Research Article

Genetic Diversity of Lowbush Blueberry throughout its U.S. Native Range in Managed and Non-managed Populations

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Abstract: Expressed sequenced tagged-polymerase chain reaction (EST-PCR) molecular markers were used to evaluate the genetic diversity of lowbush blueberry across its geographic range and to compare genetic diversity among four paired managed/non-managed populations. Seventeen lowbush blueberry populations were sampled in a general north south transect throughout eastern United States with distances between 27 km to 1600 km separating populations. Results show that the majority of genetic variation is found within populations (75%) versus among populations (25%), and that each population was genetically unique ($P \leq 0.0001$) with the exception of the Jonesboro, ME and Lubec, ME populations that were found not to be significantly different ($P = 0.228$). The effects of management for commercial fruit harvesting on genetic diversity were investigated in four locations in Maine with paired managed and non-managed populations. Significant differences were found between the populations indicating that commercial management influences the genetic diversity of lowbush blueberries in the landscape, despite the fact that planting does not occur; forests are harvested and the existing understory blueberry plants are what become established.

Keywords: EST-PCR, *Vaccinium angustifolium*, geographic range, domestication

1. Introduction

Lowbush blueberry (*Vaccinium angustifolium* Aiton) is an outcrossing, rhizomatous, tetraploid (2n=4x=48) woody perennial in the family Ericaceae. It is native to eastern North America ranging from Newfoundland, Canada south to North Carolina and west to Manitoba (USDA Plants Database; [1]). Plants are commonly found in disturbed acidic soils, especially glacial outwash plains, but also in forests, bogs, or exposed rock outcroppings [2,3]. Multiple cold hardiness zones are present throughout its geographic range. Lowbush blueberries are valued primarily for their fruit, but also for their leaves for tea, and utility as a horticultural plant in the landscaping industry. Management for commercial fruit production is primarily located in Maine, Quebec, and the Canadian Maritimes with the largest acreage in the vast barrens along the Atlantic coast [4]. Lowbush blueberry is unique among fruit crops, as fields are not planted with cultivars or varieties of known traits, qualities, or genetic lineage. Rather, an area of forest is cleared and managed with fire, mowing, and herbicides to promote the growth and spread of existing understory plants through vegetative growth of rhizomes and the colonization of bare ground by new plants via seeds [5]. The resulting field is a mosaic of genetically unique plants, referred to as "clones" by growers and researchers, that are visually distinct with varying pollination success, yield potential, cold hardiness, fertility response and pest susceptibility [6,7,8,9].

Although phenotypically diverse, the underlying genetic diversity of lowbush blueberry is relatively unknown compared to other fruit crops [8]. Recently, a variety of techniques and molecular markers have been used to quantitatively investigate the genetic heterogeneity and relatedness of *V. angustifolium* within and among populations [1,8,10,11,12,13,14,15]. These studies have shown *V. angustifolium* to be highly diverse within confined regions of the major lowbush blueberry producing areas of Maine and Canadian Maritimes. Restricted geographic sampling may not detect genetic diversity influenced by latitudinal clines and geographic separation that would be evident with increased distances between populations [16]. Latitudinal clines also drive the development of ecotypes in regards to photoperiod sensitivity and cold hardiness within a species [17,18]. Air temperatures are expected to increase 1.1-1.7°C in the primary lowbush blueberry producing regions of North America by 2050 due to climate change [19]. Already, lowbush blueberry growers in Maine are experiencing a growing season that has increased by one month since 1950 resulting in an increase in pest and pathogen pressure 20], fall bloom [21] and 50% more days with precipitation during bloom [22].

Vaccinium angustifolium is believed to be one of the first blueberry species to be managed for fruit production in North America. There is evidence to suggest that clearing and burning of forests by Native Americans for harvesting occurred before the arrival of Europeans [23]. Although attempts have been made to improve or develop cultivars of lowbush blueberry, cultivation has remained unchanged for hundreds of years, relying on wild plant populations pre-existing in fields [24,25]. It is commonly assumed that lowbush blueberry plants managed for commercial harvesting retain the same genetic diversity as plants growing in a mature forest, yet there has been no investigation to evaluate these assumptions. Loss of genetic diversity has been documented in many domesticated crops as they have been selected by humans for superior traits with an increase in genetic erosion the longer a plant has been domesticated [26,27]. Despite its relatively recent domestication (1939), commercially important rabbiteye blueberry (*Vaccinium virgatum* Aiton) cultivars can trace their lineage to 4 individual plants and as such inbreeding depression and high genetic load in cultivars have been an issue for breeders [28]. Existing lowbush blueberry genotypes in the landscape could have a founder effect on the genetic diversity of lowbush blueberry fields. A large stochastic event of removing forest cover may result in a field population of plants ultimately descended from relatively few parents [29].

