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RAPDs identify varietal misclassification and regional divergence in cranberry [*Vaccinium macrocarpon* (Ait.) Pursh]

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Abstract The majority of cultivated cranberry varieties were selected from native populations in the 1800s and early 1900s from sites in Massachusetts, New Jersey, and Wisconsin. Since their initial selections 100–150 years ago, varietal identities have become increasingly confused; primarily the result of there being a paucity of qualitative markers to effectively distinguish among varieties. Random amplified polymorphic DNA (RAPD) technology has the potential for allowing a more definitive classification of varieties and was used in this study to characterize 22 cranberry varieties. Twenty-two decamer primers amplified 162 scorable DNA fragments, of which 66 (41%) were polymorphic. On the basis of these 66 silver-stained RAPDs (ssRAPDs), 17 unique profiles were identified rather than the expected 22. Fourteen varieties had unique ssRAPD profiles, while the remaining 8 were represented by 3 ssRAPD profiles. Permuational analyses of the data suggest that the observed ssRAPD profile duplications are examples of varietal misclassification. Further analyses identified 2 ssRAPD markers that were found only in Eastern varieties (from Mass. and N. J.) and not in Wisconsin varieties. With varieties differing on average by 22 bands, ssRAPDs are shown to be effective in varietal identification and the assessment of genetic diversity in cranberry.

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Introduction

Approximately 95% of the 139 described cranberry varieties (Eck 1990) were selected in the 1800s and early 1900s from wild bogs located primarily in Massachusetts, New Jersey, and Wisconsin. Since their initial selection, varieties have been vegetatively propagated from one bog to another and from one region to another. The establishment of volunteer seedlings from fallen fruit, consequent selection under differing environments, and the lack of qualitative morphological markers has made varietal misclassifications likely. As a result, there is confusion regarding varietal identity and trueness-to-type, and a cranberry variety may be represented by more than one genotype. These genetic variants can confound the evaluation of environmental and genetic factors in cranberry research studies and also can have serious economic ramification for growers who may unknowingly establish cranberry bogs with less productive variants of a cultivar.

The use of isozymes to assess genetic diversity and to distinguish among cranberry varieties has not proven useful because of a lack of polymorphism (Hugan et al. 1993). An alternative to the use of isozymes is random amplified polymorphic DNA (RAPD) technology (Welsh and McClelland 1990; Williams et al. 1990). RAPDs have been used in the identification and classification of crop species such as cauliflower and broccoli (Hu and Quiros 1991), papaya (Stiles et al. 1993), and celery (Yang and Quiros 1993). Silver-stained RAPDs (ssRAPDs) (Huff and Bara 1993) were utilized in this study: (1) to evaluate their ability to distinguish among cranberry varieties and to establish DNA "fingerprints", (2) to assess the genetic diversity within propagated cranberry, and (3) to ascertain if ssRAPDs can identify regional genetic divergence within cranberry.

Table 1 **A** Cranberry varieties used in RAPD analysis, their source, date of introduction, and the state from which they originated. **B** Variants of 'Searles' and 'Early Richard'

A.

Varieties	Source	Date	Origin
Berry Berry	Mass. Cran.Res.Cent.	1883	Massachusetts
Black Veil	Mass. Cran.Res.Cent.	1890	Massachusetts
Matthews	Wis.-DuBays Cran. Co.	1880	Massachusetts
Early Black	Rutgers B\C Res. Cent.	1835	Massachusetts
Early Red	Mass. Cran.Res.Cent.	Unknown	Massachusetts
Howes	Rutgers B\C Res. Cent.	1843	Massachusetts
McFarlin	Rutgers B\C Res. Cent.	1874	Massachusetts
Paradise Meadow	Mass. Cran. Res. Cent.	1873	Massachusetts
Ben Lear	Wis.-DuBays Cran. Co.	Ca. 1901	Wisconsin
Gebhardt Beauty	Wis.-DuBays Cran. Co.	1893	Wisconsin
Metallic Bell	Mass. Cran.Res.Cent.	Unknown	Wisconsin
Searles	Wis-DuBays Cran. Co.	1893	Wisconsin
Thunderlake 3	Wis-DuBays Cran. Co.	1970	Wisconsin
Habelman 2	Wis-DuBays Cran. Co.	1969	Wisconsin
Biron	Wis-DuBays Cran. Co.	Ca. 1950	Wisconsin
Budd's Blues	Wis-DuBays Cran. Co.	1880	New Jersey
Cropper	Wis-DuBays Cran. Co.	1930	New Jersey
Early Richard	Wis-DuBays Cran. Co.	1870	New Jersey
Garwood Bell	Rutgers B\C Res. Cent.	1875	New Jersey
Plum	Rutgers B\C Res. Cent.	1872	New Jersey
Woolman	Rutgers B\C Res. Cent.	1897	New Jersey
Norman LeMunyon	Wis-DuBays Cran. Co.	Ca. 1960	New Jersey

