Phenotypic Plasticity and Genetic Variation of Vaccinium macrocarpon, the American Cranberry. I. Reaction Norms of Clones from Central and Marginal Populations in a Common Garden

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PHENOTYPIC PLASTICITY AND GENETIC VARIATION OF VACCINIUM MACROCARPON, THE AMERICAN CRANBERRY. I. REACTION NORMS OF CLONES FROM CENTRAL AND MARGINAL POPULATIONS IN A COMMON GARDEN

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Vaccinium macrocarpon Aiton (Ericaceae) cranberry, a dwarf shrub and a typical taxon in temperate peat bogs, has its central distribution in cool temperate regions in eastern North America. Isolated southern marginal populations are distributed along the Appalachian corridor and on the North Carolina coastal plain. A common garden (Blacksburg, Virginia) was utilized to determine whether marginal cranberry clones exhibit greater phenotypic plasticity than central clones. Three central clones from Massachusetts (MA), Wisconsin (WI), and New York (NY) and three marginal clones from North Carolina (NC), Tennessee (TN), and West Virginia (WV) were tested. A suite of phenotypic traits was measured in response to edaphic variation in the common garden. An analysis of reaction norms took the form of an analysis of covariance to test for significant differences among clones and to estimate regression slopes (plasticity) when compared with environmental (nutrient) variation. There was no regional variation in phenotypic plasticity, but there was significant clonal differentiation for 77% of nonintercorrelated traits. However, in most cases the differences were seemingly random, with little biological importance. Hence little differentiation in relation to population origin was observed among clones. Matrix comparisons were performed using a Mantel test to check for pairwise correlations among the following matrices: geographic distances, trait means, plasticity, and molecular variation assessed by random amplified polymorphic DNA (RAPD) profiling. No correspondence was found among matrices. The recent postglacial distribution of cranberry may account for the absence of phenotypic and genetic heterogeneity.

Introduction

Genetic differentiation among ecologically diverse populations has been widely documented (Turesson 1922*a,* 1922*b;* Clausen et al. 1940; Quinn 1978; Silander 1985*b*). Phenotypic plasticity, the morphological and/or physiological responses of a genotype to spatial or temporal environmental heterogeneity, has been hypothesized to be an important aspect of genetic differentiation within populations (Bradshaw 1965; Sultan 1987). Gause (1947) proposed that phenotypic plasticity could be an alternative mode of adaptation compared to "genoadaptation" or intrapopulational genetic differentiation. This inverse relationship between phenotypic plasticity and genetic variation has been rejected on empirical grounds (Moran et al. 1981; Hume and Cavers 1982; Scheiner and Goodnight 1984; Wood and Degabriele 1985; Schlichting and Levin 1986; Taylor and Aarssen 1988; Counts 1993) and on the findings that phenotypic plasticity has a genetic basis (Bradshaw 1965; Schlichting 1986; Scheiner and Lyman 1991; Scheiner 1994). However, some research supports the hypothesis of a trade-off between phenotypic plasticity and genetic variation (Cook and Johnson 1968; Pedersen 1968; Jain 1978; Wu and Jain 1978; Zangerl and Bazzaz 1983; Silander 1984, 1985*a;* MacDonald and Chinnappa 1989; Thompson et al. 1991). An alternative viewpoint is that phenotypic plasticity and genetic variation may be positively associated. If strong directional or stabilizing selection were pervasive in populations, then phenotypic plasticity could shield genetic variation from the effects of selection, thereby maintaining genetic variation (Gillespie and Turelli 1989; Goldstein and Holsinger 1992).

DeKroon and Schieving (1990) have provided a framework for characterizing clonal plant life histories. Most vegetatively spreading facultative clonal shrubs are classified as conservative growth plants. These are homologous to the category of "stress tolerators" (Grime 1979; Chapin 1980). This category includes arctic, boreal, and temperate peatland bog dwarf shrubs, such as *Vaccinium macrocarpon,* which tolerate a suite of environmental stresses such as low nutrient availability, physiological drought, and low temperatures. Conservative growers respond plastically to increased nutrient availability, potentially resulting in rapid site filling by one or a few genets, although Chapin (1980, 1987) argues that phenotypic plasticity would not be an important mode of adaptation for these plants. Guerilla growth (Lovett Doust 1981) would allow a single clone to spatially exclude possible competitors from a site, thereby rendering a possible selective advantage to plastic genets. This scenario indicates that in long-lived perennial plants, plasticity could indeed be an important mode of adaptation in locations with heterogeneous microsites. Theo-

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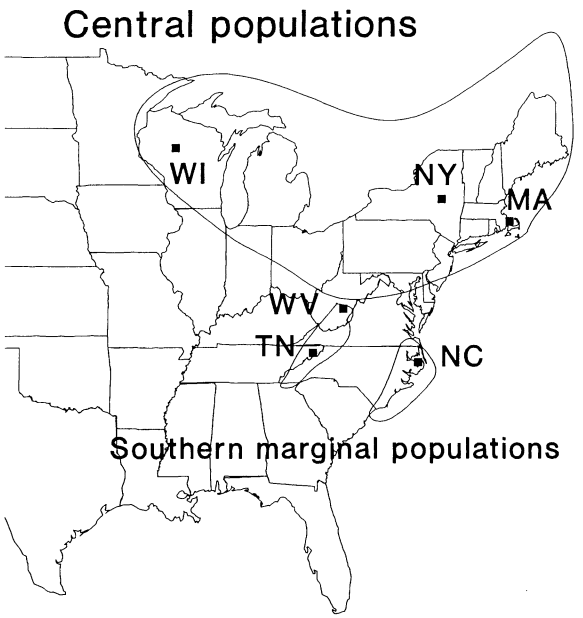