The objective of this study was to utilize EST-PCR molecular markers developed for use in *Vaccinium* species to evaluate the genetic diversity of lowbush blueberry across a latitudinal gradient in the eastern United States and compare levels of diversity between managed and non-managed populations [30]. Increasing the geographic assessment of population genetic composition will

provide a landscape-level quantification of the genetic diversity in lowbush blueberry throughout a large portion of its native range. Sampling populations representing climates warmer than those found in Maine and the Canadian Maritimes may provide insight into how diversity may change in northern populations with rising annual temperatures, fewer chilling hours, and an increased growing season due to climate change. Additionally, incorporating managed and non-managed populations will elucidate any changes in genetic diversity that occurs as forested lands are converted to an agricultural landscape managed for commercial production.

2. Materials and Methods

2.1 Plant material

Lowbush blueberry stems and leaves were collected from seven geographic locations in Maine (Lubec, Jonesboro, Old Town, Winterport, Hope, Salem, and Sebago) to represent areas of primary fruit production within the state. These locations were geographically separated by a distance of at least 27 km. Populations outside of Maine (n=6) were selected based upon prior knowledge of existing populations along a general north to south transect that would represent the geographic range in the eastern United States. These sites included Fitchburg, MA, Groton, VT, Shokan, NY, Milton, PA, Dolly Sods, WV, and New Castle, VA (Fig. 1). Several USDA cold hardiness zones were represented in the populations from 4b (-31.7 to -28.9°C) in Salem, ME to 7a (-17.8 to -15°C) in New Castle, VA (http://planthardiness.ars.usda.gov/PHZMWeb/).

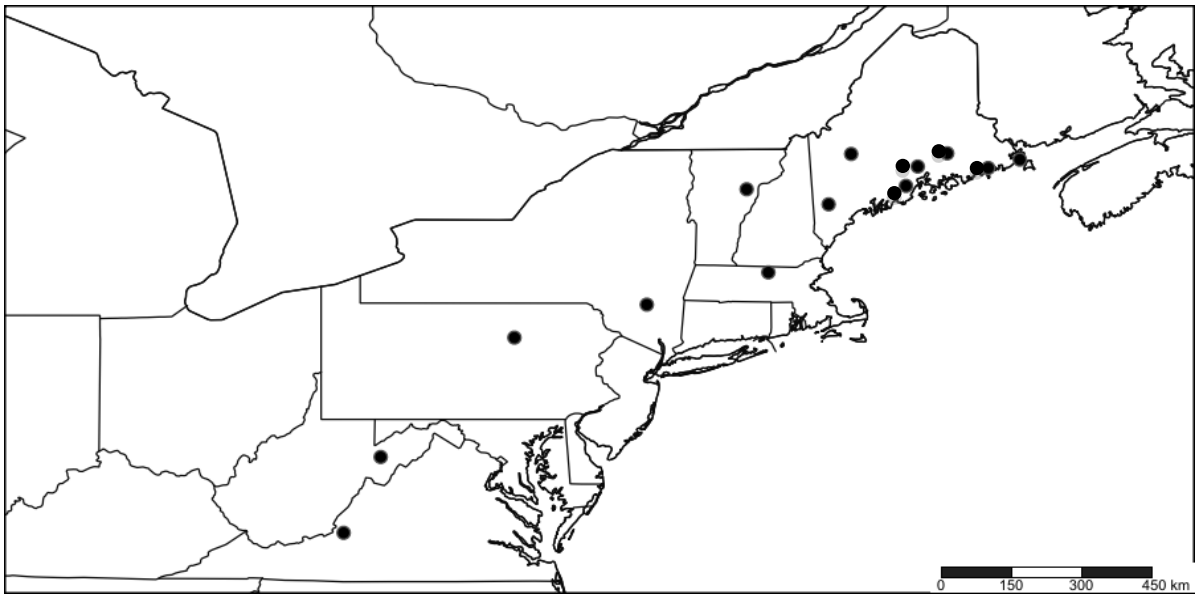

Figure 1. Sampled lowbush blueberry populations. Populations were collected along a 1600 km transect and represented a range of cold hardiness zones and a general north to south gradient which includes a large portion of the native growing range in the eastern United States (Gleason and Cronquist, 1991). Populations in Maine include plants commercially managed for fruit production (Jonesboro, Old Town, Winterport, Hope, Salem) and plants from non-managed natural forested landscape sites (Lubec, Jonesboro, Old Town, Winterport, Hope, Sebago). All locations outside of Maine were sampled from a forested landscape with no known history of commercial management.

To assess genetic diversity among managed commercial fields and wild unmanaged landscapes, we collected plants in a paired design in four growing regions in Maine making a total of 11 populations sampled in Maine (four paired and three not paired). The four "paired" populations

were sampled by collecting 20 individual plants from a field currently being managed for fruit production and 20 individual plants from an adjacent (> 500 m) forested landscape with no historical record or evidence (large mature tree stands) of management. These paired populations were sampled in Hope, Winterport, Old Town, and Jonesboro, ME. The buffer of at least 500 m between managed fields and natural areas in each pair was chosen to minimize any management "overflow" in commercial fields that could have impacted the plants in the natural areas over time, but at the same time be within the average bee foraging distance [31] and the hypothesized fruit/seed dispersal range by birds [12].

