J. Amer. Soc. Hort. Sci. 122(1):69–73. 1997.

Randomly Amplified Polymorphic DNA- (RAPD-) based Genetic Linkage Map of Blueberry Derived from an Interspecific Cross between Diploid *Vaccinium darrowi* and Tetraploid *V. corymbosum*

Luping Qu and James F. Hancock
Department of Horticulture, Michigan State University, East Lansing, MI 48824

Additional index words. blueberry breeding, inter-ploidy crosses

Abstract. **A tetraploid blueberry population resulting from a cross of US 75 {a tetraploid hybrid of Fla 4B [a selection of *Vaccinium darrowi* Camp (2n = 2x = 24) x 'Bluecrop' [(*V. corymbosum* L. (2n = 4x = 48)]} x 'Bluetta' (4x) was used to generate a genetic linkage map of US 75 by randomly amplified polymorphic DNA (RAPD) analysis. One hundred and forty markers unique for Fla 4B that segregated 1:1 in the population were mapped into 29 linkage groups that cover a total genetic distance of 1288.2 cM, with a range of 1.6 to 33.9 cM between adjacent markers. The map is essentially of *V. darrowi* because US 75 was produced via a 2n gamete from Fla 4B and only unique markers for Fla 4B were used. Therefore, all the chromosomes of *V. darrowi* could be represented in the map.**

Among woody fruit crops only apple (*Malus pumila* Mill.) (Hemmat et al., 1994), blueberry (Rowland and Levi, 1994), cherry (*Prunus avium* L.) (Stockinger et al., 1996), citrus [*Citrus sinensis* (L.) Osbeck] (Durham et al., 1992; Jarrel et al., 1992), grape (*Vitis vinifera* L.) (Lodhi et al., 1995), and peach [*Prunus persica* (L.) Batsch] (Chaparro et al., 1994; Rajapakse et al., 1995) have been mapped using molecular markers. The reason why woody perennial crop maps have lagged behind herbaceous annual plants has generally been attributed to their long periods of juvenility and the inbreeding depression that often occurs in narrow crosses of outcrossing species (Jarrel et al., 1992; Rowland and Levi, 1994). However, marker-assisted selection should be very beneficial in saving land resources and labor in woody perennial breeding programs if potentially low-value genotypes can be eliminated in the seedling stage before field planting. Linked molecular markers should be particularly useful in transferring traits from wild to cultivated species, where early generations of crosses are horticulturally poor except for the genes of interest.

In the last 2 decades, increased attention has been paid to introducing traits from wild diploid blueberries into the polyploid *V. corymbosum* (highbush) and *V. ashei* Reade (rabbiteye) species. Several cultivars have now been released with the genes of more than one species in their background (Ballington, 1990; Draper, 1977; Draper et al., 1982; Hancock et al., 1995). One of the most widely used breeding parents has been Fla 4B (*V. darrowi*), a diploid species native to the southeastern United States. Completely fertile F_1 progeny are relatively easy to produce between *V. darrowi* and *V. corymbosum*, as *V. darrowi* produces a large number of unreduced gametes (Draper et al., 1982; Ortiz et al., 1992).

The most useful interspecific hybrid parent of *V. darrowi* x *V. corymbosum* has been US 75, a tetraploid generated by crossing Fla 4B with the highbush cultivar Bluecrop (Draper et al., 1982). US 75 has been used primarily to reduce the chilling requirement of highbush types, although it also transmits high fruit quality, tolerance to mineral soils (Chandler et al., 1985; Erb et al., 1990, 1993), and a high photosynthetic rate under hot and dry conditions (Hancock et al., 1992; Moon et al., 1987).

