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Production and viability of unreduced gametes in triploid interspecific blueberry hybrids*

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Summary. Three triploid ($2n=3x=36$) blueberry hybrids were obtained by hand-pollinating approximately 7,000 flowers of tetraploid highbush blueberry cultivars (based on *Vaccinium corymbosum* L.) with pollen from the diploid species *V. elliottii* Chapm. Meiotic analysis of these triploids revealed trivalents, bivalents and univalents in all metaphase I cells, with lagging chromosomes evident at anaphase I. Pollen of the three triploids was mostly aborted and did not stain with acetocarmine. However, the three triploids did produce from 0.9%–1.3% giant pollen grains that stained with acetocarmine and were present as monads, dyads or triads, rather than the normal tetrads. Pollination of 10,853 flowers of hexaploid *V. ashei* Reade cultivars with pollen from the triploids produced 266 berries, which averaged fewer than two fully-developed seeds per berry. One triploid clone showed partial female fertility when crossed to hexaploids, self-pollinated, or intercrossed with other triploids. Ploidy levels of the resulting hybrids were determined.

Key words: *Vaccinium* – Triploids – Interspecific hybridization

Introduction

The cultivated blueberries of North America are of three major types: lowbush, highbush and rabbiteye. These correspond loosely to three species in *Vaccinium* section *Cyanococcus: V. angustifolium* Ait., *V. corymbosum* L. and *V. ashei* Reade, respectively. Interspecific hybridization has been used in cultivar breeding, especially among highbush blueberries. Section *Cyanococcus* also contains many uncultivated species (Camp 1945).

Vaccinium section *Cyanococcus* appears to be evolving rapidly. Some of the species that differ markedly in habitat preference and in morphology can readily be hybridized in the greenhouse, and form vigorous, fertile hybrids (Darrow et al. 1952; Ballington and Galletta 1978; Vander Kloet 1983). Two factors that reduce natural interspecific hybridization among sympatric species are differences in habitat preference and differences in chromosome number (Camp 1945; Darrow and Camp 1945; Galletta 1975).

Success rates from heteroploid crosses range from moderate to very low, depending upon the species and ploidy levels involved. Possibly the most successful heteroploid cross attempted to date is *V. corymbosum* $(4x) \times$ *V. ashei* $(6x)$, or the reciprocal cross, which yields partially fertile pentaploids (Moore et al. 1964; Jelenkovic and Draper 1973; Vorsa et al. 1987). Crosses between tetraploid and diploid species yield mostly tetraploid hybrids (Sharpe and Darrow 1959), and the ease with which the cross can be made varies directly with the frequency of $2n$ gametes produced by the diploid parent. Frequency of $2n$ gamete formation varies widely among *Vaccinium* species and among clones within species (Cockerham and Galletta 1976; Megalos and Ballington 1987).

The triploid block, which prevents recovery of triploid hybrids in tetraploid × diploid crosses (Woodell and Valentine 1961), is strong in *Vaccinium*. Until recently, the only triploid reported in the genus was a naturally occurring clone of *V. vitis-idaea* L. found in Finland (Ahokas 1971). Attempts to enhance production of hybrid seedlings from tetraploid *V. corymbosum* × diploid *V. elliottii* crosses by various in vitro techniques were not successful (Munoz 1985).

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As a result of numerous attempts to cross tetraploid highbush *V. corymbosum* cultivars with the native diploid species *V. elliottii,* we obtained three vigorous triploid hybrids (Lyrene and Sherman 1983). The purpose of this study was to examine the fertility of these hybrids, particularly in crosses with hexaploid *V. ashei.*

Materials and methods

The three triploids examined in this study were obtained from a population of 300 seedlings produced by pollinating 7,000 flowers of tetraploid breeding lines from the University of Florida blueberry breeding program with pollen from the diploid wild species *V. elliottii.* About 15 different tetraploid clones were used as seed parents. The three triploid hybrids were derived from three different tetraploid parents: Fla. 78-15, Fla. 65-12 and Fla. 64-76. The three triploid clones were identified by counting chromosomes from somatic cells of 35 plants that appeared to have hybrid characteristics.

