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Crossability of a Diverse Group of Polyploid Blueberry Interspecific Hybrids

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Abstract. **Five blueberry interspecific hybrids (3 tetraploids, 1 pentaploid, and 1 hexaploid) and 2 highbush (*Vaccinium corymbosum* L., 2n = 4x = 48) blueberry clones were crossed in all combinations. Seeds per pollination and seed germination were the criteria used to measure the success of these crosses. The tetraploid interspecific hybrids were fully cross-fertile with the highbush clones and with each other. The pentaploid and hexaploid interspecific hybrids were only partially cross-fertile with the highbush clones and with the tetraploid interspecific hybrids; nonetheless, they still produced an adequate amount of viable seed in most combinations. Significant reciprocal differences in crossability were detected for 4 of the 5 species hybrids.**

The cultivated highbush blueberry is a relatively new crop in the United States, and is unknown in many parts of the world. Its short history of domestication and the wealth of germplasm in native blueberry species provide potential for large strides in genetic improvement. Full exploitation of the available germplasm, however, will depend on the breeder's ability to transfer useful genes from diploid and hexaploid species to the cultivated highbush blueberry, a tetraploid.

Several types of polyploid interspecific hybrids have been produced (2, 3), but little work has been done to determine quantitatively their crossability with cultivated highbush clones or with each other. This study was initiated to determine the crossability of 5 such hybrids.

Materials and Methods

During the winter of 1981, 5 interspecific hybrids (Table 1) and 2 highbush clones, G-111 and G-362, were crossed in all combinations. The 7 clones also were self-pollinated. Seeds per pollination and seed germination were the criteria used to measure the success of these crosses.

Crosses were made in a heated greenhouse using 4 large potted plants of each clone. At least 40 pollinations (4 replications, or clusters, of about 10 flowers per replication) were made per cross. Whenever possible, these pollinations were equally divided among the 4 plants of a clone.

Unopened flowers were emasculated (to prevent any chance of self pollination) and then hand pollinated 1- to 6-days later with a pollen-coated thumbnail. Any flowers in the cluster not hand pollinated were picked off. The seeds were hand extracted from ripe berries, and the large well-filled seeds (1) were separated from the pulp and counted. Seeds were air dried on paper towels, then stored in capped glass vials in a refrigerator at 5°C for 4 months.

In Jan. 1982, seeds were sown on the surface of moist, milled sphagnum moss in 10-cm plastic pots under intermittent mist and supplemental light. Five replications of 50 seeds each were sown per cross. Germination counts were taken at 6, 8, and 10 weeks after sowing. A final germination count was taken at the time that seedlings were transplanted into peat pots (4 months after sowing).

Twelve of the original 49 crosses were repeated in 1982, in addition to several new crosses. Sibling selections of NJUS11 and NJUS64 were available and had a sufficient number of flowers for making crosses in 1982. To determine whether these sibling hybrids were equally cross-fertile with a highbush tester, we crossed both pairs of hybrids reciprocally with G-362. The full-sib crosses, NJUS10 x NJUS11 and NJUS64 x NJUS62, also were made.

A sample of pollen from each of the 5 hybrids and from the 2 highbush clones was collected and stained in acetocarmine. The number of stained pollen grains and well formed tetrads with 4 developed microgametophytes was recorded for each clone.

Results and Discussion

The tetraploid interspecific hybrids, NJUS64, US75, and US226, produced abundant stainable pollen and were cross-fertile with the highbush clones and with each other (Tables 2 and 3). The 4× x 4× interspecific crosses in this study were as successful as the intraspecific (highbush x highbush) crosses (Table 4).

