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Pedigree Analysis of Rabbiteye Blueberry Indicates Limited Genetic Diversity

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Abstract. **Limited genetic diversity, compounded by repeated use of elite parents and decreased breeding efforts, poses challenges for rabbiteye blueberry (*Vaccinium virgatum* Aiton) improvement. To facilitate more efficient rabbiteye breeding in the future, this study aims to assess the genetic diversity of rabbiteye cultivars through pedigree analysis and evaluate the impact of interspecific hybridization on modern cultivars. The pedigree data of 180 rabbiteye blueberry accessions were analyzed to calculate inbreeding coefficients, coefficients of coancestry and the genetic contribution of founders. Five founders, 'Myers', 'Black Giant', 'Ethel', 'Clara', and 'W4', collectively account for a minimum of 73% of the genetic composition of rabbiteye cultivars. The level of interspecific hybridization has been limited in rabbiteye breeding, with *Vaccinium corymbosum* contributing 3% of the genetic composition of rabbiteye cultivars and 1% from other species such as *Vaccinium constablaei* and *Vaccinium darrowii*. The narrow genetic base and repeated use of common parents led to an increase in inbreeding coefficients, from an average of less than 0.0002 before 1980 to 0.014 in the 2000s. In the future, it would be valuable to increase the use of exotic materials from other species or within the rabbiteye germplasm to broaden the genetic base while introducing valuable alleles.**

Blueberries (*Vaccinium* spp.) are a popular fruit worldwide for its pleasing taste, visual appeal, and health advantages (Ferrão et al. 2021; Kalt et al. 2020; Ru et al. 2024; Silva et al. 2020). In 2021, the global production of blueberries was 1.1M tonnes, with 0.35M metric tons produced within the United States (Food and Agriculture Organization of the United Nations 2024). The economic value of blueberries is $1.096 billion in the United States (US Department of Agriculture–Economics, Statistics and Market Information Management 2025). Cultivated blueberries can be classified into three types: northern highbush (NHB, *Vaccinium corymbosum* L.), southern highbush (SHB, hybrids between *V. corymbosum* and native species such as *Vaccinium darrowii* Camp), and rabbiteye blueberry (RE, *Vaccinium virgatum* Aiton) (Darnell et al. 1992). NHB and SHB are both autotetraploid ($2n = 4x = 48$), whereas RE is hexaploid ($2n = 6x = 72$) (Lyrene and Ballington 1986). It remains unclear whether *V. virgatum* is autohexaploid, autoallohexaploid, or allohexaploid (Lyrene 1987). Different ploidy levels between RE and other *Vaccinium* species limited introgression among RE, highbush, and wild species.

Wild RE blueberries have been reported in the southeastern United States, mainly found in northern Florida, southern Georgia, and Alabama (Lyrene 1987). The first successful cultivation of wild RE blueberries started on a farm in northeast Florida in 1887 (Camp 1945; Lyrene 1987). RE cultivars are recognized for their tolerance to biotic and abiotic stresses, such as drought and heat (Childers and Lyrene 2006). Compared with highbush cultivars, they also have a better adaptability to a broader range of soil types and pH levels (Dozier et al. 1989). In addition to their easiness of production, high yield and unique flavors also made RE the major ecotype grown in the southern United States, before the popularity of SHB cultivars (Childers and Lyrene 2006).

Breeding of RE blueberries was initiated in the early 20th century by the US Department of Agriculture (USDA)–Agricultural Research Service (ARS) and the Georgia Coastal Plains Experiment Station in Tifton, GA (Lyrene 1987). After that, blueberry breeding programs were also established in other parts of the Southeast to improve rabbiteye cultivars, including the North Carolina State University in the 1930s, the University of Georgia and University of Florida in 1940s, and the USDA-ARS in Poplarville, MS, in 1970s (Lyrene 2006; Hancock 2006; Suszkiw 2008). Intensive evaluation of wild rabbiteye collections were carried out at the USDA-ARS station in Tifton, GA, between 1925 and 1943, which identified superior wild selections such as 'Black Giant', 'Clara', 'Ethel', and 'Myers', which later became the founders for many cultivars (Austin 1984; Lyrene 1987). Early efforts of RE breeding led to the commercial adoption of the first generation of rabbiteye cultivars in the early 1960s (Hancock 2006; Stringer et al. 2023). However, the growing popularity of SHB blueberries, recognized for their superior fruit quality—including large berry size and a crispy texture—led many wholesale growers to transition to SHB production (Clinard J, personal communication). In contrast, RE blueberries are often perceived as seedy, with thick skin and late maturity, which makes them less profitable in the wholesale market (Darnell et al. 1992). As a result, most of the public breeding programs shifted their focus into southern highbush breeding to better meet the demands of major growers (Munoz P and Ashrafi H, personal communication).

