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Chromosome Homology in Tetraploid Southern Highbush x *Vaccinium elliottii* Hybrids

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Abstract. **A yellow-leaf seedling marker, r, was used to determine if there was preferential chromosome pairing in a group of tetraploid southern highbush blueberry hybrids. Plants with four copies of r (no copies of R) fail to develop anthocyanins, and cotyledons, hypocotyls, leaves, stems, and other vegetative tissues have a bright yellow-green color. In the hybrids that were studied, two genomes were from the diploid wild species, *V. elliottii* Chapman, and both carried the recessive marker r. The other two genomes were from southern highbush cultivars and both carried the dominant wildtype allele, R. When RRrr hybrids were intercrossed or crossed to rrrr yellow-leaf plants, the number of yellowleaf rrrr seedlings obtained usually equalled or exceeded the number predicted from non-preferential chromosome pairing. Since rr gametes can only be produced by RRrr plants when R and r chromosomes pair at Meiosis I, there was no evidence that the chromosomes derived from *V. elliottii* were pairing at a higher-than-random rate.**

The evolution of many species within genus *Vaccinium* has involved polyploidy. Diploid, tetraploid, and hexaploid species are found in *Vaccinium* sections *Cyanococcus* (blueberry), *Oxycoccus* (cranberry), and *Vaccinium* (bilberry), and section *Myrtillus* has diploid and tetraploid species.

Two kinds of tetraploid species are possible in plants—autotetraploids and allotetraploids, also called amphidiploids (Allard, 1960; Stebbins, 1950). The distinction is of importance to plant breeders because the type of polyploidy determines whether or not certain types of hybrids will be fertile or sterile and whether genetic recombination during meiosis will occur freely among all four sets of chromosomes or will be confined to exchanges between homologous, as opposed to homoeologous chromosomes. The difference between autotetraploidy and allotetraploidy is based on two contrasting patterns of chromosome pairing during meiosis. Both autotetraploid and allotetraploid species, as contrasted with some tetraploid plants produced in the laboratory, have regular bivalent chromosome pairing. However, in autotetraploids, the determination of which two of the four homologous chromosomes will pair during meiosis in a particular meiocyte is random, whereas in allotetraploids, chromosome pairing is strictly predetermined, occuring only between homologous and never between homoeologous chromosomes. If an autotetraploid species represented by the genome formula AAAA is crossed with a second autotetraploid species, BBBB, from which it is genetically so divergent that chromosomes of the two species cannot pair, the AABB hybrid could still be highly fertile because the hybrid contains two sets of A chromosomes and two sets of B. If, however, two allopolyploid species similarly related were crossed, e.g., AABB x CCDD, chromosomes in the ABCD F_1 hybrid would not pair, and the hybrid would be highly sterile.

Haploids derived from autotetraploids can be somewhat fertile; haploids derived from allotetraploids are highly sterile. Recessive alleles in autotetraploids normally give 35:1 segregation in the F_2 generation after a cross in which one parent of the F_1 was homozygous for the dominant allele and the other was homozygous for the recessive allele. In contrast, recessive marker genes in allotetraploid species normally segregate 3:1.

Camp (1945), who studied the taxonomy and evolution of the genus *Vaccinium* before the differences between autopolyploids and allopolyploids were fully understood, postulated that allopolyploidy was widespread in several of the sections of *Vaccinium*, including *Cyanococcus*. More recent studies of species and interspecific hybrids in *Cyanococcus* have shown that several of the species thought by Camp to be allopolyploids are actually autopolyploids. Both isozyme markers (Krebs and Hancock, 1989) and RAPD markers (Qu and Hancock, 1995; 1998) have been used to show that *V. corymbosum* and a tetraploid hybrid between *V. corymbosum* and *V. darrowi* Camp have tetrasomic inheritance, as would be expected in autotetaploids. Interspecific hybrids have been made between various diploid species in section *Cyanococcus*. High fertility in the diploid F_1 generations has indicated that the chromosomes of the crossed species were able to pair well. When *V. corymbosum* is crossed with *V. angustifolium* Ait., *V. darrowi*, *V. myrsinites* L., or *V. elliottii,* and tetraploid F_1 hybrids are backcrossed to *V. corymbosum*, the backcross-1 seedlings are usually quite fertile. This suggests that chromosomes of these species can pair and undergo recombination during meiosis.

