agronomy

MDPI

Article

Phenotypic Evaluation of a Hybrid Diploid Blueberry Population for Plant Development and Fruit Quality Traits

Lisa J. Rowland [1,*], Elizabeth L. Ogden [1] and Bryan T. Vinyard [2]

[1] Genetic Improvement of Fruits and Vegetables Laboratory, Henry A. Wallace Beltsville Agricultural Research Center, U.S. Department of Agriculture-Agricultural Research Service, Beltsville, MD 20705, USA; Elizabeth.Ogden@usda.gov

[2] Statistics Group, Northeast Area Office, U.S. Department of Agriculture-Agricultural Research Service, Beltsville, MD 20705, USA; Bryan.Vinyard@usda.gov

* Correspondence: Jeannine.Rowland@usda.gov; Tel.: +1-301-504-6654

Received: 2 July 2020; Accepted: 23 July 2020; Published: 24 July 2020

Abstract: A diploid blueberry mapping population, used previously to map quantitative trait loci (QTL) for chilling requirement and cold hardiness, was evaluated for several plant development and fruit quality traits. Specifically, the population was phenotyped in a greenhouse for timing of various stages of flower bud, leaf bud and fruit development and for fruit quality traits including weight, diameter, color, scar, firmness, flavor and soluble solids. Phenotypic data was analyzed statistically by analysis of variance, correlation tests, to examine associations of traits, and heritability. Results indicated that the traits were segregating and most were distributed normally in the population. Many of the development traits were correlated, and timing of shoot expansion, early bloom and full bloom was also correlated with the previously evaluated trait of chilling requirement. Some correlations were found among the fruit quality traits as well. For example, weight was highly correlated with diameter, and subjectively measured firmness was moderately correlated with one of the objectively measured firmness traits. In addition, most of the traits showed significant variation across genotypes and across years, and most had moderate to high heritability. Therefore, we conclude that the diploid population should be useful for identifying QTL for many of these traits.

Keywords: *Vaccinium corymbosum*; *Vaccinium darrowii*; growth traits; heritability; mapping population

1. Introduction

Blueberry is a woody perennial shrub belonging to the *Vaccinium* genus of the heath family, *Ericaceae*. The cultivated types of blueberry, which belong to the *Cyanococcus* section of the *Vaccinium* genus, are native to North America. The popularity of blueberry has risen dramatically in recent years. Production in the United States, the largest producer of blueberries, has more than doubled from 2005 to 2015 (United States Department of Agriculture-National Agricultural Statistics Service (USDA-NASS)) and has increased worldwide by 58% from 2009 to 2014 (United Nations Food and Agriculture Organization) [1]. Blueberry consumption has increased as well, by approximately 3-fold from 2000 to 2010 in the U.S., likely due to greater awareness of its high anthocyanin content and its many health benefits [2].

Blueberry is comprised of many species. The tetraploid highbush blueberry, *Vaccinium corymbosum* L., is the most important species commercially and can be classified into northern and southern types, depending on their cold hardiness levels and chilling requirements. Southern highbush cultivars, with lower chilling requirements and less cold hardiness, have been developed for the southern U.S.

by crossing the low-chilling southern diploid species *V. darrowii* Camp into the northern tetraploid *V. corymbosum* background [3].

Although much progress has been made in blueberry breeding since its domestication in the early 1900s, developing a new blueberry cultivar can still take more than ten years. Therefore, marker-assisted breeding would be particularly useful in blueberry and other woody perennials because of their long generation times and generally high levels of heterozygosity. For this reason, genetic linkage maps have been constructed for diploid and tetraploid blueberry in recent years [3–5]. Furthermore, the diploid blueberry mapping population has been used for mapping quantitative trait loci (QTL) for chilling requirement and cold hardiness [3], as it was specifically developed for that purpose.

The diploid blueberry population is a pseudo-backcross interspecific mapping population created by crossing a hybrid (F_1#10) of a low-chilling, freezing-sensitive *V. darrowii* selection Fla4B and a high-chilling, freezing-tolerant diploid *V. corymbosum* selection W85-20 to another similar diploid *V. corymbosum* selection W85-23. This resulted in a population that was segregating normally, with a wide distribution, for chilling requirement and cold hardiness. Use of a relatively low-density map (consisting of primarily simple sequence repeat (SSR) and expressed sequence tag-polymerase chain reaction (EST-PCR) markers) of this population resulted in the identification of one major QTL for chilling requirement and one major QTL for cold hardiness [3]. Efforts are currently underway to develop a high-density SNP-based map of this same population.

Although the diploid blueberry population was not created specifically for mapping other plant development and fruit quality traits, visual observations over the years have suggested that it might also be useful for these purposes. Therefore, here, we describe the evaluation of the population over several years for timing of flower bud, leaf bud, and fruit development and for several fruit quality traits including weight, diameter, color, scar, firmness, flavor and soluble solids. The phenotypic data was analyzed statistically by way of analysis of variance (ANOVA), correlation tests to examine associations of traits and heritability, and results were used to assess potential for future QTL analyses.

2. Materials and Methods

2.1. Plant Material

The blueberry interspecific diploid mapping population has been described previously [3]. Briefly, the population was developed by crossing an interspecific hybrid plant (hybrid of a low-chilling, freezing-sensitive *V. darrowii* selection Fla4B (collected in Florida, USA) and a high-chilling, freezing-tolerant diploid *V. corymbosum* selection W85-20 (collected in New Jersey, USA)) to another diploid *V. corymbosum* selection W85-23 (also collected in New Jersey). Fla4B is a genotype that has been used extensively in blueberry breeding programs. It is one of the major sources of low-chilling requirement genes in breeding southern highbush cultivars [3]; it was used as a parent in the development of 'Gulfcoast', 'Georgiagem', 'Biloxi', 'Cooper' and 'Cape Fear', among other cultivars [6,7]. Each parent and progeny of the mapping population was propagated to give approximately three to four clones of each genotype. The plants are maintained in a hoop house in 4 to 12 L pots at the Beltsville Agricultural Center-West (BARC-W) in Beltsville, MD, USA. The population is not maintained in the field, because many individuals of the population are extremely cold sensitive and would likely be severely damaged by winter temperatures.

