A Linkage Map of the Diploid Strawberry, *Fragaria vesca*

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A 445 cM long genetic linkage map consisting of seven linkage groups was constructed for the diploid ($2n = 2x = 14$) strawberry, *Fragaria vesca*. Segregation data used for linkage analysis were obtained from the F_2 generation of a cross between Baron Solemacher (BS), an Alpine *F. vesca* variety, and WC6, an *F. vesca* clone collected from the wild in New Hampshire. Segregation ratios were systematically skewed in five linkage groups, in all cases favoring the BS alleles over the WC6 alleles. The 80-marker map includes 64 dominant and 11 codominant randomly amplified polymorphic DNA (RAPD) markers, an alcohol dehydrogenase locus detected as a PCR-based sequence tagged site, the phosphoglucose isomerase and shikimate dehydrogenase isozyme loci, and the runnering and fruit color loci. A notable feature of the map is the unusually large number of codominant RAPD markers, the detection of which was due in part to the use of template mixing methods for primer testing and marker analysis. Alternate alleles of a maternally inherited RAPD marker were also detected using these methods.

A linkage map provides a unifying framework for the genetic description of a species and is a valuable resource for both basic and applied plant genetic research. Detection of close linkage between molecular markers and loci controlling traits of economic interest provides a basis for marker-assisted selection (Stuber 1992) and for map-based positional cloning (Wing et al. 1994). Comparative mapping can be used to detect linkage group and therefore chromosome rearrangement between related taxa (Moore et al. 1993; Tanksley et al. 1992). Although detailed genome maps have been developed for many major crop plants, linkage information has been largely lacking for some economically significant species including the cultivated strawberry, *Fragaria* × *ananassa* (Williamson et al. 1995).

The octoploid genomic constitution of *F.* × *ananassa* ($2n = 8x = 56$) has been a major impediment to genetic characterization of this species. Few genes of qualitative effect have been identified (Galletta and Maas 1990), and there have been no published reports of genetic linkage. In other polyploid crop species, such as alfalfa (Brummer et al. 1993; Kiss et al. 1993) and potato (Bonierbale et al. 1988), diploid relatives have been utilized as "model systems" for linkage map development. In the present study, we utilized the diploid *F. vesca* ($2n = 2x = 14$), a close relative of *F.* × *ananassa*, in the construction of the first strawberry linkage map.

The genus *Fragaria* is comprised of at least 15 strawberry species represented by four or more levels of ploidy (Hancock 1990). *F.* × *ananassa* is a hybrid species, having originated from its wild octoploid progenitors, *F. chiloensis* and *F. virginiana*, in the mid-1700s (Hancock and Luby 1993). Eight diploid species are recognized (Hancock 1990). *F. vesca*, the most broadly distributed diploid, is found in the wild throughout the northern hemisphere (Hancock and Luby 1993; Staudt 1989) and is an apparent genome contributor to *F.* × *ananassa* (Senanayake and Bringhurst 1967). *F. vesca* has been described as a predominantly selfing species (Arulsekar and Bringhurst 1981). So-called Alpine cultivars of *F. vesca* are distinguished by their perpetual, as opposed to seasonal, flowering habit and their European origin (Williamson et al. 1995).

The only two reports of linkage in strawberry (*Fragaria* sp.) are the linkages in *F. vesca* between the yellow fruit color and *Sdh* (shikimate dehydrogenase) isozyme loci (Williamson et al. 1995) and between nonrunnering and the *Pgi-2* isozyme locus (Yu and Davis 1995). The *F. vesca* fruit color and runnering loci were first identified, and assigned the symbols *c* (yellow) and

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r (nonrunnering), respectively, by Brown and Wareing (1965).

Map construction requires a segregating population and an abundance of genetic markers. Random amplified polymorphic DNA (RAPD) markers (Williams et al. 1990) are convenient for this purpose because they are easily detected by well-established PCR technology (Tingey and del Tufo 1993), and because they are usually abundant in segregating populations involving genetically distinct crossing parents. Most RAPD polymorphisms are inherited as dominant (presence-absence) markers (Tingey and del Tufo 1993). Marker dominance is a disadvantage in F_2 populations, because dominant markers provide less genetic information per F_2 individual than do codominant markers (Allard 1956). Therefore it is advantageous to maximize the identification of codominant RAPDs when utilizing F_2 data to construct a RAPD marker map.

A distinguishing feature of codominant RAPD markers is the presence of a nonparental, heteroduplex band in addition to the two "allelic" homoduplex bands in heterozygous individuals (Davis et al. 1995). Marker-associated heteroduplex bands can also be generated by combining the respective parental DNAs in a mixed template PCR reaction. Primers that generate heteroduplex bands in parent-parent mixed template reactions have been shown to detect the segregation of corresponding codominant RAPD markers in F_2 mapping populations derived from crosses between these parents (Davis et al. 1995). Similarly, heteroduplex bands generated by mixing parental and F_2 generation template DNAs in appropriate combinations can be used as an aid to scoring the segregation of certain types of codominant RAPD markers. In this study we utilized the template mixing methods described by Davis et al. (1995) to enhance the detection and interpretation of codominant RAPD markers.

