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ISOZYME INHERITANCE AND DIVERSITY IN CHERRY

By

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ABSTRACT

ISOZYME INHERITANCE AND DIVERSITY IN CHERRY

By

James Allen Beaver

Inheritance was studied at seven isozyme loci using seeds produced from crosses involving four sour cherries and one open-pollinated ground cherry. Three alleles at *6-Pgd-1* and two alleles at *Adh-1*, *Idh-2*, *Lap-1*, *Pgm-2*, *Pgi-2*, and *6-Pgd-2* accounted for the allozyme polymorphisms observed at these loci. *Idh-2*, *Pgm-2*, *6-Pgd-1*, and *6-Pgd-2* exhibited disomic inheritance confirming the allotetraploid hypothesis for sour cherry. Inheritance mode could not be determined at *Adh-1*, *Lap-1*, or *Pgi-2*. *Adh-1*, *Idh-2*, *Pgi-2*, *6-Pgd-1*, and *6-Pgd-2* were not linked. Linkage could not be determined for *Lap-1* or *Pgm-2*.

Isozyme diversity was evaluated for 67 sour, six ground, 26 sweet, and 12 interspecific hybrid cherries from the MSU germplasm collection. Tetraploid cherries exhibited 78% heterozygosity across seven enzyme loci compared to 19% for sweet cherry. Principal coordinate analysis based on isozyme diversity separated diploid sweet cherries from tetraploid cherries, but failed to separate sour and ground cherries.

To my wife Hannah
To my mother Joyce
To my little girl Nona Mae

For

Perseverance
Wisdom
Curiosity

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INTRODUCTION

Controversy exists as to whether sour cherry (*Prunus cerasus* L., 2n=4x=32) is an allotetraploid, autotetraploid, or a segmental allotetraploid. Conclusions concerning the type of polyploidy were largely based on cytogenetic and morphological criteria. Although morphological and cytological evidence is useful in understanding polyploidy, these criteria can not be conclusively related to the type of polyploidy. Both allo- and autotetraploids may exhibit regular bivalent pairing and a lack of multivalent formation (Krebs and Hancock, 1989; Soltis and Rieseberg, 1986). Allelic segregation, specifically the determination of disomic or tetrasomic inheritance, is the most definitive way to distinguish polyploid type (Krebs and Hancock, 1989; Soltis and Rieseberg, 1986).

Because of their codominant expression, isozyme loci have been commonly used as genetic markers to study inheritance in polyploids. However, allozymes have not been used to determine polyploid type in sour cherry. Isozyme studies in sour cherry to date have been limited to zymograms, patterns, or descriptions of putative alleles from different cultivars (Fernqvist and Huntrieser, 1988; Hancock and Iezzoni, 1988; Kaurisch et al., 1988, 1991).

These studies did not conduct progeny tests. Based on phenotypic segregation of allozyme patterns, genetic models can be formulated consisting of true alleles and inheritance type in tetraploid sour cherry.

An understanding of sour cherry inheritance would clearly determine sour cherry's evolutionary origin and aid in predicting the likelyhood of desired genotypes from crosses. Genotypic frequencies of progeny differ with inheritance type.

The objectives of my research were to identify alleles and loci of marker enzyme systems, and to use the segregation patterns of these loci to determine polyploid type in sour cherry.

LITERATURE REVIEW

The most widespread hypothesis for the origin of sour cherry is that it arose from hybridization between the diploid sweet cherry (*P. avium* L., 2n=2x=16) and the tetraploid ground cherry (*P. fruticosa* Pall., 2n=4x=32). Ground cherry is a spreading shrub, reaching a height of about one meter, which has small leaves 20-50 mm long, small white flowers, and small red-purple fruit (Bailey and Bailey, 1976; Hillig and Iezzoni, 1988; Olden and Nybom, 1968). Sweet cherry is a tree growing 18 to 24 m tall with leaves 60-150 mm long, white flowers larger than those of ground cherry, and fruit ranging from small in wild types to approximately 25 mm in diameter in some cultivars (Bailey and Bailey, 1976; Hillig and Iezzoni, 1988; Olden and Nybom, 1968). Morphological data for sour cherry, ranging from that of sweet cherry to ground cherry, suggests that sour cherry is an interspecific hybrid between these two species (Hillig and Iezzoni, 1988).

Ground cherry, considered the most cold hardy of the cherry species, originated and exists in maximum diversity in the former Soviet Union reaching as far north as the 60th parallel (Kolesnikova, 1975). In contrast, sweet cherry, less cold hardy than ground cherry, is found in greatest concentration between and south of the Caspian and Black

Seas, but is also found wild thoughout Europe and into southern Russia (Hedrick, 1915). The center of diversity for sour cherry is eastern Europe and Russia, where the habitat of sour cherry overlaps with that of sweet and ground cherry on the southwest and northeast, respectively.

