Derivatives of *Vaccinium arboreum* x *Vaccinium* Section *Cyanococcus*. II. Fertility and Fertility Parameters

Sylvia J. Brooks and Paul M. Lyrene

Department of Horticultural Sciences, University of Florida, Gainesville, FL 32611

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ABSTRACT. Fertility of F_1 hybrids and their open-pollinated progeny was studied for the intersectional cross *Vaccinium darrowi* Camp x *V. arboreum* Marsh as part of a project to determine the feasibility of using *V. arboreum* to breed vigorous, drought-tolerant southern highbush blueberry cultivars. The 16 F_1 hybrids that were studied were vigorous but very low in fertility. Second generation hybrids [MIKs (mother is known) obtained by open-pollination of the F_1 s] and MIK derivatives were extremely variable in vigor and fertility, but averaged far higher in fertility than the F_1 s as evidenced by pollen stainability and amount of pollen produced. F_1 s produced an average of 0.4 seedlings per 100 pollinated flowers when hand-pollinated in a greenhouse with pollen from *V. darrowi*, 0.2 when pollinated by *V. arboreum* and 3.4 when pollinated by cultivated highbush. Some MIKs that were crossed with other MIKs and with cultivated southern highbush were very high in male and female fertility. Female fertility was estimated in greenhouse crosses from fruit set, berry weight, number and weight of seeds, number of plump seeds per berry, and number of seedlings obtained. Male fertility was estimated by pollen stainability with acetocarmine and amount of pollen shed. Chromosome counts showed that three F_1 s were diploid and that four fertile MIKs were tetraploid. One MIK appeared to be aneuploid. Aneuploidy may explain much of the low fertility found in MIK populations. These results indicate that good progress is being made in returning the hybrid plants to cultivar quality in only a few generations of backcrossing.

Vaccinium arboreum Marsh ($2n = 2x = 24$), the sparkleberry, is in the same genus as cultivated blueberries ($2n = 4x = 48$), but is in a different section (*Batodendron*). The sectional or subgenus level is the point at which genetic differences that greatly interfere with hybridization become obvious in *Vaccinium*. Most intersectional hybrids are weak, and if they survive to maturity are usually sterile. However, with some intersectional combinations, if enough parental combinations are tried, it is often possible to generate a few hybrids that are vigorous and fertile enough to be useful (Ballington, 1990; Luby et al., 1990; Lyrene and Ballington, 1986).

Introgression of sparkleberry traits into cultivated highbush (HB) blueberries (cultivated taxon based largely on *V. corymbosum* L., which contains genes from other *Vaccinium* species) is desirable for many reasons, the most prominent being greater soil tolerance. Cultivated blueberries require a soil that is high in organic matter and has an acidity of pH 3.5 to 5.5. Sparkleberries will grow well on soils that are low in organic matter and have a pH as high as 6.2, as well as on standard blueberry soils. Sparkleberries are highly vigorous and upright in growth habit. They flower late and avoid most spring freezes that damage flowers and fruit on blueberry cultivars. Sparkleberry introgression should be useful in breeding late-ripening blueberry cultivars. In Florida, southern HB blueberry cultivars ripen in April and May, rabbiteye blueberry cultivars (*V. ashei* Reade) in June and July, and sparkleberries from September through November.

In 1981, Lyrene generated F_1 hybrids from intersectional crosses of *V. darrowi* x *V. arboreum* (Lyrene, 1991). Many of the hybrids grew vigorously in the field, but produced few fruit. Field observations of the F_1 plants indicated that they ranged from completely sterile to very low in male and female fertility. Considering the

large size of the plants and their copious flowering, very few berries were produced. The berries that were produced averaged less than one well developed seed per berry (Brooks, unpublished data). It was also observed that very little if any pollen was being produced.

Open-pollinated berries from the F_1 plants were harvested and the seeds were planted. The resulting generation was designated as MIK (mother is known). Field observations indicated that the MIKs ranged widely in male and female fertility, from sterile to highly fertile. Their physical appearance and the restoration of fertility suggested that the MIKs were tetraploid rather than diploid like their maternal parent. Morphological analysis indicated that this was indeed the case (Brooks and Lyrene, companion article). It was established that the most likely pollen parent of the MIKs was tetraploid HB (Brooks, 1996).