Approximately one gram of leaf material was collected from 12-20 genetically distinct individuals in each population along transects. Since lowbush blueberry is rhizomatous, care was taken to collect from samples that were visually distinct, at least 6 meters from the nearest sampled neighbor, and was large enough to survive leaf removal. Plants were taxonomically verified to ensure only *V. angustifolium* was sampled and not visually similar *Vaccinium* species (*V. myrtilloides*, *V. boreale*, and *V. pallidum*), which are commonly found occupying the same landscapes [32].

2.2 DNA isolation and amplification

Genomic DNA was isolated from approximately 2 mg of young, still expanding, leaf material using Qiagen DNeasy Plant Mini Kit (Valencia, CA, USA) or a CTAB extraction protocol developed by Doyle and Doyle [33]. DNA concentration and purity was measured with a Thermo Scientific NanoDrop 2000 spectrophotometer (Wilmington, DE, USA). Primer pairs previously reported in lowbush blueberry diversity studies were used, and additional EST-PCR molecular marker primers were developed from *V. corymbosum* EST sequences available in the blueberry genomic database (BBGD454) representing known general housekeeping and stress response genes [11,12,34]. Primer sequences were designed to amplify as much of the target gene as possible by choosing primer sequences at extreme 5′ and 3′ ends of an EST sequence using the Primer3 interface [35]. Eleven stress response [36,37], and 10 general housekeeping genes [38] were screened for polymorphisms and suitability for genetic diversity analysis. A total of 45 primer pairs were screened for this study, of which 16 were used for analysis (Table 1). Amplification protocols were performed as previously described by Rowland et al. [39]. An AdvanCE FS96 system™ (Advanced Analytical Technologies; Ames, Iowa, USA) was used for separation and digital visualization of polymorphic bands via capillary electrophoresis.

Table 1. Primer pairs used for analyses that yielded reproducible polymorphic bands throughout the dataset.

Primer name	Sequence (5′ to 3′)	Tm (°C)	Annealing temperature (°C)
02675	AAGGAAGGGGGAGGGTTTAT	58	49
	AAAAAGGGGCACAAAGAAGAA	54	
00125	AGTAGGGGACACAGCCACAC	62	55
	TGGCAGAGGGTAGAACTTGC	60	
00064	CACAGTTTTGACGGTGATGG	56	51
	TGATTGCTGCACCAAGACTC	58	
CO	AAACTACCCGATGTCGATGC	57	49

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	TCACAAAAACGATGGAACGA	54	
00313	CAGCACAAATTGCAGAGCAT	56	50
	GCATGGGAAAGGAATTCTGA	55	
NA799	TTTACCTCCCTTTGCCACAC	57	52
	GGAAATCCCACAGCTCAGAA	57	
CA448F	GTGGGCAGAGTGAGGAAGAG	60	53
	ACACAAACCAGGGAGAAACG	58	
CA15F	ACCAAAGCTGAGCAACCAAT	57	52
	GTCTGCCATGAAAACCCAAC	57	
CA65F	GTCGAATCCGAAGCCTTCTC	58	48
	AAACAGCAAATTCCCAATCG	53	
CA1463	GAAGATGTCGTGGAGGTGGT	59	51
	TAATGCGGGTTGATGTAGCA	56	
CA1423	TCATAGCCAATACACTCGAACC	56	51
	GCCCCACCTTAGCAAATC	56	
CA1785L	CACCACCACTGCGTACACC	62	50
	GCATGAGCCGAACATAATCA	55	
EST133	AAACAATCCACCAATCAACTTGT	54	49
	CCTCTCCACAGTCCGATCAA	59	
EST193	GAGGGATTCAGCACGAAGAG	58	50
	CAACATCATCAACCCCAACA	55	
EST248	TGGAGACTGGAGTGATGCAA	58	49
	AAGTGCATTAAGCATCCGAAA	54	
EST1029	GAAGTTTTCCGTTCTCTGCAA	55	50
	CTGCAGCTAGGACCGAAGAG	60	

2.3 Molecular marker analysis

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Clearly defined, reproducibly amplified fragments between 200 and 1500 base pairs were scored as dominant markers (present or absent) with PROsize™ software (Advanced Analytical Technologies; Ames, Iowa, USA). Primer pairs that failed to amplify in all individuals or that did not produce consistently reproducible bands were not used for further analysis. Binary scoring matrices for each EST-PCR primer pair were exported and combined in Microsoft Excel™ for analysis with the GenAlEx™ 6.5 statistical package add-in [40]. Distance measures (genetic and geographic) were calculated for single and combined populations to be used as the basis for analysis of molecular variance (AMOVA), spatial autocorrelation, and principal coordinate analysis (PCoA). Analysis of variance for band richness between populations was calculated with JMP™ software (SAS Institute 2017). Comparisons between managed and non-managed populations were made using non-parametric analysis of variance (PERMANOVA) measures in PC-ORD 6 with a 2-way factorial design, 4999 permutations, using Sorensen distance measures (MJM Software, Gleneden Beach, Oregon, USA). Bonferoni correction of P-values to maintain an overall experiment-wise error rate was conducted [41].