RE breeding is increasingly challenged by a narrow genetic diversity due to limited breeding efforts and repeated use of elite parents (Aruna et al. 1993; Childers and Lyrene 2006; Lyrene 1987; Rabaey and Luby 1988). Since RE is sensitive to inbreeding depression (Lyrene 1983; Hancock and Siefker 1982), a narrow genetic base can lead to compromised performance of new cultivars and

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substantially limit the progress of trait improvement. However, the actual level of genetic diversity within rabbiteye germplasm is not well understood, partially due to their hexaploidy, which makes genetic analysis more complicated. The coefficients of inbreeding and coancestry are two common parameters to evaluate inbreeding within an individual and between two individuals (Falconer 1996). The **coefficient of coancestry (*f_{XY}*)**, which is sometimes referred to as the **coefficient of kinship**, measures the probability that any single genes randomly drawn from individuals X and Y are identical by descent (Bernardo 2010; Lynch and Walsh 1998). The **inbreeding coefficient (*F*)**, on the other hand, measures the probability that the two genes at a locus in an individual are identical by descent (Lynch and Walsh 1998). Most equations for coefficients of inbreeding and coancestry were based on diploid species, as the calculation of inbreeding is less straightforward in autopolyploid species due to the higher number of homologous chromosomes and more complicated segregation patterns. Two studies evaluated the level of inbreeding in northern highbush blueberry: one using the diploid model (Hancock and Siefker 1982) and the other using a tetraploid model (Ehlenfeldt 1994). The inbreeding coefficient for northern highbush cultivars increased by more than 16-fold between 1921 (0.0017) and 1990 (0.0281) based on the tetraploid model (Ehlenfeldt 1994), which reported much lower F values compared with the diploid model used by Hancock and Siefker (1982). To better understand the level of genetic diversity in rabbiteye germplasm, the objectives of this research were: (1) to assess the inbreeding coefficient of rabbiteye blueberry cultivars based on pedigree analysis and (2) to evaluate the genetic contribution of rabbiteye founders and the impact of interspecific hybridization in modern cultivars.

Material and Methods

Pedigree and cultivar information. The pedigree information of 180 rabbiteye blueberry accessions and related parents were collected from the Fruit and Nut Cultivars Database (fruitandnutlist.org), accessed 10 Jan 2024; the Germplasm Resources Information Network (https://npgsweb.ars-grin.gov/gringlobal/search); accessed at various time points in 2024, Google Patents (https://patents.google.com/patent), accessed at various time points in 2024; and journal papers listed in Supplemental Table 1. Pedigrees of unpublished selections were collected through communication with blueberry breeders Jessica Spencer, James Ballington, and Hudson Ashrafi (North Carolina State University); Paul Lyrene (University of Florida); and Ebrahiem Babiker (USDA-ARS, Southern Horticultural Research Unit). Detailed information on pedigree, release year, and origination for each accession and 60 cultivars is presented in Supplemental Table 1 and Table 1, respectively. After removing accessions with no connection to other accessions, 148 were used in pedigree analysis. The pedigree of the 148 rabbiteye accessions or parents was visualized with Pedimap software (Voorrips et al. 2012).

Inbreeding coefficient and coancestry coefficient. The coancestry coefficient was calculated using AGHmatrix (Amadeu et al. 2023), an R package developed for diploid and polyploid coancestry coefficient analysis based on equations from Kerr et al. (2012). An additive relationship matrix (A) generated from AGHmatrix, assuming no double reduction, was later divided by 6 to calculate the kinship matrix, which contains the coancestry coefficient of any pair of individuals (Kerr et al. 2012). Furthermore, the inbreeding coefficient of an individual i was calculated as follows,

$$F_i = \frac{2vk_{ii}}{1+(2v-1)} \qquad [1]$$

where k_{ii} is the coancestry coefficient of an individual i with itself, obtained from the diagonal of the kinship matrix, and v is the gametic ploidy level (for an autohexaploid, $v=3$) (Gallais 2003). Additionally, we also confirmed the calculation from AGHmatrix with the calculation based on Eq. [2] for an inbreeding population.

$$F_X = \sum\left[\left(\frac{1}{2v}\right)^{n_1+n_2+1}(1+\ [2v-1]\times F_A)\right] \qquad [2]$$

where v is the ploidy level of the gamete, or half of the ploidy of somatic cells; A is the common founder for parents of X; n_1 is the number of generations from the common ancestor to parent 1 P; and n_2 is the number of generations from the common ancestor to parent 2. F_A is the inbreeding coefficient of the common parent A. Derivations of Eq. [2] can be found in Supplemental File 3.

For hexaploid species, Eq. [2] is equivalent to

$$F_X = \sum\left[\left(\frac{1}{6}\right)^{n_1+n_2+1}(1\ +\ 5F_A)\right] \qquad [3]$$

Genetic contribution of founders. The genetic contribution (GC) of the founders to the developed cultivars was calculated using the following equation (Sjulin and Dale 1987),

$$GC=\sum_{1}^{p}\left(\frac{1}{2}\right)^{n} \qquad [4]$$

where n represents the number of generations in a pedigree pathway between the founder and the developed cultivars, and p denotes the number of pathways between the founder and the developed cultivars. An R script was developed to trace all possible pathways from any ancestor to an offspring and later calculate GC based on Eq. [4] (R script in Supplemental Files 4 and 5).

Cultivars were clustered based on the kinship matrix, with f_{XY} on the off-diagram and F on the diagram using the R package *pheatmap* (cran.r-project.org/web/packages/pheatmap/index.html, accessed 10 Jun 2024), which also generated a dendrogram and heat map.