Our current research has focused on incorporating the fruit quality and heat tolerance of Fla 4B into highbush blueberry cultivars. A recent study by Qu and Hancock (1995) found that US 75 contains about 70% of Fla 4B's heterozygosity and its inheritance pattern is primarily tetrasomic. This would indicate that most of Fla 4B's genetic information is contained in US 75 and should readily segregate in further intercross breeding. Our previous research has shown that a small percentage of backcross progeny has improved heat tolerance (Hancock et al., 1992), but many of these progeny are poorly adapted to cold (Hancock et al., 1995). To identify recombinant progeny with high fruit quality, heat tolerance, and cold tolerance, a genetic linkage map of Fla 4B in the genetic background of US 75 would be very helpful. Herein, we report on a randomly amplified polymorphic DNA- (RAPD-) based map generated at the tetraploid level from a cross of US 75 with another commercially important highbush cultivar, Bluetta.

Materials and Methods

Plant material. US 75, 'Bluecrop', 'Bluetta', Fla 4B, and a tetraploid segregating population from US 75 x 'Bluetta' were screened for the presence of RAPD markers. The crosses were made in a greenhouse in 1988 and transplanted into a field at the Southwestern Michigan Research Experimental Center in Benton Harbor in 1990, where they were maintained under standard cultural conditions (Hanson and Hancock, 1987). 'Bluetta' is considered a highbush cultivar but is composed of 75% *V. corymbosum* and 25% *V. angustifolium* Aiton according to ancestry (Draper, 1977).

DNA extraction and amplification conditions. Total cell DNA was isolated from young leaves using a modification of the CTAB procedure (Doyle and Doyle, 1987; as modified by Rowland and Nguyen, 1993). DNA was amplified in 12.5-µL volumes using 10-base primers [Operon Technologies Inc. (OP), Alameda, Calif., and Biotechnology Laboratory, Univ. of British Columbia (BC)]. Primers were identified by the initials of their sources (OP and BC) and the company's lot number. Reaction conditions were 1 ng·µL^{-1} template DNA; buffer (50 mM KCl, 10 mM Tris-HCl pH 8.3, 0.01% gelatin), 1.6 mM $MgCl_2$; 200 µM dATP, dCTP, dGTP, and dTTP

Received for publication 13 May 1996. Accepted for publication 23 Sept. 1996. 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.

Table 1. RAPD markers not described in Qu and Hancock (1995) that fit a 1:1 (present:absent) segregation ratio in a progeny population of US 75 x 'Bluetta'.