The pentaploid interspecific hybrid, US79, produced only a scant amount of pollen, whereas the hexaploid NJUS11 produced abundant pollen. Both clones were only partially cross-fertile with the highbush clones and with the tetraploid interspecific hybrids (Tables 2 and 3). Significant differences in seeds per pollination and percentage of germination were not detected, however, between some heteroploid crosses and many of the homoploid crosses (Tables 2 and 3). US79 x G-362 and US79 x NJUS64 are 2 heteroploid crosses that compared favorably with many of the homoploid crosses in both seeds per pollination and percentge of germination. G-111 x NJUS11 and US226 x NJUS11 produced as many seeds per pollination as many of the homoploid crosses (Table 2), but the germination of seeds from these crosses was very low (Table 3).

The results presented in Tables 2 and 4 support the hypothesis (5) that, within the subgenus *Cyanococcus*, species integrity is maintained by seasonal and geographical isolation and differ-

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Table 1. Parentage, ploidy level (x = 12 chromosomes), and method of forming the polyploid hybrids used in the crossability study.

Clone	Ploidy	Parentage	Method of hybrid formation
NJUS11	6x	'Tifblue' [*Vaccinium ashei* Reade, (6x)] x US41 [US41 was produced by doubling the chromosome number of a *V. atrococcum* Heller clone (2x) with colchicine].	6x x (2x)[z]
NJUS64	4x	*V. myrsinites* Lamark (4x) x *V. angustifolium* Aiton (4x)	4x x 4x
US75	4x	Fla 4B [*V. darrowi* Camp (2x)] x 'Bluecrop' [*V. corymbosum* L. (4x)]	2x x 4x
US79	5x	Fla 4B x US56 [*V. constablaei* Gray (6x) x T 65, a *V. ashei* selection (6x)]	2x x (6x x 6x)
US226	4x	Produced by doubling the chromosome number of US126 (2x) with colchicine. US126 resulted from the cross US51 [*V. myrtilloides* Michaux (2x)] x *V. atrococcum* (2x).	(2x x 2x)[z]

[z]Chromosome number of diploid clone doubled with colchicine. (NJUS11 probably arose from the union of a 3x gamete from 'Tifblue' with a 3x gamete from the synthetic autotetraploid clone US 41.)

Table 2. Mean number of seeds per pollination from crosses involving three 4x, one 5x, and one 6x blueberry interspecific hybrids and two (4x) highbush selections.

Seed parent	Pollen parent						
	G-111[z] (4x)	G-362[z] (4x)	NJUS64 (4x)	US75 (4x)	US226 (4x)	US79 (5x)	NJUS11 (6x)
G-111 (4x)	6.0[y] hijk	24.4 bc	22.9 bcd	20.8 bcde	9.1 ghijk	0.6 k	23.5 bcd
G-362 (4x)	17.0 bcdefgh	5.6 ijk	27.5 b	16.8 bcdefgh	18.4 bcdefgh	0.3 k	8.4 ghijk
US75 (4x)	5.3 ijk	10.0 efghijk	12.7 defghij	7.8 ghijk	9.7 fghijk	0.8 k	4.5 ijk
US226 (4x)	20.4 bcdef	51.1 a	27.4 b	21.9 bcd	1.4 jk	0.5 k	15.3 cdefghi
US79 (5x)	3.3 jk	11.0 efghijk	7.3 hijk	3.0 jk	7.0 hijk	0.2 k	3.6 jk
NJUS11 (6x)	1.6 jk	1.9 jk	1.1 k	3.3 jk	2.1 jk	2.2 jk	3.1 jk

[z]Highbush selection.
[y]Means separated by Duncan's multiple range test, 5% level.

ences in chromosome number. When hybrids having the same chromosome number are brought together in a greenhouse so that they flower simultaneously, there seem to be no barriers to crossability.

Significant reciprocal differences in crossability were detected for 4 of the 5 interspecific hybrids (Table 5). Data on NJUS64 used as a female could not be collected, because its ramets failed to set any fruit. It appeared that the flower buds of this clone had been injured by cold temperatures in the unheated greenhouse where breeding clones were kept before bringing them into a heated greenhouse for crossing. US226 was more successful as a female than as a male, whereas US75 was more successful as a male than as a female.