2.4 Sequencing of amplified fragments

Amplified fragments were sequenced to determine if polymorphic bands produced by EST-PCR molecular markers were different alleles in the tetraploid lowbush blueberry. DNA from 20 clones (10 managed and 10 non-managed) was amplified with a subset of 5 EST-PCR primer pairs that reliably produced 2-7 polymorphic bands. Each amplification was repeated three times per individual and then separated by electrophoresis on a 1.5% agarose gel. Bands were excised from the gel and pooled for extraction with the Qiagen QIAquick Gel Extraction Kit (Valencia, CA, USA). Sequencing of bands was performed at the University of Maine's DNA Sequencing Facility (Orono, ME, USA). Sequences were aligned and assembled using Geneious R7 software (Biomatters, Auckland, New Zealand) before being compared to the original template EST sequence from which the primer was designed and similar sequences available in GenBank using a nucleotide BLAST.

3. Results

3.1 Managed vs. non-managed populations

The genetic make-up of a total of 175 clones were assessed with EST-PCR molecular markers. Of the 45 primer pairs tested, only 16 produced reliable bands with no missing data for further evaluation. Although GenAlEx can handle missing data (entered as -1), markers missing (not amplified) from a few locations were removed from all sites for comparing genetic diversity among populations, as further analysis in PC-ORD 6 could not incorporate missing data. In all, a total of 142 polymorphic bands were used for comparing genetic diversity between commercial fields and natural areas. AMOVA analysis with 9999 permutations estimated 75% of the total genetic variance within sampled populations and the remaining 25% was estimated to be among populations. Pairwise comparisons between populations provided evidence for significant differences ($P < 0.0001$) between all but one managed field and non-managed natural site in neighboring forest and also between location sites of the pairs, when Bonferoni corrected for the number of comparisons made (Table 2). Similarities among managed populations were observed based on PhiPT values

Table 2. Pairwise AMOVA comparison between populations currently managed for commercial blueberry production and those from a mature forest not managed for fruit production. P-values are shown (random > data) based on 9999 permutations in GenAlEx 6.5. All populations are significantly different from all others, except Jonesboro managed from Winterport managed when the P-values are Bonferoni corrected. The Old Town managed is different at $P \leq 0.10$ (Bonferoni corrected) from the Jonesboro managed. Managed populations are

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significantly different from their associated paired non-managed population (bold P-values) found in close proximity (≤500m).

		Managed				Non-managed			
		Hope	Jonesboro	Old Town	Winterport	Hope	Jonesboro	Old Town	Winterport
Non-managed	Winterport	0.0001	0.0001	0.0001	**0.0001**	0.0001	0.0001	0.0001	
	Old Town	0.0001	0.0001	**0.0001**	0.0001	0.0001	0.0001		
	Jonesboro	0.0001	**0.0001**	0.0001	0.0001	0.0001			
	Hope	**0.0001**	0.0001	0.0001	0.0001				
Managed	Winterport	0.0001	0.0056 (ns)	0.0001					
	Old Town	0.0001	0.0023†						
	Jonesboro	0.0001							
	Hope								

† *P*-value ≤ 0.10 when Bonferoni corrected to adjust for the number of comparisons

compared to their non-managed natural site counterparts. Further analysis via PerMANOVA also provided evidence of significant differences between locations, management treatment, and the interaction between location and management treatment (P = 0.0002) indicating a conclusion of significant differences in genetic diversity between managed and non-managed natural site populations (Table 3), but differences may vary with location. While there is a trend for fewer polymorphic bands in the plant populations sampled from fields managed for commercial production when compared to non-managed natural site populations, this trend is not as large for the Hope location (P = 0.077, Fig. 2). The contribution to overall genetic diversity by stress-related primer pairs within and among managed and non-managed natural sites was also assessed with AMOVA. Separate AMOVA analyses were conducted for managed and non-managed populations using only stress-related primer pairs or only "neutral" primer pairs. Results of these analyses showed no increase or decrease in genetic diversity between managed and non-managed populations based upon origin of primer pairs (P < 0.001 and P < 0.001, respectively) (data not shown).

Table 3. PerMANOVA results for paired (location) managed and non-managed population comparisons. Significant differences are found between location, management (managed or non-managed), and the interaction (P = 0.0002).

