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Inheritance and Linkage of Thirteen Polymorphic Isozyme Loci in Diploid Blueberry

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Abstract. **Diploid populations and progenies of controlled crosses of blueberry, *Vaccinium* section *Cyanococcus,* were analyzed by starch gel electrophoresis for nine enzyme systems, aconitase (ACO), aldolase (ALD), alcohol dehydrogenase (ADH), glyceraldehyde-3 -phosphate dehydrogenase (G-3 -PDH), isocitrate dehydrogenase (IDH), leucine aminopeptidase (LAP), malate dehydrogenase (MDH), 6-phosphogluconate dehydrogenase (6-PGD), and phosphoglucose isomerase (PGI). Allozyme variants indicated the existence of three alleles at *Ace-1, Ald, G-3-pdh-2,* and *Mdh-2;* four alleles at *Mdh-3* and *6-Pgd-2; five* alleles at *Aco-2, Idh, Lap-1,* and *6-Pgd-1;* six alleles at *Adh-2* and *Pgm-2;* and nine alleles at *Pgi-2.* In addition, a null allele was found at *Lap-1.* In the majority of progenies, the inheritance patterns for each of these loci were consistent with mendelian laws for single gene control. Forty-seven pairs of loci were tested for independent assortment revealing two linked pairs, *Pgi-2/Lap-1* and *Pgm-2/6-Pgd-2,* which appeared to represent two independent linkage groups.**

An extensive survey and discussion of *Vaccinium* section *Cyanococcus* A. Gray in North America by starch gel electrophoresis has revealed high levels of variability (Bruederle and Versa, 1989; Bruederle et al., 1991); 90% of the allozyme loci examined were polymorphic within the section. Inheritance studies, however, have been reported for only a few loci; Versa et al. (1988) presented evidence for mendelian inheritance at four loci among the enzyme systems malate dehydrogenase (EC 1.1.1.37; MDH) phosphoglucose isomerase (EC 5.3.1.9; PGI) 6-phosphogluconate dehydrogenase (EC 1.1.1.44; 6-PGD), and isocitrate dehydrogenase (EC 1.1.1.42; IDH); Krebs and Hancock (1989) reported tetrasomic inheritance at four loci among the enzyme systems MDH, PGI, 6-PGD, and aspartate aminotransferase (EC 2.6.1.1). Evidence for the genetic basis of more isozyme phenotypes and knowledge of the linkage relationships among isozyme loci would be useful for future genetic and breeding behavior studies and provide information on linkage conservation. The *Vaccinium* section *Cyanococcus* karyotype consists of 12 metacentric or submetacentric chromosomes (Hall and Galletta, 1971) that will potentially result in the development of maps for 12 linkage units.

The purpose of this paper is to report: i) inheritance patterns at the diploid level for nine of the isozyme loci governing the enzyme systems aconitase (EC 4.2.1.3; ACO), alcohol dehydrogenase (EC 1.1.1.1; ADH), aldolase (EC 4.1.2.13; ALD), glyceraldehyde-3 -phosphate dehydrogenase (EC 1.2.1.9; G-3-PDH), leucine aminopeptidase (EC 3.4.11.1; LAP) MDH, 6-PGD, and phosphoglucomutase (EC 2.7.5.1; PGM); (ii) linkage relationships among 13 of the loci governing these enzyme systems as well as IDH and PGI; and (iii) allozyme variants observed at these loci in *V. boreale* Hall and Aalders, *V. myrtilloides* Mich., *V. corymbosum* L., *V. pallidum* Ait., *V. tenellum* Ait., and *V. darrowi* Camp. in order to update locus and allelic nomenclature in *Vaccinium* section *Cyanococcus.*

Materials and Methods

Plant material. Thirty diploid blueberry populations collected as cuttings throughout eastern North America from Nova Scotia to central Florida were surveyed for allelic variants. The populations ranged from 25 to 55 individuals and included the six diploid species of *Vaccinium* section *Cyanococcus (sensu* Vander Kloet 1988): *V. boreale, V myrtilloides, V. corymbosum, V. pallidum, V. tenellum,* and *V. darrowi.*

For inheritance studies, crosses were made using the diploid plants described in Versa et al. (1988). The plants labeled ''dar–" and "en-" belong to the *V. darrowi* and *V. elliottii (sensu* Chap.) populations described in that study. US 392,842-16, and 842-29 are progeny of Fla4b (a selected wild clone from Ocala National Forest, Fla.) crossed with an unknown plant. Flowers were emasculated before pollination.