Materials and Methods

Plant Materials

F. vesca variety Baron Solemacher (BS), a highly homogeneous inbred line, was obtained as seed from Johnny's Select Seed (Albion, Maine). *F. vesca* accession WC6 was collected from the wild in northern New Hampshire (Williamson 1993) and maintained as a runner-propagated clone. BS and WC6 were used as parents in the cross BS × WC6, which was made by brushing the stigmas of emasculated BS flowers with dehiscent WC6 anthers. F_1 plants from this cross were allowed to naturally self-pollinate in a greenhouse, and an F_2 mapping population of 80 individuals was established.

Table 1. Primers used to detect RAPD markers

Primer[a]	Sequence	No. markers detected[b]
B2	CCTGGGCTTG	3
B17	CCTGGGCCTC	3
B84	GGGCGCGAGT	4*
B85	GTGCTCGTGC	3
B88	CGGGGGATGG	3
B89	GGGGGCTTGG	2*
B102	GGTGGGGACT	7*
B103	GTGACGCCGC	2*
B104	GGGCAATGAT	5
B106	CGTCTGCCCG	4
B122	GTAGACGAGC	1
B123	GTCTTTCAGG	3
B186	GTGCGTCGCT	4
B188	GCTGGACATC	3
B190	AGAATCCGCC	2
B191	CGATGGCTTT	2
B194	AGGACGTGCC	3*
B200	TCGGGATATG	3
PA02	TGCCGAGCTG	5
PA07	GAAACGGGTG	3
PO13	GTCAGAGTCC	3
PO20	ACACACGCTG	4
PX03*	TGGCGCAGTG	2*
PX09*	GGTCTGGTTG	1*
PX18*	GACTAGGTGG	1*
PZ04*	AGGCTGTGCT	3*
PZ14*	TCGGAGGTTC	2*
PZ18*	AGGGTCTGTG	3*

[a] The B prefix indicates the primer source (initial 18 primers) as University of British Columbia Biotechnology Center (Vancouver, British Columbia); the P prefix indicates Operon Technologies (Alameda, California) as the primer source, with the second letter indicating the Operon primer set; * indicates primers selected to detect codominant RAPDs on the basis of mixed-template testing.

[b] * indicates that one of the markers is codominant.

DNA and PCR Methodology

The isolation of genomic DNA from young, unexpanded leaf tissue, the PCR reaction components, and the PCR temperature/time profile for RAPD marker amplifications were as previously described (Davis et al. 1995). For all PCR reactions, total template DNA amount and individual primer concentrations in the 25 μl reaction volumes were 100 ng and 0.4 μM, respectively.

RAPD markers were amplified using individual decamer primers of arbitrary sequence (Table 1). The selection of RAPD primers used in this study occurred in two sequential phases. In the initial phase, 18 RAPD primers were chosen on the basis that they detected numerous polymorphisms in a separate mapping study (unpublished data). In the present study we used each of these 18 primers to screen the entire mapping population.

To maximize the detection of codominant RAPD markers, we then adopted the parent-parent template mixing approach as described by Davis et al. (1995) to test 100 additional primers from Operon (Alameda, California) sets A, G, O, X, and Z (20 primers per set). The parent-parent mixed template reactions contained a 1:1 combination (50 ng of each) of BS and WC6 DNA as template. Primers that detected nonparental, heteroduplex bands in the parent-parent mixed template reactions were chosen for use in the second phase of the mapping project. Several other primers that detected numerous polymorphisms between BS and WC6 in the process of primer testing were also chosen as mapping primers.

Segregating RAPD markers were scored as dominant markers on a band presence-absence basis unless an allelic relationship between two segregating bands generated by the same primer could be established. Two such bands were considered to be alternate alleles of a codominant marker when all of the following conditions were met: (1) One of the bands was derived from BS and the other from WC6. (2) A three-class segregation pattern was evident, such that F_2 individuals had either a BS-type band, a WC6-type band, or a heterozygote banding pattern, and no "double null" individuals were observed. (3) In the heterozygote banding pattern, in addition to both parental bands, the presence of a unique marker-associated heteroduplex band could be discerned or inferred.

Parent-progeny template mixing (Davis et al. 1995) was used to help classify codominant marker F_2 segregation patterns in cases where the two allelic bands were difficult to differentiate due to their similar gel mobilities. Each parent-progeny mixed template reaction consisted of a 1:1 mixture of an individual F_2 DNA with one or the other parental (BS or WC6) DNA.

RAPD markers were named alphanumerically using a primer number (Table 1) and band letter (dominant marker) or letters (codominant marker). For instance, primer B89 was used to detect dominant marker B89C and codominant marker B89AB.

To allow detection and mapping of the strawberry alcohol dehydrogenase (ADH) gene (Wolyn and Jelenkovic 1990) as a sequence tagged site (STS), a region spanning introns II and III of the gene was amplified with forward (5′-CCAAGGTACACATTCTTTTTTTC-3′) and reverse (5′-CTCTCCACAATCCTGAATTTTAG-3′)

primers designed using the PRIMER computer program (Lincoln et al. 1991), and synthesized by Integrated DNA Technologies (Coralville, Iowa). The ADH amplification profile began with 2′ denaturation at 94°C, followed by 30 cycles of 1′ at 94°C, 2′ at 50.4°C, and 3′ at 72°C, followed by a final 7 min at 72°C. Putative ADH bands were excised from the gel using the GeneClean II kit (Bio 101, Inc., Vista, California) according to kit instructions, and samples were sequenced by the University of New Hampshire core facility using an Applied Biosystems 373A automated sequencer.