Results

Comparisons of inbreeding coefficient based on diploid, tetraploid, and hexaploidy models. The increase in the inbreeding coefficient through selfing was much slower in the hexaploid model compared with the tetraploid and diploid models (Supplemental Fig. 1). In a diploid, biparental population derived from unrelated parents, the F value reached 0.75 after three generations of selfing. In contrast, tetraploids require nine generations, while hexaploids needed more than 10 generations of selfing to achieve the same level of inbreeding (Fig. 1). At the 10th generation, the F value for diploids is estimated to be nearly 1, compared with 0.81 for tetraploids and 0.61 for hexaploids.

Genetic contribution of founders. The pedigree data indicate that most cultivars originated from common founders and are only three to four generations away from their wild ancestors (Fig. 2). Further analysis of the GC from wild accessions identified five primary founders that collectively account for 73% of the genetic composition of RE cultivars, including Myers, Black Giant, Ethel, Clara, and W4 (Fig. 3A, Supplemental Table 2). In contrast, the remaining 22 founders contribute just 11% of the genome, with 14% from unknown accessions (open pollination with no record of paternal parents). *V. virgatum* collectively contributes to a minimum of 80% of RE genetic composition and is the primary founding species for RE breeding. The second major species is *V. corymbosum*, which contributes 3% to the genetic composition of RE cultivars. Additionally, 16% of the genetic composition originated from unknown sources due to open pollination, which may include *V. virgatum* or other species. Finally, less than 1% of the genetic composition is attributed to other species such as *V. darrowii*, *Vaccinium tenellum*, *Vaccinium constablaei*, and *Vaccinium angustifolium* (Fig. 3B, Supplemental Table 2).

Further analysis of the genetic contribution of each species in individual cultivars suggested that most of the cultivars contain a genetic background primarily from *V. virgatum*, except for 10 cultivars that contain genes from other species (Table 2). The cultivars with the highest percentage of background from *V. corymbosum* include 'Beckyblue' (50%), 'Aliceblue' (25%), 'Bonita' (25%), 'Chaucer' (25%), 'Nocturne' (16%), 'Snowflake' (13%), 'Prince' (13%), and 'Titan' (13%). 'Nocturne' (13%), 'Snowflake' (13%), 'Summer Sunset' (6%), and 'Florida Rose' (2%) are the cultivars that contain the most background from *V. constablaei*. Both 'Nocturne' and 'Summer Sunset' have backgrounds from *V. darrowii* and *V. angustifolium*.

Inbreeding coefficients of rabbiteye accessions. The inbreeding coefficients for RE cultivars ranged from 0 to 0.06, with 'Muffin Man' having the highest inbreeding coefficient (Fig. 4A, Supplemental Table 3). Of the 61 cultivars studied, 30 exhibited F values greater than zero. Following 'Muffin Man' (0.06), the next highest F values were

Table 1. Information of released rabbiteye blueberry cultivars.

