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Nature of 2n gamete formation and mode of inheritance in interspecific hybrids of diploid *Vaccinium darrowi* and tetraploid *V. corymbosum*

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Abstract RAPD markers were used to determine the level of heterozygosity transmitted via 2n gametes from *V. darrowi* selection Florida 4B (Fla 4B) to inter-specific hybrids with tetraploid *V. corymbosum* cv Bluecrop. The tetraploid hybrid US 75 was found to contain about 70% of Fla 4B's heterozygosity, a value attributed to a first-division restitution (FDR) mode of 2n gamete production. Crossovers during 2n gamete formation were evidenced by the absence of 16 dominant alleles of Fla 4B in US 75, and direct tests of segregation in a diploid population involving Fla 4B. RAPD markers that were present in both Fla 4B and US 75 were used to determine the mode of inheritance in a segregating population of US 75×*V. corymbosum* cv Bluetta. Thirty-one duplex loci were identified which segregated in a 5:1 ratio, indicating that US 75 undergoes tetrasomic inheritance.

Key words First-division restitution · Tetrasomic inheritance · Polyploidy · RAPD markers

Introduction

Polyploidy has played an important role in both plant evolution and crop breeding. It is estimated that 30–50% of all angiosperms and more than 70% of crop species are polyploid (Grant 1971; Hancock 1992). Breeding via 2n gametes has become an important aspect of many plant improvement programs, presumably due to the maintenance and transfer of higher levels of heterozygosity (McCoy 1982; Ballington 1990; Parrott et al. 1986; Ortiz and Peloquin 1991). Most polyploids have originated from sexual reproduction involving unreduced gametes (Harlan and De Wet 1975). Unreduced gametes are formed in two primary ways: (1) an incomplete first meiotic division (first-division restitution; FDR), and (2) an incomplete second meiotic division (second-division restitution; SDR) (Mendiburu and Peloquin 1971; Mok and Peloquin 1975; McCoy 1982; Hermson 1984). Other methods of 2n gamete production include pre-meiotic chromosome doubling, chromosome replication during meiotic interphase, post-meiotic chromosome doubling, and apospory (Vorsa and Ortiz 1992). Unreduced gametes via FDR are comprised mainly of the non-sister chromatids of each homologous pair of chromosomes, whereas in SDR the sister chromatids are included in the same gametes. As a result, 2n gametes formed by FDR transmit more of the parental heterozygosity into F_1 progenies than those formed by SDR.

While few direct measurements have been made on levels of heterozygosity transmitted by SDR and FDR, theoretical calculations have been carried out. If it is assumed that there is a regular distribution of the parental heterozygous loci along chromosomes and a single crossover per pair of homologous chromosomes, then in the potato FDR has been estimated to transmit approximately 80% of the parental heterozygosity to progeny, while SDR passes on about 40% (Hermson 1984). The rate at which transmitted heterozygosity ultimately assorts in polyploids is dependent on the mode of inheritance (Hancock 1992). Segregation can be quite limited in allopolyploids with disomic inheritance, due to the maintenance of "fixed heterozygosities" on non-pairing homologous chromosomes. In autotetraploids, tetrasomic inheritance allows the variability contained in the original progenitors to segregate, but at a much slower rate than in diploids.

More and more recent attention has been paid to introducing traits from diploid to polyploid blueberry cultivars via unreduced gametes (Draper 1977; Draper et al. 1982; Ballington 1990; Ortiz et al. 1992). Several cultivars have been released with the genes of multiple species in their background (Ballington 1990; Hancock et al. 1995). Particular emphasis has been placed on introducing some of the elite traits of diploid *V. darrowi* (high fruit quality, low chilling requirement, heat tolerance, high photosynthesis rate, drought resistance) into cultivars of tetraploid high-

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bush blueberry, *V. corymbosum*. Completely interfertile F_1s are relatively easy to produce between these species because some *V. darrowi* plants produce a high number of unreduced gametes. Ortiz et al. (1992) demonstrated that about 83% of the various diploid populations of blueberries contain unreduced pollen producers and the highest producer was a genotype of *V. darrowi* with a rate of almost 20%.