All PCR products were subjected to electrophoresis on 2% TBE gels made with a 1:1 blend of standard and low melt (NuSieve GTG) agarose. Gels were stained with ethidium bromide and photographed using Polaroid Type 52 film.

Isozymes and Morphological Markers

Methods for detection of SDH and PGI-2 isozymes were those described by Williamson et al. (1995) and Yu and Davis (1995), respectively. Phenotypic classification of F_2 plants as runnering versus nonrunnering was based on visual examination over a period of 6 months in the greenhouse followed by 6 months in the field.

Data Analysis

Segregation data were analyzed and pairwise linkage relationships determined with the aid of the LINKAGE-1 computer program (Suiter et al. 1983). MapMaker (Lander et al. 1987) version 3.0b was used to identify linkage groups by using the Group command at a minimum LOD of 3.00, to establish marker order within groups using the Compare and Ripple commands, and to define map distances using the Map command.

Results

RAPD Markers

In the initial phase of the project using 18 preselected primers, we identified and generated segregation data for 52 dominant and 5 codominant RAPD markers (Figure 1). In the second phase of the project, we selected additional RAPD primers based on the results of mixed template primer testing, as described below. Gel photographs documenting the results of the parent-parent template mixing procedure have been presented elsewhere (Davis et al. 1995).

For six of the primers subjected to mixed template testing, a nonparental band (presumed heteroduplex band) was observed in the mixed template lane. We then used each of these six primers to amplify the individual F_2 DNAs from the complete mapping population, and the anticipated six codominant RAPD markers (one per primer) were detected and scored. A total of (coincidentally) six dominant RAPDs were also detected by these six primers (Table 1). Four other primers that detected multiple polymorphisms but no heteroduplex bands were also chosen from among the 100 tested primers. The latter four primers detected 15 dominant markers, but no codominant markers, in the mapping population. During the second phase of the project, in which primers were selected on the basis of mixed template testing, we generated segregation data for 21 dominant and 6 codominant RAPD markers. In total, over the two phases of the project, 28 primers were used to detect 73 dominant and 11 codominant RAPD markers in the BS × WC6 F_2 mapping population (Table 1).

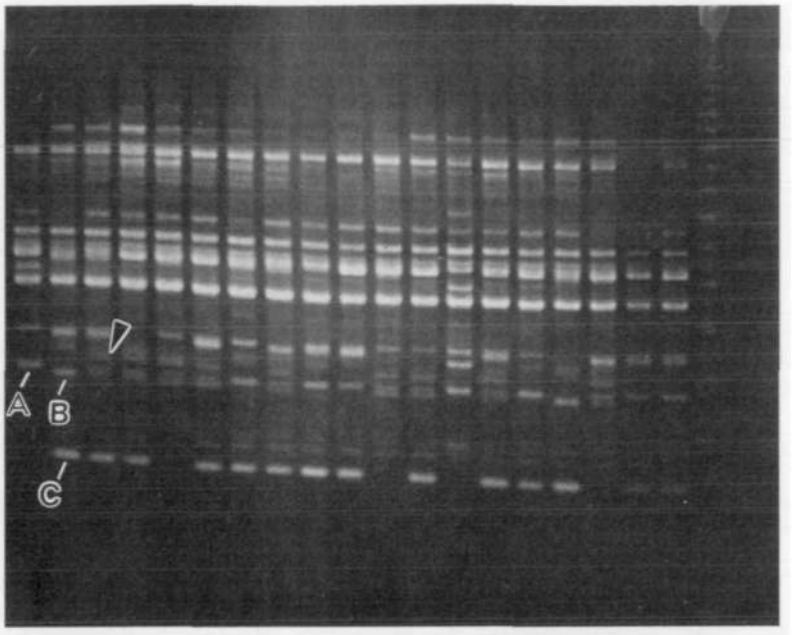

Figure 1. Dominant and codominant RAPD markers detected by primer B89. Template DNAs were (left to right) BS (lane 1), WC6 (lane 2), and individual F_2 progeny DNAs (lanes 3 through 19). Lane 20 is a 123 bp ladder (Gibco-BRL). Dominant marker B89C is indicated by the letter C. The slow and fast bands of codominant marker B89AB are indicated by the letters A and B, respectively. The heteroduplex band accompanying the parental A and B bands in B89AB heterozygotes is indicated by an arrow in lane 3.

The appearance of codominant marker banding patterns differed among markers depending on the relative mobilities of the parental and heteroduplex bands. In the most easily recognized pattern, the two parental bands and the heteroduplex band were of distinctly different mobilities, resulting in a three-banded marker pattern in heterozygotes (Figure 1, marker B89AB). With markers of this type, the three expected codominant marker banding patterns—parental (BS and WC6) and heterozygote—could be easily distinguished and classified in the F_2 mapping population (Figure 1).