Fig. 1 The distribution of *Vaccinium macrocarpon* showing the geographic location of accessions.

retically, phenotypic plasticity may be advantageous in spatially or temporally heterogeneous habitats, whereas nonplastic or canalized phenotypes may be more advantageous in stable or homogeneous habitats (Sultan 1987). Opportunity for site filling by few clones would be contingent upon long periods between colonization events (i.e., low immigration), competitive exclusion, and small site area.

Although substantial research has recently involved the nature of phenotypic plasticity and its role in plant fitness, very little work has been done using clonal plants (Silander 1985*b;* deKroon and Schieving 1990). As a subset of plasticity studies, investigations of geographic patterning of clonal plant plasticity have been undertaken (Cook and Johnson 1968; Hume and Cavers 1982; Scheiner and Goodnight 1984; Silander 1984, 1985*a;* Taylor and Aarssen 1988; Michaels and Bazzaz 1989; Thompson et al. 1991; Vasseur and Aarssen 1992). However, in many of these cases, the clonal species studied have been almost exclusively foragers or consolidators *sensu* deKroon and Schieving (1990), not conservative growers. Examples of conservative growth plants include most woody clonal species and all dwarf shrubs typical of arctic, boreal, and temperate peatlands.

Life history strategies of long-lived, clonally spreading plants indicate that phenotypic plasticity could be adaptively significant in island-like populations subject to little gene flow. For example, for any given facultatively sexual population, when emigration ceases, the only source of additional genetic variation would be by mutation and recombination. On the other hand, selection and drift would decrease the amount of genetic variation. A facultatively sexual population may turn exclusively clonal if fecundity decreases to zero because of a lack of available germination sites (Eriksson 1992). If a population was exclusively clonal, one could envisage a single adaptively plastic clone (or several closely related plastic clones) excluding nonplastic intraspecific competitors in such a setting. It would not be surprising that, if plasticity of growth is an important trait, one would observe few genets in old isolated clonal populations.

For this study we used *V. macrocarpon,* the commercially important cranberry that occurs naturally in peatlands in the northeastern United States, the Great Lakes region, and southeastern Canada, with distributionally marginal populations pocketed in the central and southern Appalachian mountains and the North Carolina coastal plain (fig. 1). These two types of populations (central and marginal) correspond generally with glaciation (central) and unglaciation (marginal) of the last Pleistocene maximum. There are genetic and ecological differences as well. Among centrally distributed populations there are higher gene flow and larger suitable habitats than in marginal populations (Ogle 1984). Marginal populations are smaller, have lower sexual reproduction, and are thought to be relicts of the Pleistocene ice age (Wieder et al. 1981; Ogle 1984; Stewart 1993*b*). Moreover, marginal cranberry populations have been shown to have lower genetic variation than central populations (Stewart and Excoffier, in press). Specifically, there were fewer discernible clones and less interclonal molecular variation in marginal populations compared to central populations. Southern clones were larger and presumably older, indicating decreased recruitment within marginal sites. Also, southern marginal sites are relatively more heterogeneous with regard to phosphorus and nitrogen availability (C. N. Stewart Jr. and E. T. Nilsen, unpublished data).

The objective of this study was to determine whether clones from marginal populations are more plastic than clones from central populations in response to nutrient variability. Nutrient availability has been shown to be an important factor in dwarf shrub ecology (e.g., Chapin and Shaver 1985; Stewart and Nilsen 1993). Furthermore, there are regional differences in nutrient availability that are positively associated with temperature in peatlands (Billings et al. 1982). As a result of low clonal variation because of historical and ecological factors in southern marginal sites, did the existing predominant clones survive because they had plastic phenotypes? Do presumably old clones from marginal populations respond differently to nutrient fluxes than young clones from central populations?