Most of our research has focused upon US 75, a tetraploid hybrid (from Arlen Draper) generated by crossing a selection of *V. darrowi* Florida 4B (Fla 4B), with a highbush cultivar, Bluecrop (Draper 1977). This particular hybrid is completely interfertile with highbush types (Draper et al. 1982) and has been used in crosses because of its high photosynthetic rate under hot and dry conditions (Hancock et al. 1992). However, little is known about the mode of 2n gamete formation in *V. darrowi* or the inheritance patterns of US 75. This information would be very useful in designing efficient breeding strategies. The type of 2n gamete formation will determine the levels of heterozygosity transmitted to US 75, and the mode of inheritance will determine how readily that heterozygosity will segregate.

The cytology of 2n pollen formation has not been reported for *V. darrowi* but, in inter-specific aneuploids (2n=4x+9=57) of *V. ashei*×*V. corymbosum* Vorsa and Ortiz (1992) found the mode of 2n pollen formation to involve three steps: desynapsis, disjunction of sister chromatids, and cytokinesis. This mechanism is genetically equivalent to FDR, and therefore should result in the transmission of most of the parental heterozygosity (Vorsa and Ortiz 1992). *V. corymbosum* has been shown to have tetrasomic inheritance at four enzyme loci (Krebs and Hancock 1990), but segregation patterns have not been examined in any of the inter-specific hybrids.

To determine the level of heterozygosity transmitted by 2n gametes of Fla 4B and the mode of inheritance in US 75, we selected genotype-specific RAPD markers and followed their inheritance from Fla 4B to US 75 and their segregation patterns in a US 75×highbush cv Bluetta backcross population. The RAPD analysis allowed us to obtain and utilize a high number of markers in a short time span (Welsh and McClelland 1990; Williams et al. 1990). Very few morphological (Draper and Scott 1971), isozyme (Breuderle et al. 1991; Van Heemstra et al. 1991; Bruederle and Vorsa 1994), or RFLP markers (Haghighi and Hancock 1992) have been described in blueberry and nonhomologous chromosomes are not easy to distinguish (Hall and Galletta 1971). RAPD markers have previously been utilized in blueberries to distinguish cultivars (Aruna et al. 1993) and to develop a diploid linkage map (Rowland and Levi 1994).

Materials and methods

Plant material

US 75, 'Bluecrop', 'Bluetta', Fla 4B, NC84 6-5, and two segregating populations were evaluated for their RAPD markers: (1) a tetraploid population of 61 individuals of US 75×'Bluetta', and (2) 15 diploid individuals of Fla 4B×NC84 6-5. 'Bluetta' is a highbush cultivar and based on its pedigree is composed of 75% *V. corymbosum* and 25% *V angustifolium* (Draper et al. 1969). NC84 6-5 is a wild selection of *V. darrowi* kindly provided by J. Ballington.

DNA extractions 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 ten-base primers (Operon Technologies Inc., Alameda, Calif., and Biotechnology Laboratory, University of British Columbia). Primers were named by the initials of their source (OP and BC) and the company's lot number. Reaction conditions were: 1 ng/µl 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, dTTP (Boehringer Mannheim), 0.2 µM primer, and 0.06 units/µl *Taq* DNA polymerase (Gibco). 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 PCR products were separated through 1.2% agarose gels and visualized by ethidium-bromide staining. Only reproducible fragments with strong bands were scored in our comparisons. All genotypes were subject to PCR at least twice.

Determining the mode of inheritance

To determine the mode of inheritance in US 75, we first located RAPD markers that were present in both Fla 4B and US 75, but absent in 'Bluecrop' and 'Bluetta'. This would mean that the genotype of Fla 4B was either AA or Aa, 'Bluecrop' and 'Bluetta' were aaaa (nulliplex), and US 75 was either AAaa (duplex) or Aaaa (simplex). We then examined the progeny ratios in the testcross population of US 75×'Bluetta'. Heterozygous pairs of alleles (Aa) transferred from Fla 4B to US 75 should segregate at a ratio of 1:1 (Aaaa:aaaa) for both disomic and tetrasomic inheritance, while homozygous pairs of alleles (AA) should segregate at a 5:1 ratio (Aaaa and AAaa:aaaa) for tetrasomic inheritance and a 1:0 ratio (all Aaaa) for disomic inheritance (Krebs and Hancock 1992). For each segregating marker, a chi-square test of the fit of progeny ratios was performed. We did not need to test for a 3:1 ratio as in Krebs and Hancock (1992), because we knew both dominant alleles in US 75 were contributed by Fla 4B, and as a result could not have been segregating as independent disomic loci.