Primer	Sequence	Fragment size (bases)	Observed ratio	χ^2	P^z
OPA16	AGCCAGCGAA	1590	28:33	0.41	0.55
OPA19	CAAACGTCGG	990	28:33	0.41	0.55
OPF04	GGTGATCAGG	850	27:34	0.80	0.42
OPF05	CCGAATTCCC	2000	28:33	0.41	0.55
OPM04	GGCGGTTGTC	980	35:26	1.33	0.26
OPM09	GTCTTGCGGA	1420	33:28	0.41	0.55
OPM13	GGTGGTCAAG	3560	36:25	1.98	0.17
OPM16	GTAACCAGCC	650	29:32	0.15	0.70
OPM17	TCAGTCCGGG	1960	23:38	3.69	0.07
OPM19	CCTTCAGGCA	530	34:27	0.80	0.42
OPM20	AGGTCTTGGG	830	33:28	0.41	0.55
OPN11	TCGCCGCAAA	680	30:30	0.00	1.00
OPN16	AAGCGACCTG	570	34:27	0.80	0.42
OPN16	AAGCGACCTG	1110	29:32	0.15	0.70
OPO02	ACGTAGCGTC	1230	27:34	0.80	0.42
OPO04	AAGTCCGCTC	1580	33:28	0.41	0.55
OPO06	CCACGGGAAG	1530	34:27	0.80	0.42
OPO07	CAGCACTGAC	1830	29:32	0.15	0.70
OPO12	CAGTGCTGTG	1200	33:28	0.41	0.55
OPO13	GTCAGAGTCC	680	30:31	0.02	0.85
OPO14	AGCATGGCTC	610	37:24	2.77	0.09
OPO16	TCGGCGGTTC	890	26:35	1.33	0.26
OPP01	GTAGCACTCC	700	33:28	0.41	0.55
OPP01	GTAGCACTCC	2090	33:28	0.41	0.55
OPP01	GTAGCACTCC	2390	30:31	0.02	0.85
OPP04	GTGTCTCAGG	700	24:37	2.77	0.09
OPP04	GTGTCTCAGG	850	30:31	0.02	0.85
OPP04	GTGTCTCAGG	1230	32:29	0.15	0.70
OPP07	GTCCATGCCA	1350	31:30	0.02	0.85
OPP16	CCAAGCTGCC	620	32:29	0.15	0.70
OPQ03	GGTCACCTCA	1000	33:28	0.41	0.55
OPQ03	GGTCACCTCA	1350	33:28	0.41	0.55
OPQ05	CCGCGTCTTG	860	32:29	0.15	0.70
OPQ09	GGCTAACCGA	2050	29:32	0.15	0.70
OPQ13	GGAGTGGACA	1470	27:34	0.80	0.42
OPR01	TGCGGGTCCT	1250	27:34	0.80	0.42
OPR09	TGAGCACGAG	1490	30:31	0.02	0.85
OPR09	TGAGCACGAG	2000	29:32	0.15	0.70
OPR11	GTAGCCGTCT	850	27:34	0.80	0.42
OPR13	GGACGACAAG	480	29:32	0.15	0.70
OPR16	CTCTGCGCGT	240	34:27	0.80	0.42
OPT12	GGGTGTGTAC	360	29:32	0.15	0.70
OPT12	GGGTGTGTAC	1650	27:34	0.80	0.42
OPU03	CTATGCCGAC	750	27:34	0.80	0.42
OPU03	CTATGCCGAC	1750	24:37	2.77	0.09
OPU07	CCTGCTCATC	1840	29:32	0.15	0.75
OPU16	CTGCGCTGGA	1350	28:33	0.41	0.55
OPV04	CCCCTCACGA	2090	29:32	0.15	0.70
OPV04	CCCCTCACGA	2210	29:32	0.15	0.70
OPV08	GGACGGCGTT	600	35:26	1.33	0.26
OPV08	GGACGGCGTT	1370	25:36	1.98	0.17
OPV14	AGATCCCGCC	470	36:25	1.98	0.17
OPV14	AGATCCCGCC	1520	27:34	0.80	0.42
OPW03	GTCCGGAGTG	310	33:28	0.41	0.55
OPW03	GTCCGGAGTG	560	28:33	0.41	0.55
OPW06	AGGCCCGATG	570	31:30	0.02	0.85
OPW06	AGGCCCGATG	860	31:30	0.02	0.85
OPW14	CTGCTGAGCA	520	26:35	1.33	0.26
OPX06	ACGCCAGAGG	1220	30:31	0.02	0.85

Table 1. Continued.

Primer	Sequence	Fragment size (bases)	Observed ratio	χ^2	P^z
OPX07	GAGCGAGGCT	1830	30:31	0.02	0.85
OPX19	TGGCAAGGCA	580	33:28	0.41	0.55
OPX19	TGGCAAGGCA	1210	27:34	0.80	0.42
OPZ01	TCTGTGCCAC	280	24:37	2.77	0.09
OPZ01	TCTGTGCCAC	2210	23:38	3.69	0.07
OPZ03	CAGCACCGCA	580	29:32	0.15	0.70
OPZ04	CAGCACCGCA	610	33:28	0.41	0.55
OPZ04	AGGCTGTGCT	980	29:32	0.15	0.70
OPZ06	GTGCCGTTCA	610	31:30	0.02	0.85
OPZ07	CCAGGAGGAC	3100	31:29	0.07	0.78
OPZ11	CTCAGTCGCA	430	37:24	2.77	0.09
OPZ15	CAGGGCTTTC	2090	30:31	0.02	0.85
OPZ16	TCCCCATCAC	890	31:30	0.02	0.85
OPZ16	TCCCCATCAC	1790	32:29	0.15	0.70
OPAG01	CTACGGCTTC	230	31:30	0.02	0.85
OPAG07	CACAGACCTG	860	31:30	0.02	0.85
OPAG16	CCTGCGACAG	910	24:37	2.77	0.09
OPAJ04	GAATGCGACC	1590	29:32	0.15	0.70
OPAJ04	GAATGCGACC	2200	31:30	0.02	0.85
OPAJ14	ACCGATGCTG	1720	30:31	0.02	0.85
OPAK05	GATGGCAGTC	1590	23:38	3.69	0.07
OPAK15	ACCTGCCGTT	1790	33:28	0.41	0.55
OPAK16	CTGCGTGCTC	380	27:34	0.80	0.42
OPAK16	CTGCGTGCTC	610	30:31	0.02	0.85
OPAM01	TCACGTACGG	1590	31:30	0.02	0.85
OPAM01	TCACGTACGG	1840	33:28	0.41	0.55

