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Isozyme diversity in sour, sweet, and ground cherry

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Abstract Thirty-six sour (*Prunus cerasus* L.), sweet (*P. avium* L.), and ground cherry (*P. fruticosa* Pall.) selections were evaluated for seven enzyme systems and principal coordinate analysis was used to examine isozyme divergence among these cherry species. The enzyme systems studied were phosphoglucose isomerase (PGI), isocitrate dehydrogenase (IDH), phosphoglucomutase (PGM), 6-phosphogluconate dehydrogenase (6-PGD), leucine aminopeptidase (LAP), shikimate dehydrogenase (SKDH), and malate dehydrogenase (MDH). The first principal coordinate, which accounted for 41% of the total variation, separated the diploid sweet cherry selections from the sour, ground, and sour × ground cherry tetraploids. An additional 86 selections were evaluated for up to six of the enzyme systems to determine the polymorphisms at the enzyme loci and the level of heterozygosity between the diploid sweet cherry and the tetraploid species and interspecific hybrids. 6-PGD was the most polymorphic enzyme exhibiting 16 patterns. The tetraploid cherry species were more heterozygous than the diploid sweet cherry with an average heterozygosity of 78% compared to 19% for the diploids.

Key words Cherry · *Prunus* · Isozyme · Polyploid

Introduction

Sour cherry (*Prunus cerasus* L. $2n = 4x = 32$) is an allotetraploid based on shared morphological, isozyme, and chloroplast restriction fragment length polymorphism homology with its proposed progenitor species, sweet cherry (*P. avium* L., $2n = 2x = 16$) and ground cherry (*P. fruticosa* Pall., $2n = 4x = 32$) (Olden and Nybom 1968; Hancock and Iezzoni 1988; Hillig and Iezzoni 1988; Iezzoni et al. 1989; Iezzoni and Hancock 1994). Additionally, segregation at four isozyme loci in sour cherry fits disomic inheritance models and rejecting tetrasomic inheritance (Beaver and Iezzoni 1993). Disomic inheritance is characteristic of allopolyploids due to non-random pairing between chromosomes from the same ancestral genome (Stebbins 1977).

Sour cherry exists in maximum diversity in Europe. Its range, extending from the Mediterranean islands off Greece and Turkey to northern Russia (Hedrick 1915), spans that of its two proposed progenitor species, sweet and ground cherry (Kolesnikova 1975). Sweet cherry, the less cold-hardy cherry species, grows wild throughout the temperate regions of mainland Europe and into southern Russia; however, the greatest concentration is between and south of the Caspian and Black Seas. Ground cherry, the more cold-hardy cherry species, exists in greatest diversity in western Russia.

The expanded ecological range of many allopolyploids compared to their diploid progenitors is usually attributed to increased heterozygosity from divergent alleles on non-pairing homologous chromosomes from their progenitor species (Stebbins 1950; Hancock and Bringhurst 1981). This heterozygosity may be expressed as multiple enzymes with distinct properties which could theoretically expand the range of environments over which normal development could take place. Allopolyploid species might then have the potential to undergo substantial differentiation in response to differing selection pressures through occasional heterogenetic associations (Stebbins 1950).

Based on morphological and cold-hardiness differences, Russian investigators have divided sour cherry cultivars into two ecotypes: western European and middle-Russian (Kolesnikova 1975; Yuskev 1975, 1977). The western European group is less winter-hardy than the middle-Russian group and morphologically re-

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sembles sweet cherry, while the middle Russian group is more winter-hardy and morphologically resembles ground cherry. The morphological variation found in sour cherry is most likely due to continued gene flow between sour cherry and both sweet and ground cherry (Yushev 1975; Hillig and Iezzoni 1988). Spontaneous hybrids between sour cherry and its progenitor species do occur and cold temperature may be the most important selection force.