Morphology and chemotaxonomy suggest that sour cherry is a polyploid hybrid of sweet and ground cherry. Olden and Nybom (1968) hybridized ground cherry with several varieties of sweet cherry in an attempt to resynthesize sour cherry. Morphological characters (flowers, leaves, fruit, and tree morphology), biochemical characters (fruit anthocyanins and leaf phenolics), and disease responses were examined in the progeny and compared to similar data collected from the parents and sour cherry. Data collected from the hybrids were intermediate to the parental data and strikingly similar to the sour cherry data.

Hillig and Iezzoni (1988) studied morphological traits of sour cherry with principal component analysis to examine variation in sixteen cultivars. Data were collected on leaf, flower, and fruit characters and assembled into multivariate observations. These observations were then plotted to create a three-dimensional scatter diagram whose three axes each correspond to a different principal component of character variance. The scatter diagram of the sixteen sour cherry cultivars presented a gradation of morphology between the two proposed progenitors, sweet and ground cherry (Hillig and Iezzoni, 1988).

Additional studies support the hypothesis that sour

cherry is derived from sexual polyploidization between ground cherry and an unreduced gamete from sweet cherry. Malate dehydrogenase bands from sweet and ground cherry were expressed codominantly in the sour cherry cultivars tested (Hancock and Iezzoni, 1988). Chloroplast RFLP's tested to date indicate that those from ground cherry are similar to the sour cherry cultivar 'Montmorency' and different from sweet cherry (Iezzoni et al., 1989). Unreduced pollen is produced in small quantities by numerous sweet cherry cultivars (Iezzoni and Hancock, 1984).

Other evidence might support an autotetraploid or segmental allotetraploid origin of sour cherry. Several landraces of sour cherry are self-incompatible; autopolyploids would favor such a breeding system. Additionally, meiotic analysis of sour cherry reveals a high percentage of infertility due to unbalanced gametes. Even quadrivalent formation occurs at a low frequency (Galletta, 1959; Hruby, 1939).

The hypothesis that sour cherry is an autotetraploid is not inconsistent with morphological and isozyme data. Gene flow between sour cherry and sweet and ground cherry may be a significant evolutionary factor regardless of the polyploid origin of sour cherry. Interspecific hybrids between sour cherry and ground cherry have been reported in regions where these species coexist. Additionally, if the three cherry species in the Eucerasus section (sweet, ground, and sour cherry) arose from a common diploid ancestor as suggested by Raptopoulos (1941), they would be

expected to share morphological and isozyme homology. An autopolyploid origin of sour cherry could be hypothesized if sour cherry exhibited tetrasomic inheritance and if tri- and/or tetra-allelic loci were identified.

Inheritance

Genetic data is essential in order to distinguish allo- from autopolyploidy. Allopolyploids exhibit disomic inheritance which may result in fixed heterozygosity, while autotetraploids exhibit tetrasomic inheritance. These different segregation ratios are dependent upon the chromosomal pairing relationships defined by their genomic origin.

Allotetraploids arise from interspecific hybridization of two diploid genetic complements, one from each of two divergent progenitors. Homoeologous chromosomes can structurally differ to some degree. During meiosis I, homologous chromosomes preferentially pair and segregate independently of their homoeologous counterparts. However, homoeologous chromosome pairings or heterogenetic associations may occur infrequently (Stebbins, 1947). Allotetraploids exhibit disomic inheritance, a two locus model for duplicate gene segregation. True allotetraploids rarely exhibit tetrasomic ratios and multivalent associations (Stebbins, 1947); they possess disomic inheritance and often exhibit fixed heterozygosity (Krebs and Hancock, 1989; Soltis and Rieseberg, 1986).

Fixed heterozygosity is one possible result of disomic

inheritance. Roose and Gottlieb (1976) define fixed heterozygosity as heterozygous phenotypes which do not segregate at meiosis. This occurs when each of the two ancestral genomes in an allotetraploid is homozygous for a different allele. For example, *aabb* produces only *ab* gametes and is therefore fixed heterozygous, while *abab* produces *aa*, *ab*, and *bb* gametes in a 1:2:1 ratio and is not fixed heterozygous.

However, a more inclusive definition for fixed heterozygosity is that only heterozygous gametes are formed at meiosis which may or may not be all the same genotype. This would include the situation where one ancestral genome in an allotetraploid is heterozygous and the other is homozygous for a different allele resulting in a tri-allelic genotype. For example, *aabc* produces only the heterozygous *ab* and *ac* gametes and is therefore also considered fixed heterozygous, while *abac* produces one-quarter homozygous *aa* gametes and is not fixed heterozygous.

Autotetraploids inherit two diploid genetic complements from a common progenitor or closely related progenitors. Their chromosomes are structurally similar; therefore, the four homologous chromosomes randomly pair during meiosis. A gene on each of four homologues segregates randomly at a single locus (Krebs and Hancock, 1989). Autotetraploids, and to a lesser extent segmental allotetraploids, exhibit tetrasomic inheritance and usually multivalent associations (Stebbins, 1947).

Criteria for diagnosing segmental allotetraploids fall

in between that for true allotetraploids and autotetraploids because the two ancestral genomes involved are less divergent than in true allotetraploids. Chromosome pairing is more random due to similarities between homoeologous chromosomes. This results in frequencies of homoeologous pairing and multivalent formation that are higher than expected in true allotetraploids and lower than expected in autotetraploids. The two ancestral genomes of a segmental allotetraploid lose their identities as a result of partial or complete homoeologous chromosome pairing and recombination of this homoeologous genetic material (Stebbins, 1947).