[z]The alternative hypothesis of 5:1 was rejected at $P < 0.01$ in all cases.

(Boehringer Mannheim, Indianapolis); 0.2 μM primer; and 0.06 units/μL *Taq* DNA polymerase (Gibco, Gaithersburg, Md.). DNA was amplified for 50 cycles in a Perkin Elmer thermal cycler programmed for a 30-s denaturation at 94 °C, 70-s annealing at 48 °C, and 120-s extension at 72 °C. The polymerase chain-reaction (PCR) products were separated through 1.2% agarose gels and visualized by ethidium-bromide staining. Only reproducible fragments with distinct bands were scored in our analysis. All genotypes were evaluated using PCR at least twice for each primer.

RAPD MARKER SELECTION. Only the markers unique to the Fla 4B genotype were used in this study. Interestingly, very few markers were detected that distinguished 'Bluecrop' and 'Bluetta'. Since US 75 was previously determined to have tetrasomic inheritance (Qu and Hancock, 1995), there were two segregation ratios expected in the tetraploid progeny, one for duplex loci [5:1 (present/absent)], and one for simplex loci (1:1). Only the 1:1 segregation RAPD markers ($P < 0.05$) were used to create the map. We will hereafter refer to these markers as single-dose amplified fragments (SDAFs), since they are analogous to the single-dose restriction fragment (SDRF) named by Wu et al. (1992) for restriction fragment-length polymorphism (RFLP) markers used in mapping polyploids. The SDAF were distinguished from the double-dose (duplex loci) amplified fragments according to their different segregation ratios. Sixty-one progeny were evaluated for their RAPD patterns in the US 75 x 'Bluetta' population. According to Mather (1951), population sizes of at least 38 are necessary [$(1 - \alpha_1 - \alpha_2)100\%$] to distinguish between 1:1 and 5:1 ratios.

LINKAGE ANALYSIS. Multimarker linkage analysis was performed using the computer program MAPMAKER (Lander et al., 1987), evaluating the data type as an F_2 backcross population. As previously mentioned, US 75 was assumed to be an autotetraploid with random pairing among homologous chromosomes (Qu and Hancock, 1995). The linkage groups were established with a minimum LOD of 3.0 and $\Theta = 0.30$ (Kosambi function).

Results and Discussion

SEGREGATING MARKERS. Five-hundred and twelve primers were screened. Of the primers tested, 143 amplified 267 polymorphic fragments that were present in Fla 4B and absent in 'Bluecrop' and 'Bluetta'. Of these, 234 (88%) were present in US 75 and 33 (12.4%) were absent. Among the markers found in US 75, 154 were SDAFs and best fitted a 1:1 ratio at $P < 0.05$ in the US 75 x 'Bluetta' population (Table 1; Qu and Hancock, 1995), while 65 markers best fitted a 5:1 ratio ($P < 0.05$). Representative gel pictures can be found in Qu and Hancock (1995). Only 15 markers displayed distorted segregation ratios that did not fit either 1:1, 3:1, or 5:1 ratios. All of these were closest to a 2:1 ratio but were not linked together. The high proportion of 5:1 ratio markers (65) found in this study further confirms that US 75 acts primarily as an autopolyploid with random pairing of homologous chromosomes (Qu and Hancock, 1995). In another tetraploid hybrid of *V. darrowi* x *V. corymbosum*, Vorsa and Novy (1995) found that only 7 of 14 RAPD markers fit tetrasomic ratios, and they suggested that the genomes of *V. darrowi* and *V. corymbosum* were divergent from each other with preferential pairing within genomes. It is not known why these results are so disparate, since our hybrids were generated from the same diploid and tetraploid parents.