Material and methods

Sites sampled

The predominant clones from three disjunct southern populations (NC, TN, WV), and from three northern, centrally distributed populations (NY, WI, MA) were used in a common garden experiment, which was performed in Blacksburg, Virginia. Sample sizes of populations were small because of the extreme rarity of bogs in the southeastern United States. In fact, *Vaccinium macrocarpon* is rare in North Carolina, Tennessee, Virginia, and West Virginia. We used single clones as physiological representatives of populations. In marginal populations genetic variation is significantly lower than in central populations (Stewart and Excoffier, in press). However, even in central populations, there is little genetic variation (Hugan et al. 1993), so using single clones to represent populations is justified at this level of inquiry. The clone from Dare County, NC, was collected from a 0.5-ha coastal pocosin. At the site, small patches of cranberry grow among larger ericaceous (typical pocosin) shrubs. The TN clone was from a small natural bog on a Johnson County, TN, farm. This 0.1-ha site had been fenced for 11 yr to exclude cattle. The WV clone was sampled from a small bog on Droop Mountain, WV. TN and WV sites have been studied with respect to vegetation (Stewart and Nilsen 1993), phenotypic plasticity, and clonal variation (Stewart and Nilsen 1995). The NY clone originated from Featherston Haugh Lake in Schenectedy County, NY. This clone was collected from the floating mat on the north side of the lake. The cranberry area was small (<1 ha) at this lake. These four clones are predominant in their respective populations, especially NC and TN, in which the sites are nearly monoclonal. The TN and WV clones correspond to the predominant clones (clone A in WV) described in Stewart and Nilsen (1995). The WI and MA clones are cultivars originally isolated from natural populations and vegetatively propagated for commercial production. The WI clone ('Searles') was originally grown in Wisconsin Rapids in 1893 and was selected from that vicinity (Dana 1983). Likewise, a Cape Cod, MA, clone ('Early Black') was first commercialized in 1857 in Harwich, MA (Dana 1983). Searles and Early Black are unimproved isolates selected from native bogs. The Searles and Early Black accessions used in these experiments came courtesy of Ocean Spray Cranberries, Inc.

Environmental conditions

The climatic conditions in Blacksburg, VA, the experimental site (mean July temperature = 21.3°C, mean yearly precipitation = 108.7 cm) were similar to northeastern U.S. commercial cranberry centers. When comparing clonal origins, Blacksburg's climate most resembled that found at the TN site (mean July temperature = 21.0°C, mean yearly precipitation = 116.4 cm), followed closely by MA (mean July temperature = 22.2°C, mean yearly precipitation = 120.7 cm) and WV (mean July temperature = 20.0°C, mean yearly precipitation = 111.8 cm). The NC site (mean July temperature = 23.8°C, mean yearly precipitation = 111.8 cm) was warmer, and NY (mean July temperature = 17.7°C, mean yearly precipitation = 96.5 cm) and WI (mean July temperature = 17.0°C, mean yearly precipitation = 78.7 cm) sites were colder (Ruffner 1985).

RAPD profiling

Clonal identities and genetic distances were assessed by random amplified polymorphic DNA (RAPD) profiling (Williams et al. 1990). DNA was isolated from leaves by either the Doyle and Doyle (1987) method or using the Stewart and Via (1993) protocol, which were of equivalent quality for RAPDs. RAPD cycling parameters have been described by Stewart and Via (1993). Reproducible band states (bands present in duplicate reactions) were scored as presence/absence data. Of the 40 primers (OPA and OPB kits from Operon, Alameda, Calif.) screened, 25% revealed interclonal polymorphisms. The following primers were used to generate 105 polymorphic bands: OPA4 (5′ AATCGGGCTG), OPA7 (5′ GGTCCCTGAC) , OPA9 (5′ GGGTAACGCC), OPA11 (5′ CAATCGCCGT), OPA13 (5′ CAGCACCCAC), OPA18 (5′ AGGTGACCGT), OPB4 (5′ GGACTGGAGT), OPB18 (5′ CCACAGCAGT), which resulted in a composite genetic profile for each sample. Reproducible characters were scored as presence or absence data.

Experimental design

Clones were propagated by homogeneous cuttings a year before the study began to eliminate home site effects and yield mature plants. Cranberry clones, each representing a population, were randomly assigned to 1600 cm^2 plastic pans containing Canadian peat, so that each pan had ramets containing all six clones. The six clones were arranged randomly within pans. The experiment was established September 1991, fertilizer added March 1992, and above-ground tissue harvested September 1992. Nutrient treatments were also randomly assigned. The nutrient treatments were (1) nitrogen addition: 1.1 g/m^2 supplied as slow release urea; (2) phosphorus addition: 1.5 g/m^2 supplied as treble superphosphate; (3) nitrogen and phosphorus added together at the same rates as above; (4) no nutrient additions. The nutrient application rates were derived from Eck (1964, 1990) and Eaton (1971*a*, 1971*b*). The fertilization rate represented amounts known to elicit

growth responses in a variety of cultivars. Nitrogen and phosphorus were chosen because they are the two most limiting nutrients to cranberry. We established a large range of nitrogen and phosphorus availabilities in which to measure reaction norms. The fertilizer was applied as recommended for commercial cranberry growers (Eck 1990). Means and standard deviations for soil nutrients were: pH, 5.37 ± 0.69; ammonium, 81.9 ± 15.8 $\mu g/cm^3$; phosphorus, 0.85 ± 0.34 $\mu g/cm^3$; potassium, 5.42 ± 0.85 $\mu g/cm^3$. Soil nutrient content fell within the ranges of commercial cranberry bogs (Fisher 1951; Eck 1990). The pans were in two raised beds (blocks). Thus, the experimental design was 6 clones × 4 nutrient treatments × 2 blocks × 4 replicates = $n = 192$ plants, with clones nested within pans. We employed a total of 32 ramets from each genet (eight per nutrient treatment). Although this was a balanced complete factorial design, because of plant overwintering mortality (probably transplant shock) the data set was not balanced.