In other cases alternate parental bands and/or the heteroduplex band were of similar gel mobilities, sometimes resulting in superimposition of parental and/or heteroduplex bands in heterozygotes and complicating F_2 classification. In such instances, parent-progeny template mixing (Davis et al. 1995) was useful in resolving ambiguities. For instance, a nearly indistinguishable band mobility polymorphism generated by primer B84 is apparent in the bands labeled D (slow) and E (fast) in Figure 2A. The polymorphism is confirmed and allelism indicated by the de novo generation of a heteroduplex band, migrating more slowly than either parental band, in the parent-parent mixed template reactions (indicated by arrows in Figure 2B, lane 3; Figure 2C, lane 2). This marker-associated heteroduplex band was present in 35 out of 80 F_2 individuals (Figure 2A, lanes 6 and 8), allowing these 35 individuals to be classified as heterozygotes. The F_2 single-banded individuals were then classified as either band D or band E homozygotes with the aid of parent-progeny template mixing, as illustrated in Figures 2B and C. When BS DNA was combined 1:1 with a sample of each of the parental

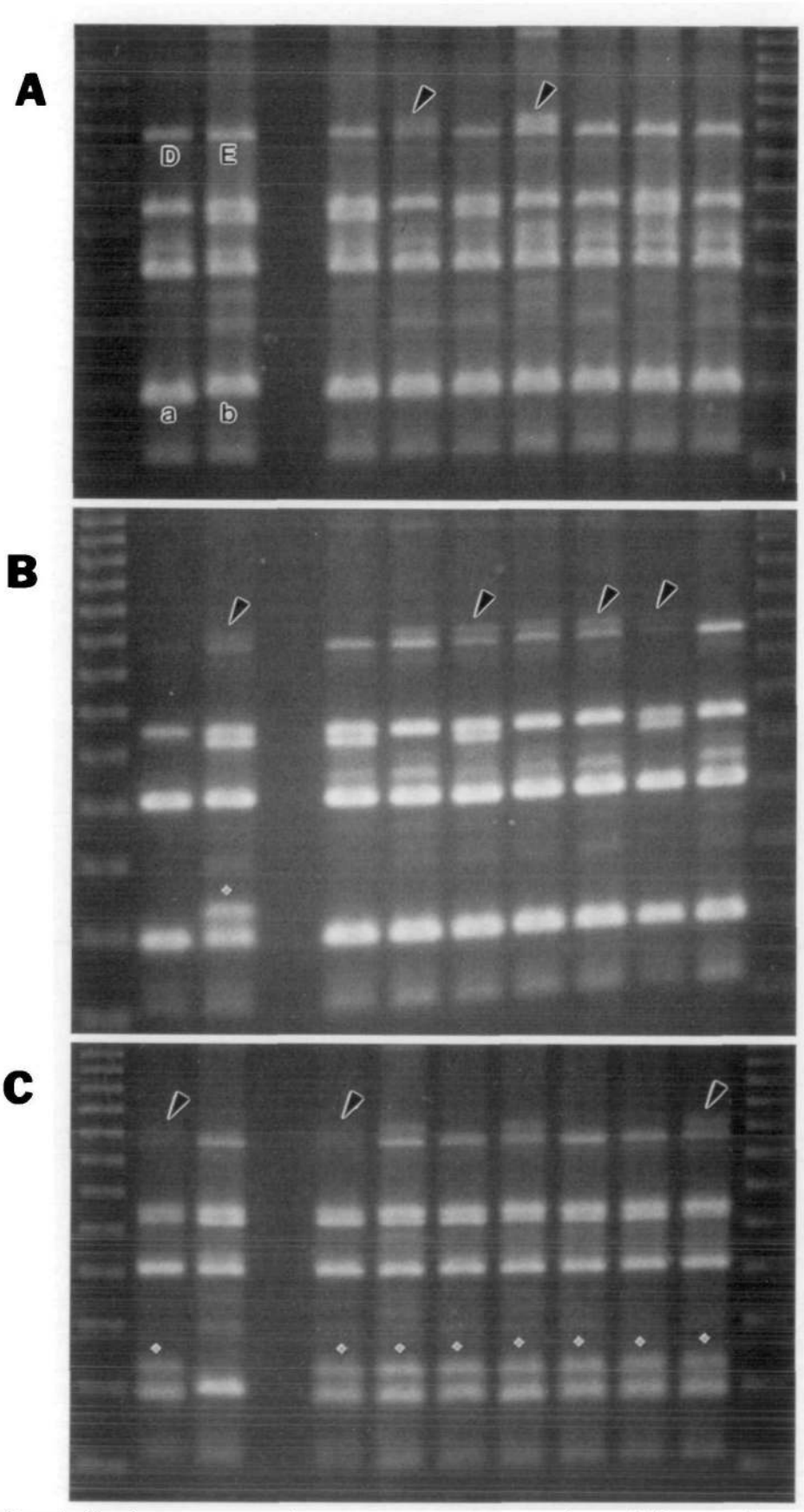

Figure 2. Use of parent-progeny template mixing to detect and interpret codominant RAPD markers. Primer BC84 was used to amplify the following template DNAs: **(A)** (left to right) BS (lane 2), WC6 (lane 3), and seven F_2 plants (lanes 5–11); **(B)** lanes same as for **(A)** but mixed 1:1 with BS DNA; **(C)** lanes same as for **(A)** but mixed 1:1 with WC6 DNA. In **(A)** the alternate bands of marker B84DE are labeled D and E, and those of marker B84ab are labeled a and b. Arrows indicate B84DE-associated heteroduplex bands in heterozygous F_2s **(A)** or generated in parents and homozygous F_2s as a consequence of template mixing **(B and C)**. Diamonds indicate marker B89ab-associated heteroduplex bands generated by template mixing in parental **(B and C)** and homozygous F_2 **(C)** lanes. Lanes 1 and 12 are a 123 bp ladder (Gibco-BRL), with the lowest two bands cropped out of the photos.

and F_2 DNAs and the resulting mixed templates were subjected to PCR with primer B84, the marker-associated heteroduplex band was generated de novo in a subset of F_2 individuals (Figure 2B, lanes with arrows). The individuals comprising this F_2 subset were classified as homozygotes for the E allele. Similarly, F_2 individuals in which a heteroduplex band was generated upon combination with WC6 DNA (Figure 2C, lanes with arrows) were classified as homozygotes for the D allele.