Estimating heterozygosity transmitted and the mode of 2n gamete production

To estimate the levels of heterozygosity maintained by 2n gamete formation in Fla 4B, we determined both the number of heterozygous allelic pairs (Aa) transferred from Fla 4B to US 75 and the number that was lost. As previously stated, FDR passes on much more heterozygosity than SDR, because FDR gametes contain the non-sister chromatids of each homologous pair of chromosomes, while SDR gametes contain only sister chromatids. The markers segregating (1:1) in the US 75×'Bluetta' population represented the heterozygous allelic pairs that were transferred from Fla 4B to US 75.

To measure the level of heterozygosity lost through crossing over during 2n gamete production, we counted the number of unique dominant markers present in Fla 4B that were absent in US 75. The most likely way that dominant alleles can be present in Fla 4B but absent in US 75 is if a heterozygous locus was lost due to crossing over during 2n gamete production. This number was then doubled to include recessive alleles in the estimate of lost heterozygous loci. It was assumed that equal numbers of dominant and recessive alleles would be lost via crossing over during 2n gamete production.

In a few instances, we were able to directly document the loss of heterozygous loci from Fla 4B. We screened the diploid population

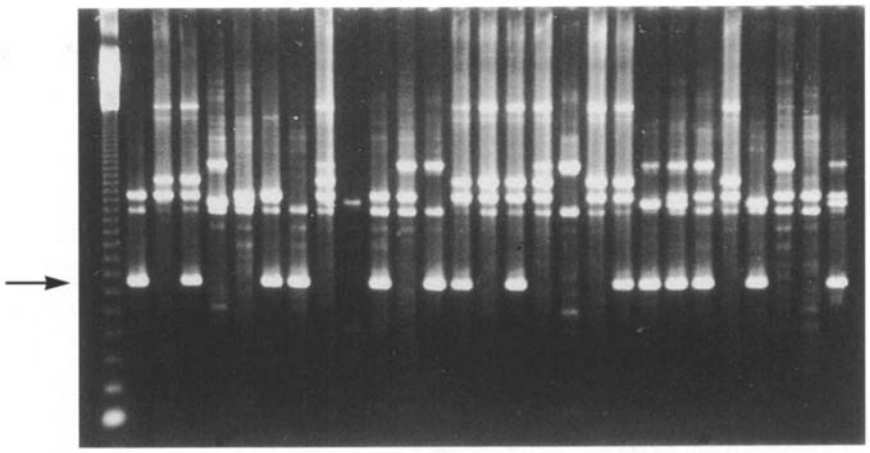

Fig. 1 DNA amplification profiles in the tetraploid progeny population US 75×Bluetta using primer OPS13. The *arrow* shows a RAPD marker segregating in a 1:1 ratio (presence:absence). From left to right, *first lane* is a 123-bp DNA ladder, *lanes 2–4* are Fla 4B, 'Bluecrop', US 75, and 'Bluetta', respectively. The *remaining lanes* are from 23 progeny individuals

of Fla 4B×NC84 6-5 with the primers producing markers that were present in Fla 4B and identified as duplex (AAaa) in US 75. We looked for markers which segregated 1:1 in the progeny when the marker was absent in NC84 6-5, and those which segregated 3:1 when the marker was present in NC84 6-5. These segregation ratios would only be possible if Fla 4B was heterozygous for that marker. Unfortunately, this approach was limited because most of the markers in Fla 4B and NC84 6-5 were shared.

The percent heterozygosity transferred from Fla 4B to US 75 was ultimately calculated as the number of heterozygous Fla 4B allelic pairs transferred to US 75 divided by the total number of heterozygous loci detected in Fla 4B. The total number of heterozygous loci in Fla 4B was calculated as the number of heterozygous allelic pairs transferred to US 75 and the number lost.