The original experimental design assigned the fertilization regimes as classification variables in a profile analysis of variance. However, unexpected complications demanded another statistical approach. The pans had holes in the bottom for water drainage to avoid soluble salts buildup. As a result, an unexpected alteration of pH and macronutrients, presumably caused by capillarity from the mineral soil underlying the pans, occurred. These nutrients swamped the effect of the experimental nutrient additions. This result was confirmed by an analysis of variance, which showed a nonsignificant relationship between applied fertilizer treatments and the measured soil nutrient levels (data not shown). Therefore, data were primarily analyzed by analysis of covariance (ANCOVA), using the measurements of soil nutrient concentrations of root medium in each pan at the end of the experiment as the covariate and clone identity as the independent variable.

Soil from three places from each pan was bulked and pH and soil macronutrient (P, K, Ca, Mg) concentrations were measured at the beginning and end of the experiment by the Virginia Polytechnic Institute and State University soil laboratory, using a double acid extraction. Ions were measured with an inductively coupled plasma spectrophotometer (Donahue and Gettier 1988). Ammonium pools were analyzed by the indophenol blue method (Keeney and Nelson 1982) on a second set of soil samples collected on the same date.

Growth

The functional plant modules in *V. macrocarpon* are uprights (phalanx traits), which are important for sexual reproduction, and runners (guerilla traits), which are important for vegetative spread only (Eck 1990). Aboveground biomass was harvested at the end of the experiment (September 1992). Leaves were counted and biomass partitioned according to organ and module type. We chose various traits because of their obvious importance as indicators of growth habit and rate (table 1). Midwinter leaf anthocyanin levels from uprights were taken February 1993 on incidental leaves that remained after harvest. Leaf anthocyanin levels are hypothesized to be physiological indicators of stress and may be associated with latitudinal patterns correlated with growth (Rabino and Mancinelli 1986).

Statistical analyses

An ANCOVA and multiple comparisons (Tukey's HSD) were used to assess clonal differentiation, plasticity, and differences in reaction norms. Clonal identity was the main effect and soil nutrients was the covariate in the model. The clone main effect tested for significant differences among clones and means of traits, i.e., tested when clonal reaction norms had different heights. Phenotypic plasticities were determined by simple linear regression by clone, i.e., tested the null hypothesis that slope = 0. To stabilize variance, and as a normalization procedure, log log (base 10) transformations were performed. Regression analysis (by clone), against one nutrient at a time, was performed to determine significant traits and nutritional factors and to estimate plasticities (slopes). The slopes (reaction norms) were compared by using an ANCOVA homogeneity of slopes model; i.e., slopes were different if the nutrient*clone interaction term was significant (SAS Institute 1990). Reaction norm analysis is appropriate since we used clonal replicates. We chose linear models to model plasticity since nonlinear models (e.g., quadratic) did not improve the fit of the data significantly. Total plasticity among clones was assessed by using a one-way ANOVA in which estimates of the linear regression slopes of all nonintercorrelated traits in response to edaphic variables were used as response variables by clone. Character correlations were determined by using a Spearman rank correlation procedure (Zar 1984). Intercorrelated characters ($r > 0.8$; $P < 0.001$) were excluded from global analyses (see next paragraph). Type III sums of squares are reported in all analyses.

RAPD data were analyzed by scoring band presence or absence for each clone. Genetic distances were estimated using the euclidean squared distance (Excoffier et al. 1992) adapted for RAPDs (Huff et al. 1993). Excoffier et al. (1992) have shown that for profile data the euclidean distance indicates the number of mismatch bands between clones (i.e., the number of mutations necessary to explain profile differences between two clones). So in this case, a euclidean distance of 20 indi-

Table 1

KEY TO TRAIT ABBREVIATIONS

Abbreviation	Trait
Phalanx traits:	
L/UP	Average leaf number per upright
ULWT	Average upright mass per leaf (mg)
LW/UP	Average mass of all leaves per upright (mg)
SW/UP	Average mass of stem per upright (mg)
TOTUPW	Mass of upright per plant (mg)
UPW[a]	Average mass of upright (mg)
UP/PT[a]	Number of upright per plant
Guerilla traits:	
L/R	Average leaf number per runner
RLW[a]	Average runner leaf mass (mg)
LW/R	Average mass of all leaves per runner (mg)
SW/R	Average mass of stem per runner (mg)
RW[a]	Average mass of runners (mg)
TOTRW	Weight of runners per plant (mg)
R/PT[a]	Number of runners per plant
Other traits and ratios:	
UW/RW	Ratio of upright mass to runner mass
U/R[a]	Ratio of upright number to runner number
PTWT	Mass of aboveground individual plant (mg)
ANTHO	Midwinter leaf anthocyanin concentrations (optical densities at 510 nm [Lloyd et al. 1992])
STOM	Average stomatal density per 0.04 mm^2 of upright leaf

[a] Traits not included in global analyses, e.g., tables 4, 5 and fig. 3, because of trait intercorrelations. See table 2.

cates about 81% RAPD profile similarity between clones. Trait means and plasticity distances among clones were determined using the Canberra metric (Rohlf 1988). Distance matrices (geographic, RAPD profile, trait means, plasticities) were standardized to a mean of 0 and a standard deviation of 1 (SAS Institute 1990). Pairwise matrix comparisons were performed using the nonparametric Mantel test (Mantel 1967; Rohlf 1988; NTSYS-PC). In this procedure one matrix is rearranged and one matrix is kept intact; then a Pearson correlation statistic is calculated. This permutational procedure is reiterated many times (in this case, 1000), yielding a null distribution. Then the actual correlation coefficient between the two matrices is compared to the null distribution as a significant test. The Mantel test was performed on the following distance matrices: geographic, genetic, trait means, and plasticity distances among clones. Trait means and plasticities distance matrices were based upon traits with no strong intercorrelations (where $r \geq 0.8$). Relationships among the various matrices were depicted by unweighted paired-group method using arithmetic averages (UPGMA) phenograms (Rohlf 1988).