To guarantee the chilling requirement was met each winter (2011 to 2019) for the population, the plants were kept in an unheated hoop house covered in opaque winter white plastic to minimize daytime temperature increases. The average low temperature was −2.4 to 0 °C. Plants received ambient light and day length. As floral buds began to swell, plants were moved to a heated hoop house (minimum 18 °C, clear plastic, ambient light) for continued development. A bumblebee hive (Class B, Koppert Biological Systems, Howell, MI, USA) was placed in the hoop house to facilitate pollination.

2.2. Phenotypic Evaluations

2.2.1. Leaf Bud, Flower Bud, and Fruit Development Traits

As vegetative and floral buds developed, plants were evaluated on a weekly basis, and the day of year (Julian day) when >50% of buds reached each stage was noted for each genotype. Stages evaluated were shoot expansion (SE), early bloom (EB), full bloom (FB), petal fall (PF), early green fruit (EG), late green fruit (LG) and >75% blue fruit (75BLUE) based on the MSU Extension Growth Stages Table [8]. Time between developmental stages was calculated for days between early bloom and full bloom (EBtoFB), early bloom and >75% blue fruit (EBto75B), and full bloom and >75% blue fruit (FBto75B). Development/growth stage traits were evaluated over 2 years (2012 and 2013).

2.2.2. Fruit Quality Traits

Fruit quality traits were evaluated when plants reached at least 50% ripe fruit. Fruit was scored subjectively for color, scar, firmness and flavor based on scales used by breeders and other researchers in the field [9–12]. Specifically, scales for fruit color and scar are depicted in the paper by Moore [9]. Fruit color was rated on a scale of 1 (black fruit) to 9 (bright, light blue fruit). Berry scar was rated from 1 (large, deep wet scar) to 9 (small, dry scar). Fruit firmness was also scored on a 1 to 9 scale (very soft fruit to very firm fruit) by gently squeezing the fruit between the thumb and index finger. Flavor was scored on a 0 to 9 scale, where 0 was very bland fruit or fruit with no flavor, 1 was very tart fruit, fruit with a score of 5 was considered balanced between tart and sweet, and very sweet fruit was scored at 9. Random samples of 10–12 berries were evaluated. Color, scar and weight were evaluated over six years (2012, 2013, 2015, 2017, 2018 and 2019). Firmness and flavor were evaluated over four years (2012, 2013, 2015 and 2017).

Objective ratings for fruit quality were also determined for weight, diameter, soluble solids and firmness from random samples of berries. Berry weight in grams (Weight) was calculated as the average of a 25-berry sample. Average berry diameter in mm (Dia) was calculated from 20 berry samples. Juice from 5-berry samples (4 reps) was squeezed through multiple layers of cheesecloth to determine average soluble solids (SS), or % Brix, as measured with a refractometer (HI 96801, Hanna Instruments, Woonsocket, RI). For firmness, two measurements were made using a texture analyzer (TA.XT, Stable Micro Systems, Surrey, UK). What we called 20S measured the force (N) to equatorially compress the berry by 20%. The score was the average of 20 berry samples. For 3 mm, the force (N) for a 1 mm probe to penetrate berries to 3 mm was determined (average of 20 berry samples). Traits were evaluated over three years (2017, 2018, and 2019).

2.3. Statistical Analyses

To estimate variance components and heritability, a random effects [Year, Genotype and Residual (=Year × Genotype)] ANOVA model was fit for each trait, using SAS v.9.4 (SAS Institute, Inc., Cary, NC, USA) PROC MIXED specifying method = type3, to avoid reliance on asymptotic estimation assumptions. Significance of year and genotype on evaluated traits was determined using Chi-square likelihood ratio tests (LRTs). For the few cases where variance components were estimated to be small negative numbers close to zero, those estimates were set to zero. Heritability estimates and 95% confidence intervals were calculated as described by Knapp et al. [13] (Equation (5), p. 193). Heritability values were classified as low ($H < 0.2$), moderate ($0.2 < H < 0.6$) and high ($H > 0.6$). Means estimates of evaluated traits were obtained by fitting generalized linear models using SAS PROC GLIMMIX with year and genotype as fixed effects, specifying a Beta distribution for soluble solids and subjective fruit quality traits, a Gamma distribution for objective firmness measurements and time between growth stages, and a Normal distribution for growth stages and weight and diameter.

Trait distribution histograms were drawn using SAS PROC UNIVARIATE. Spearman correlation coefficients were calculated with SAS PROC CORR to examine relationships between traits. For growth trait correlations, previous phenotypic data for chilling requirement and cold hardiness [3] was included

to evaluate how those traits related to bud growth, especially early in the season. Correlations were classified as high if r > 0.7, moderate if 0.4 < r < 0.7 and weak if r < 0.4.

3. Results and Discussion

3.1. Development/Growth Stage Traits

The diploid blueberry population was phenotyped for many plant development and fruit quality characteristics to determine whether the population was segregating for these traits, specifically variation of traits for year and genotype, correlation of traits and heritability. Development traits, also referred to as growth stage traits, included timing of vegetative bud (SE), flower (EB, FB, PF) and fruit (EG, LG, 75BLUE) development traits. Number of days between some of the developmental stages was evaluated as well (EBtoFB, EBto75B, FBto75B). These traits are important because many blueberry breeding programs are working on expanding the harvest season by developing earlier and later fruiting cultivars. In the case of earliness, genotypes can be selected that are earlier blooming and have shorter ripening periods. In the case of lateness, genotypes with slow rates of fruit development can be selected [12].