LINKAGE ANALYSIS. The MAPMAKER program assigned 140 of the 154 markers to 29 linkage groups, leaving 14 markers unlinked

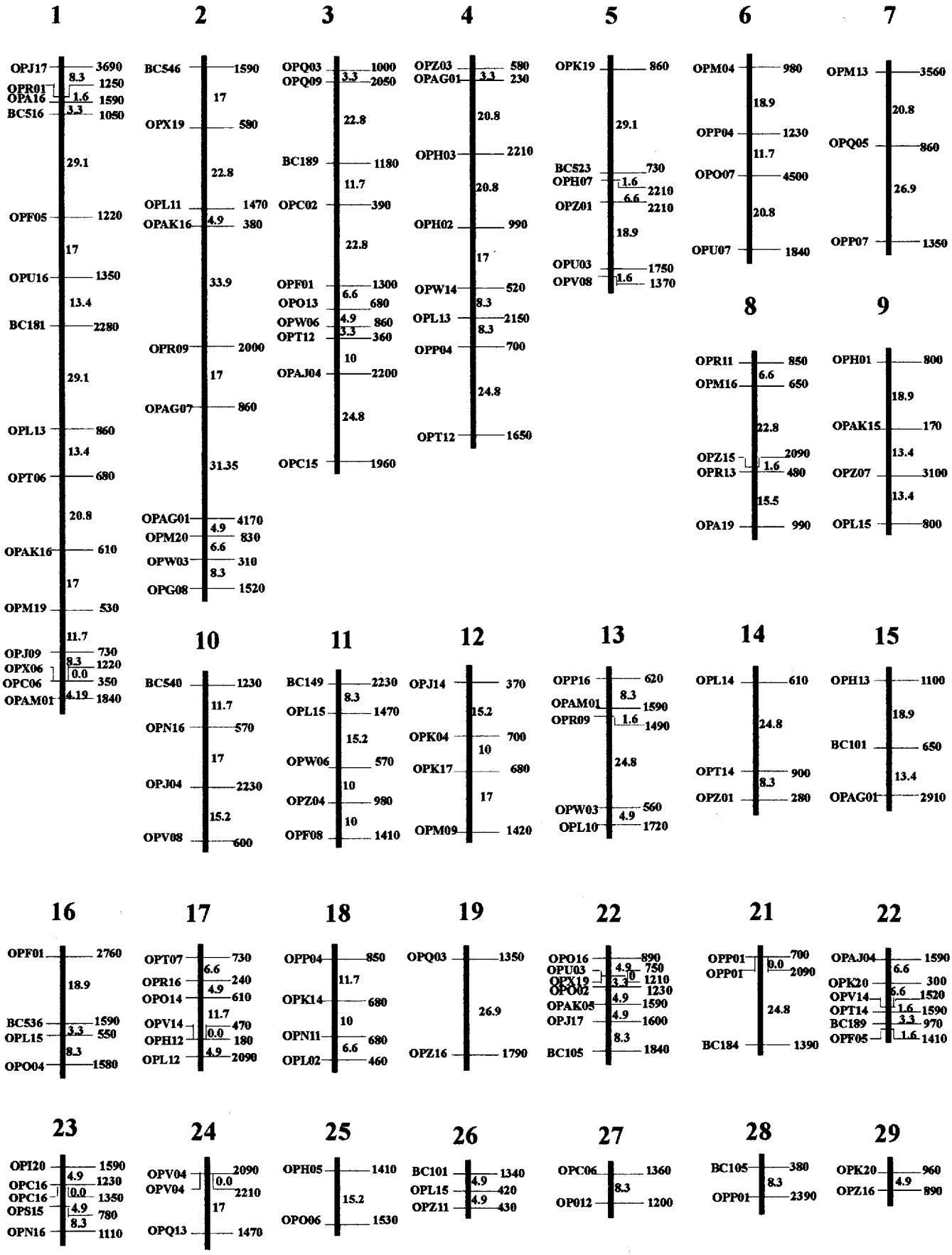
1
OPJ17 3690
8.3
OPR01 1250
OPA16 1.6 1590
BC516 3.3 1080
29.1
OPF05 1220
17
OPU16 1350
13.4
BC181 2280
29.1
OPL13 860
13.4
OPT06 680
20.8
OPAK16 610
17
OPM19 530
11.7
OPJ09 730
8.3
OPX06 1220
0.0
OPC06 350
OPAM01 4.19 1840
2
BC546 1590
17
OPX19 580
22.8
OPL11 1470
OPAK16 4.9 380
33.9
OPR09 2000
17
OPAG07 860
31.35
OPAG01 4170
OPM20 4.9 830
OPW03 6.6 310
OPG08 8.3 1520
3
OPQ03 1000
OPQ09 3.3 2050
22.8
BC189 1180
11.7
OPC02 390
22.8
OPF01 1300
OPO13 6.6 680
OPW06 4.9 860
OPT12 3.3 360
10
OPAJ04 2200
24.8
OPC15 1960
4
OPZ03 580
OPAG01 3.3 230
20.8
OPH03 2210
20.8
OPH02 990
17
OPW14 520
8.3
OPL13 2150
8.3
OPP04 700
24.8
OPT12 1650
5
OPK19 860
29.1
BC523 730
OPH07 1.6 2210
OPZ01 6.6 2210
18.9
OPU03 1750
OPV08 1.6 1370
6
OPM04 980
18.9
OPP04 1230
11.7
OPO07 4500
20.8
OPU07 1840
7
OPM13 3560
20.8
OPQ05 860
26.9
OPP07 1350
8
OPR11 850
6.6
OPM16 650
22.8
OPZ15 2090
OPR13 1.6 480
15.5
OPA19 990
9
OPH01 800
18.9
OPAK15 170
13.4
OPZ07 3100