The Michigan State University (MSU) sour cherry germplasm collection, which includes material collected from throughout the habitat range of sour cherry, exhibits morphological diversity similar to that reported by the Russian researchers (Kolesnikova 1975; Yushev 1975, 1977). The diversity for fruit and plant type in the MSU sour cherry germplasm collection suggested that the individuals were highly variable genetically (Hillig and Iezzoni 1988; Krahl et al. 1991). Therefore an isozyme study was initiated to further characterize the variation present in the sour cherry collection along with a limited collection of sweet and ground cherry. Isozymes have commonly been used for detailed studies of the genetic structure of wild populations and landraces due to their co-dominant expression and the prevalence of polymorphisms. In sweet and sour cherry, isozyme loci have been reported for nine and eight enzyme systems, respectively (Kaurisch et al. 1988, 1991; Santi and Lemoine 1990 a, b; Tobutt and Nicoll 1992; Beaver and Iezzoni 1993; Granger et al. 1993).

Our objectives were to describe the isozyme diversity within and among sweet, sour, and ground cherry cultivars and their hybrids, and to compare the level of heterozygosity between the diploid sweet cherry and the tetraploid species and interspecific hybrids.

Materials and methods

Plant material

Sixty-seven sour, 26 sweet, six ground cherry cultivars and numbered selections, and seven sour × ground cherry and five sour × sweet cherry hybrids were evaluated for up to six isozyme systems. All plant materials are from the MSU collection growing at either the Clarksville Horticultural Experiment Station, Clarksville, Mich., or the Horticultural Research Center, East Lansing, Mich. The plant material in the MSU germplasm collection was collected, beginning in 1993, in Serbia, Bulgaria, Hungary, Poland, Germany, Sweden, and Russia.

Isozyme procedures

Starch-gel electrophoresis was performed on extracts from young leaves and dormant vegetative buds. The enzyme systems studied were phosphoglucose isomerase (PGI, E.C.5.3.1.9), isocitrate dehydrogenase (IDH, E.C.1.1.1.42), phosphoglucomutase (PGM, E.C.5.4.2.2), 6-phosphogluconate dehydrogenase (6-PGD, E.C.1.1.1.44), leucine aminopeptidase (LAP, E.C.3.4.11.1), shikimate dehydrogenase (SKDH, E.C.1.1.1.25), and malate dehydrogenase (MDH, E.C.1.1.1.37). Electrophoresis and staining procedures for the first five enzymes were the same as described in Beaver and Iezzoni (1993). SKDH and MDH were resolved on morphiline citrate 6.1 gels (Clayton and Tretiak 1972) and assayed according to Arulsekar and Parfitt (1986) and Vallejos (1983), respectively.

Isozyme alleles were named based on their mobilities relative to the most anodal band which was designated as the 100 allele. However, a few cherry genotype exhibited rare isozymes anodal to 100 for PGI (110 and 105) and SKDH (120). Loci of an enzyme system were numbered progressively beginning with 1 in the most anodal position. The sour cherry cultivars, 'Montmorency' or 'Meteor', were used as controls to aid in band identification as their bands had previously been diagnosed as allelic or heteromeric for *Pgm-2*, *6-Pgd-1*, *6-Pgd-2*, *Idh-2*, and *Lap-1* (Beaver and Iezzoni 1993). Both cultivars possess the 100 allozyme for all enzyme systems except SKDH.

Analytical procedures

Thirty-six selections were analyzed for all enzyme systems (Table 1). These 36 sour, sweet, and ground cherries were compared by principal coordinate (PCO) analysis (Gower 1966; Digby and Kempton 1987). A matrix of 36 genotypes × 44 isozyme bands was constructed with a '0' or a '1' entered for each isozyme band to indicate its absence or presence for every genotype analyzed. The isozyme bands utilized are defined in Fig. 1. Data were analyzed by calculating similarity matrices using the similarity statistic of Marczewski and Steinhaus: $S = w/(a + b - w)$, where w = number of bands of common mobility and a and b are the number of bands in individuals A and B, respectively (Angus et al. 1988). The similarity matrices were used in PCO analysis using a program written in SAS/IML (SAS Inst., Cary, N.C.). The data was then plotted in two dimensions.