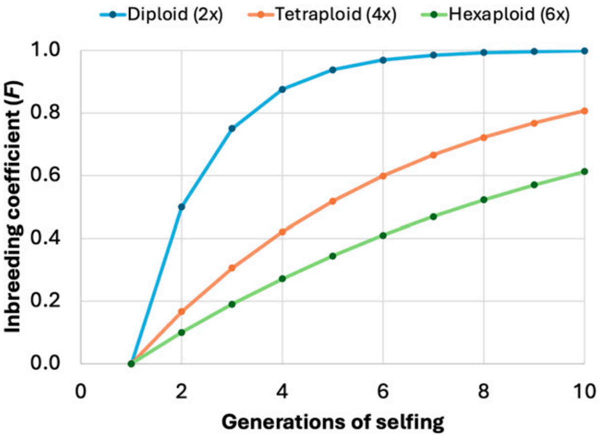
Cultivar	Alias	Female parent	Male parent	GRIN accession number	Organization	Year of release
1 Alapaha		T65	Brightwell		University of Georgia, GA, USA	2006
2 Aliceblue		Beckyblue	OP	PI 554959	University of Florida, FL, USA	1970
3 Austin	T339	T110	Brightwell	PI 618169	University of Georgia, GA, USA	1996
4 Baldwin	T117	Tifblue	Homebell		University of Georgia, GA, USA	1985
5 Beckyblue		FL6-138	E96	PI 554960	University of Florida, FL, USA	1968
6 Bluebelle		Callaway	Ethel	PI 554697	University of Georgia, GA, USA	1974
7 Bluegem		Tifton31	OP	PI 554718	University of Florida, FL, USA	1970
8 Bluesfest		T142	Powderblue	PI 679663	USDA-ARS, MS, USA	2014
9 Bonita		Beckyblue	OP	PI 554719	University of Florida, FL, USA	1985
10 Brightwell		Tifblue	Menditoo	PI 554703	University of Georgia, GA, USA	1981
11 Briteblue		Ethel	Callaway	PI 554702	University of Georgia, GA, USA	1969
12 Callaway		Myers	Black Giant	PI 554699	University of Georgia, GA, USA	1949
13 Centra Blue		Centurion	Rahi		Institute for Plant and Food Research, Auckland, New Zealand	2009
14 Centurion		W4	Callaway	PI 554715	North Carolina State University, NC, USA	1961
15 Chaucer		Beckyblue	OP	PI 657219	University of Florida, FL, USA	1985
16 Choice		Tifton31	OP	PI 554720	University of Florida, FL, USA	1985
17 Climax		Callaway	Ethel	PI 554700	University of Georgia, GA, USA	1974
18 Coastal		Myers	Black Giant	PI 554705	University of Georgia, GA, USA	1949
19 Columbus		NC758	NC911	PI 657150	North Carolina State University, NC, USA	2002
20 De Soto		T110	T107	PI 641332	USDA-ARS, MS, USA	2004
21 Delite		Bluebelle	T15	PI 554696	University of Georgia, GA, USA	1969
22 Dolce Blue		Centurion	Rahi		Institute for Plant and Food Research, Auckland, New Zealand	2011
23 Florida Rose		FL94-88	FL94-81		University of Florida, FL, USA	2004
24 Frostberry Delight	T460	T223	T258		University of Georgia, GA, USA	2017
25 Fuku berry		Woodard	Tifblue		Blueberry grower, Nerima-Ward, Tokyo, Japan	2008
26 Garden Blue		Myers	Clara	PI 657217	North Carolina State University, NC, USA	1958
27 Homebell	GA6-40	Myers	Black Giant	PI 554709	University of Georgia, GA, USA	1955
28 Ira		Centurion	NC911	PI 657152	North Carolina State University, NC, USA	1997
29 Krewer	T1101	Vernon	OP		University of Georgia, GA, USA	2014
30 Lielogu		Delite	Woodard		National Botanic Garden, Salaspils, Latvia	2010
31 Maru		Premier	OP		Institute for Plant and Food Research, Auckland, New Zealand	1992
32 Menditoo		Myers	Black Giant		North Carolina State University, NC, USA	1958
33 Montgomery		NC763	Premier	PI 657151	North Carolina State University, NC, USA	1997
34 Muffin Man	MS1190	T366	Brightwell	PI 687223	USDA-ARS, MS, USA	2018
35 Nocturne		US874	Premier		USDA-ARS, NJ, USA	2014
36 Ocean Blue		Centurion	Rahi		Institute for Plant and Food Research, Auckland, New Zealand	2011
37 Ochlockonee		Tifblue	Menditoo		University of Georgia, GA, USA/ USDA-ARS	2006
38 Onslow		Premier	Centurion	PI 657154	North Carolina State University, NC, USA	2001
39 Overtime		Centurion	Powderblue		Fall Creek Nursery, Lowell, OR, USA	2014
40 Owen		NA	NA	PI 554707	University of Georgia, GA, USA	1983
41 Powderblue		Tifblue	Menditoo	PI 554721	North Carolina State University, NC, USA	1963
42 Premier		Tifblue	Homebell	PI 554717	North Carolina State University, NC, USA	1965
43 Prince		MS598	FL80-11		USDA-ARS, MS, USA	2009
44 Rahi		Premier	OP		Institute for Plant and Food Research, Auckland, New Zealand	1992
45 Salaspils Izturiga		Tifblue	OP		National Botanic Garden, Salaspils, Latvia	2010
46 Savory		NA	NA		University of Florida, FL, USA	2004
47 Sky Blue		Centurion	Rahi		Institute for Plant and Food Research, Auckland, New Zealand	2010
48 Snowflake		Florida-K	NC1830	PI 704125	University of Florida, FL, USA	1991
49 Southland		Garden Blue	Ethel	PI 554701	University of Georgia, GA, USA	1969
50 Spiers		MS167	MS536	PI 702675	USDA-ARS, MS, USA	2023
51 Summer Sunset	T885	Climax	US1056		University of Georgia, GA, USA	2010

(Continued on next page)

Table 1. (*Continued*)

Cultivar	Alias	Female parent	Male parent	GRIN accession number	Organization	Year of release
52 Takahe	Blue Dawn	Premier	OP		Institute for Plant and Food Research, Auckland, New Zealand	1999
53 Tasty Blue	Ono	Premier	OP		Institute for Plant and Food Research, Auckland, New Zealand	1996
54 Tifblue		Ethel	Clara	PI 554698	University of Georgia, GA, USA	1955
55 Titan	T959	Frostberry Delight	FL80-11		University of Georgia, GA, USA	2011
56 Velluto Blue		Maru	Briteblue		Institute for Plant and Food Research, Auckland, New Zealand	2014
57 Vernon		T23	T260		University of Georgia, GA, USA	2007
58 Whitu	Blue Magic	Premier	OP		Institute for Plant and Food Research, Auckland, New Zealand	1996
59 Windy	FL83-97	FL79-19	FL79-27		University of Florida, FL, USA	1993
60 Woodard		Ethel	Callaway	PI 554704	University of Georgia, GA, USA	1960
61 Yadkin		Premier	Centurion	PI 657153	North Carolina State University, NC, USA	1997

GRIN = Germplasm Resources Information Network, OP = Open pollination, USDA-ARS = US Department of Agriculture−Agricultural Research Service.



Generations	Diploid (2*x*)	Tetraploid (4*x*)	Hexaploid (6*x*)
F_1	0.000	0.000	0.000
F_2	0.500	0.167	0.100
F_3	0.750	0.306	0.190
F_4	0.875	0.421	0.271
F_5	0.938	0.518	0.344
F_6	0.969	0.598	0.410
F_7	0.984	0.665	0.469
F_8	0.992	0.721	0.522
F_9	0.996	0.767	0.570
F_{10}	0.998	0.806	0.613

Fig. 1. Comparison of the inbreeding coefficients changes through selfing between diploid, autotetraploid, and autohexaploid models.

observed in 'Spiers' and 'Bluesfest' (both at 0.04), followed by 'Lielogu', 'Austin', 'De Soto', and 'Fuku Berry' at 0.03 (Fig. 4A). Notably, inbreeding coefficients increased more rapidly among recently released cultivars compared with the earlier ones. For instance, the average inbreeding coefficient and standard deviation were 0 ± 0 for cultivars released before 1960, which increased to 0.002 ± 0.004 for cultivars released between 1961 and 1980, 0.005 ± 0.008, and eventually 0.014 ± 0.016 for cultivars released after 2000 (Fig. 4B).