Vaccinium elliottii is a tall-growing, small-leafed, highly deciduous diploid blueberry species that occurs as far south as Gainesville, in the northern Florida peninsula, and is widespread in river bottoms and on rolling hills of the coastal plain and piedmont regions of the southeastern United States. It is of interest in blueberry improvement programs because of upright growth habit, very early ripening, tolerance for mineral soils and upland sites, and for its high-quality, albeit small berry. A recessive seedling marker, which removes red pigments from all plant parts, was found in two wild plants, one from northern Florida and one from southwestern Alabama (Lyrene, 1988), and the responsible allele was shown to segregate as a simple recessive in crosses within *V. elliottii*. The mutant and wildtype alleles at the locus were initially called y and R, but here are called r and R to conform with conventions for naming alleles. Crosses between plants homozygous for the yellow-leaf allele and a *V. elliottii* clone from northeast Florida that had normal foliage, but berries that were pink when ripe produced F_1 populations that were normal and segregated independently for the two alleles in the F_2 (Lyrene, 1988 and Lyrene, unpublished). Crosses between yellow-leaf *V. elliottii* and a *V. darrowi* selection that had normal foliage but pink-green mature fruit, produced an F_1 population with normal foliage but which segregated 1:1 for normal vs. pink-green fruit color (Lyrene, unpublished). This indicated that the *V. darrowi* clone was heterozygous for a dominant allele that reduced anthocyanin in the fruit, and that this allele was not at the same locus as the yellow-leaf allele in *V. elliottii*. Yellow-leaf *V. elliottii* plants have not been crossed with any of the northern highbush clones or *V. angustifolium* (Hall and Aalders, 1963) clones that have white, pink, or green berries.

The purpose of this study was to investigate the homology between the chromosomes of southern highbush blueberry and those of *V. elliottii*. This was done by hybridizing tetraploid southern highbush cultivars and diploid *V. elliottii* plants that were homozygous rr, taking advantage of 2n gamete production in *V. elliottii* to produce tetraploid hybrids. The ratio of yellow-leaf to normal seedlings in segregating generations following the crosses was used to study the pairing and segregation of the chromosomes bearing the marked locus.

Materials and Methods

Between 1987 and 1989, a large numbers of flowers of the tetraploid southern highbush blueberry selections FL 6-19 and 'Misty', neither of which carried the r allele, were pollinated with pollen from diploid *V. elliottii* selections 'Silverhill' and 'Oleno', both of which were homozygous rr. Evidence that neither FL 6-19 nor 'Misty' carried the r allele comes from the fact that no anthocyanin-free plants have been found among 200,000 related seedlings that have been fruited in the Florida

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blueberry breeding program, nor among more than a million seedlings that have been seen in germination pots. No anthocyanin-free seedlings have been reported by other blueberry breeders, nor has a *V. darrowi* or *V. corymbosum* plant that lacked vegetative anthocyanin been reported from wild populations. Such plants undoubtedly exist, but the allele frequency must be very low in the southern highbush gene pool and in native Section Cyanococcus species. Seeds from the crosses were sown in a greenhouse in November of the year in which the crosses were made, and the seedlings were transplanted to a field nursery in May of the following year. Based on intermediate hybrid phenotype, isozyme analysis, the ability to produce at least some yellow-leaf segregants in crosses with tetra-allelic rrrr plants, and production of a high percentage of potentially viable pollen as indicated by staining with 2% acetocarmine dye, nine F_1 hybrids that appeared to be tetraploid RRrr plants were identified from the 1987 crosses. In Dec. 1988, the eight largest plants were potted from the field and were chilled at 5 °C in a dark chamber for ≈6 weeks. They were then placed in a warm greenhouse and hand-crossed in various combinations after emasculation. The resulting seeds were sown in the greenhouse in Dec. 1989, and several hundred yellow-leaf seedlings were selected and transplanted to trays of peat. After several months, the 10 most vigorous were potted and maintained in the greenhouse for crossing the following fall and winter.

In Dec. 1990, 11 hybrids obtained by pollinating yellow-leaf *V. elliottii* clones 'Silverhill' and 'Oleno' with pollen from southern highbush cultivar 'Misty' were dug from the field, potted, and placed in a chamber at 5 °C. Also included were the interspecific hybrids from the previous year and five of the 10 yellow-leaf plants obtained by intercrossing the original F_1 hybrids. After ≈6 weeks, the plants were placed in a bee-proof greenhouse to flower. In Mar. 1991, additional crosses were made between pairs of F_1 hybrids, and 14 of the F_1 hybrids were pollinated with pollen from the tetraploid yellowleaf progeny of earlier F_1 x F_1 crosses.

