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RESEARCH ARTICLE

Genetic diversity and cultivar variants in the NCGR cranberry (*Vaccinium macrocarpon* Aiton) collection

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Abstract. The American cranberry (*Vaccinium macrocarpon*) is an endemic domesticated species that has become an economically important commercial fruit crop. The USDA-ARS National Clonal Germplasm Repository (NCGR) houses the national *Vaccinium* collection, which includes representatives of historical cranberry cultivars and wild-selected germplasm. The objective of this study was to examine the genotypes of 271 cranberry plants from 77 accessions representing 66 named cultivars using 12 simple-sequence repeats to assess clonal purity and cultivar relatedness. Using principal components analysis and neighbour-joining based on estimated genetic distances between individuals, we identified 64 unique genotypes and observed that intracultivar variants (i.e. subclones) existed in the germplasm collection and in the commercial bogs where some accessions originated. Finally, through a comparison of the genotypes of this study with the previous studies, pedigree analysis and the study of the geographic distribution of cranberry diversity, we identified consensus genotypes for many accessions and cultivars. We highlight the important role that the NCGR collection plays for *ex situ* conservation of cranberry germplasm for future breeders and researchers. The NCGR continues to search for historically relevant cultivars absent from the collection in an effort to preserve these genotypes before they are lost and no longer commercially grown.

Keywords. genetic resources; *ex situ* conservation; wild selections; microsatellites; simple sequence repeats; consensus genotypes.

Introduction

The American cranberry (*Vaccinium macrocarpon* Ait.) is a North American native species belonging to the family Ericaceae (Vander Kloet 1983a; Darrow and Camp 1945), which is naturally found in the acidic bogs of the northeastern United States, primarily east of the Mississippi river and southeastern Canada (Vander Kloet 1983a). The US is the global leader in cranberry production, with over 41,400 acres of an estimated 53,000 bearing acres worldwide, principally planted in Wisconsin (20,900 acres), Massachusetts (13,000 acres), New Jersey (3100 acres), Oregon (2700 acres) and Washington (1700 acres) with an estimated value of $292.29 million (USDA-NASS 2017).

Cranberry domestication and cultivation began in the mid-1800s, and since then, more than 132 wild selections have been documented (Chandler and Demoranville 1958; Dana 1983; Eck 1990). Historically, many of these wild selections were distributed and shared among growers as named cultivars, but most of them are no longer grown and have been lost or forgotten. Until the

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development of hybrid cultivars in the 1950s, wild selections dominated the cranberry industry as clonally preserved and propagated cultivars (Eck 1990). The first American cranberry genetic improvement efforts were initiated in the 1930s as a collaboration between the USDA-ARS and the Agricultural Experiment Stations of Massachusetts, New Jersey and Wisconsin. Since then approximately a dozen hybrid cultivars have been developed and released. From 1950 to 1970, the first cranberry breeding cycle was implemented with crosses between wild selections and resulted in the release of seven first generation artificial hybrid cultivars ('Beckwith', 'Bergman', 'Crowley', 'Franklin', 'Pilgrim', 'Stevens' and 'Wilcox') (Eck 1990). The second breeding cycle generated seven additional cultivars ('HyRed', 'GH1', 'DeMorranville', 'Crimson Queen', 'Mullica Queen', 'Sundance' and 'BG'). These cultivars were generated by crossing first-generation hybrids and elite wild selections, and resulted in the improvement of fruit quality (e.g. fruit anthocyanin content) and increased productivity (Fajardo *et al.* 2013). The current breeding programmes are located in New Jersey at Rutgers and Wisconsin at the USDA-ARS, University of Wisconsin-Madison.

One problem which has continually affected the industry and breeding programmes has been the uncertainty of true-to-type cranberry germplasm. Genetic inconsistencies of named cultivars are common in different growing areas and even within plantings of a single cultivar (Dana 1983; Eck 1990; Fajardo *et al.* 2013). Most of the potential genetic heterogeneity stems from the lack of control by cranberry growers in the preservation, propagation and distribution of both wild and hybrid cranberry cultivars in a clonally propagated system (Fajardo *et al.* 2013). Additionally, many commercial plantings are considerably old (i.e. 25–100 years) and have become contaminated with seedlings over time (Novy *et al.* 1994). Thus, even using modern molecular markers, it is difficult or impossible to identify a single consensus genotype for many named varieties (Novy and Vorsa 1995; Novy *et al.* 1996; Fajardo *et al.* 2013). To compound the problem, some wild cultivars were released as selection mixtures (Dana 1983; Eck 1990), which further contributed to the challenge of preserving true-to-type clonal cranberry cultivars.

The USDA-ARS National Clonal Germplasm Repository (NCGR) in Corvallis, Oregon has preserved and maintains a large collection of *V. macrocarpon* accessions. Most of the accessions in the collection are wild selections that are no longer commercially grown or are in danger of being lost. The NCGR cranberry collection is an important resource for worldwide cranberry researchers and growers. The collection preserves and freely distributes cranberry genetic resources and represents an important reservoir of genes for improving agronomic traits such as yield, quality, disease and insect resistance. Preliminary fingerprinting of 16 accessions from the NCGR collection using blueberry microsatellites or simple sequence repeats (SSRs) identified different genetic profiles in two 'Searles' accessions obtained from Wisconsin (Bassil *et al.* 2009). Also using blueberry SSRs, intracultivar variants or 'subclones' with different genetic profiles were identified from vines growing in the same pot and representing a single accession (Bassil, unpublished). Therefore, single runners from each accession were used to propagate new clones of each accession and were subsequently genetically fingerprinted as described below.

In an effort to ensure genetic purity of cultivars in the NCGR collection, the objective of this study was to determine the genetic purity and trueness-to-type of cultivars and important selections of *V. macrocarpon* in the NCGR collection. We used microsatellite markers from previous cranberry genetic diversity studies (Zhu *et al.* 2012; Fajardo *et al.* 2013; Zalapa *et al.* 2015), which are randomly distributed across nine of the 12 cranberry linkage groups (Schlautman *et al.* 2015a). The specific objectives of this study were to (i) determine clonal purity and genetic integrity of each NCGR cranberry accession and (ii) assess the genetic diversity and relatedness of the cranberry cultivar collection. This research will provide genetic information for management, maintenance and use of the accessions in the NCGR collection.

Materials and methods

Plant material and DNA isolation

Leaf tissue was collected from 271 plants belonging to 78 accessions representing 66 named cultivars from the NCGR collection (table 1). A BenchTop lyophilizer (Virtis, Gardiner, USA) was used to freeze-dry leaves from single runners of each of the 271 plants for 72 h. Total genomic DNA from 0.1 g leaf tissue was extracted using a Macherey-Nagel Plant II kit (Düren, Germany) following the manufacturer's instructions.