Results

SOIL NUTRIENTS

Nutrients and pH were significantly higher than those of most northern bogs and even marginal bogs (Stewart and Nilsen 1993). Experimental ammonium concentrations and pH means were higher than those found in ombotrophic or minerotrophic bogs, and phosphorus and potassium concentrations were higher than those found in ombotrophic bogs but comparable to minerotrophic bog (fen) soil (Heinselman 1970). In contrast to the means, the standard deviations represented field-level variation.

PLANT RESPONSES

CLONAL DIFFERENTIATION. Clonal differentiation was more pronounced than phenotypic plasticity. A Spearman rank correlation procedure (Zar 1984) revealed that, of the 19 traits measured, several strong intercorrelations existed among characters (table 2). Seventy-seven percent of nonintercorrelated traits were significantly

Table 2

INTERCORRELATIONS AMONG TRAITS

Trait	r	Trait
LW/UP	0.93	UPW
SW/UP	0.85	UPW
TOTUPW	0.88	UP/PT
LW/R	0.80	RLW
LW/R	0.90	RW
SW/R	0.90	RW
R/P	0.91	TOTRW
UW/RW	0.91	U/R

Note. Spearman correlations (Zar 1984), where $r \geq 0.80$, are shown. In such cases $P < 0.001$.

Table 3

MULTIPLE COMPARISONS OF TRAIT MEANS (untransformed) WITH CLONES

Trait	Clone					
	NC	TN	WV	MA	NY	WI
Phalanx traits:						
L/UP	30.9 ab	28.1 bc	28.0 bc	32.1 a	25.7 c	29.0 abc
ULWT (mg)	1.72 bc	1.73 bc	1.41 d	1.70 c	1.89 b	1.96 a
LW/UP (mg)	52.8 a	48.9 a	39.6 b	54.7 a	48.6 a	55.3 a
SW/UP (mg)	27.4 a	23.1 c	21.1 c	23.6 ab	25.0 ab	27.3 a
TOTUPW (mg; ns)	1701	1926	1756	2216	1229	1897
UPW (mg)	80.0 ab	72.0 b	61.0 c	78.4 ab	73.6 ab	82.9 a
UP/PT (ns)	21.1	27.8	28.8	28.0	16.9	21.4
Runner traits:						
L/R	40.3 b	36.8 bc	37.8 c	47.1 a	40.6 b	35.8 c
RLW (mg)	2.39 c	2.63 bc	2.07 d	2.76 b	3.10 a	2.58 bc
LW/R (mg)	95.7 b	97.1 b	78.5 c	129 a	128 a	93.9 b
SW/R (mg)	106 ab	81.5 c	81.8 c	121 a	129 a	88.7 bc
RW (mg)	202 b	178 bc	160 c	250 a	256 a	188 bc
TOTRW (mg)	2282 ab	1827 b	1781 b	2887 a	3151 a	1582 b
R/PT (ns)	11.0	10.2	10.8	11.6	11.4	7.79
Other traits and ratios:						
UW/RW	1.17 c	2.84 a	1.32 bc	1.19 c	0.776 c	2.78 ab
U/R (ns)	2.81	6.24	3.26	3.32	2.26	5.30
PTWT	4005 a	3991 a	3915 a	5536 b	4369 a	3950 a
ANTHO (ns; optical densities at 510 nm)	0.713	0.758	0.744	0.596	0.743	0.586
STO (ns)	28.9	27.8	29.4	29.0	27.3	28.9

Note. Different letters in rows denote significance at $\alpha = 0.05$, determined using Tukey's HSD (Zar 1984). ns = model reveals no significant differences at $P = 0.05$. Table lists trait abbreviations and text for clone provenances.

different among clones tested at $\alpha = 0.05$ (table 3). Clone MA was largest (greatest PTWT, L/UP, L/R [see table 1 for abbreviations]) and was generally most vigorous vegetatively, whereas WV was the smallest clone (least ULWT, UPW, RLW). There did not seem to be any obvious central and marginal patterns regarding clonal differentiation. However, the MA and NY clones had similar reaction norms, as did TN and WV (table 3; fig. 2). For some traits, such as LW/R, SW/R, and RW, marginal clones had generally lower runner trait means than central clones (table 3).

PHENOTYPIC PLASTICITY. At the fine scale of soil environmental manipulation, very little phenotypic plasticity was noted in the measured traits. The type III general linear models showed no significant differences, with the following exceptions: TN clone (SW/R, RW; all independent variables and interactions significant at $\alpha = 0.05$), WI clone (PTWT, TOTUPW, STOM, RLW, LW/UP, SW/UP; all covariates and interactions significant at $\alpha = 0.05$). The ANCOVA homogeneity of slopes model (nutrient*clone interaction) revealed no significant differences at $P = 0.05$.