Results and discussion

Mode of inheritance

Sixty-two of two-hundred primers produced a total of 125 polymorphic fragments that were present in Fla 4B and absent in 'Bluetta' and 'Bluecrop'. Of these, 109 (87.2%) were present in US 75, and 16 (12.8%) were absent. All the markers were separated by at least 3 cM (Qu and Hancock, in preparation). Of the markers found in US 75, 70 best fit a 1:1 ratio in the US 75x'Bluetta' population (Fig. 1, Table 1), while 31 markers best fit a 5:1 ratio (Fig. 2, Table 2) and eight markers displayed distorted segregation ratios (Table 3). For all these loci, the alternate hypothesis of 1:1 or 5:1 segregation was statistically rejected ($P<0.001$).

While the 1:1 ratios can not be used to distinguish between disomic and tetrasomic inheritance, the 5:1 segregation ratios suggest that the mode of inheritance in US 75 is tetrasomic. This is not surprising, as artificially produced interspecific hybrids between a wide range of *Vaccinium* species are highly fertile, indicating there is little genomic divergence within the genus (Draper 1977; Hancock et al. 1995). Likewise, many different types of hybrids have been observed in nature (Vander Kloet 1988; Breuderle and Vorsa 1994), and several studies have supported tetrasomic inheritance in both *V. corymbosum* (Draper and Scott 1971; Krebs and Hancock 1992) and *V. angustifolium* (Hokanson and Hancock 1993).

Eight markers displayed distorted segregation ratios in the tetraploid progeny population US 75×'Bluetta'. Five of these (BC181, OPH03, BC239, OPJ14 and OPT16) segregated but did not fit either a 1:1 or 5:1 ratio (Table 3). These markers may represent independent heterozygous loci in Fla 4B that were subject to segregation distortion and may signal incomplete homology between some of the chromosomes of *V. darrowi* and *V. corymbosum*. The remaining three markers (BC101, OPI09, OPS17) were found in US 75, but were absent in the progeny population. The loss of these markers may have been due to mutations in heterozygous rather than homozygous loci, as only single allelic alterations would be necessary to eliminate markers. It is unclear why so many mutations were observed, unless a portion of a chromosome was lost.

Levels of heterozygosity transmitted and the mode of 2n gamete production

To calculate the level of heterozygosity transferred from Fla 4B to US 75 via unreduced gamete production, we determined the number of heterozygous loci that were transferred to US 75, plus the number that were lost via 2n gamete production. The number of Fla 4B markers segregating as simplex loci in the tetraploid population was 70, i.e., those markers that segregated in a 1:1 ratio. The number of unique markers in Fla 4B that were not present in US 75 was 16 (Table 4). Therefore, 32 heterozygous allelic pairs were lost during 2n gamete production in Fla 4B, if we assume that both dominant and recessive alleles were lost at the same rate. This means that 68.6% of Fla 4B's heterozygosity was transferred to US 75 via 2n gamete production [70/(32+70)]. If we also include the markers which gave unusual segregation ratios, the level of heterozygosity transmitted was 70.9% [(78/(32+78)]. Since these values are closer to the theoretical rate of heterozygosity transfer in FDR (80%) than SDR (40%), it is likely that unreduced gametes are being formed in Fla 4B via FDR or a similar mechanism.

Table 1 RAPD markers that fit a 1:1 (present:absent) segregation ratio in a progeny population of US 75×'Bluetta' [a]