An unanticipated by-product of the use of parent-progeny template mixing with primer B84 was the identification of an apparently maternally inherited RAPD marker, B84ab (Figure 2). An allelic relationship between bands a (BS) and b (WC6) was indicated by the generation of a marker-associated heteroduplex band in parent-parent mixed template reactions (indicated by diamonds in Figure 2B, lane 3; Figure 2C, lane 2). No marker-associated heteroduplex bands occurred in the entire F_2 population in individual template reactions (Figure 2A) or in parent-progeny mixed template reactions with BS DNA (Figure 2B). However, in combination with WC6 DNA, all of the F_2 parent-progeny mixed template reactions generated a heteroduplex band (Figure 2C, diamonds). Thus all of the F_2 individuals carried the maternal BS allele of this marker and none carried the paternal WC6 allele.

STS, Isozyme, and Morphological Markers

The ADH primer pair generated single PCR products of 550 and 539 bp from BS and WC6 template DNAs, respectively. PCR product identities were confirmed as ADH and their lengths were determined by direct sequencing (data not shown). The BS and WC6 ADH bands A and B were of similar, but distinguishable, mobilities (Figure 3). In heterozygous F_2 individuals, the parental bands overlapped into a single band above which a heteroduplex band was present (Figure 3).

PGI-2 and SDH banding patterns were as expected from previous studies (Williamson et al. 1995; Yu and Davis 1995). Segregation was seen for the *Pgi-2*^{a} and *Pgi-2*^{c} alleles (Arulsekar and Bringhurst 1981; Yu and Davis 1995) derived from BS and WC6, respectively. The segregating SDH alleles derived from BS and WC6 corresponded to the fast and slow SDH alleles previously detected (Williamson et al. 1995) in BS and Yellow Wonder, respectively. The runnering versus nonrunnering phenotypes were classified unambiguously based on 1

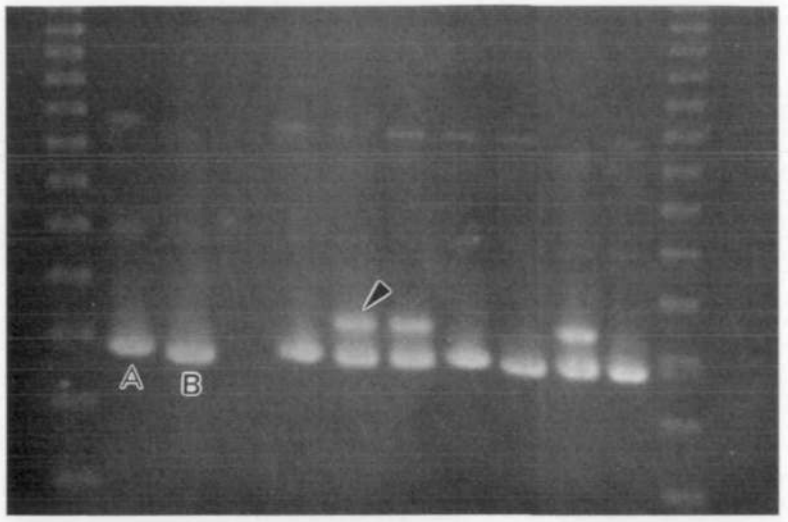

Figure 3. *Detection of a polymorphic strawberry ADH locus as a sequence tagged site.* Lanes 1 and 12 are a 123 bp ladder (Gibco-BRL) with the lower two bands cropped out of the photo. Lanes 2–11 are (left to right) BS (band A), WC6 (band B), blank lane, F_2 individuals of banding types A, AB, AB, A, B, AB, and B. Heteroduplex band (arrow) indicates marker heterozygosity.

year of observation in greenhouse and field environments.