Electrophoresis. Vegetative buds were ground in a buffer consisting of 0.1 M sodium phosphate containing 10% w/v polyvinylpyrrolidone (PVP-40) and 1% 2-mercaptoethanol at pH 7.5; extracts were absorbed onto Whatman no. 17 chromatography paper wicks (2 × 10 mm). About one vegetative bud per drop of buffer and per wick was used. Wicks were then stored in microcentrifuge tubes at – 72C until electrophoresis. Wicks were loaded onto 12% starch gels and electrophoresis was performed in a refrigerator maintained at 2C.

The gel and electrode buffer systems used were the Soltis et al. (1983) system 7 for LAP-1, ADH-2, and PGI-2 at 250 V (constant); system 8 for ACO-1, ACO-2, ADH-2, ALD, LAP-1, and PGI-2 at 250 V (constant); system 1 modified to pi-l 7.5 for ALD, G-3-PDH-2, IDH, 6-PGD-2, and PGM-2 at 100 V (constant); and system 11 modified to pH 6.5 for ACO-1, ACO-2, G-3-PDH-2, MDH-2, MDH-3, 6-PGD-1, and PGM-2 at 55 V (constant). The pH 6.1 morpholine citrate gel and electrode buffer system of Clayton and Tretiak (1972) was used for IDH, MDH-2, MDH-3, 6-PGD-1, and 6-PGD-2 at 120 V (constant). Whereas population genetic data were collected employing all of the aforementioned systems, progenies of controlled crosses were run on two or three of the systems depending on which loci were expected to segregate.

Gel slices 1 mm thick were stained for the appropriate enzymes. G-3-PDH was stained according to the following procedure: 500 mg fructose 1,6-diphosphate trisodium salt was incubated with 30 units of aldolase in 20 ml of distilled water at 37C for 30 rein, after which 50 ml distilled water, 2 ml 1.0

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M arsenic acid, 40 mg NADP, 20 mg 3-(4,5 -Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT), and 5 mg of phenazine methosulfate were added. The other enzymes were stained according to Soltis et al. (1983) with the following modifications: For ACO, the cis-aconitic acid was increased to 300 mg and the isocitrate dehydrogenase to 60 units. For LAP, the gel slice was first incubated with the substrate for 30 min and rinsed off before addition of fast black K salt dissolved in 1.0 M phosphate buffer at pH 6.0.

The migration distance of isozyme bands was measured and their mobility calculated in relation to the buffer front. The mobilitie; presented in Fig. 1 were measured on: system 7 (ADH-2, LAP-1, PGI-1, and PGI-2); system 1 at pH 7.5 (IDH and ALD); system 11 at pH 6.5 (ACO-1, ACO-2, PGM-1, PGM-2, G-3-PDH-1, and G-3-PDH-2); and on the morpholine citrate system (MDH-2, MDH-3, 6-PGD-1, and 6-PGD-2). The loci for a given enzyme were assigned sequential numbers starting with *1* for the most anodal locus; the allozymes found were assigned sequential letters starting with a for the most anodal. Any null alleles found were referred to as *n*.

Data analysis. G statistics for goodness-of-fit to expected ratios were calculated for single locus inheritance analyses (Sokal and Rohlf, 1981). Pooled and heterogeneity G statistics were calculated for progeny segregating for identical alleles. The correction for continuity for cases in which there was only one degree of freedom was used only for progeny in which a difference in alleles precluded pooling with other progeny.

Contingency table G statistics were calculated to test for independent assortment in progeny jointly segregating for two loci. Recombination values for cases of significant deviation from independent assortment were obtained from the LINKAGE-1 computer program of Suiter et al. (1983). Allozyme segregations were not included in the analysis if any of their expected frequency classes were below five. Linkage analyses in which the segregation ratio of one out of the two loci differed significantly from the expected mendelian ratio were accepted.