Polymerase chain reaction (PCR) amplification and fragment analysis

PCR was performed according to Schlautman *et al.* (2015b) using carboxyfluorescein (FAM) and hexachlorofluorescein (HEX) M13 tags (5′-CACGTTGTAAAACGAC-3′). Briefly, reactions were performed in 8 μL total volume using 3.5 μL 1× JumpStart REDTaq ReadyMix (Sigma, St Louis, USA), 1.0 μL of 15 ng/μL DNA, 2.0 μL of ddH_2O, 0.5 μL of 5 μM forward primer, 0.5 μL of 50 μM reverse primer and 0.5 μL of 0.5 μM M13-FAM, M13-HEX, or M13-NED primer. Thermocycling conditions included a 3 min melting step of 94°C, followed by 33 cycles of 94°C for 15 s, 55°C for 90 s and 72°C for 2 min, and a final extension step of 72°C for 30 min. One microlitre each of FAM and HEX labelled PCR product was mixed with 10 μL formamide and a carboxy-X-rhodamine

Table 1. List of the 78 cranberry (*V. macrocarpon*) accessions ($n = 271$) representing 66 cultivars preserved at the USDA-ARS NCGR analysed using 12 SSR loci.

Accession (PI)	Cultivar name	Origin/pedigree	*n*	Plant code
554978[a]	No. 35	Howes×Searles	6	491.001-.006
1758[b]	No. 41	Uncertain	1	1758.001
554979[a]	AJ	Searles×Early Black	6	492.001-.006
618042[b]	Bain 2	Wild, WI	1	1029.001
618043[b]	Bain 3	Wild, WI	1	1030.001
618044[b]	Bain 4	Wild, WI	1	1031.001
618045[b]	Bain 5	Wild, WI	1	1032.001
618046[b]	Bain 6	Wild, WI	1	1033.001
618047[b]	Bain 7	Wild, WI	1	1034.001
618048[b]	Bain 8	Wild, WI	1	1035.001
618049[b]	Bain 9	Wild, WI	1	1036.001
618050[b]	Bain 10	Wild, WI	1	1037.001
618041[a]	Bain Favorite No. 1	Wild, WI	6	1028.001-.006
618051[a]	Bain Favorite No. 2	Wild, WI	5	1038.001-.003,.005-.006
618052[b]	Bain McFarlin	Wild, MA	1	1039.001
657266[b]	BE 4	Uncertain	1	1825.001
554990[a]	Beckwith	McFarlin×Early Black	4	496.001,.004-.006
554983[a]	Ben Lear	Wild, WI	5	503.001-.005
554973[b]	Bennett	Wild, WI	1	112.002
657166[c]	Bennett (bog4)	Wild, WI	2	1677.001-.002
554982[a]	Bergman	Early Black×Searles	6	662.002-.007
618053[b]	Biron selection	Uncertain	1	1040.001
555008[a]	Black veil	Wild, MA	6	770.001-.006
555024[a]	Bugle: Mashpee type	Wild, MA	6	827.001-.006
555023[b]	Bugle: Wareham type	Wild, MA	1	826.001
555009[a]	Centennial	Wild, MA	6	771.001-.006
554999[a]	Centerville	Wild, MA	5	745.001-.005
555000[a]	Champion	Wild, MA	6	746.001-.006
554980[a]	Cropper	Wild, NJ	3	493.001-.003
554976[a]	Crowley	McFarlin×Prolific	6	111.001-.006
657167[c]	Crowley (bog5)	McFarlin×Prolific	2	1678.001-.002
657170[c]	Crowley (bog12)	McFarlin×Prolific	2	1681.001-.002
618054[b]	Drever	Uncertain	1	1041.001
554986[a]	Early Black	Wild, MA	5	741.002-.006
555001[b]	Foxboro Howes	Wild, MA	1	747.002
554998[a]	Franklin	Early Black×Howes	6	743.001-.006
555010[a]	Garwood Bell	Wild, NJ	6	772.001-.006
555011[a]	Gebhardt Beauty	Wild, WI	5	773.001-.005
638768[b]	Grygleski 2	Earl Rezin×McFarlin	1	1447.001
618055[b]	Habelman 2	Uncertain	1	1042.001
554995[b]	Hamilton (genetic dwarf)	Wild, MA	1	708.002
618056[b]	Hollison	Wild, MA	1	1043.001
614076[a]	Howes	Wild, MA	6	1296.001-.006
554996[a]	Langlois form	Uncertain	6	709.001-.006
554985[a]	LeMunyon	Wild, NJ	5	499.001-.005
618057[b]	Matthews	Wild, MA	1	1044.002
614075[a]	McFarlin	Wild, MA	5	1296.001-.003, .005-.006
657165[c]	McFarlin (bog3)	Wild, MA	2	1676.001-.002
618058[b]	Middleboro	Wild, MA	1	1045.001
657169[c]	Olson's Honkers (bog11)	Wild, NJ	2	1680.002
554987[a]	Olson's Honkers	Wild, NJ	6	505.001-.006
555003[a]	Paradise Meadow	Wild, MA	6	749.001-.006
555005[a]	Perry Red	Wild, MA	6	751.001-.006
614077[a]	Pilgrim	Prolific×McFarlin	5	1297.001-.005
657168[c]	Pilgrim (bog10)	Prolific×McFarlin	2	1679.001-.002
555012[a]	Potter's Favorite	Wild, WI	6	774.001-.006

Table 1 (*contd*)

Accession (PI)	Cultivar name	Origin/pedigree	*n*	Plant code
555004[a]	Pride	Wild, MA	6	750.001-.006
554993[a]	Prolific	Wild, MI	6	666.001-.006
618060[b]	Rezin McFarlin	Wild, MA	1	1047.001
618061[b]	Rezin Native	Wild, WI	1	1048.001
555002[a]	Round Howes	Wild, MA	6	748.002-.007
555013[a]	Searles	Wild, WI	10	775.001-.009,.011
555014[a]	Shaw's Success	Wild, MA	5	776.001-.005
554972[a]	Stankavich	Uncertain	5	110.001-.005
618059[b]	Stanley	Wild, MA	1	1046.001
614078[a]	Stevens	McFarlin×Potter	3	1298.001-.003
657161[c]	Stevens (bog1)	McFarlin×Potter	2	1672.001-.002
657162[c]	Steven (bog2)	McFarlin×Potter	2	1673.001-.002
657163[c]	Stevens (bog8)	McFarlin×Potter	2	1674.001-.002
657164[c]	Stevens (bog9)	McFarlin×Potter	2	1675.001-.002
657171[c]	Stevens (bog6)	McFarlin×Potter	2	1682.001-.002
657172[c]	Stevens (bog7)	McFarlin×Potter	2	1683.001-.002
555015[a]	Thunder Lake 3	Wild, WI	6	777.001-.006
555016[b]	Thunder Lake 4	Wild, WI	1	778.001
555006[a]	Wales Henry	Wild, MA	6	752.001-.006
555007[a]	Whiting Randall	Wild, MA	6	753.001-.006
614079[a]	Wilcox	Howes×Searles	6	1299.001-.006
555028[a]	Yellow Bell (mutant yellow fruit)	Wild, ME	6	832.001-.006

[a]Core cultivars ($n = 39$) represented by 3–10 plants.
[b]Cultivars ($n = 27$) represented by a single plant.
[c]Cultivars ($n = 6$) with 2–12 samples collected from 12 cultivated bogs.