To use allozyme polymorphisms in an inheritance study, the unit structure of marker enzymes must be understood. Generally, this unit structure is conserved throughout different species. Studies involving *Prunus* species report leucine amino peptidase (LAP) (Byrne and Littleton, 1988; Hauagge, Kester, Arulsekar, Parfitt, and Liu, 1987), and phosphoglucomutase (PGM) (Byrne and Littleton, 1988, 1989a, 1989b; Chaparro et al., 1987; Hauagge, Kester, Arulsekar, Parfitt, and Liu, 1987; Hauagge, Kester, and Asay, 1987) to be monomeric. Other enzymes are reported to be dimeric: alcohol dehydrogenase (ADH) (Kaurisch et al., 1991), isocitrate dehydrogenase (IDH) (Kaurisch et al., 1991; Mowrey et al., 1990a), phosphoglucose isomerase or glucose phosphate isomerase (PGI, same as GPI) (Byrne and Littleton, 1988; Hauagge, Kester, Arulsekar, Parfitt, and Liu, 1987; Hauagge, Kester,

and Asay, 1987; Parfitt et al., 1985), and 6-phosphogluconate dehydrogenase (6-PGD) (Byrne, 1989a, 1989b; Byrne and Littleton, 1989b; Chaparro et al., 1987; Mowrey et al., 1990b).

Inheritance studies involving segregating progenies diagnose the number of loci that encode isozymes and the number of alleles per locus that encode allozymes of a marker enzyme. By observing segregation, the number of zones of activity can be determined, and the bands at each zone or locus can be designated as allelic or heteromeric. Genotypes of cultivars and other clones can thus be determined for marker enzyme systems.

Allozymes are a useful tool to study tetraploid inheritance because the alleles that encode them are codominantly expressed and they can be used as genetic markers to distinguish between disomic and tetrasomic inheritance. Inheritance type has not been diagnosed in sour cherry using the allozyme phenotypes of segregating alleles at marker loci.

METHODS

Plant Material

Seeds for this study were obtained from crosses using four sour cherry parents and one open-pollinated ground cherry parent (Table 1). Standard self- and cross-pollination techniques for cherry were used to produce the seeds (Fogle, 1975).

Horizontal starch gel electrophoresis was performed on extracts from young leaves and dormant vegetative buds of parent trees. Isozyme data on progeny were obtained from individual seeds prior to germination. Leaves, buds, and seeds were stored at approximately 2 C with moist paper towels in sealed plastic bags to prevent desiccation until they were used. Seeds were removed from their exocarps and mesocarps and treated with a fungicide suspension prior to storage. Endocarps were removed just prior to extraction. All material was macerated on ice the day of electrophoresis using the procedures of Krebs and Hancock (1989) with slight modification. The extraction buffer was maintained at pH 7.5 rather than adjusted to pH 8.0 and nylon screens were not used during extraction.

Table 1. Origin of cultivars and selections used in the inheritance study.

Cultivar/Selection	Origin
Montmorency	local selection from France
Meteor	Montmorency x Vladimir
I 24(41)	*P. fruticosa* open-pollinated
II 13(36)	Cigany Meggy open-pollinated
II 15(4)	Montmorency x M63 (Pandy x Nagy Gobet)

Isozyme Procedures

Phosphoglucose isomerase (PGI, E.C.5.3.1.9), alcohol dehydrogenase (ADH, E.C.1.1.1.1), isocitrate dehydrogenase (IDH, E.C.1.1.1.42), phosphoglucomutase (PGM, E.C.5.4.2.2), and 6-phosphogluconate dehydrogenase (6-PGD, E.C.1.1.1.44) were resolved by six hours of electrical current on morpholine-citrate pH 6.1 gels (Clayton and Tretiak, 1972). Electrical current was maintained at 50 mA during elution, the first 30 minutes of electrophoresis, and as close as possible to 65 mA without exceeding 300 V for the rest of electrophoresis. Leucine aminopeptidase (LAP, E.C.3.4.11.1) was resolved by five hours of current (50 mA, not exceeding 300 V) on tris-citrate/lithium-borate pH 8.3 gels (Scandalios, 1969). Gels consisted of 12% hydrolyzed potato starch.

Stain recipes were prepared at one-half (50 ml) the volume reported per gel slice. IDH was assayed as described by Soltis et al. (1983). All other enzymes were assayed with slight modification as reported by Arulsekar and Parfitt (1986). The LAP substrate, leucyl-naphthyl amide HCl, was dissolved in 2.5 ml of N,N-dimethyl formamide per gel slice before adding it to the stain solution. Tris-HCl buffer pH 8.5 rather than pH 8.0 was used in the 6-PGD assay.