Primer	Primer sequence	Fragment size (bases)	Observed ratio	χ^2	P
BC101	GCGGCTGGAG	600	28 : 33	0.41	0.55
		1340	34 : 27	0.80	0.42
BC105	CTCGGGTGGG	1840	24 : 37	2.77	0.09
BC125	GCGGTTGAGG	380	35 : 26	1.33	0.26
BC149	AGCAGCGTGG	2230	29 : 31	0.07	0.78
BC181	ATGACGACGG	2280	37 : 23	3.27	0.07
BC184	CAAACGGCAC	1390	26 : 34	1.07	0.30
BC189	TGCTAGCCTC	970	27 : 33	0.60	0.47
		1180	29 : 31	0.07	0.47
BC244	CAGCCAACCG	550	36 : 24	2.40	0.15
BC516	AGCGCCGACG	1050	28 : 33	0.41	0.55
BC523	ACAGGCAGAC	730	27 : 33	0.60	0.47
		1720	35 : 25	1.67	0.18
BC536	GCCCCTCGTC	1590	31 : 27	0.28	0.65
BC540	CGGACCGCGT	1230	34 : 26	1.07	0.30
BC546	CCCGCAGAGT	1580	36 : 24	2.40	0.15
OPC02	GTGAGGCGTC	390	30 : 29	0.02	0.85
OPC06	GAACGGACTC	290	32 : 28	0.27	0.65
		350	29 : 31	0.07	0.78
		1360	34 : 26	1.07	0.30
OPC15	GACGGATCAG	1960	29 : 30	0.02	0.85
OPC16	CACACTCCAG	1230	35 : 26	1.33	0.26
		1350	35 : 26	1.33	0.26
OPF01	ACGGATCCTG	800	34 : 26	1.07	0.30
		1300	28 : 32	0.27	0.65
		2760	27 : 33	0.60	0.47
OPF05	CCGAATTCCC	1410	28 : 32	0.27	0.65
OPF08	GGGATATCGG	1410	30 : 31	0.02	0.85
OPF12	ACGGTACCAG	1230	32 : 28	0.27	0.65
OPG08	TCACGTCCAC	1520	30 : 31	0.02	0.85
OPH02	TCGGACGTGA	2250	30 : 30	0.00	1.00
		990	26 : 34	1.07	0.30
OPH03	AGACGTCCAC	2210	30 : 31	0.02	0.85
OPH05	AGTCGTCCCC	1100	34 : 27	0.80	0.42
OPH07	CTGCATCGTG	2210	27 : 28	0.02	0.85
OPH12	ACGCGCATGT	180	36 : 25	1.98	0.17
OPH13	GACGCCACAC	1110	27 : 32	0.42	0.54
		2220	27 : 32	0.42	0.54
OPI09	TGGAGAGCAG	590	24 : 36	2.40	0.15
OPI20	AAAGTGCGGG	1590	36 : 25	1.98	0.17
OPJ04	CCGAACACGG	2330	33 : 26	0.83	0.40
		2700	33 : 26	0.83	0.40
OPJ09	TGAGCCTCAC	730	31 : 30	0.02	0.85
OPJ14	CACCCGGATG	370	26 : 35	1.33	0.26
		1020	29 : 32	0.15	0.70
OPJ17	ACGCCAGTTC	1600	28 : 31	0.15	0.70
		3690	27 : 32	0.42	0.54
OPK04	CCGCCCAAAC	700	29 : 32	0.15	0.70
OPK14	CCCGCTACAC	300	29 : 29	0.00	1.00
		680	31 : 27	0.28	0.65
OPK17	CCCAGCTGTG	680	26 : 33	0.83	0.40
OPK19	CACAGGCGGA	860	27 : 33	0.60	0.47
OPK20	GTGTCGCGAG	960	28 : 32	0.27	0.65
OPL02	TGGGCGTCAA	2090	33 : 26	0.83	0.40
		2460	27 : 32	0.42	0.54
OPL10	TGGGAGATGG	1270	25 : 32	0.86	0.39
OPL11	ACGATGAGCC	1470	24 : 36	2.40	0.15
OPL13	AAGAGAGGGG	860	34 : 26	1.07	0.30
		2150	26 : 33	0.83	0.40
OPL14	GTGACAGGCT	610	23 : 37	3.27	0.07
OPL15	AAGAGAGGGG	420	32 : 29	0.15	0.70
		550	36 : 25	1.98	0.15
		730	29 : 32	0.15	0.70
		1470	28 : 33	0.41	0.55
OPS13	GTCGTTCCTG	810	32 : 28	0.27	0.65
OPS15	CAGTTCACGG	780	34 : 26	1.06	0.32
OPT06	CAAGGGCAGA	680	28 : 33	0.41	0.60
OPT07	GGCAGGCTGT	730	32 : 39	0.15	0.70
OPT14	AATGCCGCAG	900	22 : 37	3.81	0.06
OPT14	AATGCCGCAG	1590	27 : 32	0.42	0.54

