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Production of Dwarfs in Rabbiteye Blueberry (*V. virgatum* Aiton) Crosses

Mark K. Ehlenfeldt

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Abstract

Dwarf phenotypes segregated from an interploid 6*x* × 5*x* blueberry cross of 'Montgomery' (rabbiteye) × US 1771 [highbush × (rabbiteye × *V. constablaei*)]. To evaluate inheritance, several dwarf segregants were used as pollen sources onto both rabbiteye cultivars and species-introgressed rabbiteye hybrids. Segregation ratios of normal to dwarfs in offspring populations ranged from 6.2:1 to 8.8:1. The dwarfism appears to follow a generally recessive model; however, a limited number of dwarf × dwarf crosses segregated both normal and dwarf types, suggesting that epistatic interactions are involved. A dwarf segregant from the 'Montgomery' × US 1771 cross, US 2194-A, appears to have value for reliably generating dwarf phenotypes across a range of 6*x* material and may be useful in generating reduced-stature plants for both commercial production and for ornamental uses.

Dwarfs are not uncommon in breeding populations in species of all sorts. They typically occur at low, but variable frequencies. They may be viewed simply as oddities of modest interest, but they have the potential to be of significant value if they can modify morphology in beneficial ways that maintain or enhance productivity. The most notable such examples are the dwarf cereals of the "Green Revolution" in which modification of stature succeeded in changing plant phenotype as well as repartitioning resources such that yields and harvestability were dramatically improved (Hedden, 2003; Lumpkin, 2015). These dwarfs, which had mutations of GA regulation, expressed reduced internode lengths and increased tillering (branching) (Brian, 1959; Richards et al., 2001).

Dwarfs are also of potential interest as novelties for ornamental and landscape purposes. An increasing number of ornamentals and vegetables are being developed as patio, bush, or space-saver cultivars, to allow the consumer to grow plants previously considered too space consuming for typical backyard gardens (Bookman, 1987; Grant, 2018).

In highbush blueberry, several dwarf or semi-dwarf selections have been developed and released as ornamentals: 'Tophat' (Moulton et al., 1977), 'Cutie Pie'™ (NeSmith, 2017) and BrazelBerry® selections (zu Jeddeloh Pflanzen, 2018), and there are additional selections under development. In highbush blueberry, Draper et al. (1984) noted that dwarf segregation appeared to follow a continuous distribution in F_2 populations and did not follow any apparent single gene locus model.

Notably, in rabbiteye blueberry, two semi-dwarf types have been released as production cultivars: 'Ethel' is a semi-dwarf version of 'Satilla' (Brooks and Olmo, 1997) and 'De Soto' (Stringer et al., 2006) is also considered a semi-dwarf. Productivity ratings for 'De Soto' show its productivity as equivalent to 'Brightwell' and 'Tifblue', two commercial rabbiteye production cultivars. Such dwarfs also have a distinct advantage over rabbiteye blueberry which can have a normal stature that may exceed 3 meters in height. Dwarfs of rabbiteye blueberry may have their cane

USDA-ARS, Genetic Improvement of Fruits and Vegetables Laboratory, at P.E. Marucci Center for Blueberry and Cranberry Research & Extension, Chatsworth, NJ 08019. Email: Mark.Ehlenfeldt@usda.gov

growth reduced to a more easily manageable size, approaching that of highbush blueberry. Two broad categories of reduced stature are seen in blueberry: petites and dwarfs. Petites are essentially equivalent to midgets in mammals, being essentially regularly proportioned (often very regularly proportioned), and of uniformly reduced scale, with small uniform leaves, and internodes set at small uniform distances along the stem. These typically get no taller than about 15 cm. These are interesting plants, but are adaptively fragile and are never sufficiently vigorous (or perhaps genetically unable) to bloom. Dwarfs, following the mammalian analogy, are plants that have variously reduced features, but not all characteristics are proportionally reduced. A number of dwarfs arose in a rabbiteye-introgressed breeding population of 'Montgomery' × US 1771, and we sought to evaluate the value of these dwarfs, and determine whether they might act as starting points for further breeding and recovery of dwarf or semi-dwarf rabbiteye types possessing a reduced stature more similar to highbush.

Materials and Methods

The rabbiteye cultivars used were acquired from commercial sources (Table 1). 'Nocturne' and 'Pink Lemonade' were cultivars from the USDA-ARS breeding program. US 1771 is a selection, also from the USDA-ARS, Chatsworth, NJ breeding program, and is a complex pentaploid hybrid derived from a cross of 4*x* 'Sierra' (highbush) × 6*x* 'Little Giant' (*V. constablaei* × rabbiteye).