(ROX; GeneFlo-625 ROX; CHIMERx, Milwaukee, USA) ladder and the pool-plexed mix was sent to the University of Wisconsin Biotechnology Center DNA Sequencing Facility for fragment analysis using a ABI 3730 fluorescent sequencer (Pop-6 and a 50 cm capillary array; Applied Biosystems, Foster City, USA). Allele genotyping was performed using the GeneMarker software v 1.91 (SoftGenetics LLC, State College, USA). To avoid technical and human error during genotyping, PCR was repeated as needed (up to three times) and multiple scorers were used to ensure the repeatability of the results.

Assessing the genetic diversity of cranberry genotypes in the NCGR

We used 12 microsatellite loci to genotype all 271 plants in this study according to Fajardo *et al.* (2013) and 36 additional SSR loci from Zhu *et al.* (2012) were used to test four plants to provide a test of robustness of power to differentiate samples based on 12 versus 48 loci. Diversity statistics, such as the observed number of alleles (N_a), number of effective alleles (N_e), Shannon's information index (I) and observed (H_o) and expected (H_e) heterozygosity were calculated using GenAlEx 6.4 (Peakall and Smouse 2006). Additionally, the average nonexclusion probability for identity of two unrelated individuals per locus (NE-I) was calculated using Cervus v. 3.0 (Slate *et al.* 2000; Field Genetics Ltd.). Unique clones were initially detected by calculating a simple genetic distance matrix between all 271 plants based on the 12 microsatellite loci. Then, the alleles of each genotype were individually examined to determine clones. When two genotypes had identical allelic constitutions at all 12 microsatellite markers used, they were considered clones. When slight allelic differences were observed (1–3 alleles) among two genotypes, we repeated the PCR and confirmed the results. Missing data was almost nonexistent in this dataset with only 10 missing points in a data matrix consisting of 271 rows and 24 columns. After the clones were clearly identified, the final genetic distance between plants was estimated with R v3.2.3 using the Rogers' genetic distance formula implemented in the *adegenet* package (Rogers 1972; Jombart 2008; R Core Team 2015). Finally, for known hybrids between wild parents, we performed an analysis to confirm their pedigree based on simple allelic comparison across genotypes.

Analysing geographic patterns of diversity and identifying intracultivar variants

The 64 unique genotypes in the collection were classified into three separate groups (i.e. east of the Appalachians, west of the Appalachians and first generation hybrids) based on their assumed geographic origin from information provided in published descriptions of cranberry cultivars (Chandler and Demoranville 1958; Dana 1983;

Eck 1990). Because cranberries are not native to the Pacific Northwest (Vander Kloet 1983b), all germplasm collected from marshes in Washington and Oregon were classified as of uncertain origin because they are presumed to have been imported from elsewhere. Two methods, neighbour-joining (NJ) and principal components analysis (PCA), were used to determine the relatedness of the cranberry clones, to identify potential cultivar variants and to determine if there are any east versus west differentiation with the Appalachian Mountains potentially serving as a migration geographic barrier between wild cranberry populations. NJ trees based on the estimated Rogers' genetic distances between unique clones were generated using the *adegenet* package with 100 bootstrap replicates performed using the *ape* package (Paradis *et al.* 2004; Jombart 2008). PCA using the 12 microsatellite loci was also performed with the *adegenet* package (Jombart 2008). Because including both wild and intra-specific hybrid plants can sometimes complicate the resulting phylogenies (Camp 1945; Vander Kloet 1983b; Schlautman *et al.* 2016), PCA and NJ trees were calculated first using all unique clones and then a second time after excluding the first generation artificial hybrids (east×west crosses) from the dataset.

Results and discussion

SSR diversity in the NCGR collection

This analysis included 271 cranberry plants belonging to 78 accessions from 66 named cultivars representing all major wild selections and the seven available first-generation hybrids (table 1; Eck 1990). Of the 66 named cultivars studied in the core collection, 27 were represented by a single plant while 39 were represented by 3–10 plants obtained from single-cutting propagules. Additionally, six cultivars from 12 Oregon commercial bogs were also represented by two plants from each bog collected as single cuttings (table 1). Based on the 168 alleles in the NCGR collection observed for the 12 SSR loci, the estimated genetic distances between plants and resulting NJ tree revealed 64 unique multilocus genotypes (table 2; figure 1). The number of alleles per locus (N_a) for these 64 genotypes ranged from 7 to 21 and effective alleles per locus (N_e) ranged from 3.73 to 9.66 (table 3). Shannon's information index (I) for each locus ranged from 1.51 to 2.50. The observed heterozygosity ranged from 0.47 to 0.88. The average non-exclusion probability for the identity of two unrelated individuals (NE-I) for each locus ranged from 0.12 to 0.02. These diversity estimates are equivalent or higher than in other woody plant species (Muzzalupo *et al.* 2014; Teixeira *et al.* 2014; Brunet *et al.* 2016).

The results of this study suggest that these 12 nuclear SSRs are more useful for cranberry diversity studies than the 54 SSRs from the cranberry plastid and mitochondrial genomes developed by Schlautman *et al.* (2016), which did not distinguish nine commercial cranberry cultivars. A comparison with the only two other cranberry diversity studies using the same nuclear SSRs showed that the genetic diversity of the accessions evaluated from the NCGR collection is greater than that observed in the 21 commonly grown cultivars sample study by Fajardo *et al.* (2013) and in five wild populations from Wisconsin intensely studied by Zalapa *et al.* (2015). In fact, while the current study found 64 unique genotypes among 271 plants from 66 cultivars preserved in the NCGR collection, Fajardo *et al.* (2013) studied 164 plants from 21 commonly grown cultivars and found only 36 unique genotypes. Similarly, Zalapa *et al.* (2015) detected only 42 unique genotypes in 192 plant samples from five native populations in Wisconsin. Further, in the current study, the average number of alleles, effective number of alleles and Shannon's information index were all higher (table 3) than in the studies of both Fajardo *et al.* (2013) ($N_a = 9.75$, $N_e = 3.79$ and $I = 1.56$) and Zalapa *et al.* (2015) ($N_a = 8.09$, $N_e = 3.32$ and $I = 1.38$).