B.

Variety	Source
Searles-1	Jacob Searles Cranberry Co., Wis.
Searles-2 & 3	Claude Strozewski Marsh, Wis.
Early Richard	Ron Hill, Green Bank, N. J.

Materials and methods

Plant material

Twenty-two cranberry varieties were used in the ssRAPD analysis, of which 8 originated from Massachusetts, 7 from New Jersey, and 7 from Wisconsin (Table 1A). Leaves from each of the respective varieties were collected from greenhouse-grown plants in the Fall of 1992 and the Spring of 1993. Collected leaves were frozen in liquid nitrogen and stored at −73 °C.

DNA extraction and quantification

DNA was extracted from frozen leaf material in the Fall of 1992 and in the Spring of 1993 using the procedure described by Rowland and Nguyen (1993). This procedure was modified by the deletion of the polyethlene glycol (PEG) DNA precipitation step. DNA concentrations were quantified with a TKO-100 fluorometer (Hoefer Scientific Instruments, San Francisco) and diluted to a standard 5 ng/µl.

DNA amplification

Polymerase chain reaction (PCR) mixtures consisted of 1× buffer (Perkin Elmer Cetus, Norwalk, Conn.), 2.5 m*M* $MgCl_2$, 0.2 m*M* of each dNTP, 0.2 µ*M* of primer, 1.25 Units *Taq* polymerase, Stoffel Fragment (Perkin Elmer Cetus), and 15 ng genomic DNA. The final reaction volume of 13 µl was placed in a Perkin Elmer Cetus 480 DNA thermal cycler with a DNA amplification program of 7 min at 94 °C, followed by 40 cycles of 1 min at 94 °C, 1 min at 36 °C, and 2 min at 72 °C. A ramp time of 2 min was included during heating from 36 °C to 72 °C. At the completion of 40 cycles, reactions were kept at 72 °C for 5 min and then cooled to 4 °C. Samples were randomly assigned to wells in the thermal cyler for each PCR run. This random ordering of samples was also maintained with gel loading. Amplified DNA fragments were separated on a 7.5% acrylamide/bis gel in a 0.375 *M* TRIS, pH 8.8 buffer, using a Mini-Protean II gel system (Bio-Rad Laboratories, Hercules, Calif.). DNA was visualized using a standard silver-stain (Bio-Rad) protocol, with the exception that fixation was performed in 10% acetic acid for 30 min.

The ssRAPD analysis of the 22 cranberry varieties was initially conducted with 48 primers (University of British Columbia Biotechnology Laboratory, Vancouver, B. C., and Operon Technologies, Almeda, Calif.) using template DNA extracted from dormant Fall leaves. Of the 48 primers, 22 produced scorable ssRAPDs (Table 2). To ensure that the ssRAPDs observed were reproducible, the 22 primers were used in a second replication using DNA isolated from Spring-grown leaves of the 22 varieties. Only those DNA fragments which were consistently amplified in both replicates were used in the ssRAPD analysis.