Clones had significantly different levels of total phenotypic plasticity (table 4). WI and NC had the greatest positive plasticities in response to N and pH. After log transformation these two clones exhibited the only positive slopes in response to N and pH variation (fig. 2).

RAPD PROFILING

The UPGMA cluster analysis showed that coastal clones NC and MA formed a cluster and were joined successively with the WV and TN clones (fig. 3*B*). The two northern inland clones, WI and NY, also clustered together but were relatively dissimilar (euclidean distance of 20).

MATRIX COMPARISONS

Pairwise comparisons of the six standardized matrices showed no significant correlations, with the exception that the nitrogen-response plasticity matrix negatively correlated with RAPD, means, and geographic matrices, but with low r ($r < -0.5$) (table 5).

Discussion

GENETIC HETEROGENEITY

The seeds of *Vaccinium macrocarpon,* a temperate bog mat plant, are presumed to be bird dispersed, potentially for long distances (Ogle 1984). Following Pleistocene glaciation *V.* macrocarpon migrated from now distributionally marginal sites to currently central sites (Stewart 1993*b*). Genetic distances estimated from RAPD phenotypes show that the clones with the largest

distances between them (WI, TN) are genetically about 75% similar. This hypothesized intraspecific phylogeny is not well modeled by the UPGMA cluster analysis (data not shown), which had a dissimilar relative topology from that of a tree constructed when a larger sampling of clones is used (Stewart 1993*b;* C. N. Stewart Jr., unpublished data).

It is recognized that the species is autogamous and very homozygous, as allozyme variation within the species is depauperate (Hill and Vander Kloet 1983; Hugan et al. 1993). In contrast, blueberries (section *Cyanococcus*), which are outcrossers, contain many allozyme polymorphisms (Breuderle et al. 1991). The genetic homogeneity of *V. macrocarpon* section *Oxycoccus,* is probably, at least in part, a result of the breeding system, the recent distributional history, and the current island-like distribution. The latter is not shared with the Cyanococcus section of *Vaccinium.* Unlike the more generalist section *Cyanococcus,* section *Oxycoccus* are specialized to bog habitats. It is likely that springtime migratory birds quickly dispersed *V. macrocarpon* seeds to available bare suitable sites as glacial ice retreated (Ridley 1930; Ogle 1984). This rapid colonization and gene flow would have served to homogenize a genome that had already been through a distributional bottleneck. Although *V. macrocarpon* is now found in somewhat isolated populations, especially at its southern limits, this distribution is only a recent (last 5000 years) occurrence (Ogle 1984). For an autogamous species with this distributional history, it is not unexpected that genetic distances between now geographically distant clones are relatively low. Other work shows that the degree of genetic variation of cranberry is low compared to other autogamous insect-pollinated perennials (Ellstrand and Roose 1987; Hamrick and Godt 1989; Hugan et al. 1993; Stewart and Excoffier, in press). Strong differentiation in relation to habitat would not be expected in *V. macrocarpon* because of breeding patterns, life history, and time limitations.

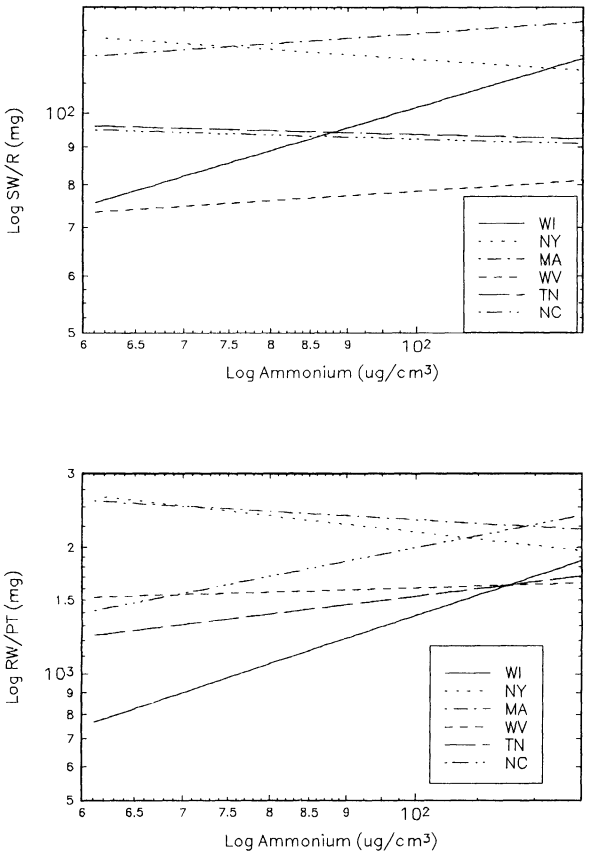


Fig. 2 Representative norms of reactions of three traits: stem mass per runner (SW/R), runner mass per plant (RW/PT), and runner mass (RW) versus soil ammonium concentration. Table 1 contains trait nomenclature. Traits and ammonium concentration were log transformed to minimize and stabilize variances.