Seed from the crosses was planted in mid-July 1991 after having been soaked in an aqueous solution of 10.4 mM gibberellin A_3 and 1.0 mM ^{6}N-benzyladenine for 24 h to enhance high-temperature germination. The germination pots were watered daily and were observed through Feb. 1992 to permit germination of seeds that did not respond to the chemical treatments. The red-leaf seedlings were counted and discarded after they emerged. The yellow-leaf seedlings were transplanted to trays of peat and were grown in the greenhouse for four additional months to make sure no red pigments appeared.

The number of red-leaf and yellow-leaf seedlings were counted from 13 AAaa x AAaa crosses and from 18 AAaa x aaaa crosses. The ratios of red-leaf : yellow-leaf seedlings were tested by Chi-square to determine if the ratios conformed to those expected from random pairing between southern highbush and *V. elliottii*-derived chromosomes.

Results and Discussion

As expected from tetraploid x diploid crosses in *Vaccinium*, which has a strong triploid block (Galletta, 1975; Sharpe and Sherman, 1971), the crosses between the tetraploid southern highbush cultivars and the diploid *V. elliottii* clones that were homozygous for rr gave only a small fraction of the number of seedlings normally obtained from homoploid crosses. However, high pollen staining, high fertility, and the phenotypes of the seedlings obtained when the F_1 hybrids were intercrossed indicated that all but one of the hybrids were tetraploid. Most of the hybrids had vigor equal to or greater than the parent species.

If the RRrr F_1 hybrids were behaving as strict allotetraploids, with the rr chromosomes from *V. elliottii* always pairing with each other, the F_1 plants would be expected to produce no rr gametes, and no yellow-leaf seedings would appear in the F_2 generation or in the progeny obtained by crossing RRrr and rrrr plants. Thus, any tendency toward preferential pairing of the *V. elliottii*-derived chromosomes in the tetraploid hybrids would result in a deficiency in the number of yellow-leaf seedlings obtained in the next generation, compared to the number expected from completely non-preferential pairing. When there were deviations from the expected values, they were mostly in the opposite direction (Tables 1 and 2), that is, more than the expected number of yellow-leaf plants were observed.

Segregation ratios were determined for 31 crosses, and 11,831 seedlings were scored for leaf color. In the F_2 intercross populations, where the 35:1 ratio expected from random chromosome pairing would have produced 72 yellow-leaf seedlings, 115 were observed (Table 1). Chi-square indicated that the probability of the discrepancy being due to sampling error was exceedingly small. In the crosses where RRrr F_1 hybrids were pollinated with pollen from yellow-leaf rrrr plants, 1539.5 yellow-leaf seedlings were expected, and 1656 were observed (Table 2). Again, the Chi-square test indicated the probability of this difference

Table 1. Yellow-leaf phenotypic segregation ratios, with Chi-square analysis, for crosses among southern highbush x *V. elliottii* hybrids heterozygous (presumably duplex tetraploids) for the yellow-leaf allele. Total seedlings = 2,594.

	Yellow-leaf : Red-leaf			
Cross	Expected[z]	Observed	χ^2	*P*
FL 88-174 x FL 89-4	0.9 : 32.1	2 : 31	1.38	<0.25
FL 88-174 x FL 89-6	5.4 : 189.6	6 : 189	0.07	>9.75
FL 88-174 x FL 91-105	5.2 : 180.8	9 : 177	2.86	>0.05
FL 89-2 x FL 88-174	5.5 : 191.5	10 : 187	3.79	>0.05
FL 89-4 x FL 89-12	2.9 : 100.1	3 : 100	0.00	>0.99
FL 89-5 x FL 88-174	5.0 : 176.0	11 : 170	7.41**	<0.01
FL 89-5 x FL 91-105	7.2 : 252.8	13 : 247	4.81*	<0.05
FL 89-6 x FL 88-174	6.2 : 216.8	14 : 209	10.10**	<0.01
FL 89-6 x FL 91-70	12.0 : 421.0	22 : 413	8.58**	<0.01
FL 89-12 x FL 88-174	3.8 : 134.2	4 : 134	0.01	>0.99
FL 89-12 x FL 89-4	2.6 : 126.4	3 : 127	0.06	>0.75
FL 89-94 x FL 89-6	6.8 : 237.2	2 : 242	3.49	<0.10
FL 89-94 x FL 91-109	7.5 : 261.5	16 : 253	9.91**	<0.01
All combined	72.1 : 2521.9	115 : 2479	26.26	<0.005

[z]Theoretical expected ratio, based on tetrasomic chromosome behavior, is 1 yellow-leaf : 35 red-leaf.