The purpose of this study was to estimate the female and male fertility of the F_1 s, MIKs and several MIK derivatives. To be commercially useful, any cultivars developed as a result of introgressing *V. arboreum* genes into HB blueberry would have to be highly fertile in both pollen and seed. A second objective was to gather further information on the probable pollen parent of the MIKs. This was done by determining the chromosome numbers of several fertile MIKs and their maternal parents and by trying to produce plants that resembled MIKs by hand pollinating their maternal parents with pollen from various sources in the greenhouse. Knowing how the MIKs arose might allow the production of better-quality MIKs by careful choice of the pollen parent.

Materials and Methods

GREENHOUSE CROSSING STUDIES. Plants used in greenhouse crosses were dug from the Horticultural Unit at the University of Florida, Gainesville in December 1991, 1992, and 1993. These plants included southern HB, *V. darrowi*, *V. arboreum*, F_1 (*V. darrowi* x *V. arboreum* hybrids), MIKs, and MIK derivatives. Throughout this paper, plants referred to as HB or southern HB were clones or selections bred largely from northern HB cultivars

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introgressed with genes from *V. darrowi*, followed by many generations of recurrent selection for high fruit quality and warm-climate adaptation. The MIKs used in this study were obtained by open pollination of five F_1 *V. darrowi* $\times$ *V. arboreum* plants that had been transplanted to the blueberry breeding field plots in Gainesville, Fla., in 1982. In the field, the F_1 hybrid parents of the MIKs were highly vigorous and flowered heavily each year, but produced only a small amount of open-pollinated fruit. Most of their berries contained only one seed. Evidence in this paper indicates that the most fertile MIKs were produced by union of an unreduced megagamete from the diploid F_1 seed parent with a normal microgamete from tetraploid southern HB selections growing nearby in the same field. The MIKs and MIK derivatives used in this study were selected to be parents based on having had high fruit set in seedling nurseries, in the field, the previous spring. MIKs that appeared to be sterile or very low in fertility, which made up more than half of the MIK population, were not used in the crossing experiments. The plants were potted in peat and placed in a cooler at $\approx 5^\circ\text{C}$ for a minimum of 1 month to satisfy their chilling requirements. This tended to synchronize blooming, and made it possible to cross plants with different chilling requirements. Following the chilling period, plants were placed in a bee-proof greenhouse where the temperature was maintained between 5 and 27°C .

Some plants were large enough to use in two or three types of crosses. These were divided into sections with approximately equal numbers of flower buds per section. Crosses were randomly assigned to sections. In some cases, it was necessary to use more than one ramet of a clone for multiple crosses. On the day of pollination, the corolla and anthers were removed. Freshly gathered pollen was applied to the stigma from the thumbnail. The number of flowers pollinated varied for each cross. Before pollination, the male parent was checked for pollen stainability. This was to ensure that low fruit set would not be due to inviable pollen. Due to pollen stainability counts of 0 for the F_1 s used in this study, no $F_1 \times F_1$ crosses were made.

Ripe berries were harvested daily and stored at 5°C . Data were collected for various crosses on fruit set, total seed weight, large seed weight, berry weight, number of plump seeds, and number of seeds germinated.

After harvest was complete, the berries were weighed. In 1993 and 1994, the number of plump seeds per berry were counted. This was done by squashing individual berries on paper towels. The seeds were left to dry overnight on a laboratory bench top. The next day, plump seeds were separated from nonplump seeds and counted. This was done for 30 berries from each cross. The remaining berries were also squashed individually to see if plump seeds were present, but the seeds were not counted. The 30 largest plump seeds were separated from other seeds for each cross, divided randomly into 2 replications of 15 and weighed. In 1992, seeds were removed using a food blender. Seeds were dried overnight on a paper towel in the laboratory. All seeds were weighed and stored.

Seeds were planted in December 1992, 1993, and 1994. The night before planting, seeds were soaked in 10.4 mM gibberellic acid for ≈ 12 h (Dweikat and Lyrene, 1989). Seeds from each cross were sown in a pot of peat. Top diameter of the pots was 10 cm. The pots were placed in a greenhouse where the temperature was maintained between 5 and 27°C . A mist system was used for watering. In February, the seedlings were counted and a maximum of 96 from each cross were transferred to flats. The flats were maintained in a greenhouse until early May. The seedlings were then transplanted into a high-density nursery at the Horticultural Unit at the University of Florida, Gainesville.

CYTOGENETICS. Vegetative apical meristem tips are the best material for examination of somatic chromosomes in blueberry. Actively growing shoot tips were collected and fixed in 3 absolute alcohol : 1 glacial acetic acid for 24 h. The fixative was then replaced with 70% ethanol and shoot tips were stored at 5°C .