NJUS11 produced more seeds as a male parent than as a female, although these seeds germinated poorly (Table 5). Embryo abortion apparently occurs at a later stage in tetraploid x NJUS11 crosses than in NJUS11 x tetraploid crosses.

US79 was a poor male parent, but it was significantly more successful as a female (Table 5). Its microgametogenesis is probably much more sensitive to the chromosomal imbalances associated with pentaploid meiosis than is its megagametogenesis.

It would be informative to know which types of tetraploid interspecific hybrids cross most successfully with highbush genotypes. In this study, NJUS64 was a more successful male than either US75 or US226 in crosses with highbush (Table 6). US75 appears to be a slightly more successful male than US226 in crosses with highbush, but when both male and female performance is considered, US226 reproductively is the more successful clone (Table 6).

Crosses productive in 1981 also were productive in 1982, and those involving US79 and NJUS11 were again relatively unproductive. An analysis of variance for the combined data did not

Table 3. Percentage of germination of seeds from crosses involving three 4x, one 5x, and one 6x blueberry interspecific hybrids and two (4x) highbush selections.

Seed parent	Pollen parent						
	G-111[z] (4x)	G-362[z] (4x)	NJUS64 (4x)	US75 (4x)	US226 (4x)	US79 (5x)	NJUS11 (6x)
G-111 (4x)	---[y]	92[x] abcd	91 abcd	90 abcde	67 ij	---[y]	5 p
G-362 (4x)	93 abcd	84 cdefg	96 ab	98 a	95 ab	---	18 no
US75 (4x)	96 ab	94 abc	97 ab	96 ab	83 cdefg	---	14 o
US226 (4x)	92 abcd	86 bcdef	96 ab	77 fghi	70 hi	---	27 mn
US79 (5x)	84 cdefg	79 efgh	82 defg	87 abcdef	70 hi	---	55 kl
NJUS11 (6x)	35 m	58 jk	48 l	68 i	73 ghi	82 defg	69 hi

[z]Highbush selection.
[y]Insufficient seeds were available for germination test.
[x]Means separated by Duncan's multiple range test, 5% level, before converting to percentages.

Table 4. Statistical comparisons of blueberry interspecific hybrid crossability data. I. Ploidy effects.

Type of interspecific cross	Seeds/ pollination	Percent germination
4x x 4x	19.7a[z]	90 a
4x x 6x	11.4 b	16 d
5x x 4x	6.3 c	80 b
6x x 4x	2.0 d	58 c
4x x 5x	0.6 d	---[y]
Intraspecific (HB[x] x HB) crosses[w]	20.2	92

[z]Means within columns separated by Fisher's LSD, 5% level.
[y]Insufficient seeds were available for germination tests.
[x]HB = highbush (4x).
[w]Not significantly different from 4x x 4x interspecific crosses.

Table 5. Statistical comparisons of blueberry interspecific hybrid crossability data. II. Reciprocal effects.

Type of cross	Seeds/ pollination	Percent germination
US226 x other tetraploids[z]	30.2	88
Tetraploids x US226	12.7	82
Significance[y]	**	*
US75 x other tetraploids	9.4	93
Tetraploids x US75	19.7	88
Significance	**	NS
NJUS11 (6x) x tetraploids	2.0	58
Tetraploids x NJUS11 (6x)	11.4	16
Significance	**	**
US79 (5x) x tetraploids	6.3	80
Tetraploids x US79 (5x)	0.6	---[x]
Significance	**	

[z]Including G-362 and G-111.
[y]NS, *,**Nonsignificant (NS) or significant at 5% (*) or 1% (**) levels.
[x]Insufficient seeds were available for germination test.

show significant differences between years or a significant year x cross interaction.

Table 6. Statistical comparisons of blueberry interspecific hybrid crossability data. III. Specific parent effects.