Scoring and analysis of RAPDs

Reproducible DNA bands were scored for their presence (1) or absence (0) in each variety. The software program PICKEM (Kobak and Smouse, unpublished) was used to calculate pairwise Euclidean genetic distances (Excoffier et al. 1992) between the row vectors of 1's and 0's generated for each variety. Monomorphic bands were excluded in the calculation of genetic distance. The matrix of genetic distance values was used in sequential, agglomerative, hierarchical, and nested (SAHN) cluster analysis (NTSYS-pc, Exeter Software, Setauket, N. Y.) as well as in the analysis of molecular variance (AMOVA) procedure (Excoffier et al. 1992). AMOVA estimates variance components for RAPD phenotypes and then partitions the variation among individuals within a regional population and between

Table 2 Primers utilized in ssRAPD analysis of cranberry. The total number of DNA fragments (bands) amplified and the number that were polymorphic are given for each primer

Operon primers	Sequence (5′ to 3′)	Total bands (no. polymorphic)	U.B.C. primers	Sequence (5′ to 3′)	Total bands (no. polymorphic)
OPA-02	TGCCGAGCTG	4 (1)	402	CCCGCCGTTG	12 (6)
OPA-04	AATCGGGCTG	7 (4)	436	GAGGGGGCCA	6 (4)
OPA-05	AGGGGTCTTG	4 (1)	471	CCGACCGGAA	9 (3)
OPA-07	GAAACGGGTG	4 (3)	472	AGGCGTGCAA	4 (2)
OPA-08	GTGACGTAGG	10 (1)	474	AGGCGGGAAC	8 (5)
OPA-09	GGGTAACGCC	10 (5)	478	CGAGCTGGTC	6 (2)
OPA-10	GTGATCGCAG	10 (6)	482	CTATAGGCCG	5 (1)
OPA-11	CAATCGCCGT	14 (6)	489	CGCACGCACA	7 (2)
OPA-12	TCGGCGATAG	3 (3)	493	CCGAATCACT	9 (2)
OPA-13	CAGCACCCAC	6 (1)	500	TTGCGTCATG	10 (4)
OPA-14	TCTGTGCTGG	9 (3)			
OPA-17	GACCGCTTGT	5 (1)			

Table 3 Pairwise ssRAPD marker differences between 22 cranberry varieties

CULTIVAR	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22
1 Berry Berry	*																					
2 Black Veil	24	*																				
3 Matthews	22	18	*																			
4 Early Black	26	18	20	*																		
5 Early Red	22	24	20	22	*																	
6 Howes	22	24	20	22	0	*																
7 McFarlin	22	24	20	22	0	0	*															
8 Paradise Meadow	19	21	19	21	27	27	27	*														
9 Ben Lear	27	23	15	23	25	25	25	24	*													
10 Gebhardt Beauty	25	23	21	19	23	23	23	26	20	*												
11 Metallic Bell	23	21	19	25	23	23	23	26	16	18	*											
12 Searles	19	25	21	21	19	19	19	26	14	24	18	*										
13 Thunderlake 3	20	26	18	22	22	22	22	19	21	17	25	27	*									
14 Habelman 2	22	18	0	20	20	20	20	19	15	21	19	21	18	*								
15 Biron	23	23	23	25	27	27	27	22	28	24	22	34	11	23	*							
16 Budd's Blues	20	20	22	18	22	22	22	23	19	21	15	15	26	22	27	*						
17 Cropper	19	21	23	23	27	27	27	20	26	26	26	24	21	23	22	25	*					
18 Early Richard	23	23	23	25	27	27	27	22	28	24	22	34	11	23	0	27	22	*				
19 Garwood Bell	18	20	20	20	18	18	18	21	21	23	21	17	22	20	27	14	23	27	*			
20 Plum	19	23	21	17	23	23	23	24	26	26	24	22	25	21	24	25	22	24	27	*		
21 Woolman	21	19	15	17	21	21	21	24	20	22	26	22	23	15	28	27	20	28	27	12	*	
22 Norman LeMunyon	22	18	0	20	20	20	20	19	15	21	19	21	18	0	23	22	23	23	20	21	15	*

regional populations (N. J.–vs–Mass.–vs–Wis.). The software program SCANEM (Kobak and Smouse, unpublished) was used to compare the efficacy of pairs and triads of primers in distinguishing among varieties.

Results

RAPD markers

Twenty-two decamer primers generated 162 scorable DNA fragments (Table 2), of which 96 (59%) were monomorphic and 66 (41%) were polymorphic. A range of 3 to 14 amplified fragments were observed, with an average of 7.4 bands per primer. Figure 1 is an example of a ssRAPD profile generated using primer U.B.C.-478.