Meiosis was studied in the three triploids. In order to estimate the frequency of unreduced gametes in the three triploid clones, pollen diameter and stainability were determined by microscopic examination after staining for 1 h with acetocarmine. Considering only the well-strained pollen, the frequency of unreduced gametes was estimated using the equation

$$\frac{A+2B+C}{T},$$

where A is the number of monads, B is the number of diads, C is the number of triads and T is the total number of pollen grains examined. Fertility of the three triploid clones was estimated by crossing them with hexaploid *V. ashei* cultivars and by intercrossing and self-pollinating the triploids. F_1 seeds were extracted from mature berries, dried and refrigerated until late October, and then germinated on the surface of peat in the greenhouse. The following May seedlings were transferred to the field. Of the 165 seedlings obtained, 111 were selected as hybrids based on vegetative, flower and fruit characteristics. Flower buds from the hybrids plants were collected for chromosome counts.

Results

Chromosome associations at metaphase I were similar for the three triploid clones and included univalents, bivalents, trivalents and quadrivalents (Fig. 1). Table 1 shows the various associations observed. Anaphase I frequently showed 1–6 lagging chromosomes that were maintained through telophase I (Fig. 2). Very few lagging chromosomes were evident in anaphase II (Fig. 3).

Less than 1.5% of the pollen in each of the three triploid clones was stainable using acetocarmine. Most of the pollen was small, irregularly shaped and apparently

Table 1. Range and mean of chromosome associations in PMCs of triploid blueberry at Metaphase I

Clone	No. of cells	Chromosome associations at metaphase I			
		Univalents	Bivalents	Trivalents	Quadrivalents
80-1	15	4–9 (7.52)	5–10 (8.00)	2–5 (3.09)	0–2 (0.80)
81-19	11	5–9 (7.59)	4–10 (6.90)	3–6 (3.02)	0–2 (1.39)
82-208	25	3–8 (6.00)	5–9 (6.60)	2–6 (3.64)	0–2 (1.47)

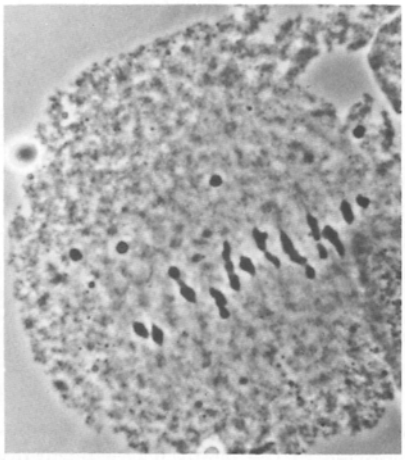

Fig. 1.

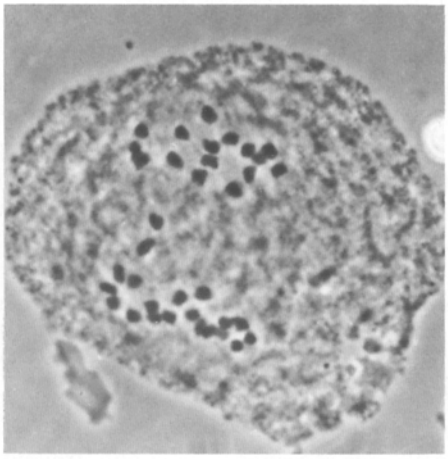

Fig. 2.

