig. 1. Distribution and approximate position of SSR markers on the genetic linkage map of 'White Angel' (WA), 'Rome Beauty' (RB), and corresponding linkage groups in 'Wijcik MacIntosh' (WM), NY 75441-58 (NY-58), 'Golden Delicious' (GD), and 'Liberty' (Li) apple cultivars. Mapped SSR markers are noted by a horizontal bar on the linkage group. The approximate location of the markers or gene not segregating in the test population are noted by (M) or gene symbols in parentheses. SSR markers mapping on more than one linkage group are noted by the size of the bands.

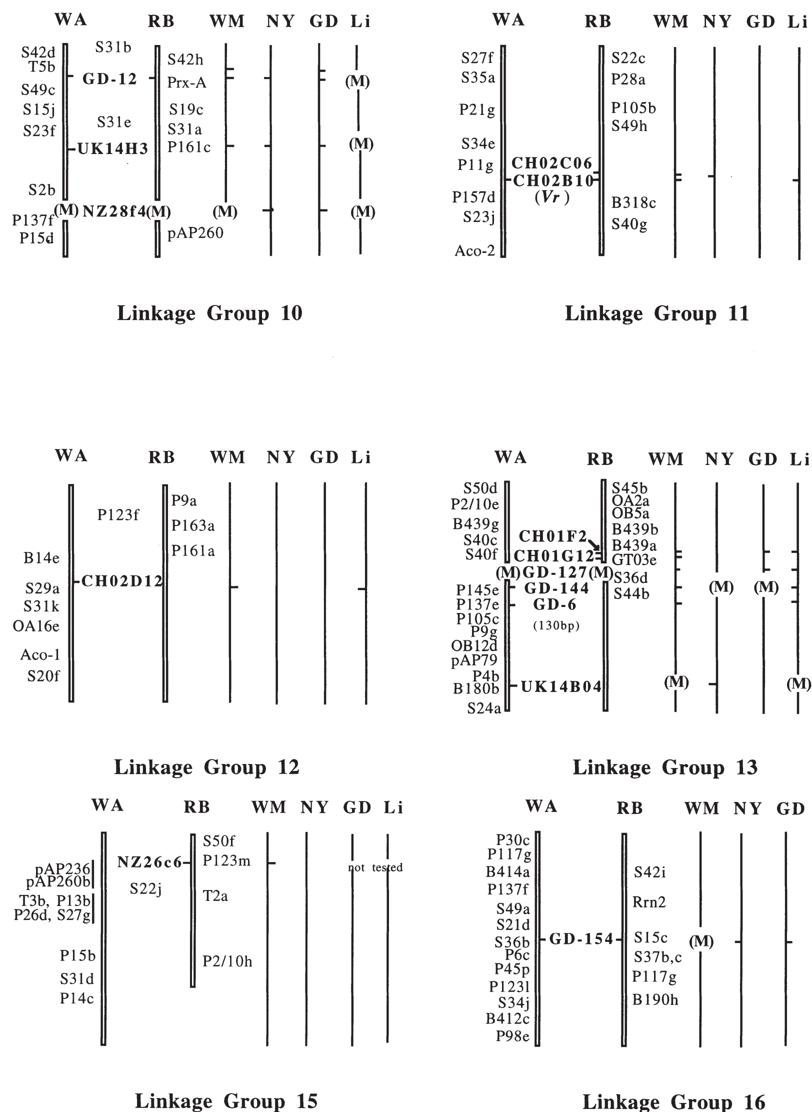

Fig. 1 (continued). Distribution and approximate position of SSR markers on the genetic linkage map of 'White Angel' (WA), 'Rome Beauty' (RB), and corresponding linkage groups in 'Wijcik MacIntosh' (WM), NY 75441-58 (NY-58), 'Golden Delicious' (GD), and 'Liberty' (Li) apple cultivars. Mapped SSR markers are noted by a horizontal bar on the linkage group. The approximate location of the markers or gene not segregating in the test population are noted by (M) or gene symbols in parentheses. SSR markers mapping on more than one linkage group are noted by the size of the bands.