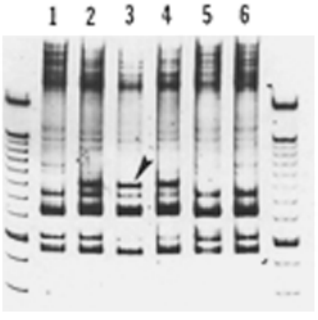

Fig. 1 ssRAPD profiles for 6 cranberry varieties generated by primer U.B.C.-478. The *arrow* points to a DNA fragment of 1000 bp that was present in 7 of the 22 varieties. *Lanes 1–6* are profiles for 'Biron', 'Habelman 2', 'Black Veil', 'Matthews', 'Plum', and 'Budd's Blues', respectively. *Lanes* at *left* and *right margins* are 100-bp DNA ladders (Gibco BRL, Gaithersburg, Md.). The three darkest bands of the ladder are 600, 1500, and 2072 bp, respectively

Genetic relationships among cranberry varieties

The Euclidean distance matrix generated following the scoring of 66 ssRAPD markers for presence/absence is shown in Table 3. Genetic distance values are analogous to a tally of band differences between varieties. Varieties differed by an average of 21 RAPD bands, with a range of 0 to 34. The dendrogram (Fig. 2) derived from these genetic distance values shows cranberry to be genetically variable, with no discrete regional clustering. Of particular interest were those varieties with genetic distances of 0, indicating that they were either genetically identical or very closely related with respect to their ssRAPD profiles. Three varietal groups with 0 band differences were observed: (1) 'Early Red', 'Howes', and 'McFarlin', (2) 'Biron' and 'Early Richard', and (3) 'Matthews', 'Habelman 2', and 'Norman LeMunyon'. Thus, rather than the expected 22 distinct ssRAPD phenotypes or "DNA fingerprints" being identified, only 17 were found. When the genetically redundant phenotypes (band differences = 0) are excluded from analysis, the 17 unique genotypes identified using ssRAPDs differ, on average, by 22 bands with a range of 11 to 34.

A histogram (Fig. 3) illustrates the distribution of the 231 genetic distances from Table 3. The cluster of 0 values separated by 11 units from the next nearest value suggests that they are not extreme values of a normal distribution, but are examples of varietal misclassification. To accept this hypothesis, however, it must be shown that values of 0 are not artifacts of a limited number of ssRAPD

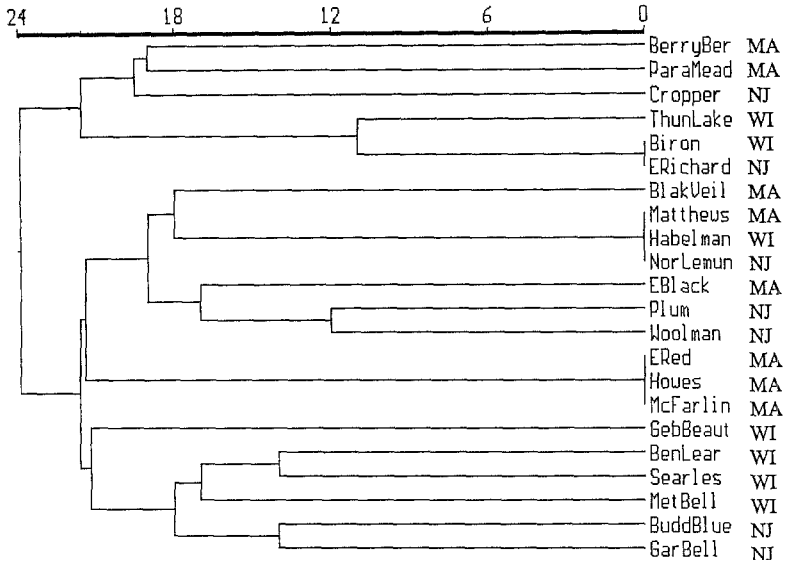

Fig. 2 Average-linkage dendrogram of varietal relationships as determined from ssRAPDs. The distance units are based upon the number of DNA band differences between cranberry varieties. The smaller the distance unit between varieties, the more closely related they are. Abbreviations next to varietal names are the state from which they originated. A cophenetic correlation value of r=0.86 indicates a high level of agreement between the dendrogram and the original dissimilarity matrix