Results

The allozyme variants reported here are discussed with respect to their migration distances, quaternary structure, and inheritance only. A discussion of the distribution of allozyme polymorphism among the species was not considered relevant to this paper and are discussed elsewhere (Bruederle and Versa, 1990; Bruederle et al., 1991).

Malate dehydrogenase. In addition to the two previously reported MDH loci (Versa et al., 1988), two weakly staining zones, one anodal and one cathodal to the former, were detected as a result of generally improved isozyme resolution. Assuming these are additional MDH loci, the former MDH-1 and MDH-2 would then become MDH-2 and MDH-3 and will subsequently be referred to as such. MDH-1 was monomorphic, consisting of one band at the same migration distance in all individuals analyzed. MDH-4 was too faint to be scored. One additional allele was found at *Mdh-3* at a migration distance between the *a* and *b* alleles reported in a former study (Versa et al., 1988) in which mendelian inheritance for this locus was reported.

Except for a few individuals that are discussed below, banding patterns at *Mdh-2* were either single-banded or three-banded, the center band being darker and about equidistant from the peripheral bands in accordance with a dimeric structure of the enzyme. Banding positions were consistent with the existence of three alleles at this locus (Fig. 1). Progenies segregating for *Mdh-2* supported mendelian inheritance (Table 1).

In certain individuals, there also were extra bands observed in proximity to the *b* allele of *Mdh-2,* the genetic basis of which cannot be determined conclusively with the present data. One pattern consisted of the *b* allele with a weaker cathodal band; another pattern consisted of the *b* allele with a weaker anodal band (Fig. 2). These patterns were reproducible and appeared to segregate in a mendelian fashion. A cross between two individuals homozygous for the *b* allele but with one having the additional cathodal band gave a progeny segregation consistent with a 1:1 ratio for the parental patterns. A cross between two individuals with the *Mdh-2b* allele, one parent possessing the additional anodal band and

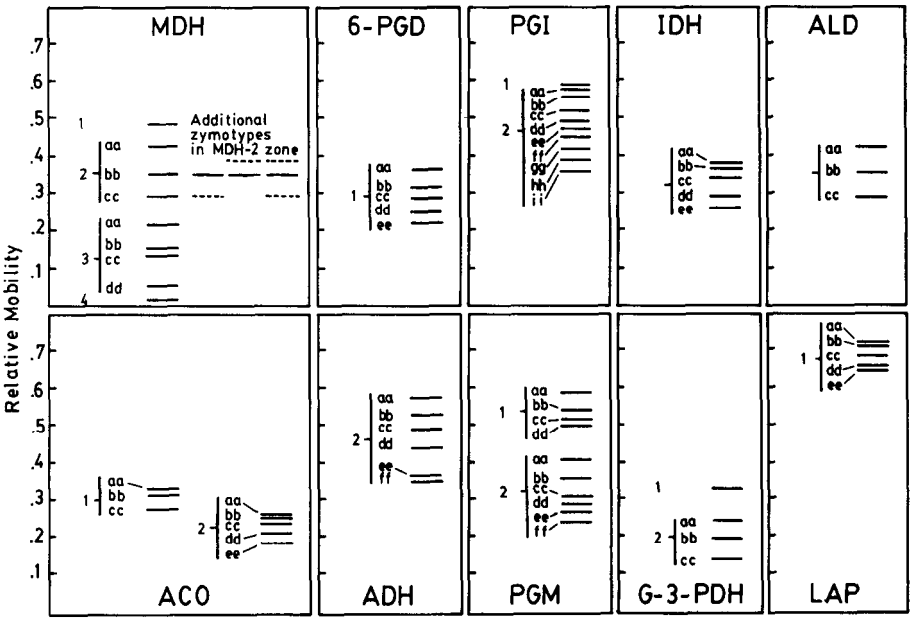

Fig. 1. Electrophoretic mobility of *Vaccinium* section *Cyanococcus* isozymes (solid lines). Numbers indicate loci. Letters indicate alleles. Dotted lines indicate bands with a weaker staining intensity relative to the solid bands.