LINKAGE MAP. All 41 SSR primer pairs tested produced markers that could be mapped in our populations (Fig. 1). At least one SSR was placed on 15 of the 17 linkage groups (LG) (Table 2). Clusters of SSRs were observed on LG 1, 2, 3, 4, 5, 6, and 13 (Table 2, Fig. 1). LG 3 and 6 were especially rich in SSR loci, possessing six and nine SSRs, respectively. UK2A01 and GD-100 on LG 6 displayed identical segregation patterns and appeared to be very tightly linked.

COMPARATIVE MAPPING. The SSR primer sets identified or confirmed homologous linkage groups in WM, NY-58, GD, and Li (Fig. 1). SSRs syntenic in WA or RB were also syntenic in the other cultivars except in two cases:

1) UKA103, UK2A01, GD-100, and SSR^{Co} [marker for columnar habit (*Co*) Hemmat et al., 1997] all mapped on LG 6 of RB, and most mapped on LG 6 of WM, NY-58, and GD. However, in WA these markers, as a group, segregated independently of the remaining markers defining LG 6. This suggests that at least one chromosomal rearrangement has occurred between Asian *Malus* species such as *M. sargentii* and *M. sieboldii* (from which 'White Angel' has been derived) and *M.* ×*domestica*.

2) In both RB x WA and Li x GD, GD-6 mapped to LG 5. GD-6 was closely linked to GD-162 in both the former progeny, and GD-162 mapped to LG 5 in WM x NY-58. However, in WM x NY-58, GD-6 mapped to LG 2. Thus, we suggest that we scored a nonhomologous GD-6 polymorphism in the WM x NY-58 progeny.

In some cases SSR markers identified a homologous linkage group or reassigned homologous linkage groups from those previously reported (Hemmat et al., 1994). GD-96 and GD-153 identified a homologous linkage group for WA LG 1**.** CH01H01 also mapped on the bottom of LG 1 and identified LG 17 as continuation of LG 1. Of four SSR markers mapped on LG 2, GD-103 and UK1A05 were particularly useful in identification of a homologous LG for WA LG 2. The V_x scab resistance gene from R12740-7A was mapped through distal markers on LG 10 of 'Wijcik McIntosh', and this same region can be assigned to the bottom of LG 6 WA (Hemmat et al., 2002).

In four cases, SSRs identified new or re-assigned linkage groups from those previously reported. For instance, GD-12 and UK14H03 were the most useful SSR markers for assigning RB LG 5 and WA LG 8 to the new LG 10. Among three SSRs mapped on LG 4, GD-147 and GD-161 were the most useful in identifying RB 16 as the homologous group for LG 4. GD-6 and GD-162 mapped to WA 5 and identified newly assigned RAPD markers for the new RB LG 5. The only SSR on LG 16, GD-154, identified RB X-3 and additional RAPD markers as the new RB LG 16. NZ26c6 mapped on LG 15 of WM and displayed weak linkage to markers on RB LG 15.

In pear, we observed the same general pattern of segregation. In most cases, a 2 to 3 °C lower annealing temperature was required for amplification of products. From 27 SSRs tested on 'Bartlett' and NY 10353-85001, 22 were polymorphic. UK2H06, UK11H08, NZ4H11 and SSR^{Co} did not amplify a fragment of the expected size and UK1A03 was monomorphic.

COMPARISON BETWEEN HEMMAT ET AL. (1994) AND MALIEPAARD ET AL. (1998) MAPS. Although researchers have numbered their linkage groups differently, the anchor loci should permit convenient inter-conversion and comparison of these two maps. We also included the map of Gianfranceschi et al. (1998) which is based on a map of 'Prima' x 'Fiesta' (Pr x Fi) (Maliepaard et al., 1998). We identified markers (isozyme, RFLP, and SSR) in 15 linkage groups that could serve as anchor markers for comparison between the maps (Table 3). For LG 1, *Aat*-1 and the self-incompatibility locus (*S*) from 'White Angel' and CH01H01 identified LG 17 as corresponding linkage group. We mapped pAP79 on LG 13 and *Me* on LG 14 of RB x WA. These two markers were mapped on LG 2 of Fi. We mapped CH01 B12 on LG 4 and CH01F2 on LG 13 of RB, while these two markers were mapped on one linkage group in 'Iduna' and 'A679/2' ('I'-5 and 'A'-11) (Gianfranceschi et al., 1998). CH01B12 was tightly linked to the markers S12g and S34i on RB 4. *Pgm*-2 and NZ2b1 both mapped on LG 9 of WA, while *Pgm*-2 mapped to the bottom of LG 7 and NZ2b1 on LG

Table 2. Distribution of SSR markers on different linkage groups of 'Rome Beauty' x 'White Angel' population.