The most inbred cultivar, Muffin Man, was released in 2018 as an ornamental cultivar.

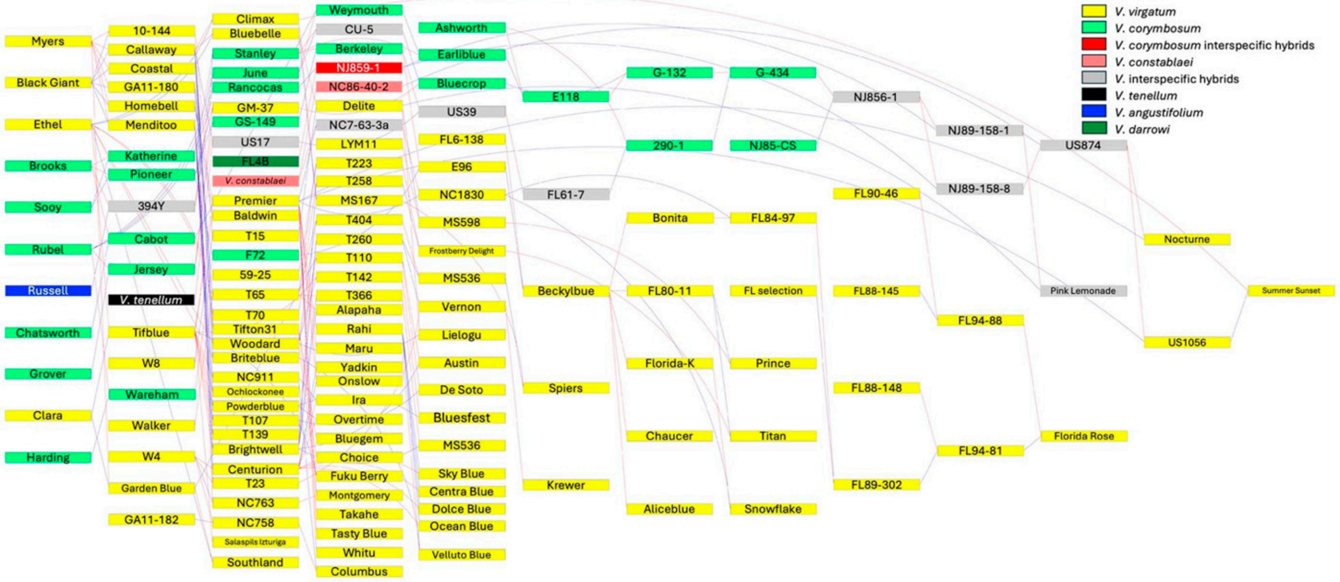


Fig. 2. Pedigree of rabbiteye cultivars and selections. Red lines indicate maternal parents, and blue lines indicate paternal parents. The species of each accession is indicated by the color of the text box: *V. virgatum* (yellow), *V. corymbosum* (green), *V. corymbosum* interspecific hybrids (red), *V. constablaei* (pink), *V.* interspecific hybrids (gray), *V. tenellum* (white), and *V. angustifolium* (blue). A pedigree chart with full resolution can be found in Supplemental Fig. 1

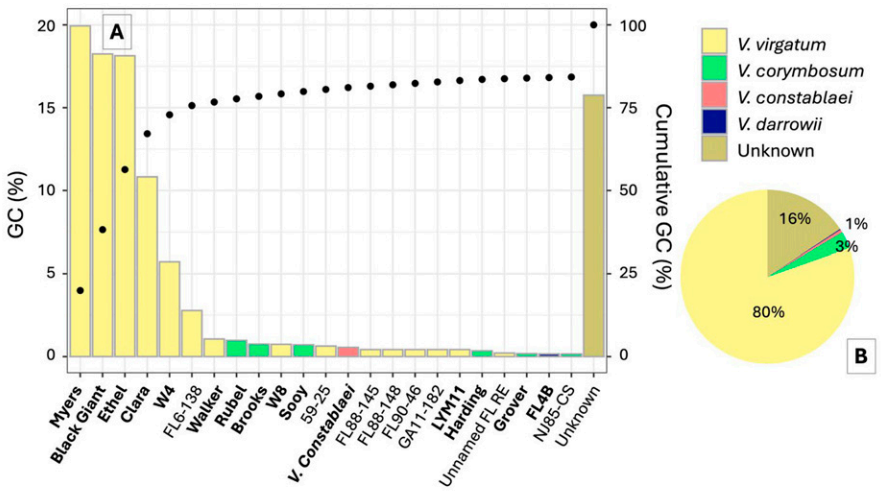


Fig. 3. Genetic contribution (GC) of the primary founders (**A**) and species (**B**). (**A**) The primary *y*-axis on the left displays the GC of the individual founders as shown in the bar plot, while the secondary *y*-axis on the right represents the cumulative GC. Founders with bold names are wild accessions. Only founders with GC > 0.1% are presented in the figure. (**B**) Pie chart depicting the GC of the primary species related to rabbiteye cultivars.