Linkage Analysis and Map Structure

Segregation data for 84 RAPD markers, the ADH STS, the PGI-2 and SDH isozyme loci, and the *r* locus were subjected to linkage analysis. Initially the analysis was conducted separately on two marker subsets, distinguished in terms of marker linkage phase. The BS and WC6 subsets incorporated all of the dominant markers derived from the BS and WC6 parents, respectively. All of the codominant markers were included in both subsets. For each marker subset, seven linkage "subgroups" were identified (Figure 4). Each subgroup contained at least one codominant marker.

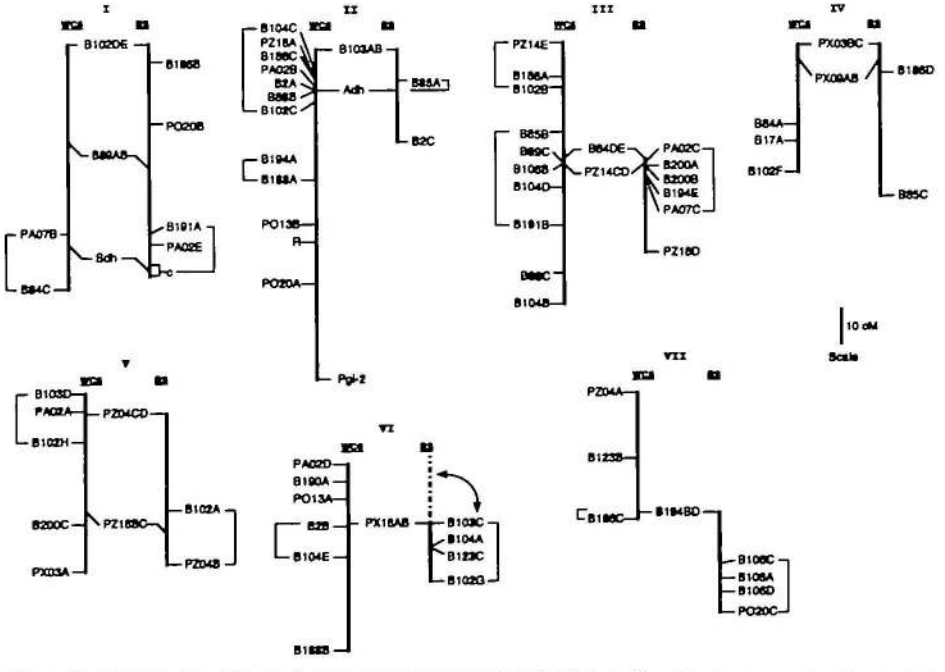

Figure 4. Linkage map of *Fragaria vesca*. Each linkage group is labeled with a Roman numeral and represented by a pair of vertical lines (linkage subgroups) incorporating dominant markers derived from WC6 (left) and BS (right). Codominant marker symbols are situated between, and connecting, linkage subgroups within each linkage group. Map regions in which the certainty of relative marker order is less than LOD 2.0 (as determined by the MapMaker "Ripple" function) are indicated by brackets. The orientation of the BS subgroup with respect to the location of codominant marker PX18AB in linkage group VI is uncertain: the alternate possible orientation is obtained by flipping the BS axis into the position indicated by the dotted line while holding constant the position of marker B103C relative to PX18AB. *R* is the dominant allele of the runnering locus (group II).

The codominant markers provided useful anchor points by which each BS linkage subgroup could be paired and cooriented with its corresponding WC6 linkage subgroup to establish a joint BS-WC6 linkage group (Figure 4). The *c* locus governing red versus yellow fruit color, although not segregating in the present mapping population, was positioned in linkage group I on the basis of its previously established 1.1 cM linkage to *Sdh* (Williamson et al. 1995).

Four RAPD markers were not associated with any linkage group at LOD 3.0. Five others were excluded from the analysis because they could not be unambiguously assigned to a single group or to a particular region within a group. Two of the excluded markers each associated with two or more otherwise discrete linkage groups. Three markers were excluded because each fit equally well as the distal marker at either end of the respective linkage group to which it was assigned. With these nine markers excluded, and the *c* locus included, the final map consisted of 80 markers, of which 14 were codominant. The linkage groups ranged in size from 43.8 cM (group IV) to 95.2 cM (group II). Total map length was 445 cM.

Individual locus segregation ratios were generally skewed (i.e., significantly different from 3:1 or 1:2:1) in linkage groups I, II, IV, V, and VI (Table 2). In every case the significant segregation bias favored the BS allele. Significantly skewed loci were excluded from the linkage analysis only if they could not be unambiguously assigned to, and localized within, a single linkage group.

Discussion

The *F. vesca* linkage map presented here is the first linkage map of a strawberry genome. The map contains 80 markers distributed over seven linkage groups, the expected number for diploid *Fragaria* ($n = x = 7$). Each of the seven linkage groups is partitioned into two subgroups according to the parental sources (BS or WC6) of dominant marker alleles (Figure 4). Corresponding subgroups could not be integrated together into single, linear groups because the relative positions of dominant markers segregating in repulsion phase could not be satisfactorily resolved from the small F_2 data set. The problem of genetic information loss associated with marker dominance in F_2 populations has been explored in detail by Allard (1956).