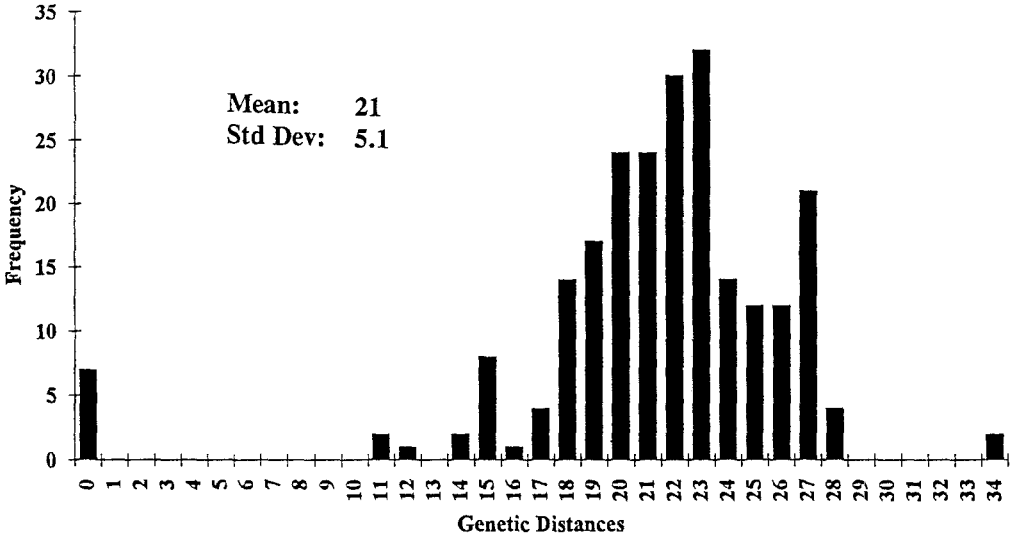

Fig. 3 Histogram showing distribution of 231 genetic distance values calculated for 22 cranberry varieties. The observed distribution suggests that the "0" values obtained are not extreme values of a normal distribution but are actually representative of varietal misclassification in cranberry

markers, i.e., more than 17 unique genotypes could be identified if a greater number of ssRAPDs were used in the analysis. Permutational testing by means of random sampling with replacement gave a probability of less than 0.001 that more than 17 genotypes existed in our dataset, leading to the rejection of the hypothesis that too few ssRAPDs were used in the analysis (Appendix 1). It would appear, therefore, that genetic distance values of 0 are representative of varietal misclassification.

Varietal-specific markers

Of the 17 unique genotypes, 9 had ssRAPD profiles with genotype-specific bands, and an additional 4 were uniquely identified by the absence of a marker present in all other genotypes. Of the 17 genotypes, 13 could therefore be distinguished from all others by the presence/absence of a band, the exceptions being 'Early Black', 'Thunderlake 3', 'Woolman', and the 'Matthews'–'Habelman 2'–'Norman LeMunyon' genotype.

AMOVA analysis and regional genetic divergence

AMOVA analysis found no significant differences (P = 0.26) between varieties on the basis of regional origin. Of the total genetic diversity, 97.9% was attributable to variance within regional populations, while 2.1% of the variation reflected regional divergence. The exclusion of redundant ssRAPD phenotypes that confounded regional differences, i.e., 'Early Richard', 'Biron', 'Norman LeMunyon', 'Matthews', and 'Habelman 2', did not significantly alter this proportioning of genetic variance (P = 0.24).