Table 2. Genetic contribution of various species in cultivars with exotic genetic background from species other than *V. virgatum*.

Cultivars	*V. virgatum* Aiton	*V. corymbosum* L.	*V. constablaei*	*V. darrowii*	*V. angustifolium* Aiton	Unknown source	Total
1 Beckyblue	50	50	0	0	0	0	100
2 Aliceblue	25	25	0	0	0	50	100
3 Bonita	25	25	0	0	0	50	100
4 Chaucer	25	25	0	0	0	50	100
5 Nocturne	50	16	13	6	2	13	100
6 Snowflake	50	13	13	0	0	25	100
7 Prince	63	13	0	0	0	25	100
8 Titan	63	13	0	0	0	25	100
9 Summer Sunset	75	8	6	3	1	7	100
10 Florida Rose	94	2	2	0	0	3	100

'Muffin Man' was developed through backcrossing 'T366' with its male parent 'Brightwell'. Notably, 'T65', the female parent of 'T366', also shares two common ancestors, 'Myers' and 'Black Giant', with 'Brightwell', further contributing to the increased inbreeding in 'Muffin Man' (Supplemental Fig. 2). 'Spiers' is another cultivar with a high inbreeding coefficient ($F = 0.04$). Its female parent, 'MS167', is the offspring of a full-sib cross between 'Climax' and 'Bluebelle', which share the same parents: 'Callaway' and 'Ethel'. Adding to inbreeding, the male parent of 'Spiers', 'MS536', shares four common ancestors, 'Myers', 'Black Giant', 'Ethel', and 'Callaway', with the mother, 'MS167' (Supplemental Fig. 3). Similarly, 'Bluesfest' ($F = 0.04$) is the result of a half-sib cross, where the 'T142' and male parent 'Powderblue' share the same parent: 'Tifblue'. Furthermore, both parents of 'Bluefest' can be traced back to four common founders: 'Ethel', 'Clara', 'Myers', and 'Black Giant' (Supplemental Fig. 4).

Limited founders and repeated use of common parents not only led to high inbreeding coefficients for individual accessions but also high coefficients of coancestry between accessions. The f_{XY} values ranged from 0 to 0.12 for all possible pairs of RE cultivars studied. A dendrogram based on the kinship matrix, with f_{XY} on the off-diagram and F on the diagram, grouped RE cultivars into two major clusters (Fig. 5). Cluster 1 includes 'Beckyblue', 'Titan', 'Bonita', 'Aliceblue', 'Chaucer', 'Florida Rose', and 'Prince', while cluster 2 includes all other cultivars. Cluster 2 was further divided into subclusters with 'Premier', 'Tifblue', 'Brightwell', 'Powderblue', 'Austin', 'Muffin Man', 'Ochlockonee', 'Bluesfest', and 'De Soto' in cluster 2.1 and other cultivars in cluster 2.2 (Fig. 5).

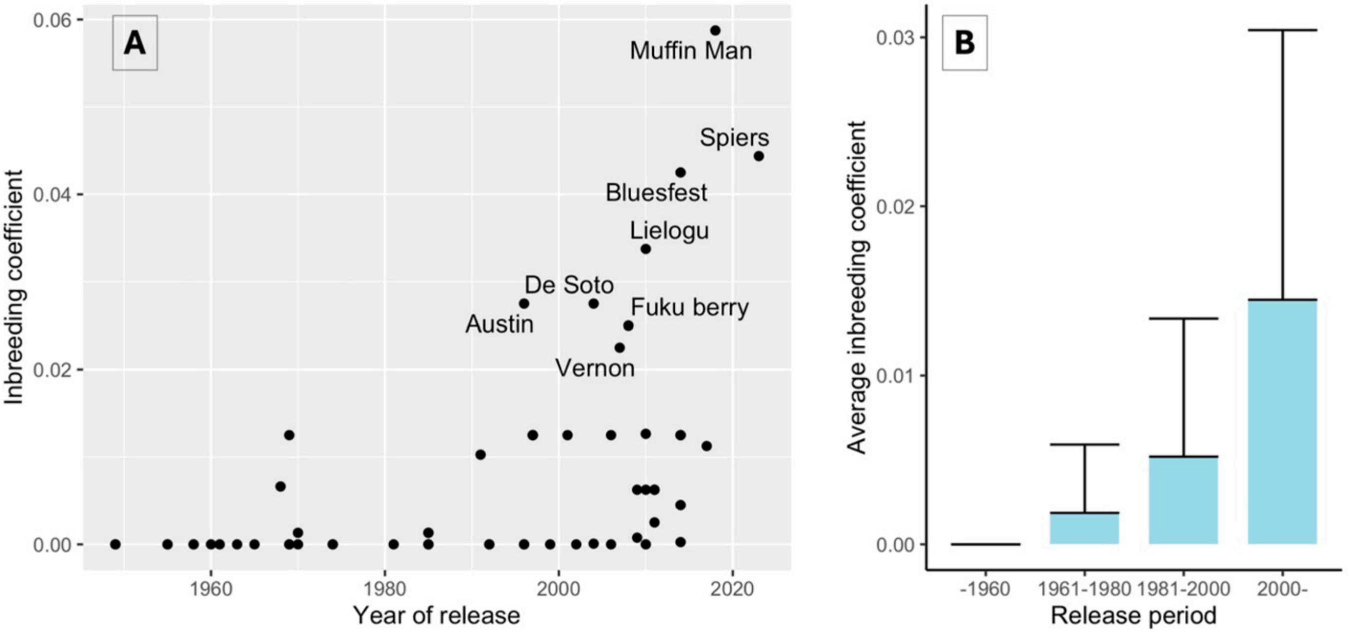


Fig. 4. Inbreeding coefficient of individual rabbiteye (RE) cultivars (**A**) and average inbreeding coefficient of RE cultivars released between various periods (**B**). Error bars represent 1 standard deviation.