13.4
OPL15 800
10
BC540 1230
11.7
OPN16 570
17
OPJ04 2230
15.2
OPV08 600
11
BC149 2230
8.3
OPL15 1470
15.2
OPW06 570
10
OPZ04 980
10
OPF08 1410
12
OPJ14 370
15.2
OPK04 700
10
OPK17 680
17
OPM09 1420
13
OPP16 620
8.3
OPAM01 1590
OPR09 1.6 1490
24.8
OPW03 560
OPL10 4.9 1720
14
OPL14 610
24.8
OPT14 900
8.3
OPZ01 280
15
OPH13 1100
18.9
BC101 650
13.4
OPAG01 2910
16
OPF01 2760
18.9
BC536 1590
OPL15 3.3 550
8.3
OPO04 1580
17
OPT07 730
6.6
OPR16 240
OPO14 4.9 610
11.7
OPV14 470
OPH12 0.0 180
OPL12 4.9 2090
18
OPP04 850
11.7
OPK14 680
10
OPN11 680
OPL02 6.6 460
19
OPQ03 1350
26.9
OPZ16 1790
22
OPO16 890
OPU03 4.9 750
OPX19 0 1210
OPO02 3.3 1230
OPAK05 4.9 1590
OPJ17 4.9 1600
8.3
BC105 1840
21
OPP01 700
OPP01 0.0 2090
24.8
BC184 1390
22
OPAJ04 1590
6.6
OPK20 300
OPV14 6.6 1520
OPT14 1.6 1590
BC189 3.3 970
OPF05 1.6 1410
23
OPI20 1590
OPC16 4.9 1230
OPC16 0.0 1350
OPS15 4.9 780
OPN16 8.3 1110
24
OPV04 2090
OPV04 0.0 2210
17
OPQ13 1470
25
OPH05 1410
15.2
OPO06 1530
26
BC101 1340
OPL15 4.9 420
OPZ11 4.9 430
27
OPC06 1360
8.3
OPO12 1200
28
BC105 380
8.3
OPP01 2390
29
OPK20 960
OPZ16 4.9 890