Source	df	SS	MS	F	P
Location	3	7.1233	2.3744	16.299	0.0002
Management	1	3.7856	3.7856	25.986	0.0002
Interaction	3	3.1031	1.0344	7.1004	0.0002
Residual	152	22.143	0.14568		
Total	159	36.155			

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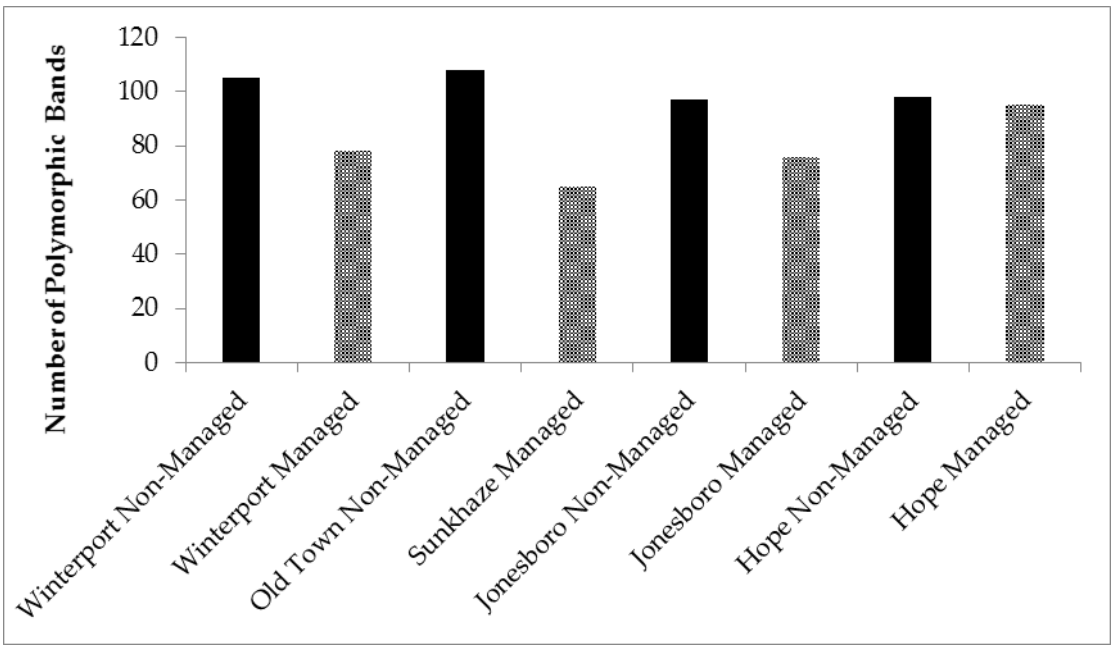

Figure 2. Number of polymorphic bands found in lowbush blueberry populations managed for commercial fruit production and those from non-managed populations in mature forests without a known history of commercial management.

3.2 Genetic diversity throughout the Eastern United States

Populations sampled for assessment of genetic diversity in lowbush blueberry across the U.S. range were separated by a minimum of 27 km and a maximum of 1600 km. Of the 45 primer pairs screened, 24 EST-PCR molecular markers were polymorphic and reliably amplified in greater than 90% of the sampled clones. Those individual plants with poor marker amplification were assigned -1 for analysis in GenAlEx [42]. A total of 202 polymorphic bands were used for analysis of 338 individuals in 17 populations. Prior results showed significant differences between managed and non-managed populations so paired populations were split and treated as individual populations. AMOVA results show 75% of the variance associated within the populations with the remaining 25% found among populations with a total differentiation between populations of 0.252 (PhiPT, $P \leq 0.001$) (Table 4). Pairwise comparisons suggest significant differences between almost all populations ($P = 0.0001$) with the exception of the non-managed Lubec, ME and managed Jonesboro, ME sites ($P = 0.228$) (Table 5). With the entire data set, populations tended to be more similar, although statistically different, among managed locations when compared to non-managed populations based on PhiPt values (PhiPt 0.037, 0.049, 0.101, 0.405, $P < 0.100$).

Table 4. AMOVA table for all sampled populations with 9999 permutations. The majority of the variance associated with our data (75%) is found within the populations with the remainder (25%) found among the populations. There are significant differences between populations (P≤0.0001) indicating a high level of genetic diversity in lowbush blueberries. This is confirmed with a relatively high PhiPT (0.252) for all populations showing little similarity among populations.

Source	df	SS	MS	Est. Var.	%
Among Pops	16	1482.821	92.676	4.063	25%

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Within Pops	321	3878.995	12.084	12.084	75%
Total	337	5361.817		16.147	100%
Stat	**Value**	***P***			
PhiPT	0.252	0.0001			

Table 5. Pairwise comparison via AMOVA with 9999 permutations for all sampled populations. Significant differences exist between sampled populations (cut-off for Bonferoni pairwise comparisons that maintains an experiment-wise error of $P = 0.05$ is $P \leq 0.0136$) with the exception of the non-managed Lubec, ME and managed Jonesboro, ME populations ($P = 0.2281$).