[a] The alternate hypothesis of 5:1 was rejected at $P<0.001$ in all cases

Table 2 RAPD markers that fit a 5:1 (present:absent) segregation ratio in a progeny population of US 75×'Bluetta'

Primer	Primer sequence	Fragment size (bases)	Observed ratio	χ^2	P
BC189	TGCTAGCCTC	615	53 : 7	1.08	0.30
BC292	AAACAGCCCG	980	45 : 13	1.37	0.25
		1850	49 : 9	0.05	0.78
BC504	ACCGTGCGTC	1520	53 : 7	1.08	0.30
BC516	AGCGCCGACG	1960	51 : 10	0.003	0.95
BC521	CCGCCCCACT	960	51 : 10	0.003	0.95
BC540	CGGACCGCGT	350	50 : 10	0.000	1.00
OPC02	GTGAGGCGTC	820	46 : 13	1.22	0.28
OPC06	GAACGGACTC	620	46 : 14	1.92	0.18
OPE03	CCAGATGCAC	460	53 : 7	1.08	0.30
OPF01	ACGGATCCTG	630	49 : 11	0.12	0.75
OPG08	TCACGTCCAC	410	52 : 9	0.09	0.78
OPH12	ACGCGCATGT	560	51 : 10	0.003	0.95
		2350	50 : 11	0.08	0.79
OPI20	AAAGTGCGGG	3180	50 : 9	0.08	0.79
OPJ01	CCCGGCATAA	730	46 : 9	0.004	0.95
OPJ09	TGAGCCTCAC	1280	50 : 11	0.08	0.79
OPJ19	GGACACCACT	490	48 : 12	0.48	0.50
OPK04	CCGCCCAAAC	610	48 : 13	0.57	0.49
OPK19	CACAGGCGGA	700	47 : 13	1.08	0.30
		2580	48 : 12	0.48	0.46
OPL07	AGGCGGGAAC	1590	50 : 9	0.08	0.79
		1840	49 : 10	0.003	0.95
OPL10	TGGGAGATGG	1580	47 : 7	0.58	0.49
OPL14	GTGACAGGCT	1150	46 : 14	1.92	0.18
OPL15	AAGAGAGGGG	700	56 : 5	3.15	0.08
		2700	48 : 13	0.57	0.49
OPS15	CAGTTCACGG	730	48 : 12	0.48	0.46
OPT06	CAAGGGCAGA	490	53 : 8	0.55	0.51
OPT14	AATGCCGCAG	2580	50 : 9	0.08	0.79
		3440	47 : 12	0.57	0.49

[a] The alternate hypothesis of 1:1 was rejected at $P<0.001$ in all cases

It is not known why our levels of transferred heterozygosity were somewhat lower than the predicted value of 80%; however, more than one crossover per arm would reduce the predicted level of transmitted heterozygosity. Also, recent studies have shown that a high proportion of the polymorphic RFLP markers commonly utilized in genetic mapping studies are found at the ends of chromosomes (Gill and Gill 1994). This would negatively bias es-

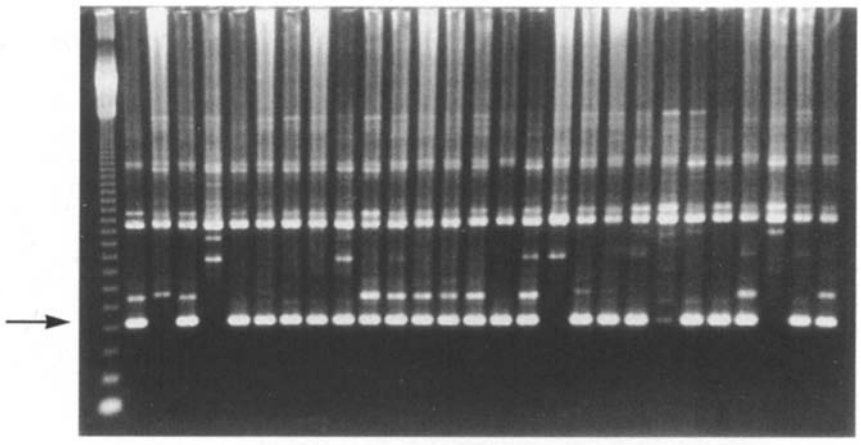

Fig. 2 DNA amplification profiles in the tetraploid progeny population US 75×Bluetta using primer OPT06. The *arrow* shows a RAPD marker segregating in a 5:1 ratio (presence:absence). From left to right, *first lane* is a 123-bp DNA ladder, *lanes 2–4* are Fla 4B, 'Bluecrop', US 75, and 'Bluetta', respectively. The remaining lanes are from 23 progeny individuals