Results

The cherry selections exhibited three and two patterns for *Pgm-2* and *Idh-2*, respectively, resulting from two previously confirmed alleles per locus (*Pgm-2*^{100} and *Pgm-2*^{75}; *Idh-2*^{100} and *Idh-2*^{64}) (Beaver and Iezzoni 1993) (Fig. 1). The patterns exhibited by *Pgi-2* and *Lap-1* were also due to two previously identified alleles per locus (*Pgi-2*^{100} and *Pgi-2*^{82}; *Lap-1*^{100} and *Lap-1*^{95}) plus one newly identified putative allele/locus. The novel *Pgi-2* band had a mobility of 110 with a heterodimeric band at 105. The newly identified variant for *Lap-1* had a mobility of 97.

Sixteen patterns encoded by nine alleles at two loci were identified for 6-PGD. *6-Pgd-1* exhibited three previously reported alleles (*6-Pgd-1*^{100}, *6-Pgd-1*^{88}, and *6-Pgd-1*^{76}) and heterodimeric bands with relative mobilities of 94, 82, and 88 (Beaver and Iezzoni 1993). At *6-Pgd-2*, two previously identified alleles (*6-Pgd-2*^{60} and *6-Pgd-2*^{48}) were found (Beaver and Iezzoni 1993) along with four newly identified putative alleles with relative mobilities of 82, 72, 38, and 28. The middle band of the five-banded patterns for both of the *6-Pgd* loci resulted from co-migration between a homodimer from one allele product and an intralocus heterodimer of two allele products. This is more diversity than previously reported by Kaurisch et al. (1988, 1991).

Thirteen patterns encoded by five putative alleles were found for SKDH. It is believed that these putative alleles are at a single SKDH locus since only one SKDH locus has been found in other *Prunus* species (Mowrey et al. 1990). Santi and Lemoine (1990a) concluded that SKDH in sweet cherry is determined by alleles at one

Table 1 Clones of sweet cherry (a), sour cherry (c), ground cherry (f) and interspecific hybrids used in the principal coordinate analysis (Fig. 2)

Clone	Species	Number Code
Emperor Francis	a	1
Windsor	a	2
Hedelfingen	a	3
Angela	a	4
Germersdorf o.p. [III17(20)]	a	5
Italia o.p. [III17(19)]	a	6
Ulster	a	7
V690616	a	8
Bing	a	9
V9062	a	10
Stark Crimson	a	11
Kristin	a	12
Lapins	a	13
Van	a	14
V690618	a	15
Rainier	a	16
Spur Lambert	a	17
Merpet	a	18
Stella	a	19
V69061	a	20
Coronation	c	21
George Glass	c	22
Meteor	c	23
Montmorency	c	24
Wolynska × Oblacinska [II7(11)]	c	25
HY 60/29 [II32(26)]	c	26
Nefris	c	27
Nana × Mari Timpurii [I51(17)]	c × (c × a)	28
Montmorency × Angela [I1(44)]	c × a	29
Montmorency × Angela [I1(58)]	c × a	30
Pitic de Iasi o.p. [II18(2)]	c × f	31
IR 883-1	f	32
IR 587-1	f	33
Dwarfrich	f	34
IR323-2	f	35
I24(41)	f(o.p.)	36