	Hope, ME	Hope, ME	Fitchburg, MA	Jonesboro, ME	Sebago, ME	Salem, ME	Old Town, ME	Winterport, ME	Jonesboro, ME	Lubec, ME	New Castle, VA	Milton, PA	Dolly Sods, WV	Shokan, NY	Groton, VT	Old Town, ME
Winterport, ME	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001
Old Town, ME	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	
Groton, VT	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	0.0004	0.0026		
Shokan, NY	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	0.0007			
Dolly Sods, WV	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001				
Milton, PA	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001					
New Castle, VA	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001						
Lubec, ME	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	0.0005	**0.2281**							

Jonesboro, ME (Managed)	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	0.0025	0.0037
Winterport, ME (Managed)	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	
Old Town, ME (Managed)	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001		
Salem, ME (Managed)	0.0001	0.0001	0.0001	0.0001	0.0001			
Sebago, ME	0.0001	0.0001	0.0001	0.0001				
Jonesboro, ME	0.0001	0.0001	0.0001					
Fitchburg, MA	0.0001	0.0001						
Hope, ME (Managed)	0.0001							

3.3 Spatial analysis and population structure

Spatial autocorrelation was performed on all 17 populations with variable distance classes representing intra-field distance classes (50 m, 100 m, 250 m) and inter-field distance classes (500 m, 1000 m, 2.5 km, 10 km, 25 km, 100 km, and 1000 km) (Fig. 3). Calculated *r* fell above the upper and lower confidence limits (95%) at 50 m and 1000 m indicating positive spatial autocorrelation within populations where genetic differentiation among clones within populations occur (> 50m) and among populations at different sites where genetic differentiation appears (> 1 km). At 250 m evidence of a slight negative spatial autocorrelation occurs where at distances less than or greater than 250 m genetic similarity increases from distances having less similarity. Principal coordinate analysis (PCoA) showed no clearly defined populations based on geographic and genetic distance (Fig. 4). Spatial autocorrelation in conjunction with PCoA show limited spatial structure in lowbush blueberry, primarily within field distances. We attempted to cluster similar individuals with STRUCTURE software using Bayesian algorithms [43]. This was ultimately unsuccessful (data not shown), as an increasingly high number (100+) of *K* populations were needed for successful clustering providing little information about population structure over a large geographic range [44].

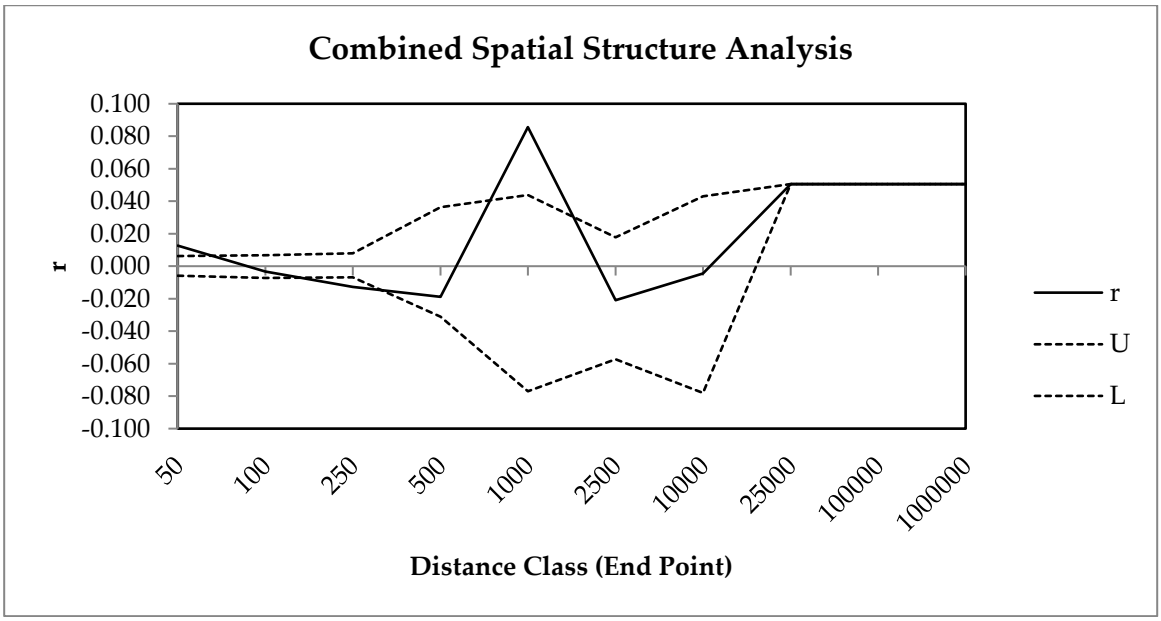

Figure 3. Spatial autocorrelation analysis. Distance classes (meters) were assigned to represent the distances between clones in a population (≤50 m) and the distances between populations at different sites (≥1000 m). Dashed lines indicate the upper and lower 95% confidence intervals while the solid line indicates the calculated *r*. Positive spatial structure appears at 50 m within a population and then again at 1 km among sites.