Chi-square likelihood ratio tests (LRTs) indicated that there was significant variation across the F_1#10 × W85-23 genotypes for most of the growth traits (Table 1). The only traits which showed no significant variation by genotype were EG, LG and EBtoFB. In addition, there was significant variation across years for all of the evaluated growth traits (Table 1). This, we assume, was at least partially due to differences in weather from year to year. Weather data indicates that 2013 was colder than 2012. Average daily temperatures in the 2012 were 3.3 °C (February) and 5.3 °C (early March) warmer than 2013. Within the hoophouse, the result was more days above 7 °C, which would have sped up development.

Table 1. This table summarizes the mean, range, and likelihood ratio test (LRT) results for year and genotype, and the heritability for each trait that was evaluated in the diploid blueberry population.

Trait	Mean	Range	Significance [1]		Heritability (CI [2])	
			Year	Genotype		
Color	5.7	2.0–7.5	***	***	0.80	(0.73, 0.86)
Scar	7.1	2.0–9.0	***	***	0.67	(0.55, 0.77)
Firm	7.3	5.0–9.0	NS	*	0.34	(0.07, 0.54)
Flavor	5	1.5–8.0	NS	NS	0.08	(−0.31, 0.36)
Weight	0.24	0.14–0.42	***	***	0.56	(0.40, 0.69)
Diameter	7.92	5.85–10.04	**	**	0.65	(0.33, 0.81)
Soluble Solids	15.2	9.1–20.3	NS	**	0.68	(0.36, 0.82)
20SFirm	1.19	0.52–1.81	NS	NS	0.47	(−0.86, 0.79)
3mmFirm	0.58	0.33–0.91	NS	**	0.72	(0.45, 0.84)
Shoot Elongation	96.7	92–106	***	**	0.43	(0.13, 0.63)
Early Bloom (EB)	96.1	86–109	***	***	0.59	(0.38, 0.74)
Full Bloom (FB)	102.6	93–113	***	***	0.69	(0.51, 0.80)
Petal Fall	109.6	100–120	***	***	0.69	(0.52, 0.80)
Early Green	134.3	123.5–148	***	NS	0.30	(−0.12, 0.56)
Late Green	153.2	148–169	***	NS	0.48	(0.15, 0.67)
75% Blue Fruit (75B)	181.4	170.5–195.5	***	*	0.46	(0.11, 0.66)
EBtoFB	6.6	4–10	***	NS	0.05	(−0.47, 0.38)
EBto75B	85.4	68–107	***	**	0.53	(0.23, 0.71)
FBto75B	79	61–100	***	**	0.51	(0.20, 0.70)

[1] Asterisks indicate significance at $p \leq 0.05$ (*), $p \leq 0.01$ (**), and $p \leq 0.0001$ (***); NS, not significant ($p > 0.05$).
[2] CI, 95% Confidence Intervals (lower, upper).

Distributions of the various growth stage traits are illustrated in Figure 1. As can be seen in Table 1 and Figure 1, bloom (EBtoFB) occurred over a short period of time (a mean of 6.6 days) in this

population. Fruit ripening (~45 days from mean of EG to mean of 75BLUE) occurred over a much longer period of time. The mean time of SE (96.7) was approximately the same as the mean time of EB (96.1). PF occurred ~7–8 days after FB. EG was ~25 days after PF, LG was ~19 days after EG, and 75BLUE was ~28 days after LG. From EBto75B was a mean of 85.4 days, with a range of 68–107 days.