For test pollinations, pollen was freshly collected from open flowers by manual manipulation, and collected on glassine weighing paper. If needed for longer term work, pollen was stored for up to a month under refrigerated, desiccated conditions. For pollinations, a graphite pencil tip was dipped into the collected pollen, and then used to apply pollen to the stigmas of unemasculated flowers in an insect-free greenhouse.

Seed from crosses were extracted manually and germinated in a 50:50, peat:sand mixture on a greenhouse mist bench. At approximately a three true-leaf stage, seedlings were transplanted to a 50:50, peat:sand mixture in 72-cell flats. Plants were grown in these packs and evaluated for dwarf stature in their second season of growth.

Results and Discussion

The initial dwarfs segregated from a population of 'Montgomery' × US 1771. 'Montgomery' is a conventional rabbiteye cultivar release from the NCSU blueberry breeding program (NC-ARS & NCSU, 1997). As noted previously, US 1771 is a complex pentaploid hybrid from a cross of 4*x* 'Sierra' × 6*x* 'Little Giant'. The initial goal in creating US 1771 had been to foster recombination between the two parents, both possessing *V. constablaei* Gray germplasm, and make it useful at the 4*x* or 6*x* level. This cross was considered a desirable combination, since both 'Sierra' (U.S. Dept. of Agriculture, 1988) and 'Little Giant' (U.S. Dept. of Agriculture, 1995) had the *V. constablaei* selection T-65 as an ancestor in their background. US 1771 was initially selected from a small field-grown population because it set buds in mid-July (presumably a reflection of the short blooming/fruiting cycle of *V. constablaei*). *V. constablaei* introgression previously had been recognized to result in later flowering and earlier ripening in rabbiteye blueberry (Ballington et al., 1986). US 1771 despite being pentaploid, is male-fertile and was hybridized as a pollen source with the cultivars 'Montgomery' and 'Premier' (NCAES & USDA, 1975), these being 6*x* × 5*x* hybridizations. US 1771 was not a dwarf.

The initial hybridization with 'Premier' produced all normal seedlings (108 transplanted seedlings); however, the hybridization with 'Montgomery' segregated 15 dwarfs out of a population of 138 plants (123 normal:15 dwarf) for a segregation ratio of 8.2:1 normal:dwarf (Table 1).

The dwarf segregants had somewhat variable morphology, but were easily distinguished from their normal siblings. These plants had short stature, and expressed a multi-shoot morphology. Leaves were reduced in size, dark, semi-glossy, and often somewhat rounded. Internodes were short, and both vegetative and floral buds occurred as multi-bud clusters at their respective nodes. After 2 years of growth, none of these plants had exceeded 20 cm in height (Fig. 1 A-D). After two seasons in 7.6 × 7.6 cm pots, several of these plants flowered and produced flowers of normal or just slightly reduced size. Pollen shed from these flowers was plentiful and was sufficient to allow them to be used in secondary crosses as pollen

Figure 1 A-D. Characteristic dwarf phenotypes: A) young dwarf segregant, B) dwarf segregant at initial flowering, ~1 year, C) dwarf segregant flowering in greenhouse (thin shoots removed), ~2 years, and D) dwarf segregant, post-flowering.

Table 1. Counts of dwarf and normal stature plants segregating from crosses of rabbiteye cultivars by rabbiteye-derived dwarfs (US 2194 selections).

Female		Male	Total	Normal	Dwarf types		Ratio
					Dwarf	Super dwarf	(Normal/Dwarf)
Initial crosses with US 1771 as pollen source							
Montgomery	x	US 1771	138	123	15	-	**8.20**
Premier	x	US 1771	108	108	-	-	-
Rabbiteye cultivars as females; dwarf US 2194-A as pollen source							
Pink Lemonade	x	US 2194-A	199	175	13	6	
Nocturne	x	"	50	45	4	-	
Powderblue	x	"	43	37	4	-	
Alapaha	x	"	10	7	1	2	
Total			**302**	**264**	**22**	**8**	**8.80**
'Powderblue' rabbiteye as female; multiple dwarf sibs as pollen sources							
Powderblue	x	US 2194-A	43	37	4	-	
"	x	US 2194-B	16	14	1	-	
"	x	US 2194-D	63	52	7	2	
"	x	US 2194-E	18	15	-	2	
"	x	US 2194-F	70	52	6	7	
Total			**210**	**170**	**18**	**11**	**5.86**
Multiple rabbiteye cultivars females; multiple dwarf sibs as pollen sources							
Alapaha	x	US 2194-A, B	34	17	4	8	
Delite	x	US 2194-B, C	20	13	5	-	
Nocturne	x	US 2194-A, C	52	45	4	-	
Pink Lemonade	x	US 2194-A, B, F	202	176	13	6	
Powderblue	x	US 2194-A, B, C, D, E, F	218	178	18	11	
Tifblue	x	US 2194-B	13	11	1	-	
Total			**539**	**440**	**45**	**25**	**6.29**
Crosses among dwarf sibs							
US 2194-A	x	US 2194-C	2	2	-	-	
"	x	US 2194-D	4	1	-	-	
US 2194-B	x	US 2194-A	7	4	1	2	
Total			**13**	**7**	**1**	**2**	**2.33**