Linkage		Segregating fragments	
group	SSR	'White Angel'	'Rome Beauty'
1	GD-96	162, 166	167, 176
1	GD-153	None	150
2	UK1A05	180	80, 210
2	GD-103	85	82, 108
2	GD-136	None	None
2	GD-126	260	None
3	NZ04H11	190	220
3	GD-153	200	230
3	GD-158	145	160
3	NZ-05g8	None	120
3	UK2H06	210	None
3	GD-142	142, 144	135, 142
4	GD-147	128	132, 142
4	GD-161	190	210
4	CH01B12	None	170
5	GD-6	145	180, 220
5	GD-162	219, 236	227, 240
6	S6PDH	None	None
6	SSR^{Co}	150, 170	185
6	GD158	135	None
6	CH02B12	145	165
6	UK1A03	200	180
6	GD-100	228, 242	228, 236
6	UK2A01	170, 200	230
6	NZ03C1	None	None
6	GD-112	None	200
7	CH01E12	None	195
7	NZ17e6	90	None
9	NZ02b1	None	None
10	GD-12	170, 200	152, 154
10	UK14h03	160	110, 150
10	NZ28F4	None	None
11	CH02B10	115,160	120,130
12	CH02D12	180	None
13	CH01F2	None	265
13	CH01G12	None	150, 190
13	GD-127	None	None
13	GD-144	220	210
13	GD-6	80	None
13	UK14B04	280	None
15	NZ26c6	None	100
16	GD-154	175, 200	180, 95

15 of Pr x Fi. NZ28f4, UKH03 and *Prx*-A all mapped on the new LG 10 of RB x WA, while UK14H03 and *Prx*-A mapped on LG 3 and NZ28f4 on LG 12 of Pr x Fi.

Discussion

We have shown that SSR markers are useful in anchoring as well as confirming previously identified homologous linkage groups in the map of 'Rome Beauty' x 'White Angel'. Certain SSR primer sets were more useful than others. Those most useful showed a high level of heterozygosity in the parents and did not generate fragments from additional loci. The combination of different kinds of markers in apple now permits good anchor loci to be identified for most of the linkage groups (Table 3).

Knowledge of map location for monogenic morphological traits and for disease resistance genes will provide better understanding and tools for genetic improvement. Availability of SSR markers on each linkage group provides better reference to monogenic traits such as resistance to powdery mildew (*Podosphaera leucotricha*) from 'White Angel' (Pl_w) (Batlle and Alston, 1996), red fruit skin color (R_f) (Cheng et al.,1996) and fruit acidity (*Ma*) (Maliepaard et al., 1998). Linkage of an SSR marker to columnar habit (*Co*) (Hemmat et al., 1997) and close markers of the V_r and V_x scab resistance genes from R12740-7A apple (Hemmat et al., 2002), and the V_g (race 7) scab resistance gene (Durel et al., 2000) will aid pyramiding of multiple genes for resistance.

The identification of an apparent chromosomal rearrangement in 'White Angel' (LG 6) suggests that we may expect to see changes in linkage relationships in pear and certainly in other *Rosaceae* genera. Some of the SSRs we examined should be useful for analyzing linkage conservation within the Maloideae. However, certain of the SSR primer sets will be of value only for fingerprinting or genetic analysis within *Malus*.

The clustering of SSR loci is interesting because the clusters often contain SSRs from different laboratories. On LG 3 and 6, SSRs from three and four different programs, respectively, are present. Certain regions of the apple genome must be particularly rich in microsatellite sequences. It is also interesting that the microsatellites tend to be more prevalent on the linkage groups that are better defined in both the American and European maps. All of the SSR polymorphisms detected in the 'Rome Beauty' x 'White Angel' progeny could be located on the standard map, indicating the map covers most of the nuclear genome. However, >80% of the SSRs mapped to the first 10 linkage groups defined, which also contain ≈90% of the expressed genes mapped in apple but relatively fewer (≈75%) of the RAPD markers mapped. It would appear that these first 10 linkage groups either contain the bulk of the nuclear genome or most of the polymorphic sequences.