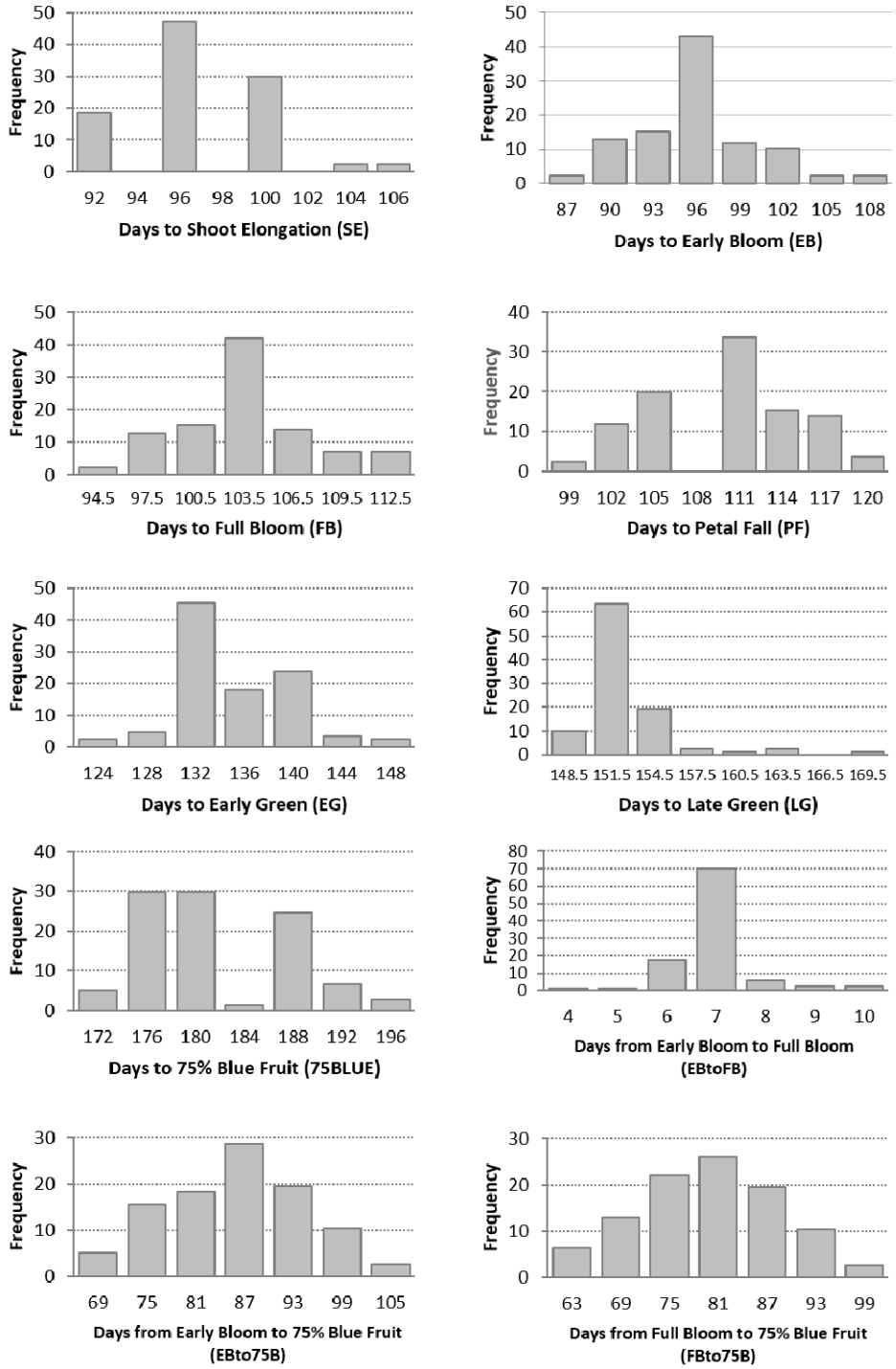

Figure 1. Distribution of the growth stage traits in the diploid blueberry population. Frequency is shown on the y-axis, and Julian day or days between two stages is shown on the x-axis.

Data was used to determine the heritability of the growth stage traits (Table 1). If we classify heritability values as low ($H < 0.2$), moderate ($0.2 < H < 0.6$) and high ($H > 0.6$), then heritability of FB

and PF was high, while heritability of SE, EB, EG, LG, 75BLUE, EBto75B and FBto75B was moderate. Heritability of EBtoFB was low in the population.

Data was also used to examine correlations among the various growth stage traits and previously analyzed traits of chilling requirement (CR) and cold hardiness (CH) (Table 2). We classified correlations as high if $r > 0.7$, moderate if $0.4 < r < 0.7$ and weak if $r < 0.4$. Using these criteria, several traits exhibited strong positive correlations as we might expect (EB and FB, EB and PF, FB and PF, 75BLUE and EBto75B, 75BLUE and FBto75B, EBto75B and FBto75B). Other traits exhibited moderate to strong negative correlations (EB and EBto75B, EB and FBto75B, FB and EBto75B, FB and FBto75B, PF and EBto75B, PF and FBto75B), simply indicating the earlier the timing of EB, FB or PF, the longer the number of days to 75BLUE, and vice versa, the later the timing of EB, FB or PF, the shorter the number of days to 75BLUE. SE, EB and FB exhibited moderate positive correlations with CR, and EG exhibited moderate positive correlations with LG and with PF. There were only a few weak correlations ($r < 0.4$) with cold hardiness (CH).

Table 2. Spearman correlation coefficients among the growth stage traits.

Variable	SE	EB	FB	PF	EG	LG	75BLUE	EBtoFB	EBto75B	FBto75B	CR	CH
Shoot Elongation (SE)		**0.32** [1] <0.01	**0.33** <0.01	**0.33** <0.01	0.16 NS [2]	0.12 NS	0.00 NS	0.08 NS	−0.18 NS	−0.18 NS	**0.54** <0.0001	**−0.35** <0.01
Early Bloom (EB)			**0.97** <0.0001	**0.93** <0.0001	**0.38** <0.01	**0.23** <0.05	**−0.28** <0.05	0.10 NS	**−0.69** <0.0001	**−0.72** <0.0001	**0.42** <0.0001	**−0.23** <0.05
Full Bloom (FB)				**0.88** <0.0001	**0.33** <0.01	0.20 NS	**−0.25** <0.05	**0.25** <0.05	**−0.65** <0.0001	**−0.69** <0.0001	**0.44** <0.0001	**−0.27** <0.05
Petal Fall (PF)					**0.41** 0.0001	**0.24** <0.05	−0.22 NS	0.05 NS	**−0.60** <0.0001	**−0.63** <0.0001	**0.38** <0.01	**−0.25** <0.05
Early Green (EG)						**0.40** <0.01	−0.17 NS	−0.07 NS	**−0.32** <0.01	**−0.30** <0.01	0.08 NS	0.03 NS
Late Green (LG)							0.05 NS	−0.03 NS	−0.15 NS	−0.15 NS	−0.07 NS	0.06 NS
75% Blue Fruit (75BLUE)								0.15 NS	**0.87** <0.0001	**0.85** <0.0001	0.03 NS	−0.01 NS
Days from Early Bloom to Full Bloom (EBtoFB)									0.04 NS	−0.04 NS	0.03 NS	−0.19 NS
Days from Early Bloom to 75% Blue Fruit (EBto75B)										**0.99** <0.0001	−0.16 NS	0.11 NS
Days from Full Bloom to 75% Blue Fruit (FBto75B)											−0.17 NS	0.12 NS
Chilling Requirement (CR)												**−0.35** <0.01
Cold Hardiness (CH)												

[1] Correlation coefficients in bold are significantly different from zero at the p level shown in the following row.
[2] NS, not significant ($p > 0.05$).

3.2. Fruit Quality Traits

In addition to the growth stage traits, the diploid blueberry population was evaluated for fruit quality traits, including some that were scored subjectively (Color, Scar, Firmness or Firm, and Flavor) and some scored objectively (Weight, Dia, SS, 20S, and 3 mm), as described in the Materials and Methods. According to a recent survey on blueberry breeding trait priorities, fruit quality traits are deemed the most important to breeders and growers [14]. The top traits in this category were fruit firmness, flavor, and shelf life [14]. Other fruit quality traits known to be important in breeding commercial blueberry cultivars include size, light blue color (due to the presence of a waxy 'bloom'), small picking scar (where the pedicel detaches from the fruit), and sweetness [9–12,14–16]. Many of these traits, as well as some of the growth traits described above, have been evaluated recently in a tetraploid *V. corymbosum* blueberry mapping population [12]. The population showed promise for

future QTL analyses, but because the population is rather small, it needs to be expanded in size before QTL mapping of these traits can be undertaken.