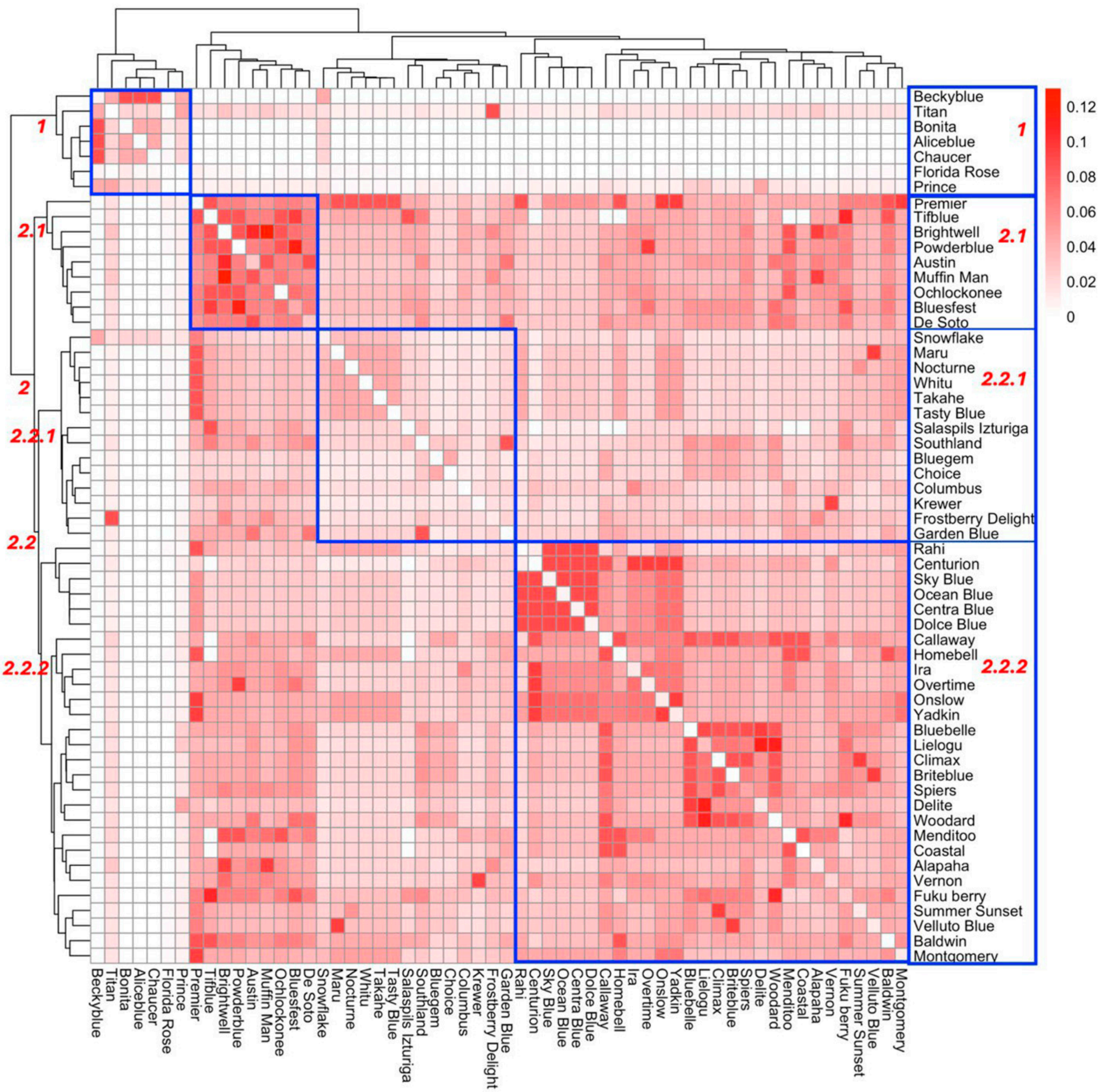


Fig. 5. Heat map and dendrogram of rabbiteye cultivars based on coefficient of coancestry. Cultivars were grouped into two major clusters: cluster 1 and cluster 2, with most of the cultivars grouped under cluster 2. Cluster 2 was further divided into cluster 2.1 and cluster 2.2, with cluster 2.2 further divided into cluster 2.2.1 and cluster 2.2.2.

Among the two highly inbred clusters, cluster 2.1 with nine cultivars had f_{XY} values ranging from 0.05 to 0.13, with a mean of 0.07. The 28 cultivars in cluster 2.2.2 had f_{XY} values ranging from 0.01 to 0.11, with a mean of 0.05.