SSR discrimination power

Although SSR loci are the molecular markers of choice for DNA fingerprinting in the plant sciences due to their reliability, repeatability and multiallelic codominant nature (Zalapa *et al.* 2012), a lack of SSR discrimination power could lead to the inability to differentiate cultivars which are truly unique. Cranberry is an outcrossing species, but it is also highly self-fertilizing (Zalapa *et al.* 2015). Thus, a high probability exists of 'volunteer' seedlings arising from self-pollination, which over time can cause inbreeding that would be difficult to detect using low numbers of SSR markers. To test the power and robustness of the 12 SSR loci to discriminate different genotypes, we re-analysed and compared two pairs of cultivars deemed genetically identical (table 2) in the current study using an existing dataset of 48 SSRs (Zhu *et al.* 2012): 'Prolific' (554993, 666.002) versus 'Crowley' (554976, 111.003) and 'Potter's Favorite' (555012, 774.005) versus 'Ben Lear' (554983, 503.001). 'Potter's Favorite' and 'Ben Lear' accessions were genetically identical based on 64 alleles from 48 SSR loci (dataset from Zhu *et al.* 2012) versus 18 alleles from the 12 SSR loci (current dataset; table 1 in electronic supplementary material at http://www.ias.ac.in/jgenet/). Similarly, 'Prolific' and 'Crowley' were also genetically identical based on 69 alleles from 44 SSR loci versus 23 alleles from 12 SSR loci. Therefore, these two pairs of accessions are genetically identical, and the 12 SSR loci appear to provide a good level of discrimination.

The high discrimination power of the loci used was also reflected by the low NE-I $= 6.20 \times 10^{-17}$ (average/locus=0.05) for the 12 SSRs (i.e. the probability that two cultivars, selected by chance, cannot be differentiated

Table 2. Unique ($n = 64$) cranberry (*V. macrocarpon*) genotypes detected among 78 cranberry plants ($n = 271$) from 78 accessions representing 66 named cultivars housed at the USDA-ARS NCGR using 12 SSR loci.

Genotype ID	Cultivar composition	Consensus cultivar
1	**McFarlin (bog3)**_1676.001, 1676.002; **Stevens (bog8)**_1674.001; **FoxboroHowes**_747.002; **Bennett (bog4)**_1677.001, 1677.002; **Crowley**_111.001, 111.002, 111.003, 111.004, 111.005, 111.006; **Pride**_750.001, 750.002, 750.003, 750.004, 750.005, 750.006; **Prolific**_666.001, 666.002, 666.003, 666.004, 666.005, 666.006	–
2	**Howes**_1296.003	Howes[a,b]
3	**Howes**_1296.001, 1296.002, 1296.004, 1296.005, 1296.006	Howes self[b]
4	**Olsons Honkers (bog11)**_1680.002	–
5	**Searles**_775.004, 775.005, 775.006, 775.007, 775.008; **Bain9**_1036.001	–
6	**Yellow Bell**_832.001, 832.002, 832.003, 832.004, 832.005	Yellow Bell[a]
7	**No. 35**_491.001, 491.002, 491.003, 491.004, 491.005, 491.006	No. 35[a,b]
8	**Stevens**_1298.001	–
9	**Wilcox**_1299.001, 1299.002, 1299.003, 1299.004, 1299.005, 1299.006	Wilcox[a,b]
10	**BE4**_1825.001	BE4[c]
11	**Franklin**_743.001, 743.002, 743.003, 743.004, 743.005, 743.006; **Centennial**_771.001, 771.002, 771.003, 771.004, 771.005, 771.006	Franklin[a,b]
12	**Pilgrim (bog10)**_1679.002	–
13	**Crowley (bog10)**_1681.001; **PerryRed**_751.001, 751.002, 751.003, 751.004, 751.005, 751.006	–
14	**Yellow Bell**_832.006	–
15	**Bugle: Mashpee Type**_827.001, 827.002, 827.003, 827.004, 827.005, 827.006	Bugle: Mashpee type[c]
16	**Stankavich**_110.001, 110.002, 110.003, 110.004, 110.005,	Stankavich[c]
17	**Black Veil**_770.001, 770.002, 770.003, 770.004, 770.006	Black Veil[c]
18	**Paradise Meadow**_749.001, 749.002, 749.003, 749.004, 749.005, 749.006	Paradise Meadow[c]
19	**Middleboro**_1045.001	Middleboro[c]
20	**Early Black**_741.003	Early Black self[b]
21	**Early Black**_741.005	Early Black self[b]
22	**Bugle: Wareham Type**_826.001; **Centerville**_745.001, 745.002, 745.003, 745.004, 745.005	–
23	**Hamilton**_708.002	Hamilton[c]
24	**Beckwith**_496.001, 496.004, 496.005, 496.006	–
25	**Cropper**_493.001, 493.002, 493.003	Cropper[c]
26	**Champion**_746.001, 746.002, 746.003, 746.004, 746.005	Champion[c]
27	**Round Howes**_748.003, 748.007; **Stanley**_1046.001	–
28	**Shaws Success**_776.001, 776.002, 776.003, 776.004, 776.005	Shaws Success[c]
29	**WhitingRandall**_753.001, 753.002, 753.003, 753.004, 753.005, 753.006; **BlackVeil**_770.005	Whiting Randall[c]
30	**Crowley (bog12)**_1681.002	–
31	**Langlois Form**_709.001, 709.002, 709.003, 709.004, 709.005, 709.006	Langlois Form[c]
32	**Bain Favorite No. 1**_1028.001, 1028.002, 1028.003, 1028.004, 1028.005, 1028.006	–
33	**Rezin McFarlin**_1047.001	McFarlin[a,b]
34	**Olsons Honkers**_505.001, 505.002, 505.003, 505.004, 505.005, 505.006	Olsons Honkers[c]
35	**Bain Favorite No. 2**_1038.001, 1038.002, 1038.003, 1038.005, 1038.006	Bain Favorite No. 2[c]
36	**LeMunyon**_499.001, 499.002, 499.003, 499.004, 499.005	–
37	**Searles**_775.002	–
38	**Thunder Lake 3**_3777.001, 3777.002, 3777.004, 3777.005, 3777.006	Thunder Lake 3[c]
39	**RoundHowes**_748.002, 748.005	–
40	**Bergman**_662.002, 662.003, 662.004, 662.005, 662.006, 662.007	Bergman[a,b]

Table 2. (*contd*)