Before examination, the ethanol was removed by soaking the shoot tips in water. At this time each shoot tip was trimmed close to the meristem. Shoot tips were then soaked in 1 N HCl for 30 min after which they were rinsed in water. Another soak followed in a pectinase-cellulase solution for ≈ 24 h. The solution consisted of 40 units pectinase and 40 units cellulase in 1 mL of water. At the end of the soaking period, the shoot tips were blotted on a paper towel, rinsed in water and blotted again. Shoot tips were then transferred to a drop of 1% acetocarmine stain (1 g carmine boiled in 100 mL 45% acetic acid) for 1 min and blotted. Tissue was transferred to a microscope slide, and a small drop of 1% acetocarmine was added. The tissue was macerated with an iron needle (iron aids the staining process). The debris was removed and a coverslip applied. The slide was heated gently over a flame for ≈ 1 min. The coverslip was tapped with a pencil eraser and pressure was applied with a paper towel between the slide and the thumb. To destain, 45% acetic acid was introduced under the cover slip, the slide was heated again, and pressed to expel the dye. Destaining was repeated three times. A phase contrast microscope (Leitz) was used to observe the chromosomes at a $1000\times$ magnification.

TOTAL SEED WEIGHT. Fifty berries resulting from open-pollination were randomly harvested from each of 20 different genotypes of each of the following taxa: HB, *V. darrowi*, *V. arboreum*, and the MIKs. All plants, except *V. arboreum*, were located at the Horticultural Unit, University of Florida, Gainesville. The *V. arboreum* plants were located in a forest 10 km north of High Springs, Fla. Only four F_1 s produced enough berries for this study. The *V. darrowi* berries were harvested from 12 to 30 May 1994. HB berries were picked on 27 Apr. 1994. *Vaccinium arboreum* berries were all harvested on Oct. 19, 1994. The F_1 s were harvested 1 June through 26 July 1994.

The character measured in this study was total seed weight of 50 berries. The seeds were removed from the berries using a food blender. The seeds were dried overnight on a laboratory bench and weighed the next morning. The *V. arboreum* berries contained sclerids that aggregated into large clumps. These were removed with a #25 sieve (0.713 mm) before the seeds were weighed.

POLLEN. Flowers were collected to use in pollen studies from plants located in the high-density nursery. These included HB, MIKs, *V. darrowi*, *V. arboreum*, MIK derivatives, and F_1 s. Collections took place February through April 1992. Flowers collected for pollen were stored in open glass vials until examination.

To determine if pollen was viable, it was stained with 2% acetocarmine glycerine jelly (Radford et al., 1974). This medium is liquid in bulk at room temperature, but on a slide becomes a jelly. The viscous nature of the jelly prevents the lighter, empty pollen grains from being pushed to the outer edges (often a problem with aqueous solutions) when the cover slip is applied. This allows for a more random distribution of normal and empty pollen grains (Radford et al., 1974). Pollen staining was used as a measure of male fertility.

Pollen was removed from the flowers by gently twirling the flower over a drop of the 2% acetocarmine glycerine jelly on a slide. Pollen from two flowers was mixed together for each clone. Each genotype was rated for the amount of pollen shed using an 6-point scale. The scale ranged from 0 to 5, with 0 representing no pollen and 5 representing a copious amount of pollen. The slides were stored in the refrigerator for 48 h before microscopic examination.

This allowed time for the pollen grains to absorb the dye. To prevent desiccation, the slides were placed in a metal pan with a damp paper towel inside a sealed plastic bag.

Pollen was examined at 400× on a Leitz light field microscope. Pollen grains that were plump and well stained were scored as viable. Grains that were granular, not stained, poorly stained, or shrunken were scored as inviable. Two-hundred grains per clone were scored. If a tetrad was positioned so that one of the grains was not clearly visible, the grain was not scored.

Results and Discussion

Crossing studies

The means for the data collected from the controlled crosses were separated using least squares mean with a Tukey-Kramer adjustment. This is the most appropriate mean separation technique when there is a difference in the number of plants used per treatment (Hochberg, 1987). Even with the use of this technique, many data sets in which the means differed substantially still indicated no significant differences among the means ($P = 0.05$). This situation is common when there are large differences in the number of plants per treatment, as is the case here.