Relative mobilities were calculated for isozymes using the ratio of the particular isozyme's migration distance in mm from the origin to that of the most anodal isozyme of the enzyme system (Mowrey and Werner, 1990). Each isozyme was

named by multiplying its relative mobility by 100. The most anodal isozyme of each enzyme system was referred to as *100*, while others of the same system were named as a fraction of this number. Loci of an enzyme system were numbered progressively beginning with *1* in the most anodal position. Letters representing alleles in Tables 2, 3, and 4 are used for convenience only and are not the assigned allelic names.

Statistical Procedures

The chi-square goodness-of-fit test was used to compare observed progeny phenotypes to expected classes (Table 2) for each inheritance mode. Phenotype rather than genotype was scored since it does not require a subjective determination of gene dosages based on visual assessment of differential band staining intensity among heterozygotes. For those loci which did not fit the expected 3:1 ratio, a 2:1 ratio was tested. The Inheritance Computer Program (Appendix C) written by me was used to aid in proposing genetic models and in calculating chi-square values. Linkage was studied using the chi-square test of independence.

Table 2. Expected gamete and progeny class frequencies for disomic and tetrasomic inheritance.

Cross type	Parental genotype	Expected gamete ratios		Expected phenotypic ratios	
		Disomic	Tetrasomic	Disomic	Tetrasomic
1	*aaab* x *aaab*	(1*aa*:1*ab*)x(1*aa*:1*ab*)	(1*aa*:1*ab*)x(1*aa*:1*ab*)	3:1	3:1
2	*aaab* x *aaaa*	(1*aa*:1*ab*)x(*aa*)	(1*aa*:1*ab*)x(*aa*)	1:1	1:1
3	*abbb* x *abbb*	(1*ab*:1*bb*)x(1*ab*:1*bb*)	(1*ab*:1*bb*)x(1*ab*:1*bb*)	3:1	3:1
4	*abab* x *aabb*	(1*aa*:2*ab*:1*bb*)x(*ab*)	(1*aa*:4*ab*:1*bb*)x(1*aa*:4*ab*:1*bb*)	0:1:0	1:34:1
5	*abbb* x *abab*	(1*ab*:1*bb*)x(1*aa*:2*ab*:1*bb*)	(1*aa*:1*ab*)x(1*aa*:4*ab*:1*bb*)	7:1	11:1
6	*aaaa* x *aabb*	(*aa*)x(*ab*)	(*aa*)x(1*aa*:4*ab*:1*bb*)	0:1	1:5
7	*aabb* x *aabb*	(*ab*)x(*ab*)	(1*aa*:4*ab*:1*bb*)x(1*aa*:4*ab*:1*bb*)	0:1:0	1:34:1
8	*abab* x *abab*	(1*aa*:2*ab*:1*bb*)x(1*aa*:2*ab*:1*bb*)	(1*aa*:4*ab*:1*bb*)x(1*aa*:4*ab*:1*bb*)	1:14:1	1:34:1
9	*abbc* x *abbc*	(1*ab*:1*ac*:1*bb*:1*bc*)x(1*ab*:1*ac*:1*bb*:1*bc*)	(2*ab*:1*ac*:1*bb*:2*bc*)x(2*ab*:1*ac*:1*bb*:2*bc*)	1:3:8:3:1	1:8:18:8:1
10	*abbc* x *abab*	(1*ab*:1*ac*:1*bb*:1*bc*)x(1*aa*:2*ab*:1*bb*)	(2*ab*:1*ac*:1*bb*:2*bc*)x(1*aa*:4*ab*:1*bb*)	1:7:6:1:1	1:17:15:2:1
11	*abbc* x *aabc*	(1*ab*:1*ac*:1*bb*:1*bc*)x(*ab*:*ac*)	(2*ab*:1*ac*:1*bb*:2*bc*)x(1*aa*:2*ab*:2*ac*:1*bc*)	1:2:5:0	1:3:7:1
12	*cddd* x *cddd*	(1*cd*:1*dd*)x(1*cd*:1*dd*)	(1*cd*:1*dd*)x(1*cd*:1*dd*)	3:1	3:1
13	*ccdd* x *ccdd*	(*cd*)x(*cd*)	(1*cc*:4*cd*:1*dd*)x(1*cc*:4*cd*:1*dd*)	0:1:0	1:34:1
14	*cddd* x *ccdd*	(1*cd*:1*dd*)x(*cd*)	(1*cd*:1*dd*)x(1*cc*:4*cd*:1*dd*)	1:0	11:1
15	*ccdd* x *cddd*	(*cd*)x(1*cd*:1*dd*)	(1*cc*:4*cd*:1*dd*)x(1*cd*:1*dd*)	1:0	11:1
16	*ccdd* x *dddd*	(*cd*)x(*dd*)	(1*cc*:4*cd*:1*dd*)x(*dd*)	1:0	5:1

NOTE: Letters represent alleles defined by their relative mobilities:

Pgi-2: *a* = 100, *b* = 82
Lap-1: *a* = 100, *b* = 95
Adh-1: *a* = 100, *b* = 56
Idh-2: *a* = 100, *b* = 64
Pgm-2: *a* = 100, *b* = 75
6-Pgd-1: *a* = 100, *b* = 88, *c* = 76
6-Pgd-2: *c* = 60, *d* = 48

RESULTS

Seven loci resolved well and exhibited good activity using horizontal starch gel electrophoresis: *Pgi-2*, *Lap-1*, *Adh-1*, *Idh-2*, *Pgm-2*, *6-Pgd-1*, and *6-Pgd-2* (Figure 1). Activity slightly anodal to *Idh-2* and *Pgm-2* was observed but was not studied due to inconsistent resolution. Kaurisch et al. (1988) designated these anodal bands *Idh-1* and *Pgm-1*.