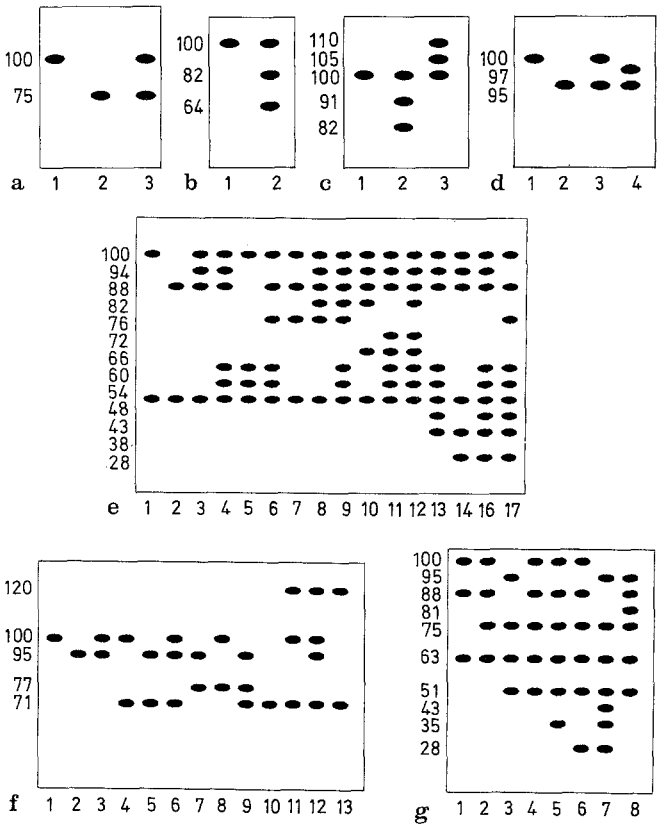

Fig. 1 Isozyme patterns for *Pgm-2* (**a**), *Idh-2* (**b**), *Pgi-2* (**c**), *Lap-1* (**d**), *6-Pgd-1* and *6-Pgd-2* (**e**) *Skdh-1* (**f**), *Mdh-1* and *Mdh-2* (**g**)

locus, *Skdh-1*; however, their results are not directly comparable to ours because their band migration resulted from isoelectric focusing.

Eight polymorphisms were discovered for MDH. The genetic basis of the banding patterns is not known; however, it is suspected that, like other *Prunus* species, there are at least two loci involved, one nuclear and one mitochondrial. In peach, *Mdh-1* and *Mdh-2* are the nuclear and mitochondrial loci, respectively (Mowrey et al. 1990). The mitochondrial form of MDH from sour cherry (Hancock and Iezzoni 1988) corresponds to our band 63. This band stained the most intense and is the only one present in all the genotypes studied. Therefore, the putative mitochondrial-encoded locus *Mdh-2* is monomorphic and the other isozyme bands may be accounted for by alleles at a putative *Mdh-1* locus. This agrees with the interpretation of Santi and Lemonie (1990a) who studied the inheritance of MDH in sweet cherry.

Sour cherry and the other tetraploid cherry species were more polymorphic than sweet cherry for all the isozymes studied. The most polymorphic isozyme was 6-PGD (Table 2). Sour cherry exhibited 12 patterns and possessed all the *6-Pgd-1* and *6-Pgd-2* alleles and putative alleles found in sweet and ground cherry, three alleles at *6-Pgd-1* and five alleles at *6-Pgd-2*). Pattern four was the most common for sour cherry representing 30% of the individuals tested. The sweet cherries tested were all homozygous for $6\text{-}Pgd\text{-}2^{48}$ and either homozygous or heterozygous for $6\text{-}Pgd\text{-}1^{100}$ and $6\text{-}Pgd\text{-}1^{88}$. All of the ground cherries possessed $6\text{-}Pgd\text{-}1^{76}$ in addition to $6\text{-}Pgd\text{-}1^{100}$ and/or $6\text{-}Pgd\text{-}1^{88}$. In addition to $6\text{-}Pgd\text{-}2^{48}$, ground cherry had $6\text{-}Pgd\text{-}2^{60}$ and the putative allele $6\text{-}Pgd\text{-}2^{28}$.

Sour cherry exhibited 11 of the 13 SKDH patterns identified, and possessed all five putative alleles (Table 2). The sweet cherry selections exhibited only two putative alleles while the ground cherry selections exhibited all the putative alleles except $Skdh\text{-}1^{77}$.