Chi-square likelihood ratio tests (LRTs) in the diploid blueberry population indicated that there was significant variation across the genotypes for all of the fruit quality traits except for Flavor and 20S (Table 1). In addition, four of the fruit quality traits exhibited significant variation across years (Color, Scar, Weight, Dia), while five did not (Firm, Flavor, SS, 20S, 3 mm) (Table 1). Distributions of the various fruit quality traits are illustrated in Figure 2.

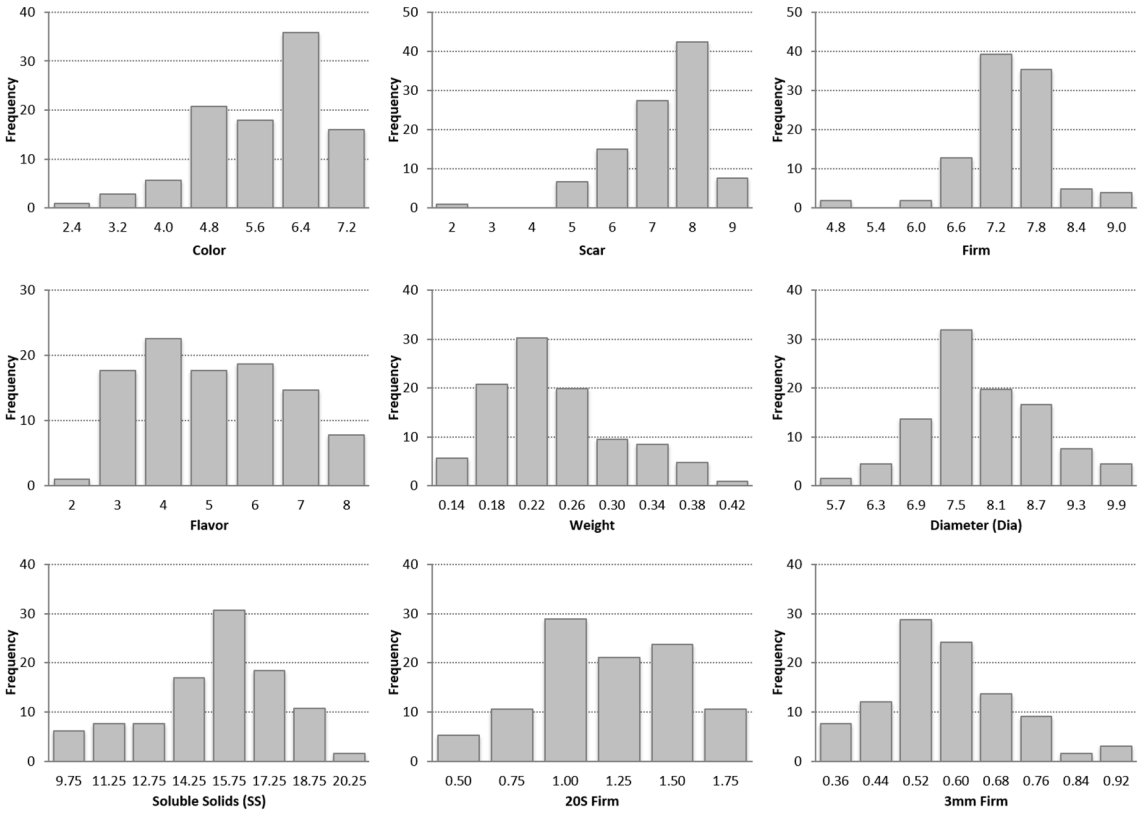

Figure 2. Distribution of the fruit quality traits in the diploid blueberry population. Frequency is shown on the y-axis, and scores for each trait are shown on the x-axis.

The fruit quality data was also used to determine the heritability of these traits (Table 1). Heritability was high for Color, Scar, Dia, SS, and 3 mm. Heritability was moderate for Firm, Weight, and 20S, and low for Flavor, which is probably the most subjective of all the traits.

Furthermore, data was used to examine correlations among the various fruit quality traits (Table 3). Results indicated that Weight was highly correlated with Dia, as would be expected. The subjectively scored trait of Firm (measured by squeezing the berries with the thumb and forefinger) was moderately correlated with the objectively scored firmness trait of 3 mm (measured with a texture analyzer by puncturing the fruit skin with a small probe). Surprisingly, however, the Firm trait was not correlated with the objectively scored firmness trait of 20S, which was measured with a texture analyzer by pressing the fruit with a large probe. Further, the 3 mm trait was only weakly correlated with the 20S trait. Perhaps, the lack of correlation or weak correlation between some of the firmness measurements in this study is due to the extremely small size of the diploid blueberry fruit. The mean weight of the diploid fruit in this population was only 0.24 g (Table 1), as compared to over 2.0 g in the tetraploid mapping population [12]. In other studies of our own using large tetraploid blueberry fruit, we have seen better correlations between the different ways of measuring firmness (unpublished data). The firmness of the small diploid fruit may be more of a reflection of the toughness of the skin than the fruit pulp, whereas for tetraploid fruit, it may be a reflection of both. In the current study, we also

found that Weight and Dia were moderately correlated with 20S, indicating that it takes more force to press larger size fruit. Other weak correlations, shown in Table 3, are those between Firm and Scar and between Firm and SS. A positive correlation between firmness and scar has also been reported in the tetraploid blueberry mapping population [9]. SS was also weakly correlated with 3 mm in the diploid population. Interestingly, Scar exhibited weak negative correlations with Weight and Dia, and a weak positive correlation with 3 mm.

Table 3. Spearman correlation coefficients among the fruit quality traits.