CROSSES WITH *F₁* *V. darrowi* × *V. arboreum* HYBRIDS AS THE FEMALE PARENT. The fruit set of the F_1 × HB crosses was 20× greater and significantly different ($P = 0.04$) from fruit set on the F_1 × *V. arboreum* and the F_1 × *V. darrowi* crosses (Table 1). The fruit set for the 7 F_1 × *V. arboreum* crosses ranged from 0% to 5.1%. Only one cross out of four in the F_1 × *V. darrowi* crosses produced any fruit, 4.6% fruit set. The percent fruit set for the F_1 × HB crosses ranged from 2.8% to 67.9%.

Mean berry weight did not differ significantly for the three crosses (Table 1), but mean berry weight for the cross using HB pollen was ≈2× greater than the mean for the crosses using *V. arboreum* and *V. darrowi* pollen. Many factors influence berry weight, including the genetic constitution of the mother plant for berry size, the number of viable ovules present, availability of genetically compatible pollen, and the number of viable seeds. Since all the F_1 clones that were used in crosses produced berries with seeds in at least one of the crosses, it is reasonable to assume that all of these F_1 s produced some viable ovules. The most likely

reason here for the differences in berry weight resulting from different pollen sources was variation in the number of viable seeds produced.

Over all, in crosses in which the F_1 s were the female parent, the mean seed weight per berry was positively correlated with mean berry weight (Table 1). The crosses using *V. darrowi* pollen had the lowest mean berry weight and the lowest mean seed weight. The crosses with HB pollen had the highest mean berry weight and the highest mean seed weight. The crosses with *V. arboreum* pollen were between the other two crosses for mean berry weight and mean seed weight. The mean berry weight for the F_1 × HB cross was close to the mean berry weight found for open-pollinated F_1 berries (Brooks and Lyrene, 1997).

The number of seedlings per 100 pollinated flowers is the best measure of success of a cross (Galletta, 1975). This character takes into account the fertility of the parents and the ability of the parental genotypes to work together to produce a viable seedling. The F_1 (*V. darrowi* × *V. arboreum*) × HB crosses produced an average of 3.4 seedlings per 100 pollinated flowers (Table 1). This number was much larger than for the crosses in which the F_1 was crossed with *V. arboreum* and *V. darrowi*, 0.2 and 0.4 respectively.

The crosses with HB pollen produced significantly more plump seeds per 100 pollinated flowers ($P = 0.01$) and more mg seed per 100 pollinated flowers ($P = 0.02$) than the other two crosses (Table 1). It seems reasonable that the number of plump seeds per 100 pollinated flowers or berries and the mg of seeds per 100 pollinated flowers or berries would give the same estimate of crossing success as the number of seedlings per 100 pollinated flowers. This however was not always true. In this case, the *V. arboreum* cross produced twice as many plump seeds per 100 pollinated flowers as the *V. darrowi* cross. The opposite was true for these two crosses for seedlings per 100 pollinated flowers and mg seed per 100 pollinated flowers; the figures for *V. darrowi* were twice those for *V. arboreum*. These data indicated that many plump seeds were inviable and that the number of plump seeds per 100 pollinated flowers was not a good estimate of cross success for these wide hybrids. A prime example occurred when the F_1 85-131 was pollinated by 'Avonblue' (a HB cultivar). This cross produced 192.7 plump seeds per 100 pollinated flowers but only 14.3

Table 1. Summary of fertility data (mean^c, with range in parentheses) collected from F_1 (*Vaccinium darrowi* × *V. arboreum*) controlled crosses made in 1992, 1993, and 1994.

Cross	n ^d	Pollinations (no.)	Fruit set (%)	Seedlings/100 pollinated flowers (no.)	Berry wt (g)	Seeds/ berry (mg)	Plump seeds/100 pollinated flowers (no.)	Plump seeds/ berry (no.)	Seeds/100 pollinated flowers (mg)
F_1 × Arb ^x	7	521.7 (475–645)	1.3 b (0–5.1)	0.2 ^{ns} (0–0.8)	0.13 ^{ns} (0.11–0.18)	1.8 ^{ns} (0.6–4.3)	1.4 b ^w (0–5.8)	1.0 b ^w (0–3.0)	1.2 b ^v (0–4.4)
F_1 × Dar ^z	4	500 (500–500)	1.2 b (0–4.6)	0.4 (0–1.4)	0.08 (0–0.08)	0.6 (0–0.6)	0.7 b (0–2.9)	0.2 b (0–0.6)	0.7 b (0–2.7)
F_1 × HB ^t	11	656.7 (28–1363)	20.8 a (2.8–67.9)	3.4 (0.3–14.3)	0.24 (0.13–0.35)	2.9 (0.2–7.2)	70.4 a (13.2–192.7)	3.3 a (0.9–4.7)	51.0 a ^v (0.6–142.5)

^aMean separation within columns by least square means with a Tukey-Kramer adjustment on log transformed data, $P = 0.04$. Actual means presented here.