Sour cherry exhibited all of the eight MDH patterns (Table 2). All but one of the sweet cherry selections exhibited pattern one. The putative mitochondrial allele $Mdh\text{-}2^{63}$ was present in all the cherry selections.

All cherry species and species hybrids exhibited the two *Pgi-2* alleles, $Pgi\text{-}2^{82}$ and $Pgi\text{-}2^{100}$ (Table 2). Only one sour cherry selection, an open-pollinated seedling from Russia, exhibited the putative allele $Pgi\text{-}2^{110}$ along with $Pgi\text{-}2^{100}$ and the heteromeric band at 105.

All cherry species and species hybrids had both *Idh-2* alleles (Table 2). Individuals homozygous for $Idh\text{-}2^{64}$

Table 2 Number of sweet, sour, ground and hybrid cherry selections which possessed patterns for seven isozymes. The patterns are described in Fig. 1

Isozyme	Pattern	Species				
		P. avium	*P. cerasus*	*P. fruticosa*	*P. cerasus* × *P. avium*	*P. cerasus* × *P. fruticosa*
Pgm-2	1	26	–	–	–	–
	2	–	–	3	–	–
	3	–	10	3	3	1
Idh-2	1	18	23	1	2	6
	2	2	44	4	3	1
Pgi-2	1	10	39	2	2	6
	2	11	27	3	3	1
	3	–	1	–	–	–
Lap-1	1	21	–	–	1	–
	2	–	1	2	–	–
	3	–	46	2	4	6
	4	–	–	1	–	–
6-Pgd	1	11	–	–	–	–
	2	4	–	–	1	–
	3	11	9	–	–	1
	4	–	20	–	2	5
	5	–	–	–	1	1
	6	–	10	3	–	
	7	–	4	–		–
	8	–	6	–	1	–
	9	–	9	2	–	–
	10	–	2	–	–	–
	11	–	1	–		–
	12	–	2	–	–	–
	13	–	1	–	–	–
	14	–	1	–	–	–
	15	–	1	–	–	–
	16	–	–	1	–	–
Skdh-1	1	18	4	–	–	–
	2	–	1	–	1	–
	3	8	1	–	–	–
	4	–	19	2	–	1
	5	–	18	1	1	6
	6	–	16	–	3	–
	7	–	1	–	–	–
	8	–	2	–	–	–
	9	–	1		–	–
	10	–	–	2	–	–
	11	–	3	–		–
	12	–	1	–	–	–
	13	–	–	1	–	–
MDH	1	25	1	–	1	–
	2	1	3	–	1	1
	3	–	4	3	–	–
	4	–	43	2	3	5
	5	–	10	1		1
	6	–	2	–		–
	7	–	1	–	–	–
	8	–	2	–	–	–
	9	–	1	–	–	–

were not found. However, Kaurisch et al. (1991) identified sweet cherry selections homozygous for *Idh-2*^{64}. Granger et al. (1993) identified a third putative *Idh-2* allele with a slower mobility than *Idh-2*^{64} in 4 out of 77 *P. avium* cultivars.

For *Lap-1*, sweet cherry was monomorphic for *Lap-1*^{100} while the tetraploid species were primarily heterozygous (Table 2). The putative allele *Lap-1*^{97}, along with *Lap-1*^{95}, was only found in one ground cherry selection.

All sour cherries and species hybrids were heterozygous for the two *Pgm-2* alleles (Table 2). Sweet cherry was monomorphic for *Pgm-2*^{100} while ground cherry was either homozygous for *Pgm-2*^{75} or heterozygous for both alleles. Kaurisch et al. (1991) did find some sweet cherry selections that were heterozygous for *Pgm-2*, probably due to the diversity of their collection.