Table 1. Single-locus goodness-of-fit tests (G statistics) at nine polymorphic isozyme loci in *Vaccinium* section Cyanococcus.

Locus	Cross[z]	Parental genotypes	Progeny genotypes	Expected ratio	df	G
Aco-1	2	*ab* × *bb*	15*ab*:19*bb*	1:1	1	0.47
	7	*bb* × *ab*	22*ab*:17*bb*	1:1	1	0.64
				G pooled	1	0.01
				G heterogeneity	1	1.10
Aco-2	2	*ab* × *aa*	41*aa*:23*ab*	1:1	1	4.57*
	6	*ab* × *ab*	10*aa*:24*ab*:14*bb*	1:2:1	2	0.67
	7	*bb* × *ab*	30*ab*:27*bb*	1:1	1	0.16
Adh-2	1	*bb* × *bd*	21*bb*:26*bd*	1:1	1	0.53
	2	*bd* × *bb*	34*bb*:34*bd*	1:1	1	0.00
	7	*bb* × *bd*	38*bb*:40*bd*	1:1	1	0.05
	14	*bb* × *bd*	17*dd*:17*bd*	1:1	1	0.00
				G pooled	1	0.21
				G heterogeneity	3	0.37
	12	*dd* × *bd*	39*bd*:23*dd*	1:1	1	3.66
Ald	1	*bc* × *bb*	40*bb*:52*bc*	1:1	1	1.57
	2	*bb* × *bc*	39*bb*:55*bc*	1:1	1	2.74
	12	*bb* × *bc*	48*bb*:44*bc*	1:1	1	0.17
	14	*bc* × *bb*	24*bb*:29*bc*	1:1	1	0.47
				G pooled	1	2.54
				G heterogeneity	3	2.41
	5	*bc* × *bc*	29*bb*:47*bc*:23*cc*	1:2:1	2	0.95
G3pdh-2	1	*bb* × *bc*	34*bb*:47*bc*	1:1	1	2.09
	2	*bc* × *bb*	57*bb*:41*bc*	1:1	1	2.62
	5	*bb* × *bc*	45*bb*:51*bc*	1:1	1	0.37
				G pooled	1	0.03
				G heterogeneity	2	5.06
	7	*ab* × *ab*	21*aa*:44*ab*:28*bb*	1:2:1	2	1.27
Lap-1	6	*ac* × *bn*	39*ab*:37*bc*:27*an*:17*cn*	1:1:1:1	3	10.98*
	10	*bd* × *dd*	61*bd*:74*dd*	1:1	1	1.07
	11	*ac* × *bb*	34*ab*:34*bc*	1:1	1	0.00
	12	*ac* × *cc*	28*ac*:24*cc*	1:1	1	0.17
	14	*ab* × *cn*	9*an*:15*ac*:14*an*:14*bn*	1:1:1:1	3	1.82
Mdh-2	12	*bb* × *bc*	36*bb*:33*bc*	1:1	1	0.13
	13	*bb* × *bc*	41*bb*:59*bc*	1:1	1	3.26
	14	*bb* × *bc*	31*bb*:21*bc*	1:1	1	1.93
				G pooled	1	0.11
				G heterogeneity	2	5.21
6-Pgd-1	3	*ab* × *ab*	9*aa*:19*ab*:9*bb*	1:2:1	2	0.03
	4	*ab* × *ab*	24*aa*:32*ab*:14*bb*	1:2:1	2	3.18
	9	*ab* × *ab*	30*aa*:41*ab*:13*bb*	1:2:1	2	6.95*
				G pooled	2	7.71*
				G heterogeneity	4	2.45
	7	*ab* × *bb*	48*ab*:44*bb*	1:1	1	0.17
	8	*ab* × *bb*	39*ab*:41*bb*	1:1	1	0.05
				G pooled	1	0.02
				G heterogeneity	1	0.20
	6	*ab* × *bc*	27*ab*:19*bb*:29*bc*:24*ac*	1:1:1:1	3	2.36
Pgm-2	1	*ab* × *bb*	38*ab*:55*bb*	1:1	1	3.12
	2	*bb* × *ab*	45*ab*:54*bb*	1:1	1	0.82
	3	*ab* × *bb*	24*ab*:14*bb*	1:1	1	2.66
	4	*ab* × *bb*	48*ab*:43*bb*	1:1	1	0.27
				G pooled	1	0.38
				G heterogeneity	3	6.50
	5	*ab* × *ab*	32*aa*:48*ab*:17*bb*	1:2:1	2	4.68
	9	*ab* × *ab*	31*aa*:35*ab*:26*bb*	1:2:1	2	5.75
				G pooled	2	6.60*
				G heterogeneity	2	3.82
	10	*dd* × *ab*	75*ad*:65*bd*	1:1	1	0.58