Fig. 1. Randomly amplified polymorphic DNA- (RAPD-) based genetic linkage map of blueberry derived from a cross of US 75 (*V. darrowi* x *V. corymbosum*) x 'Bluetta' (*V. corymbosum* x *V. angustifolium*). Linkage groups are numbered from longest to shortest. Marker names are shown to the left of each linkage group and the base pair number of the analyzed fragments is shown on the right. Distances between adjacent markers (in cM) are indicated between the brackets. The unlinked markers and sizes were BC244-550, BC523-1720, OPC06-290, OPF04-850, OPF12-1230, OPJ04-2700, OPJ14-1020, OPK19-640, OPM17-1960, OPQ04-5000, OPS13-860, OPX07-1830, OPZ04-610, and OPZ06-610.

(Fig. 1). The number of markers assigned to individual groups ranged from 2 to 15. The total length for the map is 1288.2 cM, with the linkage groups ranging in length from 4.9 to 178.1 cM. The distance between adjacent markers ranged from 1.6 to 33.9 cM, with an average distance of 9.2 cM. In six cases, two markers were mapped to the same position. Of these, three primers (OPC16, OPP01, and OPV04) amplified both markers together, while the other three pairs were amplified by two different primers (OPH12 and OPV14, OPU03 and OPX19, and OPC06 and OPX06).

While this map was generated using US 75, it probably represents a linkage map of *V. darrowi,* because US 75 was produced via a 2n gamete from Fla 4B, and only unique markers for Fla 4B were used in this study. The actual map distances depend on whether crossing over is equal between DD (*D* = *V. darrowi*) vs. DC (*C* = *V. corymbosum*) pairings; however, the gene orders should be the same in our map and in one generated from a cross of Fla 4B with another diploid or from an autopolyploid of Fla 4B. We detected five more linkage groups than the diploid number of *V. darrowi* (24). Since eleven small linkage groups were identified with only two or three markers, the evaluation of more markers may reduce the total number of linkage groups observed. It is also possible that some of the extra linkage groups represent the chromosomal pairing abnormalities suggested by Vorsa and Novy (1995).

The previous RAPD-based map generated for Fla 4B was done at the diploid level (Rowland and Levi, 1994) and, as a result, cannot be used to identify directly linkages with tetraploid traits. However, these associations can be located using our map of US 75, since this interspecific hybrid undergoes tetrasomic inheritance. This may make it possible to use marker-assisted selection to identify blueberry segregants with high heat tolerance and high fruit quality that also have sufficient cold tolerance and a sufficient chilling requirement for northern climates. Since US 75 carries most of Fla 4B's alleles, it should prove to be a very useful parent in transferring traits from Fla 4B into the highbush background. The simplex RAPD dominant markers can be used to facilitate this process if they are tightly linked to traits of interest. A similar approach to tagging agronomically important traits can probably be used in all polyploid crop breeding programs using unreduced gametes.

Literature Cited

Ballington, J.M. 1990. Germplasm resources available to meet future needs for blueberry cultivar improvement. Fruit Var. J. 44:54–63.

Chandler, C.K., A.D. Draper, G.J. Galletta, and J.C. Bouwkamp. 1985. Combining ability of blueberry interspecific hybrids for growth on upland soils. HortScience 20:257–258.

Chaparro, J.S., P.J. Werner, D. O'Malley, and R.R. Sederoff. 1994. Targeted mapping and linkage analysis of morphological, isozyme and RFLP markers in peach. Theor. Appl. Genet. 87:805–815.

Doyle, J.J. and J.L. Doyle. 1987. A rapid DNA isolation procedure for small quantities of fresh leaf material. Phytochem. Bul. 19:11–15.

Draper, A.D. 1977. Tetraploid hybrids from crosses of diploid, tetraploid and hexaploid *Vaccinium* species. Acta Hort. 61:33–37.

Draper, A.D., G.J. Galletta, and J.R. Ballington. 1982. Breeding methods for improving southern tetraploid blueberries. J. Amer. Soc. Hort. Sci. 96:791–792.