Genotype ID	Cultivar composition	Consensus cultivar
41	**Hollison**_1043.001; **Olsons Honkers (bog11)**_1680.001; **Habelman2**_1042.001; **Drever**_1041.001; **Champion**_746.006; **Bennett**_112.002; **Bain McFarlin**_1039.001	Putative Potter's Favorite[b]
42	**Grygleski2**_1447.001	GH2[a,b]
43	**No. 41**_1758.001; **Crowley (bog5)**_1678.001, 1678.002; **Stevens**_1298.002, 1298.003; **Stevens (bog1)**_1672.001, 1672.002; **Stevens (bog2)**_1673.001, 1673.002; **Stevens (bog8)**_1674.002; **Stevens (bog9)**_1675.001; **Stevens (bog7)**_1683.001, 1683.002	Stevens[a,b]
44	**Rezin Native**_1048.001	Rezin Native[c]
45	**McFarlin**_1295.001, 1295.002, 1295.003, 1295.005, 1295.006	–
46	**Stevens (bog9)**_1675.002	–
47	**Wales Henry**_752.001, 752.002, 752.003, 752.004, 752.005, 752.006	Wales Henry[c]
48	**Garwood Bell**_772.001, 772.002, 772.003, 772.004, 772.005, 772.006	Garwood Bell[c]
49	**Round Howes**_748.004, 748.006; **Gebhardt Beauty**_773.001, 773.002, 773.003, 773.004, 773.005	–
50	**Biron Selection**_1040.001	–
51	**ThunderLake 4**_4778.001	–
52	**Early Black**_741.002; **Thunder Lake 3**_3777.003	–
53	**Early Black**_741.004, 741.006; **Stevens (bog6)**_1682.001, 1682.002	–
54	**Matthews**_1044.002	Matthews[c]
55	**Searles**_775.003, 775.009, 775.010, 775.011	–
56	**Bain 8**_1035.001	Bain 8[c]
57	**Bain 10**_1037.001	Bain 10[c]
58	**Bain 2**_1029.001; **Bain 4**_1031.001, **Bain 5**_1032.001;	–
59	**Bain 7**_1034.001	Bain 7[c]
60	**Bain 6**_1033.001	Bain 6[c]
61	**Bain 3**_1030.001	Bain 3[c]
62	**Pilgrim**_1297.001, 1297.002, 1297.003, 1297.004, 1297.005; **Pilgrim (bog10)**_1679.001	Pilgrim[b]
63	**Ben Lear**_503.005	–
64	**AJ**_492.001, 492.002, 492.003, 492.004, 492.005, 492.006; **Ben Lear**_503.001, 503.002, 503.003, 503.004; **PottersFavorite**_774.001, 774.002, 774.003, 774.004, 774.005, 774.006	Ben Lear[a,b]

The cultivar composition column indicates accessions, according to the NCGR plant codes in table 1, with the same genotype. The consensus cultivar for each genotype was determined as a match with a genotype from Fajardo *et al.* (2013), contains alleles consistent with its expected pedigree, and/or is a cultivar represented by a single genotype that grouped with genotypes of similar geographic origin in principal component and NJ analyses as hypothesized in Schlautman *et al.* (2015b).

[a]Matches with a genotype from Fajardo *et al.* (2013).

[b]Contains alleles consistent with its expected pedigree.

[c]Cultivar represented by a single genotype that grouped with genotypes of similar geographic origin in principal component and NJ analyses as hypothesized in Schlautman *et al.* (2015b).

by their SSR profile). Using 48 SSRs, the NE-I was 3.46×10^{-32} (average/locus=0.31) in 25 cranberry cultivars from Zhu *et al.* (2012). According to Polashock and Vorsa (2002), differentiation of a cranberry parental genotype from its selfed progeny is possible using only 10–20 dominant markers. In the current study, 12 SSR markers would be expected to differentiate selfed progeny from their parents nearly 100% ($(0.50)^{12}$) of the time assuming that markers are heterozygous, unlinked and exhibit Mendelian segregation. Outcrossed progeny resulting from intercrosses between a mother genotype and different pollen genotype/s should be also easy to differentiate using 12 SSR loci with 168 alleles due to the segregation of a large number of alleles per locus (Polashock and Vorsa 2002).

Analysing geographic patterns of cranberry diversity

Because cranberries are a recently domesticated crop, less than 200 years ago, and since the collection represents mostly wild selections with a few cultivars product of artificial breeding, it is possible that the cranberry NCGR collection could be useful to study the natural differentiation

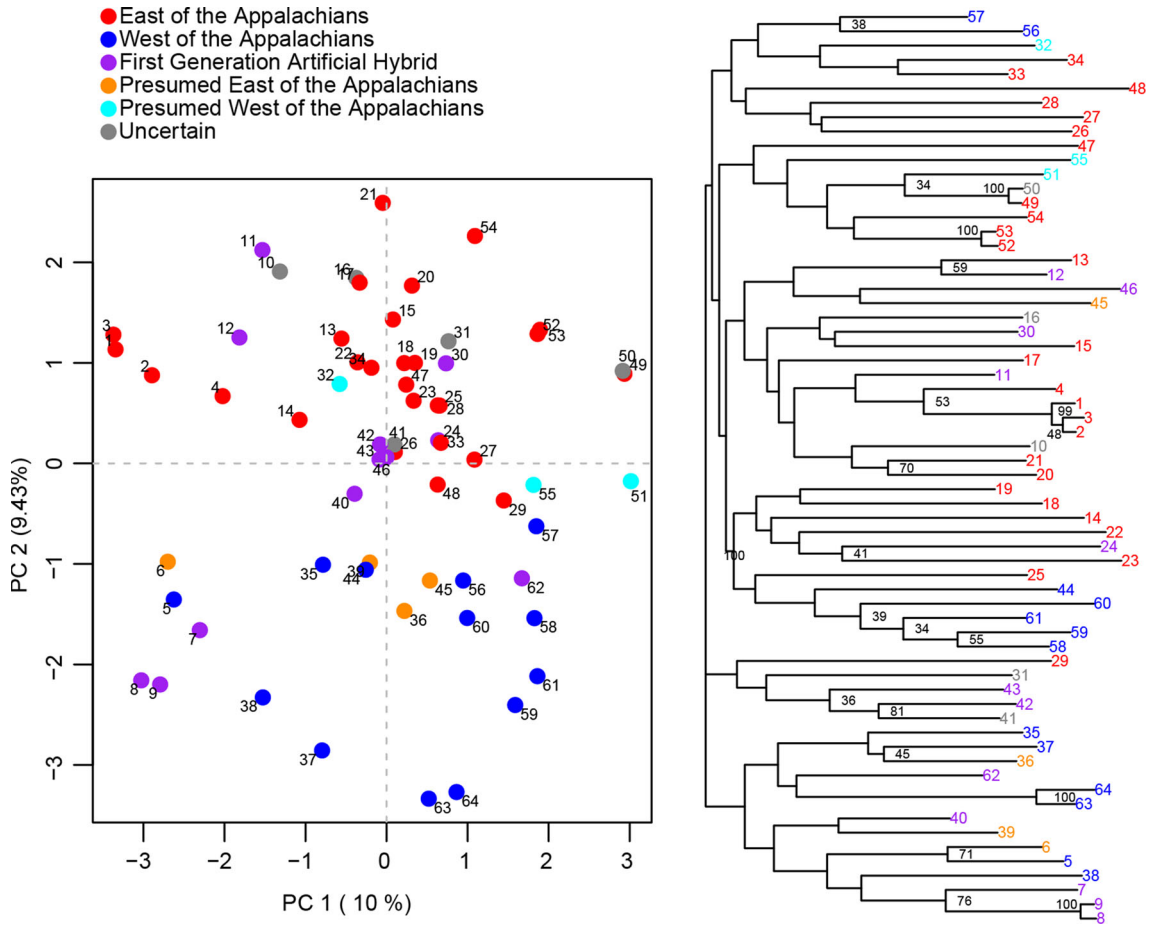

Figure 1. PCA (left) and NJ (right) tree based on the 12 SSR loci used to genotype the 64 unique cranberry (*V. macrocarpon*) genotypes from the USDA-ARS NCGR. Each genotype is coloured based on the assumed location where it was originally collected (table 1) to test the hypothesis that the Appalachian mountains may serve as a barrier to cranberry migration. The numbers at nodes are the proportion of occurrence in 100 bootstrap replicates. Genotypes which appear to violate the hypothesis (table 2 in electronic supplementary material) are given their own colour.