Two of the crosses for *Pgi-2* (Table 3) segregated in a 1:1 phenotypic ratio and 'Meteor' selfed fit a 3:1 ratio, supporting the existence of two alleles. 'Montmorency' selfed fit a 2:1 segregation ratio for *Pgi-2*, rather than the expected 3:1. Two *Lap-1* phenotypes (Table 3) segregated in expected 3:1 ratios indicating the presence of two alleles.

Segregation for two alleles at the *Adh-1* locus (Table 3) fit expected 3:1 ratios for 'Montmorency' selfed and 'Meteor' selfed. Insufficient progeny were available to reject either mode of inheritance for the crosses 'Meteor' x I 24(41) and I 24(41) x II 13(36) (Table 4). However, progeny were all heterozygous for the *100* and *56* alleles at *Adh-1* for I 24(41) x II 13(36) because II 13(36) exhibited fixed heterozygosity at this locus.

Figure 1. Allozyme phenotypes of sour cherry demonstrate di-allelic segregation at (A) *Pgi-2*, (B) *Lap-1*, (C) *Adh-1*, and (D) *Idh-2*; di-allelic fixed heterozygosity at (E) *Pgm-2* and (F) *6-Pgd-2* (alleles *60* and *48*); and tri-allelic segregation at (F) *6-Pgd-1*. PGI, ADH, IDH, and 6-PGD are dimeric enzymes producing intralocus heterodimers for heterozygous genotypes. LAP and PGM are monomeric.

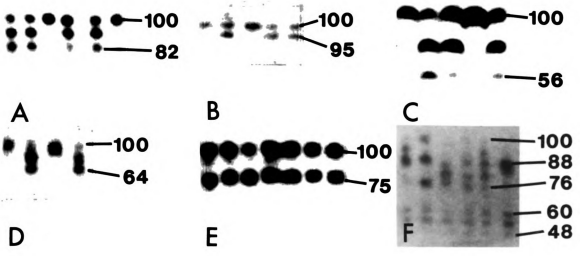
100
82
A
100
95
B
100
56
C
100
64
D
100
75
E
100
88
76
60
48
F

Table 3. Segregation and chi-square values at five polymorphic loci in sour cherry where the expected phenotypic ratios for disomic and tetrasomic inheritance are the same.

Locus	Cross	Cross Type[1]	Observed Progeny Phenotypes	Ratios Tested[2] (X^{2y})	
			ab a		
Pgi-2	Montmorency selfed	1	162:76	3:1 (6.10*)	2:1(0.20)
	Meteor selfed	1	181:73	3:1 (1.90)	
	Meteor x I 24(41)	2	75:85	1:1 (0.63)	
	Meteor x II 15(4)	2	86:62	1:1 (3.89*)	
			ab a		
Lap-1	Montmorency selfed	1	83:24	3:1 (0.38)	
	Meteor selfed	1	26: 6	3:1 (0.67)	
			a *ab* b		
Adh-1	Montmorency selfed	1	42:165	1:3 (2.45)	
	Meteor selfed	3	133:43	3:1 (0.03)	
			a *ab*		
Idh-2	Montmorency selfed	1	55:152	1:3 (0.27)	
	Meteor selfed	1	79:175	1:3 (5.04*)	2:1 (0.55)
	Meteor x I 24(41)	2	73: 84	1:1 (0.77)	
	Meteor x II 15(4)	1	43:105	1:3 (1.30)	
			cd d		
6-Pgd-2	Montmorency selfed	12	153:85	3:1 (14.57*)	2:1 (0.62)

[1]Cross types and expected phenotypic ratios are defined in Table 2.

[2]Yate's Correction for Continuity was used for chi-square tests with 1 degree of freedom.

$$\chi^2 = \sum \frac{(|obs - exp| - 0.5)^2}{exp}$$

[3]* Significance at 5% for deviation from expected ratio.

Table 4. Segregation and chi-square values at five polymorphic loci in sour cherry where the expected phenotypic ratios for disomic and tetrasomic inheritance differ.