A regional AMOVA analysis was also conducted with Eastern varieties (Mass. and N. J.) contrasted with Western varieties (Wis.). Again, no significant (P = 0.17) dif-

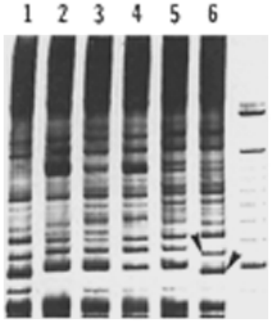

Fig. 4 Representative ssRAPD profiles generated using primer U.B.C.-474. *Arrows* indicate fragments of 575 and 750 bp that are present in the Eastern var 'Howes' (*lane 1*) and Budd's Blues' (*lane 6*), but which are absent in the Wisconsin var 'Ben Lear', 'Gebhardt Beauty', 'Metallic Bell', and 'Thunderlake 3' (*lanes 2–5*). *Lane* at *right* margin is a 100-bp DNA ladder (Gibco BRL, Gaithersburg, Md.). The three darkest bands of the ladder are 600, 1500, and 2072 bp, respectively

ferences were seen. However, the exclusion of redundant ssRAPD phenotypes resulted in significant ($P< 0.01$) East-West differences.

Further analysis was undertaken to identify specific ssRAPD markers that accounted for the observed regional differences. Two bands, generated with primer U.B.C.-474, were found to be a major source of the regional variance (Fig. 4). Always associated with one another, these bands appeared to originate from the same genomic region (Appendix 2). These markers were present in all of the Eastern varieties except 'Early Richard' and were missing in 5 of the 7 Wisconsin varieties; the exceptions being 'Searles' and 'Habelman 2'. Two of the three anomalous varieties had already been identified as being potentially misclassified, i.e., 'Early Richard' and 'Habelman 2'. The results with 'Searles', however, which is a major Wisconsin variety, were puzzling and indicated that it too might be a misclassified type.

To determine if misclassification of 'Searles' had occurred, 3 additional 'Searles' variants were obtained from two sites in Wisconsin (Table 1B). One variant (designated 'Searles-1') was collected from a bog of the Searles family whose ancestor, Andrew Searles, was the originator of this variety; the other two variants (designated 'Searles-2' and '-3')were collected from another Wisconsin site. The U.B.C.-474 ssRAPD profiles obtained for the 3 variants indicated they were not the same clone; their band patterns also differed from the original 'Searles' used in the study. 'Searles-1' was found to be missing the "Eastern" markers, while 'Searles-2' and '-3' had them.

Additional 'Early Richard' selections (4 vines) from a 90-year-old New Jersey bog (Table 1B) were also analyzed with primer U.B.C.-474. Three of the four selections appeared to be identical based upon their band profiles. All 4 selections differed from the 'Early Richard' used in the initial study, in that the "Eastern" bands were present in their profiles.

Table 4 Combinations of 3 primers able to uniquely distinguish or "fingerprint" all 17 observed ssRAPD phenotypes on the basis of 12–13 polymorphic bands

Primer combinations	Polymorphic markers	Total markers
OPA-11, 12, & 14	12	26
OPA-11, 14, & U.B.C.-436	13	29
OPA-12, 14, & U.B.C.-402	12	24
OPA-14 & U.B.C.-402, 436	13	27

Increasing the efficiency of the ssRAPD procedure

The use of the software programs SCANEM and PICKEM also proved useful in determining the minimum number of primers that could uniquely distinguish among the 17 phenotypes. Several groups of 3 primers were identified (Table 4) that could accomplish this task on the basis of 12 or 13 polymorphic bands rather than the 66 bands initially used in the study.

Discussion

Cranberry varieties differed on average by 22 ssRAPD bands, establishing ssRAPDs as an effective means of "fingerprinting" and assessing genetic variation in cranberry. This is in direct contrast to isozymes, which were less informative in this regard (Hugan et al. 1993). ssRAPDs also revealed the difficulty in maintaining the genetic identity of a cranberry variety over time. On the basis of 66 polymorphic markers, 8 of the 22 cranberry varieties examined had ssRAPD phenotypes identical to those of another variety. Although it would seem unlikely that 2 genetically distinct varieties could have an identical combination of 66 ssRAPD markers, it could be argued that it is possible. However, permutational analysis of the ssRAPD data indicated there was less than a 0.001 probability that 2 uniquely distinct varieties would be classified as identical on the basis of 66 ssRAPDs. Instead, it appears that a misclassification of cranberry varieties has occurred, with several varieties being represented by 1 genotype. On the basis of the few morphological descriptors present in cranberry, the misclassification hypothesis appears to be valid in that varieties with 0 band differences were phenotypically similar.