Discussion

The pedigree analysis demonstrates that RE cultivars originated mainly from five founders, ‘Myers’, ‘Black Giant’, ‘Ethel’, ‘Clara’, and ‘W4’, which collectively account for a minimum of 73% of the genetic composition of RE cultivars. The actual contribution of these five founders is likely to be much higher than 73% for two reasons. First, the unknown pollen sources for open-pollinated accessions could also come from offsprings of the five founders. Second, some RE founders, such as ‘FL6-138’, ‘59-25’, and ‘FL88-145’, lacked pedigree records and are likely related to the five founders. Considering all the possible connections to the five founders, the total genetic contribution of ‘Myers’, ‘Black Giant’, ‘Ethel’, ‘Clara’, and ‘W4’ in RE cultivars could be as high as 93%, indicating the extreme narrow genetic base of RE germplasm.

In contrast to NHB and SHB, in which interspecific hybridization has played an important role in cultivar improvement, the level of interspecific hybridization has been limited in RE breeding (Lyrene 1987). Differences in ploidy levels and the tendency of offspring losing the blue color were among the obstacles for crossing highbush with RE cultivars (Darnell et al. 1992). Regardless, evidence of limited interspecific hybridization can be observed from the pedigree data. For example, NHB contributes 50% to the genome of ‘Beckyblue’ and 25% each to ‘Aliceblue’, ‘Bonita’, and ‘Chaucer’, respectively. ‘Nocturne’ is the most exotic RE cultivar, which contains 50% of *V. virgatum*, 16% of *V. corymbosum*, 13% of *V. constablaei*, 6% of *V. darrowii*, and 2% of *V. angustifolium*.

Individuals with the highest level of interspecific hybridization were also the ones with the lowest coefficient of coancestry with common RE cultivars.

Inbreeding has a significant negative effect on blueberries, including reduced vigor (Dorr and Martin 1966; Hancock and Siefker 1982; Lyrene 1983; Morrow 1943; White and Clark 1939), reduced fruit set (Rabaey and Luby 1988) and berry size (Aalders and Hall 1961; Rabaey and Luby 1988), as well as postponed maturity (Rabaey and Luby 1988). Many studies emphasized the importance of broadening the genetic base of breeding germplasm to improve cultivars for better resilience against biotic and abiotic stresses (Conner et al. 2023; Ehlenfeldt 1994; Noiton and Alspach 1996; Pérez de los Cobos 2021; Sjulin and Dale 1987). To introduce desirable characteristics from other species while broadening the genetic base for RE, future efforts should focus on interspecific hybridization and increasing the use of exotic cultivars such as Nocturne, Bekyblue, and Aliceblue in new crosses. Additionally, some wild accessions, such as PI 638759 and PI 695363, which did not appear in the pedigree of modern cultivars (Supplemental Table 1), can serve as valuable resources to broaden the genetic base of RE breeding. Additionally, since blueberry is native to the southeastern United States, systematic collection, evaluation, and integration of these valuable natural genetic resources holds promise to overcome the bottleneck of RE breeding.

Despite the narrow genetic base and high level of inbreeding in RE cultivars, the values of F and f_{XY} based on hexaploid models tended to be lower than diploid and tetraploid models. For example, the average F value of the NHB cultivar Elliott was estimated to be 0.19 based on a diploid model (Hancock and Siefker 1982) but only 0.006 based on a tetraploid model (Ehlenfeldt 1994). NHB cultivars released between 1951 and 1960 was estimated to have an average F of 0.14 based on a diploid model (Hancock and Siefker 1982) but only 0.003 based on a tetraploid model (Ehlenfeldt 1994). The slower increase in inbreeding in polyploid species can be attributed to more pathways for allele transmission from one generation to the next, which reduced the probability of having identical alleles within an individual or between a pair of individuals. Therefore, it is important to use the appropriate equations/models to derive F and f_{xy} values for polyploid species and only compare those values within the same ploidy level.

This study used pedigree information to analyze the genetic diversity and population structure of RE cultivars. However, relying solely on pedigree data for such analyses has its limitations. Firstly, pedigree records can contain errors due to oversight in record keeping or outcrossing events that occurred naturally or through artificial means. Secondly, pedigree information is missing in founders and open-pollinated families to reveal the genetic composition of those individuals. Thirdly, while pedigree analysis estimates the relatedness between individuals based on average allele transmission from parents to offspring (Falconer 1996; Lynch and Walsh 1998), it does not accurately capture the actual genetic transmission in any single individual. To enhance our understanding of the genetic diversity and population structure of RE germplasm, future research should incorporate genomic information from RE accessions to validate the accuracy of pedigree data. Genomic data can provide a more accurate perspective for population structure analysis in several ways. For instance, it can help infer the genetic composition of open-pollinated offspring whose paternal parentage is unknown. Additionally, genomic information can validate the accuracy of pedigree records. In a study of potato clones, for example, single nucleotide polymorphism (SNP) marker-based genotypic data were used to verify pedigree information, revealing discrepancies in records for several clones (Pandey et al. 2021). Similarly, genome-wide SNP data have been employed to elucidate the relatedness among SHB, NHB, RE, and other blueberry types (Nishiyama et al. 2021), suggesting that RE cultivars are genetically distinct from both SHB and NHB cultivars, which aligns with the findings of this study.

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