A distinguishing feature of our map is its

Table 2. Segregation ratios of mapped markers organized by linkage group and parental allele source

Group[a]	Marker	Observed segregation[b] ratio	Expected ratio	Marker source[c]
I	B186B	70:10*	3:1	BS
I	PO20B	71:9*	3:1	BS
I	B191A	76:2*	3:1	BS
I	PA02E	77:3*	3:1	BS
I	B102DE	20:47:13	1:2:1	BS/WC6
I	B89AB	36:40:4*	1:2:1	BS/WC6
I	*Sdh*	45:30:4*	1:2:1	BS/WC6
I	PA07B	31:49*	3:1	WC6
I	B84C	36:44*	3:1	WC6
II	B85B	65:15	3:1	BS
II	B2C	64:16	3:1	BS
II	B103AB	26:34:17	1:2:1	BS/WC6
II	*Adh*	30:38:12*	1:2:1	BS/WC6
II	*Pgi-2*	27:41:11*	1:2:1	BS/WC6
II	B104C	52:28	3:1	WC6
II	PZ18A	51:29*	3:1	WC6
II	B188C	51:29*	3:1	WC6
II	PA02B	52:28	3:1	WC6
II	B2A	49:30*	3:1	WC6
II	B88B	50:28*	3:1	WC6
II	B102C	48:32*	3:1	WC6
II	B194A	44:36*	3:1	WC6
II	B188A	44:36*	3:1	WC6
II	PO13B	44:36*	3:1	WC6
II	*R*	43:35*	3:1	WC6
II	PO20A	39:41*	3:1	WC6
III	PA02C	56:24	3:1	BS
III	B200A	56:24	3:1	BS
III	B200B	56:24	3:1	BS
III	B194E	55:25	3:1	BS
III	PA07C	54:26	3:1	BS
III	PZ18D	51:29	3:1	BS
III	B84DE	22:35:23	1:2:1	BS/WC6
III	PZ14CD	21:35.24	1:2:1	BS/WC6
III	PZ14E	22:58	3:1	WC6
III	B186A	61:19	3:1	WC6
III	B102B	63:17	3:1	WC6
III	B85B	53:26	3:1	WC6
III	B89C	59:21	3:1	WC6
III	B106B	59:21	3:1	WC6
III	B104D	55:24	3:1	WC6
III	B191B	56:22	3:1	WC6
III	B88C	64:14	3:1	WC6
III	B104B	63:17	3:1	WC6
IV	B186D	69:11*	3:1	BS
IV	B85C	66:13	3:1	BS
IV	PX03BC	31:38:11*	1:2:1	BS/WC6
IV	PX09AB	31:38:11*	1:2:1	BS/WC6
IV	B84A	53:27	3:1	WC6
IV	B17A	52:27	3:1	WC6
IV	B102F	59:21	3:1	WC6
V	B102A	62.18	3:1	BS
V	PZ04B	60:20	3:1	BS
V	PZ04CD	30:32:18*	1:2:1	BS/WC6
V	PZ18BC	22:44:14	1:2:1	BS/WC6
V	B103D	53:26	3:1	WC6
V	PA02A	49:31*	3:1	WC6
V	B102H	51:29*	3:1	WC6
V	B200C	56:24	3:1	WC6
V	PX03A	62:18*	3:1	WC6
VI	B103C	76:3*	3:1	BS
VI	B104A	74:6*	3:1	BS
VI	B123C	74:6*	3:1	BS
VI	B102G	71:9*	3:1	BS
VI	PX18AB	45:32:3*	1:2:1	BS/WC6
VI	PA02D	42:38*	3:1	WC6
VI	B190A	39:41*	3:1	WC6
VI	PO13A	37:43*	3:1	WC6
VI	B2B	34:46*	3:1	WC6
VI	B104E	31:48*	3:1	WC6
VI	B188B	42:38*	3:1	WC6
VII	B106C	63:17	3:1	BS
VII	B106A	62:18	3:1	BS
VII	B106D	61:19	3:1	BS
VII	PO20C	59:21	3:1	BS
VII	B194BD	16:47:17	1:2:1	BS/WC6
VII	PZ04A	55:25	3:1	WC6
VII	B123B	62:18	3:1	WC6
VII	B186C	66:14	3:1	WC6
U	B190B	67:13	3:1	BS
U	B17B	52:28	3:1	WC6
U	PO13C	52:28	3:1	WC6
U	PA07A	71:9*	3:1	BS

[a] Linkage group. U = unlinked.

[b] For all codominant markers, observed numbers are presented as BS type:heterozygote:WC6 type. Asterisks indicate significant chi-square ($P < .05$) as calculated using Yates correction.

[c] For dominant markers, the marker band was derived from either BS or WC6. For codominant markers, each parent contributed a marker band.

unusually high number of codominant RAPD markers. This important feature resulted from the discovery that marker-associated heteroduplex bands were a diagnostic feature of codominant RAPD markers (Davis et al. 1995). Upon reexamination of early gel photos in light of this discovery, we detected heteroduplex bands and recognized allelic relationships that had initially gone unnoticed (e.g., marker B84DE). The inclusion of parental PCR products on all gels containing F_2 PCR products aided in this comparison.