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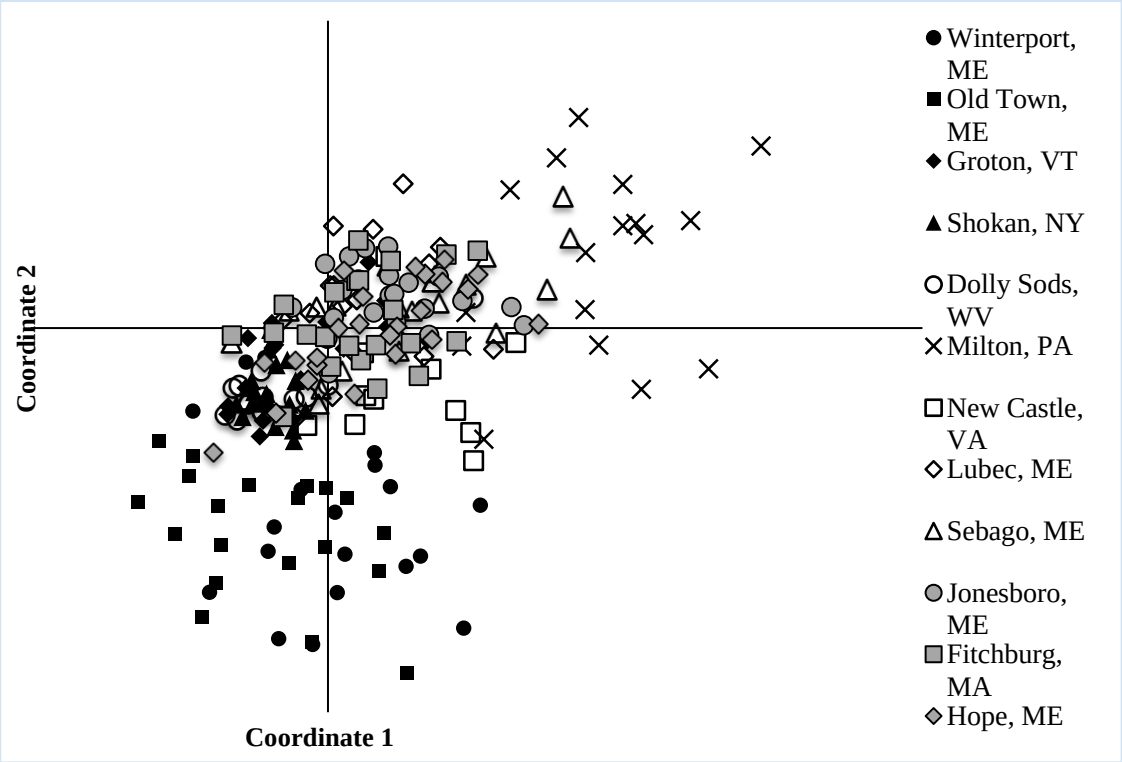

Figure 4. Principal coordinates (PCoA) analysis of genetic composition of non-managed lowbush blueberry populations (n=11).

3.4 Evaluation of stress related EST-PCR molecular markers

EST-PCR primers were developed from EST sequences of housekeeping and stress-related genes with the hypothesis that EST-PCR molecular markers would produce a higher rate of polymorphic bands due to the relaxed selective pressure compared to housekeeping genes [45]. Our results found this not to be the case as the average number of bands per primer pair designed from stress genes and housekeeping genes did not differ significantly, 7.0 and 7.16 respectively (P = 0.863). We also observed no difference in the frequency of the number of bands between management. EST-PCR markers developed from stress-related genes proved useful for genetic diversity analysis, but they did not have an advantage over the existing EST-PCR markers in terms of increased number of bands or frequency between populations. The effect of latitude and cold hardiness zone on the number of stress-related bands were evaluated with linear regression analysis. Neither latitude (r^2 = 0.244, P = 0.126) nor cold hardiness zone (r^2 = 0.452, P = 0.494) had a significant effect on the number of stress related bands present in any of the sampled populations (data not shown).

3.5 Sequencing of selected polymorphic bands

All sequenced bands were homologous to primer sequences at the ends, but BLAST results varied in regards to similarities with the original EST sequence from which they were designed. Generally, sequences of the same size and primer pair were highly homologous (>90%) between all 20 lowbush blueberry clones with most variation between sequences associated with gaps in the refined sequence resulting from background noise in the raw sequence contigs. At least one polymorphic band from each primer pair was homologous to the original EST sequence with other bands having little or no homology. Multiple polymorphic bands of differing size with homology to the original sequence, such as the case with primer 00064 bands of 210 bp and 250 bp, were highly similar (>98%) but the shorter sequence aligned to the middle of the longer sequence. Thus, we conclude that polymorphic bands for a particular EST marker do not generally represent different alleles.