^dNumber of crosses.

^x*Vaccinium arboreum*.

^w $P = 0.01$.

^v $P = 0.02$.

^z*Vaccinium darrowi*.

^tHighbush.

^{ns}Nonsignificant at $P = 0.05$.

Fig. 1. Fruiting MIK (mother is known; open-pollinated progeny of *F₁* *Vaccinium darrowi* × *V. arboreum* hybrids) showing characteristically long pedicels and peduncles.

seedlings per 100 pollinated flowers (data not shown).

MIK AND MIK DERIVATIVE CROSSES. The MIKs (Fig. 1) were used as female parents in two types of crosses, MIK × MIK and MIK × HB. One (MIK × MIK) clone was crossed with HB. Five (MIK × HB) clones were backcrossed to HB. Ten HB × HB crosses were made to have a standard of comparison for the MIK and MIK derivative crosses.

In 1992, seven MIK × MIK and MIK × HB crosses had fruit sets >100% (Brooks, 1996). It was later determined that these exceptionally high fruit sets were probably the result of environmental conditions in the greenhouse that year. Fruit sets of >100% were not obtained again in the following years. For the purposes of this paper, the fruit sets of these crosses were adjusted to 100%. Three of the five (MIK × HB) × HB crosses had percent fruit sets within the range of the HB × HB crosses (data not shown). The ranges of fruit set for the MIK and MIK derivative crosses was large (Table 2). Both types of crosses produced progeny with very high percent

fruit sets and with very low percent fruit sets. By comparison, the HB × HB percent fruit sets had no extreme lows and were all clustered between 65% and 100%.

Mean berry weight increased as more HB genes were incorporated into the hybrid plants (Table 2). There was no significant difference ($P=0.01$) between the MIK × MIK, MIK × HB and (MIK × MIK) × HB crosses in mean berry weight. There was, however, a trend toward increasing mean berry weight from 0.71 to 0.88 to 1.00 g. The (MIK × HB) × HB crosses were significantly different ($P=0.01$) from the other MIK crosses, but not from the HB × HB crosses. Although not statistically different from the HB × HB mean berry weight, the (MIK × HB) × HB mean berry weight was still too low for commercial standards. Individual plants however, had berries large enough to meet cultivar standards (data not shown). The data indicate that good progress is being made

toward increasing the berry size of MIK derivatives after only a few generations of backcrossing.

Plump seeds per 100 pollinated flowers, seed weight per berry, number of plump seeds per berry and mg seed per 100 pollinated flowers are all estimates of female fertility. There was no significant difference ($P=0.05$) between the MIK × MIK, MIK × HB, (MIK × MIK) × HB and HB × HB crosses for mean milligrams seed per berry and mean milligrams seed per 100 pollinated flowers (Table 2). The (MIK × HB) × HB means for these two characters were less than half that of the other taxa, but this may have been due to sampling error. In general, plants that produce more seed by weight are more fertile than similar plants that produce less. It was shown in the companion article that individual seed weight of the hybrid taxa was much larger than that of southern HB, probably due to genes for large seeds deriving from the sparkleberry (Brooks and Lyrene, 1997). Because of this, mg of seed per berry and mg of seed per 100 pollinated flowers were not very useful in estimating female fertility, because they confounded variations in the number and the weight of viable seed produced.

There were no significant differences ($P=0.05$) between any of the crosses for mean number of plump seeds per berry and mean

Table 2. Summary of fertility data (mean, with range in parentheses) collected from controlled *Vaccinium* crosses made in 1992, 1993, and 1994.