of populations in the wild. Although a more in-depth sampling of the entire cranberry natural range will be required in the future to investigate the population structure of cranberries in the wild, we conducted PCA and NJ analyses based on the origin of the accessions as a preliminary assessment of the population structure. We chose these two methods due to the focus of the collection to preserve cultivated and breeding materials. We found some evidence of two groupings consistent with population structure east versus west with the Appalachian Mountains possibly serving as a major geographical barrier to gene flow, which together with the dispersal of bog ecosystems may make it difficult for insect pollinators to carry cranberry pollen across long distances (Schlautman *et al.* 2015b). Information regarding different genetic lineages across geographical regions could be used for conservation efforts and to create complementary genetic pools and heterotic crosses. In terms of breeding, significant heterosis for yield has already been observed in certain crosses between east versus west parents (e.g. 'Stevens' (Massachusetts×Wisconsin) released in 1950 represents 40% of total US acreage).

The 12 SSR loci separated all 64 clonal genotypes in the NJ and PCA analysis (table 2; figure 1). Principle component 2, which showed 9.43% of the total genetic variation separated the genotypes with a presumed origin east of the Appalachian Mountains (quadrants 1 and 2) from the genotypes with a presumed origin west of the Appalachian Mountains (quadrants 3 and 4) (figure 1). However, the NJ tree did not provide conclusive evidence supporting the east versus west differentiation hypothesis (figure 1) possibly due to confounding of 1st generation artificial hybrids in the dataset which appear randomly distributed among genotypes in both the NJ tree and PCA (figure 1).

Removing the artificial hybrids improved the separation of genotypes of eastern and western origin in the NJ tree; however, bootstrapping branch support was still lacking to support significant population structure east versus west (figure 2). In the PCA that excluded hybrids, the wild selections collected east of the Appalachian mountains were

Table 3. Genetic estimates for 12 SSR loci (168 alleles) based on 64 unique cranberry (*V. macrocarpon*) genotypes among 78 cranberry accessions ($n = 271$) housed at the USDA-ARS NCGR.

Locus	N	N_a	N_e	I	H_o	H_s	NE-I
vm04084	64	14	7.41	2.22	0.81	0.87	0.03
vm25796	64	21	5.77	2.19	0.47	0.83	0.05
vm26877	64	13	6.18	2.07	0.78	0.84	0.05
vm28527	64	16	4.00	1.92	0.66	0.75	0.08
vm31701	63	15	6.31	2.16	0.70	0.84	0.04
vm38401	64	15	8.31	2.31	0.75	0.88	0.03
vm39030	63	12	7.66	2.19	0.67	0.87	0.03
vm40600	64	9	4.20	1.68	0.80	0.76	0.08
vm51985	64	7	3.73	1.51	0.77	0.73	0.12
vm52682	64	15	6.07	2.09	0.75	0.84	0.05
vm55441	64	19	9.66	2.50	0.88	0.90	0.02
vm78806	64	12	6.62	2.16	0.61	0.85	0.04
Average	63.83	14.00	6.33	2.08	0.72	0.83	0.05

N, sample size; N_a, number of alleles; N_e, number of effective alleles; I, Shannon's information index; H_o, observed heterozygosity; H_e,expected heterozygosity; NE-I, average nonexclusion probability for identity of two unrelated individuals.

separated from the selections that originated west of the Appalachian mountains by the line $y = 1x$ (figure 2). Seven of the wild selections appeared to violate the barrier (figures 1 and 2; table 2 in electronic supplementary material). However, we cannot ascertain if this inconsistency is due to genetic heterogeneity or to contamination or error during the preservation of the clonal genotypes in the commercial cranberry industry. A total of 69 alleles across the 12 loci were shared among the wild selections made from east and west of the Appalachians mountains; there were 70 private alleles held only by eastern selections and 11 private alleles held only by western selections (table 3 in electronic supplementary material). The limited number of private alleles in the western selections could reflect the fact that fewer western genotypes (all which were from Wisconsin) were included in this study or lack of gene flow and concomitant genetic differentiation between eastern and western populations.

The observed genetic diversity among the wild cranberry selections from multiple geographic regions currently held within the NCGR collection highlights the need for an expansion of this study in the future. Thorough