[z]Crosses are: dar20 × dar18 (1); dar18 × dar20 (2); 842-16 × Ten (3); 842-29 × Ten (4); dar20 × dar22 (5); ellFL6 × dar16 (6); dar10 × dar18 (7); ellFL19 × W85-6(8); US392 × US136 (9); darl4 × ellNC19 (l0); ellFL6 × dar10 (11); ellFL6 × dar12 (12); ellFL19 × dar4 (13); dar5 × dar6 (14).
*Significant at $P = 0.05$.

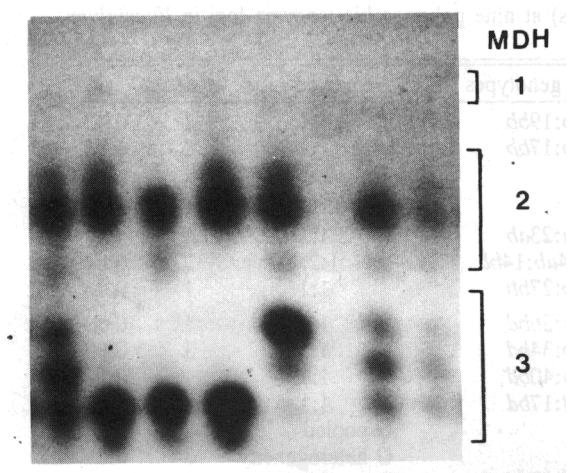

Fig.2. MDH zymograms showing additional variation found in proximity of MDH-2. From left to right the first three genotypes are interpreted to be: **(1)** Mdr-2bb with both extra anodal and cathodal bands, Mdh-3ac; **(2)** Mdh-2bb with extra anodal band, Mdh-3cc; **(3)** *Mdh-2bb* with extra cathodal band, *Mdh-3cc.*

the other the additional cathodal band, segregated in a 1:1:1:1 fashion for four patterns: the *Mdh-2bb* allozyme alone, the *Mdh-2bb* allozyme with both extra anodal and cathodal bands, and the two parental patterns. The *Mdh-2bb* band always stained with the same intensity in the parents and in the progeny whether or not the extra anodal and/or cathodal bands were present. A cross between a plant exhibiting only the *Mdh-2bb* band and a plant with a three-banded *Mdh-2bc* pattern gave progeny segregating in a 1:1 ratio for the three-banded *Mdh-2bc* pattern and the *Mdh-2bb* band with the additional cathodal band. A cross between a plant possessing the *Mdh-2bb* band with the extra cathodal band and a plant with the three-banded pattern *Mdh-2bc* resulted in three types of progeny: 14 exhibited only the *Mdh-2bb* band, 18 had the *Mdh-2bc* bands with the additional cathodal band, and 21 had the three-banded *Mdh-2bc* pattern. One hypothesis that is consistent with the data would be the existence of another MDH locus that produced a monomeric enzyme with three alleles co-migrating with *Mdh-2.* The *bb* allozyme of this hypothesized locus would co-migrate with the *Mdh-2bb* band, the *aa* allozyme would be the extra anodal band, and the *cc* allozyme would be the extra cathodal band that would co-migrate with *Mdh-2cc.* Although MDH has usually been found to be a dimer, two-banded MDH phenotypes have been reported in organelles of other plants (Longo and Scandalios, 1969; Sternberg and Ting, 1979). The subcellular localization of the various blueberry MDH loci has not been done and may help to explain these observations.