Results of the PCO analysis are presented in Fig. 2. Only the first two principal coordinates, accounting for 41% and 13% of the total isozyme variation, respectively, are shown; however, all eigenvalues were greater than, or equal to, zero. The distances between pairs of observations are only approximations, since the first two principal coordinates were not dominant (Digby and Kempton 1987). Additionally, the respective rela-

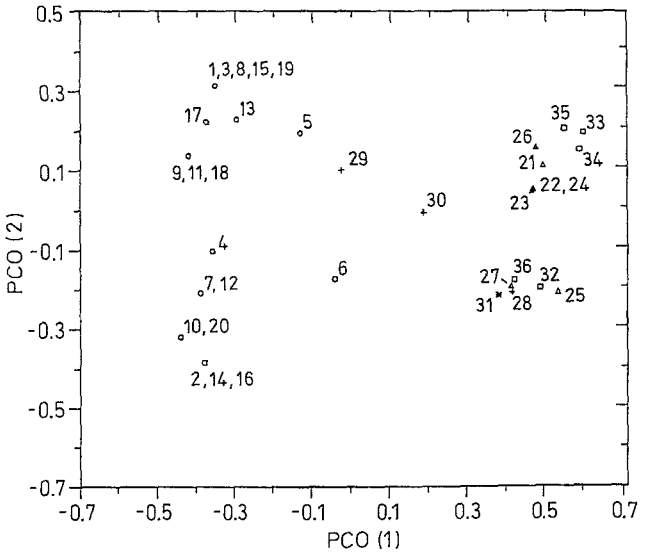

Fig. 2 PCO analysis of sweet, sour and ground cherry and their hybrids. (Legend: ○ = *P. avium*, △ = *P. cerasus*, □ = *P. fruticosa*, + = *P. cerasus* and *P. avium* in pedigree, * = *P. cerasus* and *P. fruticosa* in pedigree)

tionships between the species and species hybrids should be considered preliminary, since only a limited number of cherry selections were used in this analysis.

A two-dimensional plot of PCO scores separated the diploid sweet cherry selections from the sour cherry, ground cherry, and sour × ground cherry tetraploids along the first axis (Fig. 2). *Idh-2*^{64}, *Lap-1*^{95}, *Pgm-2*^{75}, *6-Pgd-1*^{76}, *6-Pgd-2*^{60}, putative allele *Skdh-1*^{71}, and MDH bands 75 and 51 were primarily responsible for the separation along PCO(1) (Fig. 2). These alleles and bands were generally present in the tetraploids with positive values for PCO(1) and generally absent in the sweet cherry diploids with negative values for PCO(1). Most other alleles and bands were present in diploids and tetraploids.

Sweet cherry genotypes 5 and 6, 'Germersdorf' o.p. and 'Italia' o.p., respectively, which exhibited both *Idh-2* alleles, *Idh-2*^{100} and *Idh-2*^{64}, were closer to the tetraploids on the first axis. 'Italia' o.p., unlike the other sweet cherries, also had MDH band 75.

The 'Montmorency' × 'Angela' (sour × sweet) tetraploids 29 and 30 have near zero values on PCO(1); they plot between the diploid sweet cherries and the tetraploids. They resembled the other tetraploids for *Pgm-2* and *Skdh-1*, and the sweet cherries for *Idh-2*. Genotype 29 resembled the sweet cherries for MDH and 6-PGD and the tetraploids for LAP, while genotype 30 showed the opposite condition.

The presence or absence of *Pgi-2*^{82} separated both the sweet cherry diploids and the tetraploid cherry species along PCO(2). Individuals with positive values of PCO(2) exhibited both *Pgi-2* alleles, *Pgi-2*^{100} and *Pgi-2*^{82}, while those with negative values for PCO(2) were homozygous for *Pgi-2*^{100}.