Table 2. Yellow-leaf phenotypic segregation ratios, with Chi-square analysis, for crosses between F_1 interspecific hybrids heterozygous (presumably RRrr) for the yellow-leaf allele, used as female parent, and homozygous (rrrr) yellow-leaf hybrids, derived from F_1 x F_2 crosses, used as pollen parent.

	Yellow-leaf : Red-leaf			
Cross	Expected[z]	Observed	χ^2	*P*
FL 88-174 x FL 91-212	19.7 : 98.3	18 : 100	0.18	>0.75
FL 89-6 x FL 91-203	461.3 : 2306.7	478 : 2290	0.73	>0.50
FL 89-12 x FL 91-213	91.3 : 456.7	78 : 470	2.33	>0.25
FL 91-70 x FL 91-213	102.2 : 510.8	121 : 429	3.95*	<0.05
FL 91-104 x FL 91-211	50.5 : 252.5	61 : 242	2.62	>0.10
FL 91-104 x FL 91-213	47.5 : 237.5	37 : 248	2.78	>0.10
FL 91-105 x FL 91-203	163.7 : 818.3	191 : 791	5.46±	<0.05
FL 91-105 x FL 91-214	238.5 : 1192.5	286 : 1144	11.35**	<0.01
FL 91-106 x FL 91-212	77.3 : 386.7	30 : 434	34.73**	<0.01
FL 91-107 x FL 91-203	101.3 : 506.7	123 : 465	5.58*	<0.05
FL 91-107 x FL 91-213	23.3 : 116.7	26 : 114	0.38	>0.75
FL 91-108 x FL 91-209	6.2 : 30.8	9 : 28	1.52	<0.25
FL 91-109 x FL 91-205	37.5 : 187.5	42 : 183	0.65	>0.25
FL 91-110 x FL 91-213	17.0 : 85.0	23 : 79	2.54	>0.10
FL 91-111 x FL 91-201	59.2 : 295.8	61 : 294	0.07	>0.75
FL 91-111 x FL 91-215	9.8 : 49.2	9 : 50	0.08	>0.75
FL 91-112 x FL 91-213	28.3 : 141.7	38 : 132	3.99*	<0.05
FL 91-113 x FL 91-209	18.8 : 94.2	25 : 88	2.45	>0.10
All combined	1539.5 : 7697.5	1656 : 7581	10.58	<0.005

[z]Theoretical expected ratio, based on tetrasomic chromosome behavior, is 1 yellow-leaf : 5 red-leaf.

being due to random error was <0.005.

Possible reasons for the deviations from expected results include misclassification of some seedling phenotypes, reduced survival of the red-leaf seedlings, or a less-than-random tendency for the *V. elliottii* chromosomes to pair with each other in the tetraploid F_1 hybrids. None of these explanations seems very likely. With regard to possible misclassifications of seedlings, the visual contrast between the two phenotypes was very great. It also seems unlikely that the yellow-leaf alleles increased the viability of the seedlings. Plants with the yellow leaf characteristic that have been maintained for 10 years in the field seem similar to normal plants in their vigor and survival. There is also no theoretical basis for expecting the *V. elliottii* chromosomes to pair with each other less often than expected with random pairing. The cause of the small but consistent excess of yellow-leaf seedlings is unknown.

In summary, evidence from the large number of seedlings scored for the yellow-leaf marker indicate that the chromosomes of *V. elliottii* pair readily with those of *V. corybosum*, *V. darrowi,* and the other species in *Vaccinium* section *Cyanococcus* that have contributed in small part to the southern highbush gene pool. This finding agrees with studies of other species combinations that indicate that the chromosomes of the species in *Vaccinium* section *Cyanococcus* are similar enough to produce high fertility and genetic recombination in the interspecific hybrids (Dweikat and Lyrene, 1989; Lyrene and Ballington, 1986; Vorsa, 1997).

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