6-phosphogluconate dehydrogenase. Progeny segregations for *6-Pgd-1* gave either one-banded or three-banded patterns consistent with a dimeric structure for the enzyme. Banding positions suggested the existence of five alleles at *6-Pgd-1* (Fig. 1). Segregation ratios were in accordance with mendelian inheritance (Table 1) except for cross 9. Mendelian inheritance for 6-*Pgd-2* has already been shown (Versa et al., 1988); no new alleles were found at this locus.

Phosphoglucose isomerase. Mendelian inheritance has been reported for *Pgi-2* (Versa et al., 1988). In that study, one pattern obtained for *Pgi-2* contained only two discernible bands, the third band having been hypothesized to co-migrate with PGI-1. This hypothesis has now been confirmed; with electrophoresis of longer duration, the *a'* allele was resolved from PGI-1. Four new alleles were identified, thus converting the former *Pgi-2 a', a, b, c,* and *d* alleles to *Pgi-2 a, b, d, e,* and g alleles.

Isocitrate dehydrogenase. Mendelian inheritance for *Idh* has already been published (Versa et al., 1988). Two additional *ldh* alleles were found, one with a migration distance between the former *a* and *b* alleles and the other migrating between the former *b* and c alleles. Thus, the former *Idh-b* and c alleles become *ldh-c* and e alleles, respectively.

Aldolase. Staining for ALD showed one zone of activity with either one-banded or five-banded electrophoretic patterns. This result is consistent with a tetrameric structure for this enzyme in blueberry. Banding positions indicated the existence of three alleles (Fig. 1). Segregation ratios were in accordance with expected ratios (Table 1) for single locus control.

Aconitase. Two zones of staining for aconitase could be distinguished; banding patterns for each zone consisted of either one or two bands. This observation is consistent with a monomeric structure for both *Aco-1* and *Aco-2* (Fig. 1). ACO has been found to be a monomer in other plant species (Griffin and Palmer, 1987; Hyun et al., 1987). Banding positions in blueberry indicated the existence of three alleles at Ace-l and five at Aco-2. Staining for ACO in the progeny analyses was faint, making it more difficult to read than the other enzymes. Therefore, progeny numbers for segregation and linkage analyses were generally reduced compared to other enzymes. However, the available data support mendelian inheritance (Table 1).

Alcohol dehydrogenase. ADH exhibited two zones of activity, the first of which was very weak. The second zone, although sometimes weak in leaf tissue, was storable; banding patterns consisted of either one or three bands in agreement with a dimeric structure for *Adh-2.* ADH has been found to be a dimer in other plant species (Suiter, 1988; Weeden and Marx, 1987). Six alleles were found at Adh-2 in blueberry (Fig. 1). Segregation ratios were in accordance with mendelian inheritance (Table 1).

Phosphoglucomutase. In general, PGM showed two zones of activity, the zymotypes for each zone being either one-banded or two-banded; this pattern is consistent with a monomeric structure for *Pgm-1* and *Pgm-2.* PGM has been found to be a monomer in many plant species (Arus et al., 1982, Hyun et al., 1987; Manes and Fairbrothers, 1987; Suiter, 1988). *Pgm-1* will not be discussed here as we are not confident in the scoring of the progeny data 'for this locus. Six alleles were found at Pgm-2 (Fig. 1). Progenies with expected segregation ratios of 1:1 for Pgm-2 did not deviate significantly from the expected ratio but progenies with expected segregation ratios of 1:2:1 did (Table 1).

Glyceraldehyde-6-phosphate dehydrogenase. Two zones of activity for G-3-PDH could be distinguished. G-3-pdh-l was monomorphic and consisted of one band in all species and progenies analyzed. *G-3-pdh-2* was polymorphic, zymotypes being either single-banded or expressed as a diffuse zone of staining extending between the migration distances of two of the single bands. The diffuse zone was interpreted to represent the closely migrating bands of heterozygotes for a multimeric enzyme, and the migration distance of the zymotypes found was consistent with the existence of three alleles (Fig. 1). Following this interpretation, segregation ratios calculated for progeny were in accordance with mendelian inheritance (Table 1).