Table 3 Percent of diploid and tetraploid individuals which are heterozygous for each of seven enzyme loci

Locus	Ploidy			
	Diploid[a]	Tetraploid[b]		
		Total	Di-allelic	Tri-allelic
Pgm-2	0	85	85	0
Lap-1	0	94	94	0
6-Pgd-2	0	74	67	7
Idh-2	10	62	62	0
Skdh-1	31	90	62	28
6-Pgd-1	42	96	75	21
Pgi-2	52	42	42	0

[a] *Prunus avium*
[b] *P. cerasus*, *P. cerasus* × *P. avium*, *P. cerasus* × *P. fruticosa*, and *P. fruticosa*

The tetraploid cherry species were more heterozygous than diploid sweet cherry for six out of the seven enzyme loci studied (Table 3). The sweet cherry selections were homozygous and monomorphic for *Pgm-2*, *Lap-1*, and *6-Pgd-2* while only 10% were heterozygous for *Idh-2*. Slightly more sweet cherry selections were heterozygous for *Pgi-2* compared to the tetraploid selections. In the tetraploids, the average heterozygosity over the loci was 78% compared to 19% for the diploids. Most of the tetraploid heterozygotes were di-allelic, some were triallelic at *6-Pgd-1* and *Skdh-1*, and none were tetra-allelic.

Discussion

Sour cherry exhibited the most isozyme polymorphism having all the alleles or bands identified in its presumed progenitor species, sweet and ground cherry, except *Lap-1*^{97}. In addition, sour cherry had three unique bands for MDH: 28, 43, and 81 (presumably alleles at *Mdh-1*). It is expected that polyploids would contain the alleles of their ancestral species plus unique alleles not present in the progenitor species, particularly if the polyploid is not of recent origin.

Sweet cherry was monomorphic for three loci and lacked many of the tetraploid alleles for *Mdh-1*, *6-Pgd-1*, *6-Pgd-2* and *Skdh-1*. It is possible that the diversity exhibited by sour cherry may be due to a wider geographic sampling of the sour cherry collection as compared to the sweet cherry collection. For example, our 60 sour cherry selections evaluated showed more diversity than the 45 sour cherries evaluated by Kaurisch et al. (1991) for *6-Pgd-1*, *6-Pgd-2*, and *Lap-1*. However, in a study by Santi and Lemoine (1990b), who analyzed 286 sweet cherry selections and 33 sour or hybrid cherries for nine isozyme systems, all the sweet cherry bands were also found in the tetraploids. Unique bands in the tetraploids were only identified for LAP, SKDH, and acid phosphatase.

The origin of polyploid species is most easily understood when the progenitor species are fixed for distinct allelic variants. This is not the case between sweet and ground cherry. All the alleles and bands identified in sweet cherry were also present in ground cherry. Sour and ground cherry did not plot to distinct separate regions in the two-dimensional PCO analysis. Therefore, the diversity data suggests that sweet, sour, and ground cherry share a common gene pool and/or are continually sharing alleles through introgression. Sour and ground cherry are both tetraploids and they frequently hybridize in the wild. Additionally, sweet cherry is not reproductively isolated from the tetraploid species due to the occurrence of unreduced gametes (Iezzoni and Hancock 1984). However, the ploidy difference between sweet cherry and the tetraploids may limit the introgression in comparison to the ease of gene exchange among the tetraploids.

Tetraploid cherries exhibited more enzyme multiplicity compared to diploid cherries for six out of seven enzyme systems evaluated. Our study, like many others (Roose and Gottlieb 1976; Lack and Kay 1986; Soltis and Rieseberg 1986; Soltis and Soltis 1989), demonstrates that polyploids can maintain higher levels of heterozygosity than their diploid relatives. These and other studies suggest that enzyme multiplicity may provide polyploids with an adaptive advantage over diploid ancestors (Adams and Allard 1977).

Sour cherry maintains heterozygosity either by fixed heterozygosity (homozygous ancestral genomes) or by disomic inheritance (heterozygous ancestral genomes) (Beaver and Iezzoni 1993). The tri-allelic heterozygotes would have both heterozygous and homozygous genomes. More than four putative alleles were identified at two loci, *6-Pgd-2*, and *Skdh-1*. Yet, the tetra-allelic state, requiring two heterozygous genomes, was not found. Most cherry selections are self-compatible and historically have been grown in clonal groups. This could account for the level of inbreeding.

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