Variable	Color	Scar	Firm	Flavor	Weight	Dia	SS	20S	3 mm
Color		0.14 NS [2]	0.01 NS	−0.02 NS	−0.02 NS	0.03 NS	−0.1 NS	−0.05 NS	−0.31 [1] <0.05
Scar			**0.31** <0.01	−0.14 NS	**−0.3** <0.01	**−0.35** <0.01	0.07 NS	−0.26 NS	**0.25** <0.05
Firm				−0.03 NS	0.09 NS	0.09 NS	**0.31** <0.05	0.03 NS	**0.44** <0.01
Flavor					0.09 NS	**0.24** <0.05	0.2 NS	0.01 NS	0.09 NS
Weight						**0.74** <0.0001	0.24 NS	**0.45** <0.01	0.13 NS
Diameter (Dia)							0.1 NS	**0.62** <0.0001	−0.07 NS
Soluble Solids (SS)								0.01 NS	**0.27** <0.05
20SFirm (20S)									**0.37** <0.05
3mmFirm (3 mm)									

[1] Correlation coefficients in bold are significantly different from zero at the p level shown in the following row.
[2] NS, not significant ($p > 0.05$).

3.3. Relationship to the Big Picture

Blueberry is one of the most rapidly expanding fruit crops in the world today, both in terms of production and consumption. Production currently exceeds 525,000 metric tons worldwide (United Nations, Food and Agriculture Organization). The development of germplasm resources for traditional breeding of new blueberry cultivars and in pre-breeding of new breeding lines is essential for keeping up with this demand. Development of germplasm resources are also necessary for generating new genetic and genomic tools for use in future marker-assisted selection and phenomics for more efficient breeding of blueberry [17,18]. Such resources include biparental mapping populations and association panels for identifying QTL for traits important to the blueberry industry, like development and fruit quality traits.

According to the most recent survey conducted to assess blueberry industry breeding priorities, the most important trait cluster overall was fruit quality [14]. This included traits of firmness, flavor and shelf life, among others. Machine harvestability was also found to be very important in certain regions. Machine harvestability is affected by such fruit quality factors as firmness and scar, as well as plant development traits like time of ripening and uniform ripening period. The earliest ripening and latest ripening cultivars tend to bring in the highest prices, while machine harvestability reduces labor costs. Therefore, combining the growing of early and late ripening cultivars with machine harvestability increases profitability [14].

Genomic resources (mapping populations) have been recently used to map QTL for development and fruit quality traits in other berry crops, like raspberry [19], blackberry [20], cranberry [21–24] and strawberry [25]. The first genetic linkage maps for red raspberry were generated from

a 'Glen Moy' × 'Latham' population [26,27]. This biparental mapping population has been used to identify QTL for several growth stage traits [26,27] and fruit quality traits, including texture [28], color [29], flavor volatiles [30], anthocyanins [31] and fruit softening [28]. Two QTL for the fruit texture disorder in raspberry, called crumbly, have also been identified [32]. Castro et al. [20] have mapped QTL for primocane-fruiting (annual fruiting) and thornlessness in blackberry. Fruit quality traits related to fruit development, texture, color, sugar, anthocyanin and organic acid contents have been mapped in strawberry as well [25]. In cranberry, which is a member of the *Vaccinium* genus and is closely related to blueberry, QTL for fruit rot resistance [21,22], total anthocyanin content, titratable acidity, proanthocyanidin content, Brix and mean fruit weight [23], and fruit shape and size [24] have all been identified.

Unfortunately, few mature biparental mapping populations are currently available for identifying blueberry QTL, but more are being generated. To date, phenotypic data for only two has been published [3,12], and this includes the chilling requirement and cold hardiness data [3] for the diploid mapping population described herein. Although phenotypic data related to growth traits and fruit quality of the tetraploid blueberry mapping population, 'Draper' × 'Jewel', is now available [12], the size of the population needs to be increased before it will be useful for QTL identification. Besides these biparental mapping populations, a large collection of genotypes has recently been used in a genome-wide association study (GWAS) to identify candidate genes affecting fruit-related traits in tetraploid southern highbush blueberries [33]. A panel of 1575 southern highbush individuals were genotyped and phenotyped for eight fruit-related traits. Fifteen single nucleotide polymorphisms (SNPs) associated with five traits (fruit size, scar diameter, soluble solids, pH and flower bud density) were identified. The ability to map some of these same traits and others in the diploid blueberry mapping population will allow us to compare the QTL and associated genes in the diploid and tetraploid blueberry populations and either confirm the same genes are involved or identify new genes previously not identified. In addition, we will be able to compare the QTL and associated genes to those identified in other related species, like cranberry.

4. Conclusions

Most of the growth and fruit quality traits that were evaluated in our diploid blueberry mapping population showed significant variation across genotypes and across years. Because most appeared to segregate, to be distributed normally, and to have moderate to high heritability, the population should be useful for identifying QTL for these growth and fruit quality traits. However, because there is variation across years for most traits, QTL should be mapped for each year separately. Many of these same traits have recently been evaluated in a tetraploid *V. corymbosum* blueberry mapping population [12], and the population showed promise for future use in QTL analyses. Some of the same fruit quality traits have been evaluated in a tetraploid southern highbush panel for use in GWAS [33]. Therefore, it should be possible to eventually compare the QTL results in the diploid and tetraploid populations. Efforts are currently underway to develop a high-density map of the diploid blueberry population. A high-density map, together with the recent availability of the chromosome-level blueberry genome [34], should enable us to not only map the QTL, but also identify candidate genes for these traits by their vicinity to QTL. This work will eventually allow the development of markers for use in marker-assisted breeding of blueberry, and the combining of marker-assisted selection for growth traits with those of fruit quality traits in the development of new cultivars.

Author Contributions: Conceptualization, L.J.R. and E.L.O.; methodology, L.J.R., E.L.O. and B.T.V.; investigation, E.L.O. and B.T.V.; formal analysis, E.L.O. and B.T.V.; writing—original draft preparation, L.J.R., E.L.O. and B.T.V.; writing—review and editing, L.J.R., E.L.O. and B.T.V.; visualization, E.L.O.; supervision, L.J.R.; funding acquisition, L.J.R. All authors have read and agreed to the published version of the manuscript.