Type of cross	Seeds pollination	Percent germination
Highbush x NJUS64	25.5 a[z]	94 a
Highbush x US75	18.5 b	94 a
Highbush x US226	14.4 b	81 b
US226-highbush[y]	25.8	85
US75-highbush[x]	12.7	94
Significance[w]	**	**

[z]Means within columns separated by Fisher's LSD, 5% level.
[y]Includes US226 x highbush crosses and highbush x US226 crosses.
[x]Includes US75 x highbush crosses and highbush x US75 crosses.
[w]**Significant at 1% (**) level.

Table 7. Statistical comparisons of blueberry interspecific hybrid crossability data. IV. Sibling effects.

Cross	Seeds/ pollination	Cross	Seeds/ pollination
NJUS10 x G-362	2.2	NJUS62 x G-362	7.6
NJUS11 x G-362	5.4	NJUS64 x G-362	0.8
Significance[z]	NS		**
G-362 x NJUS10	13.3	G-362 x NJUS62	27.9
G-362 x NJUS11	9.1	G-362 x NJUS64	23.0
Significance[z]	NS		NS

[z]NS, **Nonsignificant (NS) or significant at 1% (**) level.

Sibling hybrids NJUS10 and NJUS11 and sibling hybrids NJUS62 and NJUS64 performed similarly in crosses with the highbush clone G-362 (Table 7), except that the cross NJUS62 x G-362 produced significantly more seeds per pollination than the cross NJUS64 x G-362.

Blueberry breeders may find it useful to produce F_2 populations of certain interspecific hybrids. This can be accomplished by selfing the interspecific hybrid or by crossing it with a full sibling. The use of selfing in blueberry breeding schemes has been hindered, however, by widespread self-incompatibility (4). The interspecific hybrids NJUSl0, NJUSll, NJUS62, and NJUS64 appear to be partially self-incompatible, but produced abundant seed when mated with a full sib (Table 8).

Despite the relatively poor crossability of US79 and NJUSll with tetraploids, poor crossability should not be a major obstacle to the use of any of the interspecific hybrids in this study. All are vigorous clones that produce large numbers of flowers that are relatively easy to emasculate and pollinate. Large progenies can be produced by making many hand pollinations.

The results of this study are encouraging, but the hybrids used represent only a very small amount of the germplasm available within the subgenus *Cyanococcus*. Lyrene and Sherman (6) recommended that attempts to use *V. darrowi, V. elliottii* Chapman, *V. fuscatum* Aiton, and *V. myrsinites* in breeding should exploit the genetic variation among plants within species as well as differences among the species. Their variance analysis suggested that for most of the traits studied, selecting the best clone within a species is almost as important as selecting the best species. Ballington and Galletta (1) also cited the importance of the individual plant chosen to represent a species in hybridizations when intercrossing diploid blueberry species *V. atrococcum, V. caesariense* Mackenzie, *V. darrowi* and *V. tenellum* Aiton.

Table 8. Statistical comparisons of blueberry interspecific hybrid crossability data. V. Sibling self- and cross-pollination effects.

Cross	Seeds/ pollination	Cross	Seeds/ pollination
NJUS10 x self	0.6	NJUS62 x self	2.2
NJUS10 x NJUS11	16.4	NJUS64 x NJUS62	10.0
Significance	**		**
NJUS11 x self	3.8	NJUS64 x self	2.1
NJUS10 x NJUS11	16.4	NJUS64 x NJUS62	10.0
Significance	**		**

**Significant at 1% (**) level.

The transfer of useful traits from NJUSll, and other hexaploid interspecific hybrids, into commercially acceptable highbush-type clones will require at least 2 cycles of crossing and selection. Offspring resulting from crosses between NJUS11 and highbush are most likely to be pentaploids of low fertility. If these pentaploids are backcrossed to highbush, their progeny will probably consist primarily of aneuploids; however, fertile tetraploids can be obtained from this type of cross (7). The transfer of useful traits from US79 into commercially acceptable highbush-type clones may require 1 less cycle of crossing and selection than will NJUS11 because US79 is already at the pentaploid level.

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