Cross	n ²	Pollinations (no.)	Fruit set (%)	Seedlings/100 pollinated flowers (no.)	Berry wt (g)	Seeds/ berry (mg)	Plump seeds/100 pollinated flowers (no.)	Plump seeds/ berry (no.)	Seeds/100 pollinated flowers (mg)
MIK ³ × MIK	10	278.2 (183–301)	66.7 ^{NS} (27.3–100)	83.7 ^{NS} (0–218.0)	0.71 b ⁴ (0.47–1.01)	12.8 ^{NS} (1.1–28.5)	312.4 ^{NS,w} (290.0–334.81)	6.1 ^{NS,w} (6.0–6.1)	1170.9 ^{NS} (27.6–3234.9)
MIK × HB ^w	15	237.9 (112–311)	70.3 (6.4–100)	85.6 (0–268.0)	0.88 b (0.46–1.38)	14.1 (1.8–31.9)	1081.1 ^v (158.4–2419.0)	13.3 ^v (2.2–29.0)	1153.6 (65.9–2700.0)
(MIK × MIK) × HB	1	200	93.0	68.0	1.00 ab	12.2	995.1	10.7	1136.2
(MIK × HB) × HB	5	200 (200–200)	69.0 (29–95.5)	44.0 (7.5–113.3)	1.49 a (0.64–1.94)	4.9 (2.3–7.2)	719.0 (201.6–1317.9)	9.8 (3.2–13.8)	352.6 (119.9–598.5)
HB × HB	10	99.3 (78–114)	81.4 (67.6–98.7)	230.5 (39.2–627.2)	1.73 a (1.15–2.40)	13.1 (4.0–21.3)	1991.4 (522.3–5316.0)	23.8 (7.7–65.61)	1081.9 (272.7–2098.1)

²Number of crosses.

³Open-pollinated progeny of *F₁* hybrids.

⁴Mean separation within columns by least square means with a Tukey-Kramer adjustment on log transformed data, $P=0.04$. Actual means presented here.

^wHighbush.

^{NS}Nonsignificant at $P=0.05$.

Table 3. Chromosome counts for plants representing the F₁ (*Vaccinium darrowi* x *V. arboreum* hybrids) and MIK (open-pollinated progeny of F₁ hybrids) taxa.

Plant	Taxon	Chromosome no.
85-127	F ₁	24
85-134	F ₁	24
85-130	F ₁	24
93-135	MIK	<48
93-128	MIK	48
91-333	MIK	48
93-136	MIK	48

Table 4. Mean total seed weight from 50 berries of the 5 *Vaccinium* taxa used in this study; 50 berries were harvested from each of 20 plants in each taxon.

Taxon	Plants (no.)	Total seed wt (mg) of 50 berries
<i>V. darrowi</i>	20	498 ab ^z
<i>V. arboreum</i>	20	366 b
Highbush	20	878 a
F ₁ ^y	5	67 c
MIK ^x	20	783 a

^zMeans within columns separated by least square means with a Tukey-Kramer adjustment on transformed data, $P = 0.01$. Actual means are presented here.

^y*Vaccinium darrowi* x *V. arboreum* hybrids.

^xOpen-pollinated progeny of F₁ hybrids.

number of plump seeds per 100 pollinated flowers (Table 2). However, there was a general trend toward increasing seediness for both characters as more HB genes were added to the hybrid taxa through backcrossing. Approximately twice as many plump seeds per berry were produced by the MIK x HB crosses as the MIK x MIK crosses. The HB x HB crosses produced approximately twice as many plump seeds per berry as the MIK x HB crosses. The number of plump seeds per 100 pollinated flowers followed the same trend.

As mentioned before, the number of seedlings produced is the best measure of the success of a cross. The MIK x MIK and MIK x HB crosses produced similar numbers of seedlings, averaging 83.7 and 85.6 seedlings per 100 pollinated flowers respectively (Table 2). The single (MIK x MIK) x HB cross produced 68 seedlings per 100 pollinated flowers. The (MIK x HB) x HB crosses produced an average of 44.0 seedlings per 100 pollinated flowers (Table 2), which was half that of the MIK x MIK and MIK x HB crosses.

Cytogenetics

Chromosomes were counted for three *V. darrowi* x *V. arboreum* F₁s: 85-127, 85-134, and 85-130. A minimum of two separate counts of 24 chromosomes were made for each clone (Table 3), indicating that these clones were diploid as expected.

Chromosome counts were made for four MIKs that were chosen for their high fertility. Forty-eight chromosomes were counted for each of three MIKs: 93-128, 91-333, and 93-136. These MIKs were tetraploid as was suggested by a comparison of their morphology to the F₁ parents. Seven counts were made for clone 93-135. Chromosome counts for this clone ranged from 42 to 47. The variable counts may have been due to no perfect cells being found to count, or the clone may have been mixaploid. Aneuploidy may explain some of the low fertility in the MIKs.