Funding: This research was partially funded by in-house USDA-ARS funding and partially by USDA-CSREES Specialty Crop Research Initiative, grant# 2008-51180-04861, entitled "Generating Genomic Tools for Blueberry Improvement".

Acknowledgments: We would like to thank our student interns, George J. Thomas and Christian Neri, for help carrying out phenotypic evaluations of the blueberry population. We also thank Eunhee Park for assistance with using the texture analyzer.

Conflicts of Interest: The authors declare no conflict of interest. The funders had no role in the design of the study; in the collection, analyses, or interpretation of data; in the writing of the manuscript, or in the decision to publish the results.

References

1. Qi, X.; Ogden, E.L.; Die, J.V.; Ehlenfeldt, M.K.; Polashock, J.J.; Darwish, O.; Alkharouf, N.; Rowland, L.J. Transcriptome analysis identifies genes related to the waxy coating on blueberry fruit in two northern-adapted rabbiteye breeding populations. *BMC Plant Biol.* **2019**, *19*, 460. [CrossRef] [PubMed]
2. Yousef, G.G.; Brown, A.F.; Funakoshi, Y.; Mbeunkui, F.; Grace, M.H.; Ballington, J.R.; Loraine, A.; Lila, M.A. Efficient quantification of the health-relevant anthocyanin and phenolic acid profiles in commercial cultivars and breeding selections of blueberries (*Vaccinium* spp.). *J. Agric. Food Chem.* **2013**, *61*, 4806–4815. [CrossRef] [PubMed]
3. Rowland, L.J.; Ogden, E.L.; Bassil, N.; Buck, E.J.; McCallum, S.; Graham, J.; Brown, A.; Wiedow, C.; Campbell, A.M.; Haynes, K.G.; et al. Construction of a genetic linkage map of an interspecific diploid blueberry population and identification of QTL for chilling requirement and cold hardiness. *Mol. Breed.* **2014**, *34*, 2033–2048. [CrossRef]
4. McCallum, S.; Graham, J.; Jorgensen, L.; Rowland, L.J.; Bassil, N.V.; Hancock, J.F.; Wheeler, E.J.; Vining, K.; Poland, J.A.; Olmstead, J.W.; et al. Construction of a SNP and SSR linkage map in autotetraploid blueberry using genotyping by sequencing. *Mol. Breed.* **2016**, *36*, 1–24. [CrossRef]
5. Schlautman, B.; Diaz-Garcia, L.; Covarrubias-Pazaran, G.; Schlautman, N.; Vorsa, N.; Polashock, J.; Ogden, E.L.; Brown, A.; Lin, Y.-C.; Bassil, N.; et al. Comparative genetic mapping reveals synteny and collinearity between the American cranberry and diploid blueberry genomes. *Mol. Breed.* **2018**, *38*, 9. [CrossRef]
6. Draper, A.D.; Galletta, G.J. Breeding methods for improving southern tetraploid blueberries. *J. Am. Soc. Hortic. Sci.* **1982**, *107*, 106–109.
7. Gough, R.E. *The Highbush Blueberry and Its Management*, 1st ed.; Food Products Press, an Imprint of the Haworth Press, Inc.: Binghamton, NY, USA, 1994; pp. 93–107.
8. Michigan State University Extension. Growth Stages Table. Michigan Blueberry Facts. Available online: http://www.Canr.msu.edu/blueberries/growing_blueberries/growthstages (accessed on 25 April 2018).
9. Moore, J.N. Improving highbush blueberries by breeding and selection. *Euphytica* **1965**, *14*, 39–48.
10. Edwards, T.W., Jr.; Sherman, W.B.; Sharpe, R.H. Evaluation and inheritance of fruit color, size, scar, firmness and plant vigor in blueberry. *HortScience* **1974**, *9*, 20–22.
11. Finn, C.E.; Luby, J.J. Inheritance of fruit quality traits in blueberry. *J. Am. Soc. Hort. Sci.* **1992**, *117*, 617–621. [CrossRef]
12. Hancock, J.F.; Olmstead, J.W.; Itle, R.A.; Callow, P.W.; Neils-Kraft, S.; Wheeler, E.J.; Mangandi, J.; Sooriyapathirana, S.S.; Rowland, L.J.; Mackey, T.A.; et al. Performance of an elite, hybrid family of a northern x southern highbush cross ('Draper' x 'Jewel'). *Euphytica* **2018**, *214*, 95. [CrossRef]
13. Knapp, S.J.; Stroup, W.W.; Ross, W.M. Exact confidence intervals for heritability on a progeny mean basis. *Crop Sci.* **1985**, *25*, 192–194. [CrossRef]
14. Gallardo, R.K.; Zhang, Q.; Dossett, M.; Polashock, J.J.; Rodriguez-Saona, C.; Vorsa, N.; Edger, P.P.; Ashrafi, H.; Babiker, E.; Finn, C.E.; et al. Blueberry trait priorities of the blueberry industry in the United States and Canada. *HortScience* **2018**, *53*, 1021–1028. [CrossRef]
15. Rowland, L.J.; Hancock, J.F.; Bassil, N.V. Blueberry. In *Genetics, Genomics and Breeding of Berries*; Folta, K., Kole, C., Eds.; Science Publishers: Enfield, NH, USA, 2011; pp. 1–40.
16. Hancock, J.F.; Lyrene, P.; Finn, C.E.; Vorsa, N.; Lobos, G.A. Blueberries and cranberries. In *Temperate Fruit Breeding: Germplasm to Genomics*; Hancock, J.F., Ed.; Springer: Dordrecht, The Netherlands, 2008; pp. 115–150.
17. Rowland, L.J.; Bell, D.J.; Alkharouf, N.; Bassil, N.V.; Drummond, F.A.; Beers, L.; Buck, E.J.; Finn, C.E.; Granham, J.; McCallum, S.; et al. Generating genomic tools for blueberry improvement. *IJFS* **2012**, *12*, 276–287. [CrossRef]