Locus	Cross	Cross Type[z]	Observed Progeny Phenotypes	X^{2y} Disomic[z]	X^{2y} Tetrasomic[z]
			a ab b		
Adh-1	I 24(41) x II 13(36)"	4	0: 75: 0	0	4.41
	Meteor x I 24(41)	5	145:15	1.43	0.23
			a ab		
Idh-2	I 24(41) x II 13(36)"	6	2:73	--	10.58*x
			a ab b		
Pgm-2	Montmorency" selfed	7	0:122:0	0	7.18*
	Meteor" selfed	7	0:128:0	0	7.53*
			a ac ab abc bc b		
6-Pgd-1	Montmorency selfed	8	13: 206: 19	1.40	30.96*
	Meteor selfed	9	10: 48:137:40:18	4.48	25.14*
	I 24(41) x II 13(36)	10	6: 29: 32: 6: 2	3.25	9.35
	Meteor x I 24(41)	9	11: 26: 76:33:12	1.53	25.76*
	Meteor x II 15(4)"	11	22: 32: 79: 0	1.99	21.91*
			c cd d		
6-Pgd-2	Meteor" selfed	13	0:254: 0	0	14.94*
	I 24(41) x II 13(36)"	14	75: 0	0	6.79*
	Meteor" x I 24(41)	15	156: 4	--	7.13*
	Meteor" x II 15(4)	16	137:11	--	9.09*

[z]Cross types and expected phenotypic ratios for disomic and tetrasomic inheritance are defined in Table 2.

[y]Yate's Correction for Continuity was used for chi-square tests with 1 degree of freedom.

$$\chi^2 = \sum \frac{(|obs - exp| - 0.5)^2}{exp}$$

[x]* Significance at 5% for deviation from expected ratio

"Exhibits a fixed heterozygous genotype

Three of the *Idh-2* crosses (Table 3) fit the proposed 3:1 or 1:1 models. 'Meteor' selfed fit a 2:1 alternate ratio. Tetrasomic inheritance was rejected at *Idh-2* (Table 4) for the cross I 24(41) x II 13(36). Goodness-of-fit could not be tested for disomic inheritance due to the observation of two unexpected homozygotes resulting in an undefined chi-square equation. The two homozygotes may be the result of pollen contamination or heterogenetic associations in II 13(36). Without these two homozygotes, the data fit the proposed model for fixed heterozygosity in II 13(36).

All *Pgm-2* and *6-Pgd-1* crosses (Table 4) fit the proposed models for disomic inheritance involving two and three alleles per locus, respectively. 'Montmorency' and 'Meteor' exhibited fixed heterozygosity at *Pgm-2*. Tetrasomic inheritance was rejected for all crosses except I 24(41) x II 13(36) at *6-Pgd-1*. Insufficient progeny were obtained to reject either mode of inheritance for this cross.

None of the crosses tested segregated for *Pgm-2*. However, all of the 26 sweet cherry cultivars studied had only the $Pgm\text{-}2^{100}$ allele and three of four ground cherry clones studied had only the $Pgm\text{-}2^{75}$ allele (Chapter II). Therefore, the two bands of *Pgm-2* in 'Montmorency' and 'Meteor' were considered allelic with both cultivars exhibiting fixed heterozygosity.

'Montmorency' selfed data for *6-Pgd-2* (Table 3) fit a 2:1 instead of the 3:1 ratio expected with both disomic and

tetrasomic inheritance. The *6-Pgd-2* locus (Table 4) in 'Meteor' selfed and in I 24(41) x II 13(36) exhibited fixed heterozygosity due to disomic inheritance. The final two crosses involving 'Meteor' were again potentially diagnostic of disomic inheritance if the homozygotes are considered to be the result of pairing among homoeologous chromosomes. Tetrasomic inheritance was rejected for all *6-Pgd-2* crosses.

PGI, ADH, IDH, and 6-PGD are dimeric in cherry as indicated by the presence of heteromeric bands (Figure 1). LAP and PGM did not exhibit heteromers and are thus monomers in cherry.

Linkage between loci was tested using five segregating loci in selfed 'Montmorency' progeny. *Lap-1* and *Pgm-2* were not tested for linkage because they were assayed from different seeds than the other loci and because *Pgm-2* was fixed heterozygous in 'Montmorency' and 'Meteor.' None of the loci examined were linked, indicating that they may be located on different chromosomes or chromosome arms in the sour cherry genome.

DISCUSSION

Polymorphisms and putative alleles presented by Kaurisch et al. (1991) for *6-Pgd-2*, *Idh-2*, *Pgm-2*, *Adh-1*, and *Lap-1* for 'Montmorency' and 'Meteor' are similar to my results. However, in my analysis, 'Montmorency' and 'Meteor' have three bands corresponding to two alleles for *Pgi-2* rather than one allele as proposed by Kaurisch et al. (1991). Their *Pgi-2* band appears to correspond to my $Pgi\text{-}2^{100}$.

In my analysis, 'Meteor' has five bands for *6-Pgd-1* representing three alleles and three intralocus heterodimers. Kaurisch et al. (1991) presented only three bands which most likely correspond to my $6\text{-}Pgd\text{-}1^{100}$ and 1^{88} alleles and the heterodimer at 1^{94}. Fernqvist and Huntrieser (1988) only presented two bands for 'Meteor' at the putative *6-Pgd-1* locus. These discrepancies could either be caused by variation due to different buffer systems or differences between the clones used. However, unlike the previous studies, progeny segregation was used in my study to diagnose true alleles and reliably determine the allele dosage of the parents at all the loci presented. Segregation data for 'Meteor' at the *Pgi-2* and *6-Pgd-1* loci were consistent with two and three segregating alleles, respectively.