Environmental stress and marginality

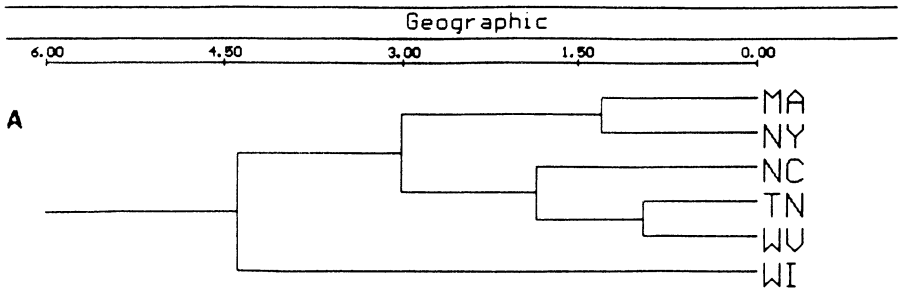
Peatlands have relatively low nutrient status and dissolved oxygen as a result of high water tables (Small 1972*b*). Sclerophyllous leaves may provide a high nutrient use efficiency to cope with chronically low nutrient status (Monk 1966; Small 1972*a;* Chapin 1980). In many commercial cran-

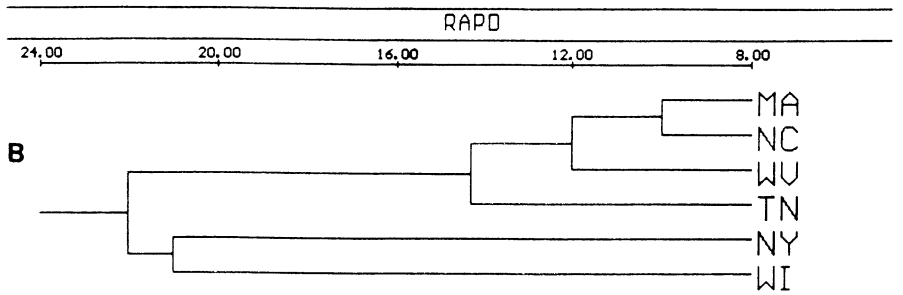
Table 4

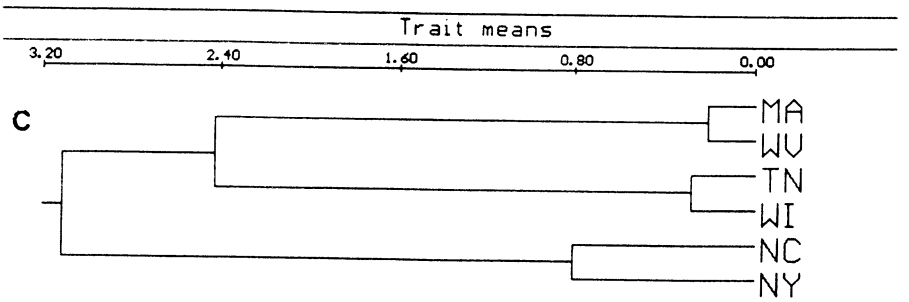
Clonal differentiation for plasticity (means of slopes) in response to soil nutrient concentrations and log-transformed traits and nutrients

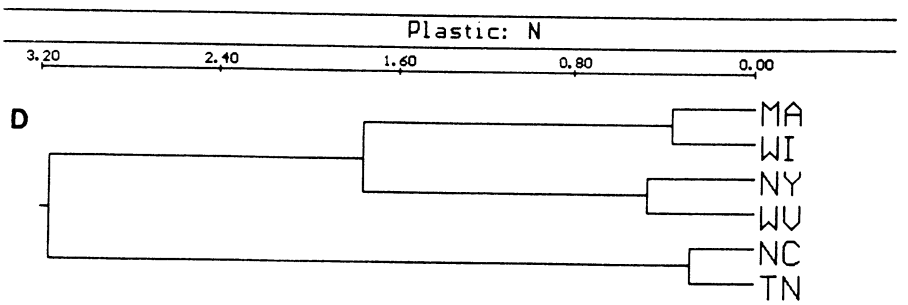
	Soil nutrient factor					
	Log log transformed			Untransformed		
Clone	pH	P	N	pH	P	N
WI	0.54 a	0.12 a	0.45 a	178.1 a	−2.76 b	2.73 ab
NY	−0.09 abc	0.21 a	−0.31 c	54.0 ab	5.61 ab	−2.76 bc
MA	−0.46 c	0.08 a	−0.23 c	−227.8 c	4.15 ab	−6.43 c
WV	−0.25 bc	0.29 a	−0.16 bc	−120.4 bc	13.72 a	−5.04 bc
TN	−0.25 bc	−0.24 c	−0.24 c	−96.3 bc	0.46 ab	0.78 abc
NC	0.33 ab	0.05 a	0.28 ab	105.5 ab	0.56 ab	8.26 a

Note. Different letters in columns denote significant differences at the $\alpha = 0.05$ level.