The development and use of mixed template primer testing (Davis et al. 1995) contributed significantly to the identification of codominant RAPDs in this study. Although adopted late in the study, the approach allowed us to quickly and efficiently test 100 primers and select six primers, each of which detected a codominant RAPD marker. The addition of these markers to the map resulted in the inclusion of at least one codominant marker in every linkage group and facilitated coorientation of the corresponding pairs of BS and WC6 dominant marker subgroups within each linkage group.

The parent-progeny template mixing method, first described by Davis et al. (1995), was useful in classifying codominant RAPDs (e.g., B48DE) in which the parental bands were of similar or indistinguishable mobilities on the 2% agarose gels employed in this study. We later found that polyacrylamide gels could also be used to resolve similarly migrating parental and/or marker-associated heteroduplex bands (data not shown); however, all of the segregation data used in map construction was based on marker visualization on agarose gels.

The detection of an apparent maternally inherited RAPD marker (B84ab) was an unanticipated consequence of parent-progeny template mixing. The maternal inheritance pattern exhibited by marker B84ab indicates that the B84a and B84b bands were amplified from either the mitochondrial or chloroplast genome. RAPD markers derived from organelle genomes have received little attention (Lorenz et al. 1994), in part because they are irrelevant to linkage map construction. However, their use for other purposes such as cultivar identification and phylogenetic analysis could be explored if such markers could be readily identified. Our results with marker B84ab suggest that systematic application of template mixing methods might aid in the detection and analysis of other maternally inherited RAPDs by providing a means to establish allelic relationships between polymorphic markers that do not segregate in the progeny of a cross.

Monogenic segregation ratios were significantly skewed for many markers in linkage groups I, II, IV, V, and VI (Table 2). The skewing was systematic in that alleles derived from the Alpine parent (BS) were always in excess of expectation. Yu and Davis (1995) previously reported that Alpine alleles of the *r*, *Pgi-2*, and *Sdh* loci were selectively favored in the segregating progenies of several crosses between Alpine and non-Alpine parents. However, segregations of *Sdh* and the closely linked *c* locus were normal in a cross between two Alpine varieties (Williamson et al. 1995).

In this study, which involved a cross between an Alpine (BS) and non-Alpine (WC6) parent, skewed segregation was shown to encompass entire linkage groups. We speculate that the maternal BS cytoplasm may have provided a gametic or zygotic cellular environment more conducive to transmission of certain BS as opposed to WC6 alleles. As has been pointed out by others (Guo et al. 1994), selection acting to skew the segregation of one locus will necessarily result in skewing of closely linked loci, even though the latter may be selectively neutral. Therefore the skewed segregations of entire linkage groups seen in the present study may reflect selection acting on only one or a few loci within each group. Because skewed monogenic segregations may be misinterpreted as indicating dihybrid or other forms of multigenic segregation inheri-

tance, strawberry geneticists should use caution when attempting to fit genetic models to segregation data for morphological-physiological traits in Alpine × non-Alpine crosses, and perhaps in other wide crossing combinations (Yu and Davis 1995).

The issue of whether to exclude markers that segregate abnormally from data sets used for map construction has been raised by Tulsieram et al. (1992), who excluded markers that deviated from a 1:1 ratio from their linkage analysis of white spruce (*Picea glauca*). If we had excluded markers that deviated from the 3:1 or 1:2:1 segregation ratios expected for dominant and codominant markers, respectively, in our *F. vesca* mapping population, we would have excluded 37 of the 79 mapped markers, resulting in the complete elimination of linkage group VI and most of the markers in linkage groups I and II. Most authors reporting segregation distortion in their mapping population have retained the respective markers in their mapping data sets (e.g., Bonierbale et al. 1988; Brummer et al. 1993; Kiss et al. 1993; McCouch et al. 1988). We conclude that markers exhibiting distorted monohybrid ratios should not be excluded from data sets unless such markers cannot be unambiguously mapped.

As the first map of a strawberry genome, the *F. vesca* linkage map presented here will provide a foundation for further investigation of the strawberry genome structure. Comparative mapping among species of *Fragaria* will contribute to an understanding of the germplasm base available to strawberry breeders. As a condition for comparative mapping in *Fragaria*, it will be necessary to identify markers that are conserved between different species. Our preliminary investigations suggest that RAPD markers may not be sufficiently conserved among *Fragaria* species for this purpose, but that PCR-based STS markers such as the ADH marker mapped in this study may be quite useful (Yu H and Davis TM, submitted). Accordingly, the addition of conserved molecular markers such as STS and RFLP markers to the *F. vesca* map will be an important priority. Once this goal has been achieved, it may also be feasible to compare strawberry linkage group structure to that of other Rosaceae family members now being mapped, including peach and almond (Foolad et al. 1995; Rajapakse et al. 1995; Viruel et al. 1995) and apple (Hemmat et al. 1994).

Construction of the *F. vesca* map is also an important first step toward map-based (positional) cloning of genes of economic interest in strawberry. For this purpose diploid *F. vesca* will be a much more tractable subject than will octoploid *F.* × *ananassa*. Positional cloning is feasible in *F. vesca* because of its small genome size, estimated at approximately 10^8 bp by Ahmadi et al. (1988). The mapped *c* (yellow fruit color) and *r* (nonrunnering) genes, disease resistance, flowering habit, and other genes of economic importance are potential candidates for positional cloning in *F. vesca*.

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