18. Lobos, G.A.; Hancock, J.F. Breeding blueberries for a changing global environment: A review. *Front. Plant Sci.* **2015**, *6*, 782. [CrossRef] [PubMed]
19. Foster, T.M.; Bassil, N.V.; Dossett, M.; Worthington, M.L.; Graham, J. Genetic and genomic resources for *Rubus* breeding: A roadmap for the future. *Hortic. Res.* **2019**, *6*, 116. [CrossRef] [PubMed]
20. Castro, P.; Stafne, E.T.; Clark, J.R.; Lewers, K.S. Genetic map of the primocane-fruiting and thornless traits of tetraploid blackberry. *Theor. Appl. Genet.* **2013**, *126*, 2521–2532. [CrossRef] [PubMed]
21. Georgi, L.; Johnson-Cicalese, J.; Honig, J.; Das, S.P.; Rajah, V.D.; Bhattacharya, D.; Bassil, N.; Rowland, L.J.; Polashock, J.; Vorsa, N. The first genetic map of the American cranberry: Exploration of synteny conservation and quantitative loci. *Theor. Appl. Genet.* **2013**, *126*, 673–692. [CrossRef] [PubMed]
22. Daverdin, G.; Johnson-Cicalese, J.; Zalapa, J.; Vorsa, N.; Polashock, J. Identification and mapping of fruit rot resistance QTL in American cranberry using GBS. *Mol. Breed.* **2017**, *37*, 38. [CrossRef]
23. Diaz-Garcia, L.; Schlautman, B.; Covarrubias-Pazaran, G.; Maule, A.; Johnson-Cicalese, J.; Grygleski, E.; Vorsa, N.; Zalapa, J. Massive phenotyping of multiple cranberry populations reveals novel QTLs for fruit anthocyanin content and other important chemical traits. *Mol. Genet. Genom.* **2018**, *293*, 1379–1392. [CrossRef]
24. Diaz-Garcia, L.; Covarrubias-Pazaran, G.; Schlautman, B.; Grygleski, E.; Zalapa, J. Image-based phenotyping for identification of QTL determining fruit shape and size in American cranberry (*Vaccinium macrocarpon* L.). *PeerJ* **2018**, *6*, e5461. [CrossRef]
25. Lerceteau-Kohler, E.; Moing, A.; Guerin, G.; Renaud, C.; Petit, A.; Rothan, C.; Denoyes, B. Genetic dissection of fruit quality traits in the octoploid cultivated strawberry highlights the role of homoeo-QTL in their control. *Theor. Appl. Genet.* **2012**, *124*, 1059–1077. [CrossRef] [PubMed]
26. Graham, J.; Smith, K.; MacKenzie, K.; Jorgenson, L.; Hackett, C.; Powell, W. The construction of a genetic linkage map of red raspberry (*Rubus idaeus* subsp. idaeus) based on AFLPs, genomic-SSR and EST-SSR markers. *Theor. Appl. Genet.* **2004**, *109*, 740–749. [CrossRef] [PubMed]
27. Hackett, C.; Milne, L.; Smith, K.; Hedley, P.; Morris, J.; Simpson, C.G.; Preedy, K.; Graham, J. Enhancement of Glen Moy x Latham raspberry linkage map using GbS to further understand control of developmental processes leading to fruit ripening. *BMC Genet.* **2018**, *19*, 59. [CrossRef] [PubMed]
28. Simpson, C.G.; Cullen, D.W.; Hackett, C.A.; Smith, K.; Hallett, P.D.; McNicol, J.; Woodhead, M.; Graham, J. Mapping and expression of genes associated with raspberry fruit ripening and softening. *Theor. Appl. Genet.* **2017**, *130*, 557–572. [CrossRef]
29. McCallum, S.; Woodhead, M.; Hackett, C.A.; Kassim, A.; Paterson, A.; Graham, J. Genetic and environmental effects influencing fruit colour and QTL analysis in raspberry. *Theor. Appl. Genet.* **2010**, *121*, 611–627. [CrossRef]
30. Paterson, A.; Kassim, A.; McCallum, S.; Woodhead, M.; Smith, K.; Zait, D.; Graham, J. Environmental and seasonal influences on red raspberry flavor volatiles and identification of quantitative trait loci (QTL) and candidate genes. *Theor. Appl. Genet.* **2013**, *126*, 33–48. [CrossRef]
31. Kassim, A.; Poette, J.; Paterson, A.; Zait, D.; McCallum, S.; Woodhead, M.; Smith, K.; Hackett, C.A.; Graham, J. Environmental and seasonal influences on red raspberry anthocyanin antioxidant contents and identification of quantitative trait loci (QTL). *Mol. Nut. Food Res.* **2009**, *53*, 625–634. [CrossRef]
32. Graham, J.; Smith, K.; McCallum, S.; Hedley, P.E.; Cullen, D.W.; Dolan, A.; Milne, L.; McNicol, J.W.; Hackett, C.A. Towards an understanding of the control of 'crumbly' fruit in red raspberry. *SpringerPlus* **2015**, *4*, 223. [CrossRef]
33. Ferrao, L.F.V.; Benevenuto, J.; de Bem Oliveira, I.; Cellon, C.; Olmstead, J.; Kirst, M.; Resende, M.F.R., Jr.; Munoz, P. Insights into the genetic basis of blueberry fruit-related traits using diploid and polyploid models in a GWAS context. *Front. Ecol. Evol.* **2018**, *6*, 107. [CrossRef]
34. Colle, M.; Courtney, P.L.; Wai, C.M.; Ou, S.; Bird, K.A.; Wang, J.; Wisecaver, J.H.; Yocca, A.E.; Alger, E.I.; Tang, H.; et al. Haplotype-phased genome and evolution of phytonutrient pathways of tetraploid blueberry. *GigaScience* **2019**, *8*, 1–15. [CrossRef]