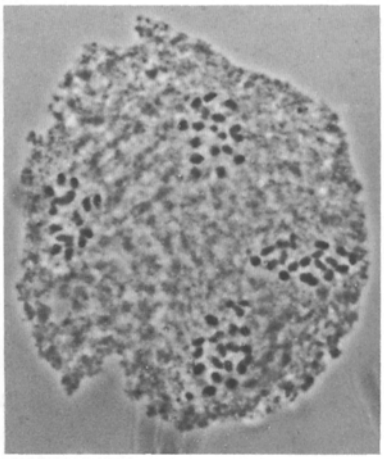

Fig. 3

Fig. 1. Meiotic metaphase I in triploid 82-208 ($2n=3x=36$) with 5 I, 4 II, 5 III and 2 IV

Fig. 2. Anaphase I in triploid 82-208 with 2 lagging chromosomes

Fig. 3. Late Anaphase II in 82-208 with no lagging chromosomes

Table 2. Frequency of sporad types and estimated unreduced gamete frequency for 3 triploid clones

Triploid clone	Total examined	No. of sporads[a] consisting of				Estimated unreduced gamete frequency
		four spores	one large stained spore	two large stained spores	one large stained spore plus two small spores	
Fla. 80-1	7,024	6,721	32	61	210	1.3%
Fla. 81-19	2,170	2,070	4	15	61	1.1%
Fla. 82-208	3,854	3,741	12	25	76	0.9%

[a] A sporad comprises the post-meiotic products of one pollen mother cell, which in *Vaccinium* are bound together as a unit

Table 3. Fertility of blueberry triploids and viability of resulting seeds and progeny

Seed parent	Pollen parent	Flowers pollinated	Fruit set (%)	Mean plump seed/fruit	No. of seedlings	No. of hybrids[a]
V. ashei cultivars (6*x*)	Fla. 82-208 (3*x*)	5,206	0.6	1.8	23	16
V. ashei cultivars (6*x*)	Fla. 81-19 (3*x*)	2,311	3.0	1.9	42	22
V. ashei cultivars (6*x*)	Fla. 80-1 (3*x*)	3,336	5.0	1.6	74	53
Fla. 82-208 (3*x*)	*V. ashei* (6*x*)	809	10.5	1.7	61	61
Fla. 81-19 (3*x*)	*V. ashei* (6*x*)	2,160	0.0	0.0	0	0
Fla. 80-1 (3*x*)	*V. ashei* (6*x*)	3,174	0.0	0.0	0	0
Fla. 82-208 (3*x*)	Fla. 81-19 (3*x*)	717	0.0	0.0	0	0
Fla. 81-19 (3*x*)	Fla. 82-208 (3*x*)	390	3.3	1.0	2	2
Fla. 81-19 (3*x*)	Fla. 80-1 (3*x*)	1,711	0.0	0.0	0	0
Fla. 80-1 (3*x*)	Fla. 82-208 (3*x*)	1,270	0.0	0.0	0	0
Fla. 80-1 (3*x*)	Fla. 81-19 (3*x*)	997	0.0	0.0	0	0
Fla. 82-208 (3*x*)	Self-pollinated	810	0.6	1.0	3	0
Fla. 80-1 (3*x*)	Self-pollinated	800	0.0	0.0	0	0
Fla. 81-19 (3*x*)	Self-pollinated	919	0.0	0.0	0	0

[a] Showed elements of parent species morphology

abortive. Each of the three triploid clones produced 0.9%–1.1% large, well-stained microspores, occurring as monads and dyads (Table 2). These were assumed to contain $2n$ or $4n$ gametes.

Fertility of these triploids, measured as percent fruit set, number of large seeds per fruit and percent seed germination, was very low in all crosses attempted (Table 3). Hexaploid *V. ashei* cultivars pollinated with pollen from the three triploids produced 0.6%–5.0% fruit set and averaged fewer than two full-size seeds per fruit (Table 3). Compared to the average fruit set percentage (46) and the average number of seed per berry (9) in hexaploid × hexaploid crosses (El-Agamy et al. 1981), fertility of hexaploid × triploid crosses was approximately 1.2% as high. Only one triploid clone, Fla. 82-208, set fruit when pollinated by hexaploid *V. ashei*, whereas the other two clones showed complete female sterility regardless of pollen source. Intercrosses among the three triploid clones and self-pollinations also failed to set seed, except Fla. 82-208. All seedlings from *V. ashei* × triploids were hexaploid, as determined by chromosome counts, whereas triploid × hexaploid, triploid × triploid, and self-pollination of triploids produced progenies with chromosome numbers ranging from 60-72 (Table 4).