The skewed 2:1 ratios for *Pgi-2*, *Idh-2*, and *6-Pgd-2* may have resulted from gametophytic selection. These ratios only occurred when the progeny were produced by selfing 'Montmorency' and 'Meteor.' 'Montmorency' and 'Meteor' have been shown to be partially self-incompatible (Lansari and Iezzoni, 1990) which is defined as a majority of the pollen grains stopping tube growth prematurely in the style, resembling gametophytic incompatibility. This partial self-incompatibility may have caused the progeny class frequencies to deviate from the expected models. Additionally, the 2:1 ratios could be caused by zygotic lethality due to inbreeding depression.

Enzyme unit structure in cherry is monomeric for LAP and PGM and dimeric for ADH, IDH, PGI, and 6-PGD. This confirms the results of other *Prunus* studies and demonstrates that enzyme unit structure is conserved in the genus *Prunus*.

Segregation data confirmed the existence of two alleles at the *Pgi-2*, *Lap-1*, *Adh-1*, *Idh-2*, *Pgm-2*, and *6-Pgd-2* loci and three alleles at the *6-Pgd-1* locus. Linkage to *Lap-1* and *Pgm-2* was not testable, while the other loci were found to independently assort from one another. Disomic inheritance has been clearly shown to occur at *Idh-2*, *Pgm-2*, *6-Pgd-1*, and *6-Pgd-2*, consistent with an allotetraploid origin of sour cherry.

The possibility remains that sour cherry could be a segmental allotetraploid exhibiting occassional homoeologous chromosome pairing. Infrequent homoeologous pairing could

account for the low level of homozygous offspring for *Idh-2* from the cross I 24(41) x II 13(36) and for *6-Pgd-2* from the crosses Meteor x I 24(41) and Meteor x II 15(4). However, true allotetraploids can also exhibit heterogenetic associations (Stebbins, 1947).

A maximum of four nonhomologous chromosomes in sour cherry (x=8) have been clearly demonstrated to undergo disomic inheritance during meiosis. The molecular data presented herein support the previously published morphological, chemotaxonomical, and geographical evidence that sour cherry is an allotetraploid.

Chapter II. Comparative Isozyme Diversity in *Prunus cerasus*, *P. avium*, and *P. fruticosa*

INTRODUCTION

Sour cherry is an allotetraploid based on allozyme inheritance data from Chapter I, with sweet and ground cherry as its proposed progenitor species based on shared isozyme, chloroplast RFLP, and morphological homology between the three cherry species.

Unlike self-pollinating allopolyploids such as wheat, the two ancestral genomes in sour cherry are heterozygous at many loci. In Chapter I, some genotypes clearly exhibited fixed heterozygosity characterized by homozygosity within the ancestral genomes and others exhibited assortment in agreement with disomic inheritance due to heterozygous genomes at four isozyme loci.

The mating behavior in cherry, regulated by a gametophytic self-incompatibility system in sweet cherry and commonly a self-incompatibility system in sour cherry, suggests that sour cherry would exhibit a high level of heterozygosity due to outcrossing. Therefore, sour cherry could potentially have four different alleles at a locus.

To assess isozyme diversity in sour cherry, it is necessary to screen a very diverse collection. The MSU sour cherry germplasm collection includes material collected in Yugoslavia, Bulgaria, Hungary, Romania, Poland, and portions

of the former Soviet Union.

The objectives were twofold. Can sour cherry individuals be identified which exhibit a tri- or tetra-allelic condition at various isozyme loci? How does the isozyme diversity in sour cherry compare to that identified in the limited sweet and ground cherry collection?

LITERATURE REVIEW

Prunus species have been analyzed for isozyme polymorphism using horizontal starch gel electrophoresis by numerous authors. The number of polymorphisms observed and a genetic description of the diversity are presented for each enzyme system studied (Table 5). The symbol "-/-" denotes that the reference did not indicate the number of alleles per locus and the number of loci encoding the enzyme system, most likely due to the lack of diagnostic segregation data, and often in the case of MDH, the complex nature of its inheritance. Enzymes evaluated include acid phosphatase (APS), aconitase (ACON), ADH, aspartate amino transferase or glutamate oxaloacetate transaminase (AAT, same as GOT), catalase (CAT), diaphorase (DIA), esterase (EST), glutamate dehydrogenase (GDH), glutathione reductase (GRD), IDH, LAP, malate dehydrogenase (MDH), peroxidase (PX), PGM, PGI, 6-PGD, shikimate dehydrogenase (SKDH), and triose phosphate isomerase (TPI).

Results for ten enzyme systems have been reported in almond (*P. amygdalus*) leaf tissue and eight enzyme systems involving pollen (Table 5). AAT, IDH, LAP, PGM, and PGI were studied in the sporophytic and gametophytic generations. The number of polymorphisms varied from one

Table 5. Summary of isozyme diversity in the genus *Prunus*.