The high level of reproducibility of these SSR markers on six different apple cultivar maps allows us to use them as anchor points between maps from different laboratories. Using isozyme, RFLP and SSR markers, we identified 15 corresponding linkage group between our maps and those of Maliepaard et al. (1998).

However, there are some differences between the maps. We mapped both NZ2b1 and *Pgm*-2 on LG 9, while these were mapped on LG 7 and 15 of Maliepaard et al. (1998). We mapped *Prx*-A, UK14H03, and NZ28f4 on LG 10 of several different apple cultivars, yet these were mapped on LG 3 and 12 of Maliepaard et al. (1998). In two of our maps the SSR markers UK 14H03 and NZ28f4 are linked and belong to the same linkage group. These also linked to the SSR GD-12, which was closely linked to *Prx*-A on LG 10.

Another point of disagreement among the maps is between LG 2 of Pr x Fi and our corresponding linkage group. Gianfranceschi et al. (1998) mapped CH01B12 and CH01f2 on one linkage group, 'I'-5 x 'A'-11. We mapped pAP79 and CH01F2 on LG 13 corresponding to LG 2 of Fi and 'I'-5 x 'A'-11, respectively. However, we examined the possibility of linkage between CH01B12 and the markers on LG 13. We found a weak linkage (over 25 cM) between S45a and CH01B12. Maliepaard et al. (1998) also mapped *Me* on Fi 2. However, we mapped *Me* on LG 14 of RB. Neither the two SSRs (CH01B12 and CH01f2) nor isozyme *Me* were linked on one linkage group in our maps. In addition, three linkage groups in our map have closely mapped markers with no major gap. However, there is a 30 cM gap between *Me* and pAP79 on LG 2 of Fi. Therefore, we preferred to err on the side of caution and did not use linkage weaker than 25 cM.

Success in amplification and polymorphism of *Malus* primers

Table 3. Suggested anchor markers and comparison of the apple maps.

Hemmat et al.(1994) linkage groups	Maliepaard et al. (1998) linkage groups[z]
1 (*S*, *Aat*-1, *Fdh*, GD-153)	17, and ‘I’-1 & ‘A’-12
2 (*Pgd*-1, UK1A05, GD-103)	14
3 (R_f, *Idh*-2, NZ04H11, GD-142)	9
4 (*Mdh*-4, GD147, GD-161, CH01B12)	top of ‘I’-5 & ‘A’-11
5 (Cathodal *Prx, Est*-4, GD-6, GD-162)	4
6 *Rrn1*, *Adh*, SSR^{Co}, GD-100, UK2A01	10
6 CH02B12	10 and ‘I’-14, & ‘A’-6
7 (Aat-2, Lap-2, powdery mildew Pl_w resistance)	8
8 (V_f, *Pgm*-1, Tpi-c2)	1
9 NZ02b1	15
9 (*Pgm*-2)	7
10 Anodal *Prx*, (GD-12, UK14H03)	3
10 NZ28f4 (GD-12, UK14H03)	12, and ‘D’-12,
11 (V_r, CH02B10, CH02C06)	‘I’-8 & ‘A’-4
12 (CH02D12)	‘I’-7 & ‘A’-3
13 (pAP79, UK14B4, GD-144, CH01F2)	2 and ‘I’-5 & ‘A’-11
14 (*Me*, Lap-4)	2
15 (pAP236, pAP260b, NZ26c6)	16
16 (GD-154, *Rrn2*)	?

[z]All linkage groups, unless otherwise specified, are from ‘Prima’ x ‘Fiesta’ (Maliepaard et al., 1998). ‘I’ and ‘A’ represent ‘Iduna’ and ‘A679/2’ (Gianfranceschi et al., 1998).

in pear population (81%), is an indication of conserved microsatellite flanking regions that exist between *Malus* and *Pyrus* as opposed to Cipriani et al. (2000) who found only 18% of the peach SSRs amplified in apple. Our results suggesting a high degree of transferability of microsatellite primers across the two genera are in agreement with Yamamoto et al. (2001) and Monte-Corvo et al. (2001). However, we do not know if these are homologs until the linkage map for pear is prepared. Identified microsatellites on *Malus* linkage groups can be utilized as anchor loci in comparative mapping among *Malus* species and between *Malus* and *Pyrus*.

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