The varietal confusion in cranberry is not surprising in that it reproduces both sexually and asexually through seeds and stolons. Given its two modes of reproduction, a bog of a given "variety" may actually be a heterogeneous population of genotypes, especially in older plantings. Considering that most of the varieties examined in this study were selected from the wild 100–150 years ago, there appears to have been ample time for misclassifications to occur.

The inability of the three cranberry research programs which provided the plant material to identify such varietal

misclassifications emphasizes the difficulty in distinguishing among cranberry varieties solely on the basis of morphological descriptors. These findings have serious implications for cranberry research in that results obtained pertaining to a given "variety" may have little or no value if misclassification has occurred. Commercial growers may also be renovating or establishing bogs with something other than the variety they had intended to use.

The removal of varieties with identical ssRAPD phenotypes helped to identify 2 ssRAPD markers that are putatively present in all Eastern cranberry varieties (originating from Mass. and N. J.) but are absent in Wisconsin varieties. 'Searles' was an anomaly among Wisconsin varieties in that the "Eastern" bands were present. However, the lack of "Eastern" bands in 'Searles-1', a clone most likely to represent an "original" 'Searles', leads one to question the identity of the 'Searles' clone initially analyzed.

The lack of "Eastern" bands in the 'Early Richard' used in the first analysis suggests that it was most likely a 'Biron' that had been misclassified. Cluster analysis also supports this statement, in that the variety most closely related to the 'Early Richard'–'Biron' genotype was the Wisconsin variety 'Thunderlake 3'.

With respect to 'Hableman 2', the presence of the "Eastern" bands indicates that this selection is most likely not of Wisconsin origin. Instead it appears to be either a 'Matthews' or 'Norman LeMunyon' genotype. Records indicate that 'Habelman 2' was selected from a bed of native cranberries in 1969 (M. Dana, unpublished data). A bog established with Eastern varieties was nearby this native bed (D. M. Boone, personal communication), and it is possible that Eastern "escapes" may have comprised the native population. Another explanation is that misclassification of 'Habelman 2' had occurred following its initial selection from the wild. It is not known which of the two explanations is more plausible.

The presence of "Eastern" bands in the 'Early Red'–'Howes'–'McFarlin' ssRAPD profile was of no use in ascertaining which varieties had been misclassified, for all 3 originated from Massachusetts. However, subsequent studies (unpublished data) indicate that this profile is representative of 'Howes'.

Software developed by Kobak and Smouse was instrumental in determining the minimum number of primers (3) needed to distinguish among cranberry varieties. The use of 3 rather than 22 primers to fingerprint a cranberry variety is a substantial savings of both time and cost. As additional cranberry varieties are analyzed, the ssRAPD phenotypes generated with the use of only 3 primers may be identical to previously generated phenotypes. In such a situation, it will be necessary to utilize varietal-specific markers in conjunction with additional primers to ascertain whether the new variety is genetically identical or uniquely different from the other variety.

The discovery of varieties with identical ssRAPD fingerprints illustrates the confusion present in cranberry varietal identification. A limited number of physical characteristics, often environmentally influenced, has likely resulted in varietal misclassifications. ssRAPDs have provided valuable qualitative markers in cranberry and are a useful method of identifying and assessing genetic relatedness among cranberry varieties without the uncertainty contributed by environmental factors. Although the production and reproducibility of RAPDs can be influenced by factors such as primer and template concentration, or the type of thermocycler or *Taq* polymerase used (Kernodle et al. 1993; Penner et al.1993; Schierwater and Ender 1993), the use of a standardized RAPD protocol in conjunction with silver-staining and replication ensures the production of reproducible molecular markers. These markers have been consistently generated with DNA extracted from cranberry material harvested in the Spring (during active growth) and in the Fall (dormant period), indicating that the physiological state of the plant does not influence RAPD amplification. The 17 ssRAPD fingerprints generated in this study will be useful standards for further varietal classifications in cranberry.

The identification of cranberry varieties with identical genotypes leads to the conclusion that misclassifications may result in a variety being represented by several genotypes. This appears to be the case with 'Searles' and 'Early Richard' in which variants with different ssRAPD profiles were identified. In such a situation, a ssRAPD survey of the questionable variety using clonal samples from different growing regions would need to be conducted to identify a concensus fingerprint that would typify that cultivar. A study of intra-cultivar genetic variation in cranberry has been undertaken to address this question.