4. Discussion

We found that the commonly held assumption that lowbush blueberries managed for commercial production retain the same genetic diversity as found in an established forest does not hold true. Although fields of lowbush blueberries are not planted with selected cultivars, a series of selective pressures associated with management might remove genotypes from the landscape. The application of herbicides, such as hexazinone, for weed control can remove sensitive genotypes, this has been well documented in weedy species, but herbicide application can also have phytotoxic or lethal effects on lowbush blueberry plants [46,47,48]. Lowbush blueberry is managed on a two-year cycle, with a cropping year followed by a pruning year where the fields are mowed or burned [49]. The common practice of burn pruning for lowbush blueberry fields is an effective disease control measure as it removes pathogens in the stems and on the soil surface, but the intensity (heat and duration) can have negative effects on the plants and the organic layer of the soil [49,50,51]. Although the negative effects of burn pruning have been contested, the increase in price of oil used for most burn applications has provided incentive for many growers to adopt flail mowing as an alternative pruning method with lower risk to the plants but again may negatively impact some genotypes [52].

The management practices for lowbush blueberry would suggest a decrease in genetic diversity over time relative to non-managed populations. We did find significant differences in the genetic composition of populations between managed and non-managed areas although only a significant trend when = 0.10 (P = 0.077) of fewer bands in managed fields that might indicate a loss of genetic diversity. However, this trend appears to be driven from the similarity between the managed and non-managed populations at the Hope, ME location. If the Hope site populations are taken out for an analysis, there is significantly less genetic diversity in the managed populations in Old Town, Winterport, and Jonesboro when compared to the non-managed populations (P = 0.044). We are unable to draw conclusions about functional genetic diversity in managed populations since polymorphic bands did not generally appear to represent alleles, as many of the sequenced polymorphic bands did not have homology to the original EST sequence from which the primer was designed, except at the ends. Other studies have found that the selection process under stress conditions results in a more tightly refined stress response pathway [45].

Domestication of wild crops (maize, wheat, apple, etc.) by the selection and planting of individuals with superior traits, and at the same time removing plants of lower quality from the gene pool, has resulted in a genetic bottleneck in many of our current traditionally-bred crops [26,53]. In contrast, intentional rogueing of lowbush blueberry plants is not a current practice among lowbush blueberry growers, so plants with less desirable traits remain in the gene pool retaining a higher-level genetic diversity relative to domesticated.

Overall genetic diversity for lowbush blueberry was retained for all sampled populations reflecting the results of previous studies investigating genetic diversity in Maine and the Canadian Maritimes [12,54,10]. Bell et al. [12], in particular described significant differences among four managed fields in the Down East region of Maine. Similar to our findings, Bell described a higher level of variance (91.6%) associated within populations relative to among populations (8.4%). Positive spatial autocorrelation (SA) within fields at the 50 m-distance class found in our study was also reported by Bell et al. [12] with within field SA being high before decreasing with distance. Reasoning for significant differences between lowbush blueberry populations has been attributed to the last receding ice sheet that covered much of Maine [12]. Our results would not entirely support this hypothesis, as we found no correlation with genetic diversity and latitude as would be expected with receding ice sheets [55]. Genetic differentiation as a result of extreme discontinuous geographic isolation has been found in bilberry (*Vaccinium myrtillus*) throughout Scandinavia, and the same may be true for lowbush blueberry here in North America but greater geographical separation than is reported here is likely to be a necessity [56]. The large geographic range, outcrossing, and animal seed dispersal traits associated with lowbush blueberry likely contributes to this high level of genetic diversity [57].

Our sampled populations from 8 states spanning 1600 km represent, generally, the native growing range of lowbush blueberry in the eastern United States. Spanning multiple cold hardiness zones, the populations are a good representation of differing climatic zones that could provide insight into how an increased average temperature may influence the lowbush blueberry industry in Maine. Climate change has increased the growing season for lowbush blueberry by approximately one month in the past 50 years in Maine [20]. The revised USDA cold hardiness map in 2012 reflected warmer average minimum temperatures compared to the last map update in 1990 with the majority of Maine's blueberry growing region shifting from predominantly zone 5a (-28.9 to -26.1°C) to predominantly zone 5b (-26.1 to -23.3°C) and to a lesser extent zone 6a (-23.3 to -20.6°C). Populations sampled from warmer locations (New Castle, VA, Dolly Sods, WV, Milton, PA) maintained a high level of diversity similar to cooler, northern populations. Pairwise comparisons between each site and also comparisons between northern (ME, VT, MA, NY) and southern (VA, WV, PA) populations did not reveal any population structure based on latitude or prevailing hardiness zones. The numbers of stress-related marker bands also were not correlated with latitude or hardiness zone.

5. Conclusions

These findings suggest that while growers in Maine may need to adapt management practices to a warmer and longer growing season, the overall genetic diversity of the plants would remain high, but might not provide the genetic ability to adapt rapidly to extreme climate change.

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