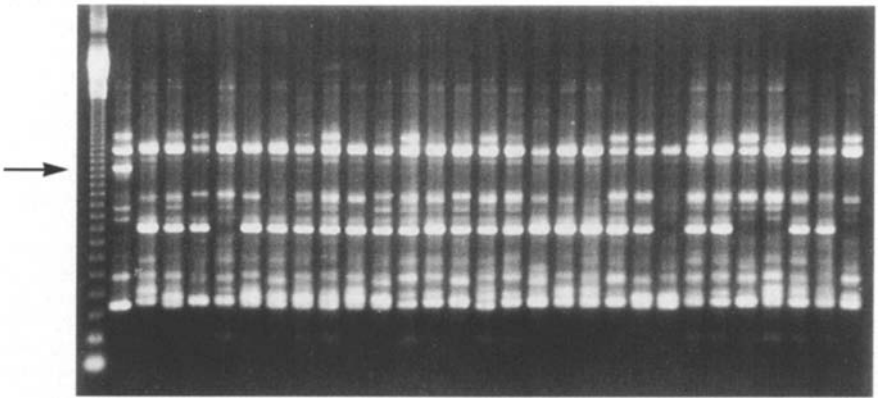

Fig. 3 DNA amplification reactions in the tetraploid progeny population US 75×Bluetta using primer OPP17. The *arrow* shows a RAPD marker that was present in FL4B, but absent in US 75. From left to right, *first lane* is a 123-bp DNA ladder, *lanes 2–4* are Fla 4B, 'Bluecrop', US 75 and 'Bluetta', respectively. The remaining lanes are from 25 progeny individuals

timates of transferred heterozygosity via FDR 2n gamete production, since a higher proportion of heterozygosity would be lost due to crossing over than would be predicted from a random distribution of genes along a chromosome.

Several other abnormal meiotic behaviors can produce levels of heterozygosity that mimic FDR, such as synaptic mutants, lack of homology, unbalanced ploidy or any other cause that hampers homologous pairing; but in all these instances, only 2n gametes are functional (Hermsen 1984; Iwanaga 1984). Vorsa et al. (1992) observed a desynaptic mechanism in an interspecific blueberry aneuploid (2n=4x+9=57) that produced almost solely 2n pollen. This is not the case in Fla 4B, however, as the majority of its gametes are reduced and viable (Ortiz et al. 1992).

Our data also indicate that numerous crossovers have occurred during 2n gamete production in Fla 4B, which is not consistent with mechanisms associated with synaptic mutations or limited homology. We identified 16 dominant markers in Fla 4B that were absent in US 75 (Table 4, Fig. 3); this most likely occurred due to crossovers between the centromere and heterozygous loci. Likewise, several recessive markers were not transferred from heterozygous loci in Fla 4B to US 75. These were discovered when the diploid population of Fla 4B×NC84 6-5 was screened for markers that segregated 5:1 (duplex loci-AAaa) in the tetraploid population or gave skewed segregation ratios (Fig. 4). Of the 39 markers that fit this category, six of those that segregated 5:1 and one with a skewed tetraploid ratio were found to segregate in the diploid population, signalling the loss of the recessive allele during 2n gamete production via crossing over. Four of these were present in Fla 4B and absent in NC84 6-5 and segregated 1:1 in the progeny. Three were present in both parents and segregated 3:1 (Table 5). One of these (OPH03) had produced an unexpected ratio in the tetraploid population. Several other heterozygous loci in Fla 4B probably remained undetected, because NC84 6-5 was homozygous for the same dominant marker as Fla 4B and could not be subjected to a segregation analysis. In total, these data provide clear evidence for the formation of multiple chiasmata during 2n gamete production.