Geographic
6.00
4.50
3.00
1.50
0.00
A
MA
NY
NC
TN
WV
WI


RAPD
24.00
20.00
16.00
12.00
8.00
B
MA
NC
WV
TN
NY
WI


Trait means
3.20
2.40
1.60
0.80
0.00
C
MA
WV
TN
WI
NC
NY


Plastic: N
3.20
2.40
1.60
0.80
0.00
D
MA
WI
NY
WV
NC
TN

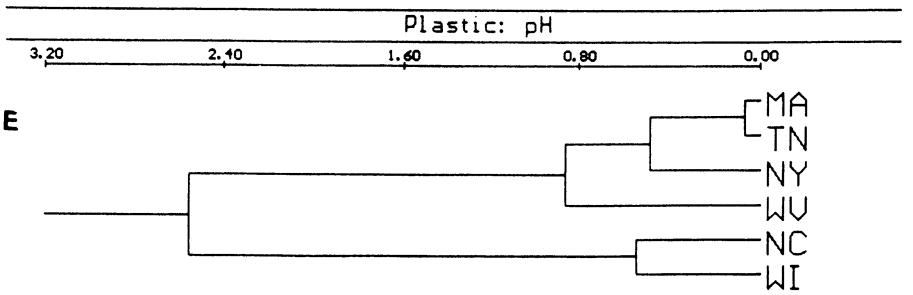

Plastic: pH
3.20
2.40
1.60
0.80
0.00
E
MA
TN
NY
WV
NC
WI

Table 5

PAIRWISE MATRIX COMPARISONS OF RAPD PROFILES, GEOGRAPHIC DISTANCES, TRAIT MEANS, AND PLASTICITIES; THE MANTEL TEST WAS USED TO TEST FOR PAIRWISE SIMILARITY BETWEEN EACH MATRIX

Factor	RAPD	Geographic distance	Trait mean	Slope, X = pH	Slope, $X = P$
Geographic distances	0.18				
Trait means	0.34	0.36			
Slopes, X = pH	0.41	0.26	0.29		
Slopes, $X = P$	0.23	0.15	0.21	0.32	
Slopes, X = N	0.22	0.07	0.18	0.37	0.09

Note. P values of Mantel test (1000 iterations) are listed. For slopes, the variable on the X axis is listed. See text for details about tests and factors. Note that all P values are not significant at $\alpha = 0.05$.

berry growing regions, such as in Wisconsin, there is a yearlong possibility of frost (Curtis 1959). Since *V. macrocarpon* is a dominant member of the vegetation where these conditions are prevalent, it is presumably adapted to these abiotic factors. One could argue that at its southern limit, where it exists as a relict, *V.* macrocarpon is exposed to stresses for which it is not well adapted, such as heat and interspecific competition with more vigorous plants. Therefore, the few clones that inhabit these marginal relict sites could conceivably possess traits not present in central sites, e.g., phenotypic plasticity, which have allowed them to survive this suite of "foreign" stresses.

Several studies with clonal plants have found that clones from ecologically marginal (stressful) sites have greater phenotypic plasticity. Grant and Antonovics (1978) found that *Anthoxanthum odoratum* plants from adjacent marginal sites responded more quickly to environmental change compared with a central population. However, ecotypic differences were greater than plasticity. Lotz and Blom (1986) found that in *Plantago major,* plants from stressful environments showed greater plasticity. Few studies have examined bog plant plasticity, and none have compared genetic variation and phenotypic plasticity. *Vaccinium vitis-idaea* was found to be the least plastic of bog dwarf shrubs in a high arctic nutrient study (Chapin and Shaver 1985). In a common garden study, Riebesell (1981) found that more stressed alpine populations of *Ledum groenlandicum* had higher plasticity in photosynthesis rates than central bog populations. Ohlson (1989) compared low nutrient (stressed) and higher nutrient populations of the mire plant *Saxifraga hirculus* and found higher plasticity in marginal clones. She found large amounts of ecotypic differentiation as well.

In *V. macrocarpon,* there generally seems to be no geographic pattern regarding phenotypic differentiation. Although many clonal trait means are significantly different, there is no pattern in the differences, and the differences in many instances are biologically slight (table 3). There are also no obvious patterns when comparing geographic, genetic, trait mean, or plasticity distance matrices (table 5; fig. 3). In other instances, where these types of comparisons have been made, the matrices or trees for genetic distances and geographic and trait means also did not correlate (Schlichting and Levin 1988; Vasseur and Aarssen 1992). These authors concluded that phenotypic plasticity was independent of traits and was a trait itself (Schlichting and Levin 1988), or that plasticity was not necessarily adaptive (Vasseur and Aarssen 1992). The nonsignificance of matrix correlations could be a product of the limited sample size. Perhaps if a larger sample were gathered, geographic patterns would emerge. In *V. macrocarpon* the most parsimonious explanation is that the genetic and phenotypic similarities among clones are a result of the recent clonal ancestry and relatively low genetic differences among clones. The low genetic divergences are likely, in turn, to be a result of a glacial bottleneck and rapid colonization coupled with autogamous breeding habit.

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Fig. 3 UPGMA phenograms showing clonal relationships using various distance matrices: geographic (*A*), RAPD (*B*), trait means (*C*), and trait plasticity in response to N (*D*) and pH (*E*). Euclidean distances (Excoffier et al. 1992) were used for RAPDs, and the Canberra metric was used for phenotypic data (Rohlf 1988).

River for cranberry accessions. Thanks to Carl Schlichting, James Hancock, Gregory Cheplick, and three anonymous reviewers for helpful comments on earlier drafts. This work was supported by grants from Sigma Xi, the Research Society, and Ocean Spray Cranberries, Inc. Full tables (e.g, correlation and ANOVA) may be found in Stewart (1993*a*).

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