sources with conventional rabbiteye cultivars and rabbiteye-derivatives as females.

In the secondary crosses, two categories of reduced stature segregants were recorded, dwarfs and super-dwarfs (Table 1). Dwarfs in most respects were similar to the dwarf progenitors in terms of having recognizably reduced stature, although their chlorophyll pigmentation, by subjective estimation, more closely approached normal pigmentation. The super-dwarf segregants were small, bushy, and typically only 2.5 to 5 cm tall. It is unclear whether the super-dwarfs represent a meaningfully different type, and are reported as such mostly for their distinct size. In all subsequently reported segregation ratios however, reduced-stature types have been grouped and dealt with simply as dwarfs.

Several groupings were considered in the evaluation of the secondary crosses: 1) crosses using various 6*x* cultivars and selections as females with a single dwarf, US 2194-A, as pollen source; 2) crosses using a single rabbiteye cultivar, 'Powderblue' as female with a range of several sibling dwarf selections (US 2194-A through US 2194-F) as pollen sources; and 3) crosses among all hexaploid females and all dwarf sib pollen sources.

In the first of these advanced-cross groupings, 'Pink Lemonade', 'Nocturne', 'Powderblue', and 'Alapaha' were hybridized as females with the most vigorous dwarf, US 2194-A, serving as the pollen source. Across these females, we generated a total of 310 offspring, which were categorized as 268 normal types, 23 dwarfs, and 8 super-dwarfs (Table 1).

In the next set of these advanced crosses, 'Powderblue' was hybridized as the sole female with five dwarfs that flowered that same season, US 2194-A, -B, -D, -E, and -F as pollen sources. Across these males we evaluated a total of 210 offspring, which were categorized as 170 normal types, 18 dwarfs, and 11 super-dwarfs (Table 1).

In the final grouping, across all females and all pollen sources, we considered a total of 539 offspring, which were categorized as 440 normal types, 45 dwarfs, and 25 super-dwarfs (Table 1).

Amongst these three groupings, the segregation ratios of normals to dwarfs were respectively 8.8:1, 5.86:1, and 6.29:1. These ratios are suggestive of a mode of disomic recessive inheritance. In contrast, the simplest-case hexaploid polysomic segregation of a full heterozygote × full recessive would yield 19 normals: 1 dwarf. It should also be noted that the original segregation of normals to dwarfs in the 'Montgomery' × US 1771 crosses, was within a similar range, with a ratio of 8.2:1 normal:dwarf. The fact that dwarfs are produced in first generation crosses is somewhat unexpected, and suggests that many cultivars may already be heterozygous for the genes involved.

A low-count, but equally important set of crosses, were those among several dwarf sib genotypes. These crosses, all involving US 2194-A, expressed limited fruit set and seed set, and therefore generated few offspring. This low level of success was not surprising, since this cross was significantly inbred, as well as being a cross of two morphologically variant types. Across three cross-combinations, we generated only 13 offspring, which were categorized as 7 normal types, 1 dwarf, 2 super-dwarfs, and 3 ambiguous (Table 1). What is notable about these crosses is the fact that the dwarfs did not behave as typical recessives. In the dwarf × dwarf crosses, normal types segregated at a ratio of 2.33:1. Typically, a cross of two recessive types would be expected to generate all recessive types, and no normals. In evaluating which among these segregation groupings is most informative, one should consider homogeneity. It is perhaps relatively safe to assume that all of the original dwarfs were of a similar genotype with respect to dwarfing genes since they were generated from a specific rabbiteye × hybrid cross, albeit a 6*x* rabbiteye × 5*x* hybrid cross. Therefore, the set of crosses that used several of these dwarfs as pollen sources onto a single genotype, 'Powderblue', would seem to be the most uniform combination for estimating segregation. This cross followed a 170 normal:18 dwarfs: 11 super-dwarf segregation, or more broadly, a 6.29:1 segregation (Table 1).