Table 3 RAPD markers which did not statistically fit a 5:1 ratio or 1:1 segregation ratio

Primer	Primer sequence	Fragment size (bases)	Observed ratio
BC181	ATGACGACGG	1470	40 : 21
OPH03	AGACGTCCAC	2090	43 : 18
BC239	CTGAAGCGGA	1540	42 : 18
OPJ14	GTGACAGGCT	3200	43 : 18
OPT16	GGTGAACGCT	750	45 : 16

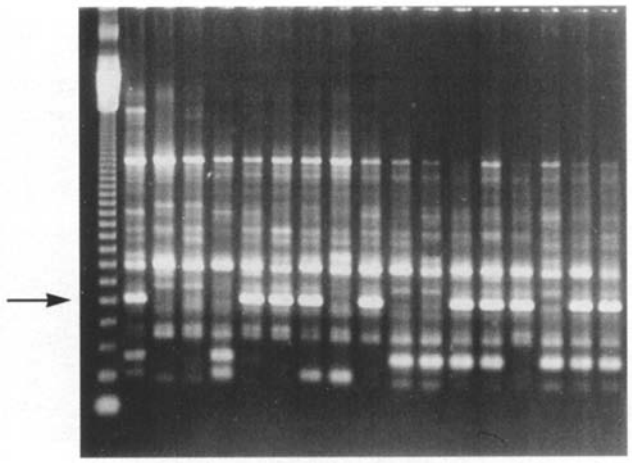

Fig. 4 DNA amplification reactions in the diploid progeny population Fla 4B×NC84 6-5 using primer OPC06. The *arrow* shows a RAPD marker segregating in a 1:1 ratio (presence:absence). This same marker segregated 5:1 in the tetraploid population US 75× 'Bluetta'. From left to right, *first lane* is a 123-bp DNA ladder, *lanes 2 and 3* are Fla 4B and NC 6-5, respectively. The *remaining lanes* are from 15 progeny individuals

Table 4 Primers which produced amplification products in Fla 4B that were absent in US 75

Primer	Primer sequence	Fragment size (bases)	Number of markers
BC127	ATCTGGCAGC	730	1
BC222	AAGCCTCCCC	420	1
BC149	AGCAGCGTGG	4100	1
OPJ01	CCCGGCATAA	3200	1
OPK04	CCGCCCAAAC	2100	1
OPK11	AATGCCCCAG	2460, 2700	2
OPK14	CCGCTACAC	1470, 1590	2
OPK20	GTGTCGCGAG	1860	1
OPP17	GTCCATGCCA	1720	1
OPS18	CTGGCGAACT	450, 4200	2
OPT07	GGCAGGCTGT	3350	1
OPT12	GGGTGTGTAG	2210	1
OPT14	AATGCCGCAG	2220	1

Table 5 Segregation ratios in the diploid population Fla 4B×NC 6-5 for the RAPD markers which segregated 5:1 or had skewed segregation ratios in the tetraploid population US 75×'Bluetta'

Primer	Fragment size (bases)	Observed ratio	Expected ratio	χ^2	P
BC504	1520	6 : 9	1 : 1[b]	0.27	0.65
OPH12	2350	6 : 9	1 : 1[b]	0.27	0.65
OPC06	620	9 : 6	1 : 1[b]	0.27	0.65
OPF01	630	6 : 9	1 : 1[b]	0.27	0.65
OPH03	2090	12 : 2	3 : 1[c]	0.38	0.52
BC516	1960	8 : 6	3 : 1[c]	1.52	0.21
OPL15	2700	10 : 5	3 : 1[c]	0.20	0.68

[a] The chi-square tests were done using the Yates correction for small population size (Strickberger 1985)
[b] Marker present in Fla 4B and absent in NC84 6-5
[c] Marker present in both Fla 4B and NC84 6-5

Conclusions

With these results, it is not surprising that Fla 4B has been such a useful parent in breeding highbush blueberries. Fla 4B is highly heterozygous and this heterozygosity was readily transmitted to its tetraploid progeny US 75. We only examined one product of an unreduced gamete, but this hybrid appeared to carry about 70% of Fla 4B's heterozygosity. This heterozygosity was shown to readily segregate in the tetraploid background through predominantly tetrasomic rather than disomic inheritance. This makes a high proportion of the genome of Fla 4B available for tetraploid highbush breeding.

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