Leucine aminopeptidase. Two zones of activity were observed, the second zone being too faint to be scored. Zymotypes found for *Lap-1* were either one-banded or two-banded, consis-

Table 2. Number (and size) of progenies analyzed by G statistics contingency tests for independent assortment of jointly segregating isozyme loci (above the diagonal) and number of progenies deviating from independent assortment (below the diagonal) in *Vaccinium* section Cyanococcus.[z]

	Aco1	*Aco2*	*Adh2*	*Ald*	*G3pdh2*	*Idh*	*Lap1*	*Mdh2*	*Mdh3*	*6Pgd1*	*6Pgd2*	*Pgi2*	*Pgm2*
Aco1		2(72)	2(68)	---[y]	1(32)	2(70)	---	---	---	---	---	1(64)	---
Aco2	NS		2(106)	---	2(118)	2(117)	---	---	---	---	---	3(167)	---
Adh2	NS	NS		2(76)	3(181)	2(143)	---	2(82)	---	---	1(62)	4(253)	---
Ald	---	---	---		1(96)	---	1(52)	2(165)	---	---	1(88)	3(238)	3(275)
G3pdh2	NS	---	1*	NS		2(187)	---	---	1(93)	1(91)	---	4(355)	1(94)
Idh	---	NS	NS	---	NS		---	---	1(72)	---	2(193)	5(430)	1(139)
Lap1	---	---	---	NS	---	---		---	2(171)	1(94)	1(68)	3(186)	---
Mdh2	---	---	NS	NS	---	---	---		1(100)	---	1(63)	2(158)	---
Mdh3	---	---	---	---	NS	NS	NS	NS		4(394)	2(177)	6(664)	3(292)
6Pgd1	---	---	---	---	NS	---	NS	---	NS		1(81)	3(253)	2(152)
6Pgd2	---	---	1*	NS	---	NS	NS	NS	NS	NS		5(424)	2(118)
Pgi2	NS	NS	NS	NS	NS	NS	3***	NS	NS	1*	NS		3(320)
Pgm2	---	---	---	1*	NS	NS	---	---	---	NS	2***	NS	

[z]( )Total number of individuals analyzed in all progenies combined.
[y]---No progenies available jointly segregating for these two loci.
*,***,[NS]Number of progenies significant at P = 0.05 and 0.001 or nonsignificant at P = 0.05, respectively.

Table 3. Isozyme loci showing deviations from independent assortment in joint segregation analysis in *Vaccinium* section *Cyanococcus.*

Locus pair	Cross	Progeny with respective genotype[z] (no.)	G	Recombination value ± SE
Pgi-2/Lap-1	6	36,1,34,0/3,26,3,17	111.77***	n.d.
Lap-1/Pgi-2	10	31,29,4,0/2,4,28,27	120.08***	n.d.
Pgi-2/Lap-1	12	14,0,13,2/1,9,1,12	46.06***	n.d.
6Pgd-2/Pgm-2	3	11,0/6,12	16.42***	0.21 ± 0.07
6Pgd-2/Pgm-2	9	25,17,4/5,18,20	26.13***	0.17 ± 0.05
Adh-2/G3pdh-2	2	25,9/15,18	5.57*	0.36 ± 0.06
Ald-1/Pgm-2	2	18,17/15,35	3.97*	0.38 ± 0.05
Pgi-2/6-Pgd-1	6	19,15,19,6/8,9,9,13	8.31*	n.d.
6-Pdg-2/Adh-2	12	26,9/13,14	4.48*	0.35 ± 0.06

[z]A slash (/) and a comma (,) separate genotypic classes that differ at the first and second locus of the locus pair, respectively; on either side of the slash the sequence of genotypic classes is the same.
*,*** Significant at P = 0.05 and 0.001, respectively.

tent with a monomeric structure for this enzyme. A monomeric structure for LAP has been reported in other plant species (Arus et al., 1982; Manosand Fairbrothers, 1987; Suiter, 1988; Wendel and Parks, 1982). The banding positions for *Lap-l* in the populations analyzed indicated the existence of at least five alleles at Lap-1 (Fig. 1). In addition, the segregation patterns in progenies 6 and 14 revealed the presence of at least one null allele. Segregation ratios for *Lap-1* gave mendelian ratios except for cross 6 (Table 1).