Discussion

The fact that few triploids have been reported from large-scale $4x$–$2x$ crossing efforts in *Vaccinium*, despite the recovery of the fairly large number of $4x$ hybrids (Sharpe and Darrow 1959; Sharpe and Sherman 1971), indicates that the triploid block is well developed in *Vaccinium*. Selection pressure favoring the evolution of such a block would probably be high in nature due to the frequent sympatric occurrence of diploid and tetraploid *Vaccinium* species (Camp 1945; Vander Kloet 1977; Lyrene and Sherman 1980), coupled with the high degree of sterility observed in triploids. The recovery of triploid hybrids from tetraploid *V. corymbosum* × diploid *V. elliottii* crosses probably does not reflect a weakening of the triploid block with this species combination, but results instead from the very large number of flowers that were

Table 4. Distribution of chromosome number in progeny from crosses using blueberry triploids as male and female parent

Cross		No. of progeny	Chromosome no.					
Female parent	Male parent		72	71	70	69	68	60
V. ashei (6*x*)	Fla. 80-1 (3*x*)	53	51		2			
V. ashei (6*x*)	Fla. 81-19 (3*x*)	22	22					
V. ashei (6*x*)	Fla. 82-208 (3*x*)	16	16					
Fla. 82-208 (3*x*)	*V. ashei* (6*x*)	61	35	9	9	3	2	3
Fla. 82-208 (3*x*)	Fla. 80-1 (3*x*)	2	1	1				
Fla. 82-208 (3*x*)[a]	Fla. 82-208 (3*x*)	3	2		1			

[a] Manual self pollination

pollinated, along with the relatively low frequency of tetraploid hybrids produced.

The three triploids studied were similar in meiotic behavior, although there was considerable variation in seed set. The high frequency of trivalents (2–6 per meiocyte) in the three triploids suggested close homology among the three sets of chromosomes present.

Chromosome association in quadrivalents appeared to be common in the three triploids. It was not certain whether these were loose secondary associations, reported previously in blueberry (Jelenkovic and Hough 1970), or whether they were true multivalents resulting from translocation. The possibility has been raised by Ahokas (1971) and by Goldy (1983) that the basic chromosome number in *Vaccinium* might be 6 rather than 12 as has generally been assumed. If $x=6$ in *Vaccinium*, quadrivalents and higher multivalents could be expected in a 36-chromosome plant where normal pairing relationships had been disrupted by wide hybridity.

The estimated unreduced gamete frequency for the three triploid clones was near 1%, and the very low fertility of the triploids in crosses with hexaploids was surprising. Two of the three triploids (Fla. 82-208 and Fla. 80-1) shed pollen rather copiously, and stigmas of the seed parents were heavily coated with pollen. It is likely that most stigmas received at least one ($3x=36$) gamete. Therefore, it appears that $3x$ gametes from the triploids were not very efficient at fertilizing $3x$ eggs from the hexaploids or from the triploids.

Because the number of flowers pollinated was great, a fairly large number of full-size seeds was obtained. In *Vaccinium*, $6x-3x$ and $3x-3x$ crosses and $3x$ self-pollinations have not been previously reported. Chromosome number of progeny from these crosses suggest that a selective advantage exists for male gametophytes having approximately the same ploidy as the eggs. Most of the aneuploids from $3x-6x$ crosses had fewer than $2n=6x=72$ chromosomes. Evidently, female gametophytes from triploids may function even they are deficient for more than one chromosome, whereas most aneuploid male gametophytes did not function.

It is hoped that by using these triploids to bridge diploid and tetraploid species, progeny can be selected that will combine the early fruit ripening of *V. elliottii* and *V. corymbosum* with the large berry size of *V. corymbosum* and *V. ashei*, and the high vigor and heat tolerance of *V. elliottii* and *V. ashei*. Studies on inheritance of these important characteristics are now underway.

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