The concern however with modeling segregation in these offspring is two-fold. First, to the best of our knowledge, no detailed determination has been made of mode of inheritance in 6*x* *V. virgatum* material. Lyrene (2017) noted that pink fruit segregated at an approximately 3:1 (blue:pink) ratio from a rabbiteye population that gave rise to the pink-fruited rabbiteye cultivar 'Florida Rose' (Lyrene, 2004), and he speculated that it was an autoallohexaploid.

		Male gametes 2 $a_1\,b$	Male gametes 2 $a_2\,b$
Female gametes	1 $A_1\,B$	$A_1\,a_1\,B\,b$	$A_1\,a_2\,B\,b$
	1 $A_1\,b$	$A_1\,a_1\,b\,b$	$A_1\,a_2\,b\,b$
	1 $a_1\,B$	$a_1\,a_1\,B\,b$	$a_1\,a_2\,B\,b$
	1 $a_1\,b$	$a_1\,a_1\,b\,b$	$a_1\,a_2\,b\,b$

	Progeny	
12 (2 each)	$A_1\,a_1\,B\,b$	normal
	$A_1\,a_1\,b\,b$	"
	$a_1\,a_1\,B\,b$	"
	$A_1\,a_2\,B\,b$	"
	$A_1\,a_2\,b\,b$	"
	$a_1\,a_2\,B\,b$	"
2	$a_1\,a_2\,b\,b$	dwarf
2	$a_1\,a_1\,b\,b$	lethal

Figure 2. A two-locus multi-allelic recessive model proposed for dwarf inheritance, 'Powderblue' (normal stature = $A_1 / a_1 / B / b$) × US 2194 clones (dwarf = $a_1/a_2/b/b$).

Based upon his observation, rabbiteye most likely has disomic rather than polysomic inheritance considering its excellent male fertility and cross-fertility, but barring more detailed analysis, meiosis and inheritance could conceivably lay intermediate between disomic and polysomic depending upon the specific allele and/or trait. A second concern is the possibility that the dwarfs generated from the original 6*x* 'Montgomery' × 5*x* US 1771 cross were not fully euploid (6*x*), and thus might in fact not be genotypically identical. Moreover, even segregations from a single dwarf clone, if non-euploid, may produce random dosage effects that defy simple, classical modeling of gene segregation. Vorsa (1998) working with rabbiteye material noted differential transmission of extra chromosomes in similar ploidy crosses of hexaploid by pentaploid blueberry.

These considerations in mind, a bi-locus diallelic recessive model can be suggested for 'Powderblue' category crosses. If the dwarfs are considered to be full recessives with different recessive alleles at one of two loci (because of their mixed-species background), in this case, a dwarf might be $a_1 / a_2 / b / b$. 'Powderblue' might be proposed to be a heterozygote at both loci, $A_1 / a_1 / B / b$ (Fig. 2). If just one further assumption is made, that being that an $a_1 / a_1 / b / b$ genotype (i.e. homozygous recessive at both the a_1 and b locus) is lethal, a segregation of 6 normal : 1 dwarf : 1 lethal can be modeled. This model represents a plausible explanation for this set of crosses; however, it significantly fails to explain dwarf × dwarf crosses that segregate a portion of normal types. No model examined adequately explains all of the observed results.

Conclusions

The critical conclusion from these crosses is that using the primary dwarf segregants as parents, dwarfs can be produced in relative abundance, they can be produced in crosses to many standard rabbiteye types, and they can be produced in the first generation of crossing. These dwarfs pending further evaluation may be useful for either production-oriented or ornamental purposes. What is not yet known is the full range of expression of these dwarfs under field conditions. Considering that we categorized some plants as extreme dwarfs, it is unlikely that those plants will develop into intermediate-stature plants under field conditions. However, among less extreme dwarfs, it is unknown what range of dwarfing will be expressed under field conditions, although it is reasonable to think that intermediate types will be expressed. It is also unknown at this point what levels of productivity might be expected from these reduced-stature types. Further study is needed to fully evaluate this material.

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About the Cover:

Common Elderberry [*Caprifoliaceae Sambuscus nigra L. susp. Canadensis (L.) Bolli*] is sometimes called Arizona elderberry, American elder, sweet elder, tree of music, Danewort, and hairy blue elderberry. There are about 50 species. Some species are native to Europe and others are native to North America and were used by Native Americans. Most named cultivars are clones selected from the wild but there are some small programs breeding for high yield and uniform ripening. Elderberries are high in antioxidants and several health-promoting products contain elderberry extract. Photo by Michele Warmund.