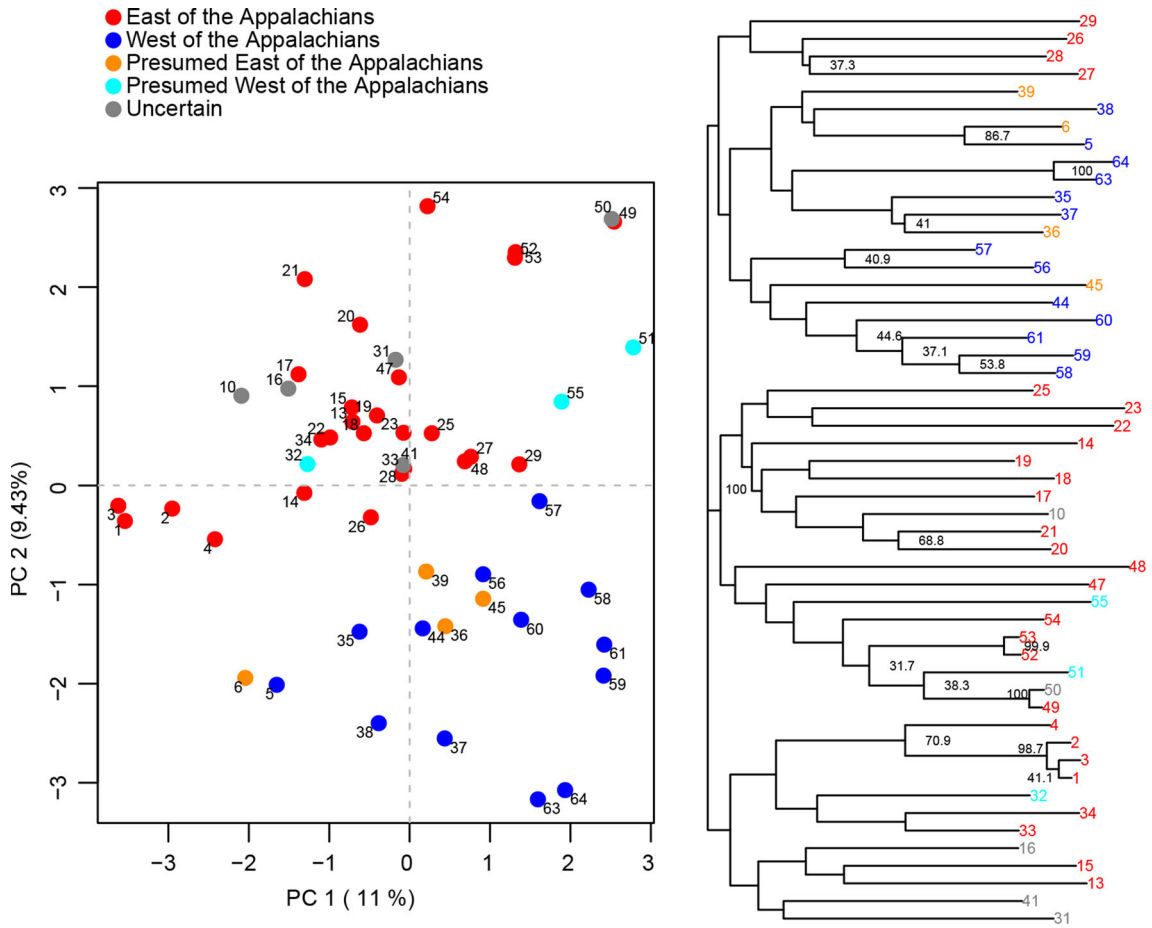

Figure 2. PCA (left) and NJ (right) based on the 12 SSR loci used to genotype the unique genotypes of wild cranberry (*V. macrocarpon*) selections held in the USDA-ARS NCGR. Each genotype is coloured based on the assumed location where it was originally collected (table 1) in order to test the hypothesis that the Appalachian Mountains may serve as a barrier to cranberry migration. Genotypes which appear to violate the hypothesis (table 2 in electronic supplementary material) are given their own colour.

collection and sampling of wild cranberry germplasm from across its entire native range could lead to better understanding of the genetic relationships and geographic barriers to migration which exist among wild cranberry populations and could have important implications for breeding. A study of this sort would not only improve the NCGR collection *ex situ* conservation efforts and provide potential new sources of genetic variation for commercial cranberry genetic improvement, but also provide valuable insights about the locations of unique cranberry diversity hotspots which are candidates for *in situ* conservation and breeding efforts (Pavek *et al.* 2003; Meilleur and Hodgkin 2004).

Identification of intra-accession and intra-cultivar variants in the NCGR collection

In addition to the genetic diversity observed among the 64 unique genotypes in the NCGR collection, we also observed within accession diversity and intra-cultivar variation (table 2; figure 1). Of the 39 core cultivars represented by multiple plants, 27 were homogeneous with no variation in their genetic profiles based on the three–six representative plants fingerprinted (table 2; figure 1). The remaining 12 core cultivars had at least one genetic variant among the cuttings sampled with the majority (seven) displaying two genetic variants. However, 'Round Howes' (27, 39, 49) and 'Searles' (5, 37, 55) showed three distinct genotypes while 'Early Black' (20, 21, 52, 53) showed four (table 2). Additionally, 14 of the 64 unique genotypes were shared by multiple cultivars, ranging from two–seven cultivars per genotype. Nine of these genotypes were associated with samples from two cultivars (5, 'Searles' and 'Bain9'; 11, 'Franklin' and 'Centennial'; 13, 'Crowley' and 'Perry Red'; 22, 'Bugle Wareham' type and 'Centerville'; 27, 'Round Howes' and 'Stanley', 29, 'Whiting Randall' and 'Black Veil', 49, 'Round Howes' and 'Gephardt Beauty', 52, 'Early Black' and 'Thunder Lake', 53, 'Early Black' and 'Stevens') (table 2). Similarly, three genotypes were associated with up to three cultivars (43, 'No. 41', 'Crowley', and 'Stevens'; 58, 'Bain2', 'Bain4', and 'Bain5'; 64, 'AJ', 'Potter's Favorite' and 'Ben Lear') (table 2). Finally, two genotypes were associated with seven cultivars each: (1, 'McFarlin', 'Stevens', 'Foxboro Howes', 'Bennet', 'Crowley', 'Prolific' and 'Pride'; and 41, 'Hollison', 'Olson Honkers', 'Hableman2', 'Drever', 'Champion', 'Bennet' and 'Bain McFarlin') (table 2).

Such apparent inconsistencies and intra-cultivar variation are likely due to sampling or labelling errors prior to clonal preservation at the NCGR collection. In essence, the cranberry cultivation model requires a near perfect system of clonal preservation, propagation and distribution of cultivars, but the species has no morphological characters capable of reliably distinguishing among different cultivars (Novy *et al.* 1994; Novy and Vorsa 1995). In practice, depending on the purity of the starting material, cranberry beds are established using a single genotype and serve as genetic stocks for propagation. Historically, cranberry growers have served as both the keepers and propagators of cultivars and wild selections, but these plantings are managed as production fields and not to maintain genetic purity. Establishment of volunteer seedlings resulting from sexual reproduction or contamination with native and other cultivated clones can occur over many decades (up to 100 years) as long as the commercial cranberry bed remains in production. Over time, genetically diverse or heterogeneous populations can give rise to unique and completely different genetic compositions than the original named cultivar planting (Novy and Vorsa 1995; Novy *et al.* 1996). The spread of these 'error' genetic variants is likely to happen when the contaminated vegetative materials are used to establish new plantings of the named cultivars. Genetic contamination has been virtually impossible to track in the field or in collections such as NCGR by conventional phenotypic methods until recently with the advent of molecular markers (Novy *et al.* 1994; Novy and Vorsa 1995; Fajardo *et al.* 2013).

In an effort to ensure genetic purity of cultivars in the NCGR collection, new clones were propagated from each accession based on the results of the genetic fingerprint analysis. During the propagation of clones, two cuttings were sampled from each of the core cultivars, which were repotted into a 2 gallon-deep pot. Subsequently, we genotyped the two cranberry plants representing each of the 10 accessions that had multiple variants. All but three (Early Black, Round Howes and Thunder Lake 3) accessions had the same genotypes found in the majority of the clones genotyped that represented that cultivar.