Appendix 1

Cranberry varieties having identical ssRAPD profiles suggest that either the number of ssRAPDs was insufficient to effectively distinguish among closely related but non-identical varieties or that varietal misclassification has occurred. To determine which of the two possibilities was more probable, an 18th ssRAPD phenotype that differed by 1 to 66 (66 being the number of ssRAPDs used in the study) bands from the 'Early Red'-'Howes'-'McFarlin' phenotype was artificially generated to give a total of 66 modified datasets. One thousand random samples of 66 band profiles were drawn from each of the modified datasets with replacement (Efron 1979) in order to calculate the probabilities that the 18th RAPD phenotype would not be identified, but instead be classified as identical to another ssRAPD phenotype. By construction, these probabilities were conditional upon the original dataset having ssRAPD phenotypes that differed between 1 to 66 bands. The probabilities of finding ssRAPD phenotypes that differ by 1 to 66 bands were calculated from 1000 random samples of 66 band profiles taken with replacement from the original dataset of 66 ssRAPDs. On the basis of the definition of conditional probability, associated conditional and prior probabilities were multiplied to generate joint probabilities. These values are the likelihood that 2 varieties will be classified as identical when they actually differ by a known number of bands. The summation of all 66 joint probabilities was less than 0.001, which was the probability that more than 17 ssRAPD phenotypes existed in the original dataset.

The greatest likelihood of individuals being classified as identical would be if they differed by only 1 band. The conditional probability in such a situation was 0.379, indicating that there was a real likelihood that the 18th RAPD phenotype would not be identified. However, when this value is multiplied by its associated prior probability of less than 0.001, there is less than a 0.0004 chance that 2

Table 5 Primers with non-independent bands. For each primer, the polymorphic bands remaining after removal of associated bands as well as the percent reduction are given

Primers with redundant bands	Actual number of independent polymorphic bands	% reduction in polymorphic bands
U.B.C.-402	5	16
U.B.C.-436	3	25
U.B.C.-474	4	20
U.B.C.-478[a]	1	50
U.B.C.-500[b]	2	50
OPA-04[c]	3	25
OPA-07	1	66
OPA-09	4	20
OPA-10	4	33

[a] One band was associated with 3 bands of OPA-7
[b] Two pairs of non-independent bands were observed
[c] One band was associated with a band from OPA-10

varieties differering by 1 band would have been classified as identical. The probability of this occurring six additional times is tantamount to zero. On the basis of this finding and the small joint probability value of 0.001, it appears that the observed 0 distance values are not the result of too few ssRAPD markers, but are representative of varietal misclassification in cranberry.

Appendix 2

The two "Eastern" bands generated with primer U.B.C.-474 were an example of a class of ssRAPDs in which distinctly different bands produce an identical profile of "1's" and "0's" across all 22 varieties. The identical RAPD profiles suggested that these bands were either tightly linked with one another, examples of associated heteroduplex bands, or that a primer-recognitions site was inside a larger template sequence (Hunt and Page 1992). Analyses of the ssRAPD data identified 10 such instances of non-independence, 8 of which resulted from the amplification of bands from the same primer, with the remainder occuring between primers (Table 5). It could be argued that these bands are representing different regions and by random chance they provide the same ssRAPD profile. This is a possibility in those instances where non-independent bands were observed across primers, but 80% of such bands occurred within a given primer suggesting amplification of affiliated chromosome regions had occurred.

This class of ssRAPDs appeared to provide redundant information with respect to the genome, which could inflate genetic distance estimates between individuals and bias inferences concerning genetic relationships. Genetic distance estimates and cluster analyses were performed with and without the non-independent bands to assess their effect. Although some genetic distance estimates differed by as much as 27% between the datasets, the correlation coefficient between the two datasets was 0.96. This high correlation indicates that the non-independent ssRAPDs did not substantially impact this study. However, this class of markers has the potential to bias genetic distance estimates and should be taken into consideration when RAPDs are used in assessing genetic relatedness.

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