Total seed weight

The total seed weight of 50 berries was determined for 20 plants in each of the following taxa: *V. darrowi*, *V. arboreum*, southern HB, F₁s, and the MIKs (Table 4). Only five of the F₁ clones produced enough berries for this study. Although seed weight per berry is sometimes used as an indicator of female fertility, its value in this study was reduced by the fact that individual seeds were much larger for *V. arboreum* than for HB and *V. darrowi* (Brooks and Lyrene, 1997). Southern HB and *V. darrowi* typically have many small seeds, whereas *V. arboreum* has fewer seeds that are much larger. The F₁s, MIKs, and MIK derivatives inherited the large seed size of *V. arboreum*. Total seed weight of 50 berries would be a good index of fertility only if there was no difference in seed size.

The mean total seed weight of 50 berries for the F₁s, 67 mg, was significantly different ($P = 0.01$) from the *V. arboreum* parent, 366 mg (Table 4). Even though individual seeds were much larger in the F₁ hybrids than in the *V. darrowi* parent, total seed weight per 50 berries was much higher for *V. darrowi* due to low seed numbers in the F₁s. The total seed weight was much higher for MIKs, 783 mg, than for their F₁ parents, 67 mg. The difference in seed size of the MIKs and southern HB makes a comparison of total seed weight difficult to interpret. Despite the confounding factor of seed size and the limitations it imposes, these data show a drastic decrease in female fertility of the F₁ hybrids compared to both the *V. darrowi* and the *V. arboreum* parent. They also demonstrated a return to much higher fertility levels in the next generation, the MIKs.

Pollen

Pollen stainability is a technique used to investigate male fertility. As expected, the F₁s had the lowest mean pollen stainability at 0.9% (Table 5). In addition to low stainability, most of the pollen grains were shrunken (Fig. 2). The MIKs and (MIK x MIK) clones did not differ significantly ($P = 0.01$). These 2 taxa showed a large increase in mean pollen stainability (Table 5 and Fig. 3) over the F₁s. All of the hybrid taxa, except the F₁s, contained individuals that had percent pollen stainabilities in the nineties. There was no significant difference ($P = 0.01$) between *V. arboreum*, *V. darrowi*, southern HB and the (MIK x HB) taxa. This indicates

Fig. 2. Shrunken, nonstaining (2% acetocarmine) pollen grains characteristic of F₁ (*Vaccinium darrowi* x *V. arboreum*) hybrids.

Table. 5. Mean pollen stainability and amount of pollen shed from *Vaccinium* species and hybrids.

Taxon	Plants (no.)	Pollen stainability (%)	Pollen amount ^z
Highbush	20	87.6 a ^y	4.2
<i>V. arboreum</i>	20	78.3 a	2.7
<i>V. darrowi</i>	20	84.9 a	4.2
(MIK ^x x HB ^w)	111	75.1 a	3.2
(MIK selfed)	16	48.8 b	2.4
(MIK x MIK)	56	45.8 b	2.5
MIK	64	43.7 b	2.5
F ₁ ^v	15	0.9 c	1.7

^zScored on a scale of 0–5, with 0 being no pollen and 5 being a copious amount of pollen.

^yMeans within columns separated by least square means with a Tukey-Kramer adjustment on transformed data, $P = 0.01$. Actual means are presented here.

^xOpen-pollinated progeny of F₁ hybrids.

^wHighbush.

^v*Vaccinium darrowi* x *V. arboreum* hybrids.

a return to normal male fertility levels for the MIK backcrosses to southern HB in only two generations.

The amount of pollen produced by a plant is also a measure of male fertility. The amount of pollen was rated on a scale of 0 to 5, with 0 indicating no pollen and 5 indicating a copious pollen flow. The F₁s produced the least pollen, averaging 1.7 on the pollen-abundance rating scale used (Table 5). All of the F₁ clones produced at least a small amount of pollen. The amounts ranged from 1 to 3, with 1 being a very low amount of pollen and 3 being a medium amount of pollen. Not only was the average amount of pollen produced very low, but also most pollen grains were inviable as indicated by nonstaining. The mean amount of pollen for the MIKs and (MIK x MIK) taxa was greater than the F₁s and less than the (MIK x HB) taxa. HB and *V. darrowi* had the highest mean amount of pollen. Surprisingly, *V. arboreum* had a lower mean amount of pollen than the other nonhybrid taxa, and it was close to that of the MIKs and (MIK x MIK) taxa.