Genotypic variation within commercial cranberry bogs

Presumably, the error variants in the cranberry NCRG collection originated through cultivar contamination or misclassification by grower propagators prior to being recorded in the NCGR collection. This idea is supported by the fact that all six core NCGR cultivars for which two samples were collected from 12 commercial cranberry bogs displayed intra-cultivar and intra-accession heterogeneity. No variation in cultivar fingerprints was found in seven out of the 12 bogs (bogs 1–7) sampled (table 2; figure 1). However, five of the 12 bogs (bogs 8–12) contained a mixture of genotypes. Of the six 'Stevens' bogs, three were true-to-type to the consensus 'Stevens' of Fajardo *et al.* (2013) (bogs 1, 2 and 7), and one bog was homogeneous, but its genetic fingerprint matched one of the five 'Early Black' (not true 'Early Black') genetic variants in the NCGR core collection rather than 'Stevens' (table 2; figure 1). In the remaining two 'Stevens' bogs (8 and 9), one of the

two samples matched 'Stevens' while the other contained genotype 1 (which is shared among 'Crowley', 'Prolific', 'Pride' and 'Foxboro Howes' from the main NCGR collection) in bog 8 and genotype 49 in bog 9. The 'Crowley' bog contained 'Stevens' while the genetic profiles of the 'Bennett' and 'McFarlin' bogs (3 and 4) were identical and also corresponded to genotype 1 (table 2; figure 1). In Bog 10, one of the 'Pilgrim' samples was true-to-type to the 'Pilgrim' accession (62) held at NCGR while the other had a unique genetic profile (12). Neither of the two samples in the 'Olson's Honkers' bog 11 (4) or the 'Crowley' bog 12 (30) matched the NCGR cultivar with the same name (34 and 1, respectively).

Consensus cultivar genotypes and proposed updates to the NCGR collection

We compared the 64 unique genotypes identified in the NCGR collection to fifteen reference consensus cultivar genotypes identified by Fajardo *et al.* (2013). Among the reference consensus cultivars used for comparison were all seven second-generation hybrids ('BG', 'Crimson Queen', 'Demoranville', 'GH1', 'HyRed', 'Mullica Queen' and 'Sundance'), four wild selections ('Ben Lear', 'Howes', 'Yellow Bell' and 'No. 35'), four first-generation hybrids ('Bergman', 'Franklin', 'Stevens' and 'Wilcox') and two other hybrids ('GH2' and 'LoRed'). By comparing genotypes obtained in this study to previously established consensus genotypes and additional pedigree analyses based on allele calls conducted herein, several NCGR genotypes were designated as true-to-type: 'Howes'=2, 'Yellow Bell'=6, 'No. 35'=7, 'Wilcox'=9, 'Franklin'=11, Putative 'McFarlin'=33, 'Bergman'=40, Putative 'Potter's Favorite'=41, 'GH2'=42, 'Stevens'=43 and 'Ben Lear'=64 (table 2). Based on pedigree analysis, several genotypes appear to have resulted from selfing including: 'Howes' self=3, 'Early Black' self=20 and 'Early Black' self=21. 'No. 35', 'Bergman', 'Howes', 'GH2', 'Yellow Bell' and 'Wilcox' had unique no other named cultivars associated with them. However, the 'Franklin' consensus genotype (11) included plants from 'Franklin' and 'Centennial'. Similarly, the 'Stevens' consensus genotype matched the 'No. 41' and 'Crowley' accessions. Additionally, many of the named cultivars which were represented by a single unique genotype and seemed to match the hypothesized geographic origin where the selection was originally made were also considered putative consensus genotypes (table 2; figure 1).

Although the 'Potter's Favorite' accession held by the collection (555012) turned out to be 'Ben Lear', we found a putative 'Potter's Favorite' genotype (41), whose alleles complement those of the putative 'McFarlin' genotype (33=McFarlin Rezin at NCGR) to match our consensus 'Stevens' genotype (43), as expected since 'Stevens' is a hybrid of 'Potter's Favorite' and 'McFarlin'. Genotype 41 was composed of plants from seven named cultivars, most of them from eastern provenance ('Hollison', 'Olsons Honkers', 'Habelman2', 'Drever', 'Champion', 'Ben Lear' and 'Bain McFarlin') (table 2). The putative 'Potter's Favorite' genotype (41) did not group with other western genotypes in the PCA and NJ trees (figures 1 and 2) as expected based on its reported Wisconsin origin (Dana 1983; Eck 1990). However, if our putative 'Potter's Favorite' genotype is true, it is possible that the original 'Potter's Favorite' genotype collected in Wisconsin (1895) could have been lost or become contaminated by eastern genotypes prior to being used as a parent in the crosses (1930s) which generated 'Stevens'. Another possibility is that the original 41 genotype actually originated in the east and somehow was renamed 'Potter's Favorite' in Wisconsin. Both ideas are supported by the eastern grouping of genotype 41 and the eastern provenance of most of the named cultivars that correspond to this genotype, from which at least one cultivar, 'Hollison' or 'Holliston' (Dana 1983; Eck 1990), is older (1885) than 'Potter's Favorite' (1895). Therefore, the different named cultivars represented by genotype 41 highlight the possibility of genetic contamination, misclassification or even that the same genotype was selected for domestication and named in multiple occasions by different people and at different times, all of which complicate the preservation of cranberry germplasm.

The remaining unique genotypes which were composed of multiple named cultivars or which did not contain the expected allele combinations in the pedigree analysis are still valuable sources of diversity and will be maintained in the NCGR collection. For example, no consensus genotype was found for the cultivar Searles, which has historically been one of the most important commercial cultivars and parent in cranberry breeding (Peltier 1970; Eck 1990; Fajardo *et al.* 2013). However, multiple accessions listed as 'Searles' (i.e. 5 and 37) did have 'Searles' alleles at many loci which are also present in first generation hybrids with 'Searles' in their pedigree. Further, these genotypes fit the hypothesized geographic distribution based on their assumed origin (figures 1 and 2). Therefore, the genotypes could potentially be related to 'Searles' or offspring of 'Searles' and still harbour some of the diversity from the historic cultivar.

Although consensus and true-to-type cranberry genotypes were found within the NCGR collection, a few historically important wild selections and cultivars were missing (e.g. 'Beckwith', 'Crowley', 'Early Black', 'LeMunyon', 'Prolific' and 'Searles') (Dana 1983; Eck 1990). In the past few decades, commercial cranberry growers have been steadily replacing plantings of these native selections or the 1st generation hybrids with newer 2nd and 3rd generation hybrid varieties; therefore, new collections should be made as soon as possible, genetically analysed, and the most likely genotypes need to be preserved from the few remaining commercial beds of these missing cultivars before the genotypes are lost or no longer available.

In conclusion, this study revealed that the *V. macrocarpon* collection held at the NCGR contains highly diverse cranberry germplasm which is useful to cranberry researchers and breeders. More importantly, the collection serves as a means of *ex situ* conservation for many historically important wild cranberry selections which were once commercial cultivars in the cranberry industry but are now in danger of being lost or forgotten. Additionally, the distribution of genetic diversity in the PCAs and NJ trees based on 12 SSR loci used to genotype wild selections held at NCGR provided evidence supporting the hypothesis that genetic diversity is spread across the native range of cranberry with genetic differentiation in extant wild populations east versus west. Intra-cultivar and intra-accession genotypic variants were observed, a problem that is likely due to past misidentification or contamination (mixed clones) of the accessions acquired by NCGR. Consensus and true-to-type genotypes were found for many cultivars and wild selections; however, others were apparently absent suggesting that the collection can still be improved by sampling genotypes in cranberry bogs on commercial marshes in the Pacific Northwest, Wisconsin, along the East Coast and in Canada.

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