Nonsegregating progenies. For all loci discussed, crosses between homozygous genotypes having alleles with identical Rf values exhibited true breeding behaviors: 100% of the progeny exhibited one band at the respective Rf value, Plants homozygous for different alleles yielded 100% heterozygotes for those two alleles.

Linkage analyses. Forty-seven pairs of loci were tested for independent assortment (Table 2). Departures from independent assortment (Table 3) revealed linkage for two pairs: *Lap-1* with *Pgi-*2 and Pgm-2 with *6-Pgd-2;* these were each confirmed in at least two progeny. A few other cases with significant departures ($P \leq$ 0.05) from independent assortment occurred: *Adh-2* with *G-3-pdh-*2 in cross 2, *Ald* with *Pgm-2* in cross 1, *Pgi-2* with *6-Pgd-l* in cross 6, and *6-Pgd-2* with *Adh-2 in* cross 12. In the first three cases, however, these deviations were not confirmed in the other progeny jointly segregating for these pairs of loci.

Discussion

The mendelian segregation patterns observed at the eight allozyme loci discussed above bring to 13 the number of allozyme loci shown to be inherited as single nuclear genes in blueberry. The observed number of aberrant segregations constitute 6% of the 65 segregation ratios tested, if segregations for *Idh, Mdh-3, 6-Pgd-2,* and *Pgi-2,* not shown in Table 1, are included. This is about the number of distorted segregations one would expect to observe due to chance as a Type I error considering the 0.05 level of significance chosen. Two crosses, 2 and 9, could account for most of the deviations from mendelian segregation (Table 1). Cross 9 is a cross between half-siblings and it is possible that the parents in cross 2 also share common ancestry. This situation may have contributed to segregation distortion through the unmasking of any deleterious recessive genes linked to allozyme loci.

The two linkage groups found, *Lap-1/Pgi-2* and *6-Pgd-2/Pgm-2,* appeared to be independent from one another as no significant

deviations from independent assortment between *6-Pgd-2* and *Pgi-2*, *Pgi-2* and Pgm-2, or *6-Pgd-2* and *Lap-1* were found. These two pairs of linked loci are the first to be reported in blueberry. Linkage between loci coding for the same pairs of enzyme systems has been found in a variety of plant species: *Gpi-c* and *Lap-1* in grape (Weeden et al., 1988) and *Pgi-1* and *Lap-2* in cotton (Suiter, 1988), *Pgd-p* and *Pgm-c* in pea (Weeden and Marx, 1987), *6-Pgd-1* and *Pgm-2* in tomato (Tanksley, 1985), *6-Pgd-2* and *Pgm-2* in *Camellia japonica* (Wendel and Parks, 1982), and *Pgd-p* and *Pgm-c* in lentil (Muehlbauer et al., 1989). Although these taxa are highly divergent, these could possibly represent regions of linkage conservation. First, however, the genes involved would need to be proven homologous. One method for analyzing relationships is to compare subcellular locations of isozymes. An initial effort to determine subcellular localization of the enzymes by using pollen leachates (Weeden and Gottlieb, 1980) was unsuccessful; pollen leachates did not exhibit activity for any of the enzymes. Also, the linkages reported here were determined in the *V. darrowi* and *V. elliottii* species only and should be investigated in other *Vaccinium* species as well.

The three deviations from independent assortment for *Adh-2/G-3-pdh-2*, *Ald/Pgm-2*, and *Pgi-2/6-Pgd-1* do not allow us to confirm linkage yet as they were not significant in two of the three progenies jointly segregating for these loci. The analysis of more progenies involving different parents should indicate whether these deviations represent simple random variation or weakly linked loci. As there was only one progeny jointly segregating for *6-Pgd-2/Adh-2*, the analysis of more progenies would be desirable to ascertain whether its deviation from independent assortment for these pairs of loci can be confirmed.

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