Durham, R.E., P.C. Liou, F.G. Gmitter Jr., and G.A. Moore. 1992. Linkage of restriction fragment length polymorphisms and isozymes in *Citrus*. Theor. Appl. Genet. 84:39–48.

Erb, W.A., A.D. Draper, G.J. Galletta, and H.J. Swartz. 1990. Combining ability for plant and fruit traits of interspecific blueberry progenies on mineral soil. J. Amer. Soc. Hort. Sci. 115:1025–1028.

Erb, W.A., A.D. Draper, and H.J. Swartz. 1993. Relation between moisture stress and mineral soil tolerance in blueberries. J. Amer. Soc. Hort. Sci. 118:130–134.

Hancock, J.F., A. Erb, B. Goulart, and J. Scheerens. 1995. Utilization of wild germplasm in blueberry breeding: The legacy of Arlen Draper. J. Small Fruit and Viticult. 3:1–16.

Hancock, J.F., K. Haghighi, S.L. Krebs, J.A. Flore, and A.D. Draper. 1992. Photosynthetic heat stability in highbush blueberries and the possibility of genetic improvement. HortScience 27:1111–1113.

Hanson, E. and J.F. Hancock. 1987. Hints on growing blueberries. Ext. Bul. E-2066, Michigan State Univ., East Lansing.

Hemmat, M., N.F. Weeden, A.G. Manganaris, and D.M. Lawson. 1994. Molecular marker linkage map for apple. J. Hered. 85:4–11.

Jarrell, D.C., M.L. Roose, S.N. Traugh, and R.S. Kupper. 1992. A genetic map of citrus based on the segregation of isozymes and RFLPs in an intergeneric cross. Theor. Appl. Genet. 84:49–56.

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

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.

Mather, K. 1951. The measurement of linkage in heredity. Methuen, London.

Moon, J.W., J.F. Hancock, A.D. Draper, and J.A. Flore. 1987. Genotypic differences in the effect of temperature on CO_2 assimilation and water use efficiency in blueberry. J. Amer. Soc. Hort. Sci. 112:170–173 .

Ortiz, R., N. Vorza, L.P. Bruederle, and T. Laverty. 1992. Occurrence of unreduced pollen in diploid blueberry species, *Vaccinium* Sect. *Cyanococcus*. Theor. Appl. Genet. 85:55–60.

Qu, L.P. and J.F. Hancock. 1995. Nature of 2n gamete formation and mode of inheritance in interspecific hybrids of diploid *Vaccinium darrowi* and tetraploid *V. corymbosum*. Theor. Appl. Genet. 91:1309–1315.

Rajapakse, S., L.E. Belthoff, A.E. Hegestanger, R. Scorza, I. Verde, R.E. Ballard, W.V. Baird, A. Challahan, R. Monet, and A.G. Abbott. 1995. Genetic linkage mapping in peach using morphological, RFLP and RAPD markers. Theor. Appl. Genet. 90:503–515.

Rowland, L.J. and A. Levi. 1994. RAPD-based genetic linkage map of blueberry derived from a cross between diploid species (*Vaccinium darrowi* and *V. elliottii*). Theor. Appl. Genet. 87:863–868.

Rowland, L.J. and B. Nguyen. 1993. Use of PEG for purification of DNA from leaf tissue of woody plants. Biotechniques 14:735–736.

Stockinger, E.J., C.A. Mulinix, C.M. Long, T.S. Brettin, and A.F. Iezzoni. 1996. A linkage map of sweet cherry based on RAPD analysis of a microspore-derived callus culture population. J. Hered. 87:214–218.

Vorsa, N. and R. Novy. 1995. Meiotic behavior and RAPD segregation in a tetraploid hybrid (*V. darrowi* x *V. corymbosum*) suggests genomic divergence in blueberry. HortScience 30:808 (Abstr.)

Wu, K.K., M.E. Burnquist, M.E. Sorrells, T.L. Tew, P.H. Moore, and S.D. Tanksley. 1992. The detection and estimation of linkage in polyploids using single-dose restriction fragments. Theor. Appl. Genet. 83:294–300.