Summary

One possible reason for the low fertility of many MIKs is aneuploidy. The chromosome counts of MIK clone 93-135 showed that aneuploidy was probably present in the MIK population. Another possible explanation for low fertility could be ploidy level. Both *V. darrowi* and *V. arboreum* are diploid and their F₁ hybrids are presumed to be diploid as well. During the bloom time of the F₁ hybrids, possible pollen sources included diploids, tetraploids, and hexaploids. Thus, the MIK population could have included monoploid, diploid, triploid and pentaploid plants as well as tetraploids. Because this study was done using MIKs selected for high fertility, tetraploid MIKs were probably favored over any monoploids, triploids or pentaploids that may have been present.

The F₁ crosses with southern HB were much more successful than the F₁ crosses with *V. arboreum* and *V. darrowi* for every character measured. Mean percent fruit set was 20× greater

for the HB crosses than for the other 2 types of crosses. The F₁ x HB crosses produced 8.5× more seedlings than the F₁ x *V. darrowi* crosses and 17× more seedlings than the F₁ x *V. arboreum* crosses.

Normally, in *Vaccinium*, homoploid crosses give better results than heteroploid crosses (Galletta, 1975). As shown above, the crosses using the diploid F₁ hybrids gave the opposite result, since pollination with *V. darrowi* and *V. arboreum* (which gave low success) constituted homoploid crosses and pollinations with tetraploid HB (which gave more success) were heteroploid crosses. The most likely explanation for this anomaly lies in the wide-hybrid background of the diploid F₁ seed parents. Even though these plants were diploid, they apparently produced few or no viable monoploid megagametes, probably due to abnormal meiosis. On the other hand, any unreduced gametes formed during meiosis could be viable because of having a full complement of genes from *V. arboreum* and *V. darrowi*. Thus, even though the F₁ *V. darrowi* x *V. arboreum* hybrids were probably diploid, most of their viable gametes may have been 2x or near 2x.

Male and female fertility of the MIK derivatives increased with increasing amounts of southern HB in the parentage. Mean percent fruit set, mean number of seedlings per 100 pollinated flowers, mean berry weight, mean pollen stainability and mean amount of pollen all increased through this series of crosses. None of the populations as a whole were as high as the southern HB crosses in any of the fertility indicators measured. The closest to an exception was mean pollen stainability for the (MIK x HB) taxa, which was not statistically different ($P = 0.01$) from the HB x HB crosses. Yet, every taxon had individual plants that were within the ranges established by the HB x HB crosses as normal fertility levels.

It was shown here that mg of seed per berry, number of plump seeds per 100 pollinated flowers, number of plump seeds per berry, and milligrams of seed per 100 pollinated flowers were not good estimates of female fertility. The characters that involved weight measurements of seeds were confounded by the difference in seed size between the MIKs, MIK derivatives and HB. The number of plump seeds per 100 pollinated flowers might be expected to have a direct correlation with number of seedlings per 100 pollinated flowers. This, however, did not appear to be true for any of the taxa, even HB. Only about an eighth of the plump HB seeds germinated.

Fig. 3. Plump, stained pollen grains found in the fertile MIKs (mother is known; open-pollinated progeny of the F₁ *Vaccinium darrowi* x *V. arboreum* hybrids).

It should be noted that a 12.5% germination rate for HB was lower than what has been reported in the literature. A germination rate of 35% (Lyrene, 1988) is closer to normal. This may indicate that not all viable seeds germinated in the test year. However, since all seeds were subjected to the same environment, comparisons should still be valid.

There was a dramatic increase in fertility from the F_1 s to the MIKs. The increase in female fertility was shown in the large increase in total seed weight for 50 berries, percent fruit set, berry weight, and number of seedlings per 100 pollinated flowers. There was also a dramatic increase in milligrams of seed per berry, number of plump seeds per 100 pollinated flowers, number of seeds per berry and milligrams of seed per 100 pollinated flowers. Since the morphological study reported in the companion article (Brooks and Lyrene, 1998) showed no significant difference between seed size for the F_1 s and MIKs, characteristics involving seed weight were not confounded by seed size. Thus, fertility comparisons involving seed weight were valid in this instance.

Male fertility also increased dramatically from the F_1 s to their MIK progeny as evidenced by pollen stainability and amount of pollen produced.

The dramatic increase in fertility along with increased flower and leaf size was a good indicator that the MIKs are tetraploid, rather than diploid like their F_1 parent. This hypothesis was supported by the chromosome counts made on several MIKs.

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