Species	Enzyme	Number of Polymorphisms	Number of Alleles / Locus	References[1]
Subgenus *Amygdalus*				
dulcis or *amygdalus*[2] (ALMOND)	APS[3]	5	-/1	1
	ADH[3]	3	-/1, -/2, -/3	1
	AAT	4	2/1, 2/2	2, 3, 4, 19
	AAT[3]	3	2/1	1
	CAT[3]	6	-/1[4]	1
	IDH	2	3/1	19
	IDH[3]	3	-/1[5], -/2	1
	LAP	6	3[6]/1, 2/2	2, 3, 4, 13, 19
	LAP[3]	3	2/1	1
	MDH	3	2/1, 2/2	2, 13
	PX	1	1/1, 1/2[4]	13, 19
	PGI	3	1/1, 2/2	2, 3, 4, 13, 19
	PGI[3]	3	1/1, 3/2	1
	PGM	7	2/1, 3/2	2, 3, 4, 13, 19, 20
	PGM[3]	8	2/1, 2/2	1
	SKDH	1	1/1	19
	6-PGD	2	1/1, 2/2	2, 4, 13, 19, 20
	TPI	1	1/1, 1/2	13
argentea	AAT	1	1/1, 1/2	4
	PGI	1	1/1, 1/2	4

Table 5 (cont'd).

Species	Enzyme	Number of Polymorphisms	Number of Alleles / Locus	References
bucharica	AAT	1	1/1, 1/2	4
	PGI	1	1/1, 1/2	4
davidiana	AAT	1	1/1, 1/2	19
	IDH	1	2/1	19
	LAP	2	1/1, 2/2	19
	PX	2	2/1, 1/2[4]	19
	PGI	2	1/1, 2/2	19
	PGM	2	2/1, 1/2	19
	SKDH	2	2/1	19
	6-PGD	1	1/1, 1/2	19
kansuensis	AAT	2	1/1, 2/2	19
	IDH	1	1/1	19
	LAP	1	1/1, 1/2	19
	PX	1	1/1, 1/2[4]	19
	PGI	1	1/1, 1/2	19
	PGM	1	1/1, 1/2	19
	SKDH	1	1/1	19
	6-PGD	1	1/1, 1/2	19
mira	AAT	1	1/1, 1/2	19
	IDH	1	1/1	19
	LAP	1	1/1, 1/2	19
	PX	1	1/1, 1/2[4]	19
	PGI	1	1/1, 1/2	19

Table 5 (cont'd).

Species	Enzyme	Number of Polymorphisms	Number of Alleles / Locus	References
	PGM	1	1/1, 1/2	19
	SKDH	1	1/1	19
	6-PGD	1	1/1, 1/2	19
persica (PEACH)	ACP[3]	2	2/1	17
	ADH[3]	1	-/-	17
	AAT	1	1/1, 1/2	2, 9, 19
	AAT[3]	1	-/-	17
	CAT	3	2/1	14
	CAT[3]	1	-/-	17
	DIA	3	3/1	9
	EST	1	-/-	9
	EST[3]	3	2/1	17
	GDH[3]	1	-/-	17
	GRD	1	-/-	9
	IDH	3	2/1	9, 15, 19
	IDH[3]	3	-/1[5], 2/2, -/3[5]	17
	LAP	1	1/1, 1/2	2, 9, 13, 19
	LAP[3]	1	-/-	17
	MDH	6	3/1	2, 9, 10, 13, 15
	MDH[3]	4	-/1, -/2, -/3, -/4[4]	17
	PX	3	1/1, 2/2[4]	9, 13, 19
	PGI	1	1/1, 1/2	2, 9, 13, 19, 21
	PGI[3]	1	-/-	17

Table 5 (cont'd).

Species	Enzyme	Number of Polymorphisms	Number of Alleles / Locus	References
	PGM	1	1/1, 1/2	2, 9, 13, 19, 20, 21
	PGM[3]	1	-/-	17
	SKDH	2	2/1	9, 15, 19
	SKDH[3]	1	-/-	17
	6-PGD	1	1/1, 1/2	2, 9, 13, 19, 20
	6-PGD[3]	1	-/-	17
	TPI	1	1/1, 1/2	13
persica ssp. *ferganensis*	AAT	1	1/1, 1/2	19
	IDH	1	1/1	19
	LAP	1	1/1, 1/2	19
	PX	1	1/1, 1/2[4]	19
	PGI	1	1/1, 1/2	19
	PGM	1	1/1, 1/2	19
	SKDH	1	1/1	19
	6-PGD	1	1/1, 1/2	19
tangutica	AAT	1	1/1, 1/2	4
	PGI	1	1/1, 1/2	4
Subgenus *Cerasus*				
avium (SWEET CHERRY)	ACON	4	1/1, 3/2	6, 18
	ADH	1	1/1	18
	IDH	3	1/1, 2/2	6, 18
	LAP	1	1/1	18

Table 5 (cont'd).

Species	Enzyme	Number of Polymorphisms	Number of Alleles / Locus	References
	MDH	1	-/-	7
	PGI	2	1/1, 2/2	6, 18
	PGM	3	1/1, 3/2	18
	6-PGD	6	2/1, 2/2	6, 8, 18
canescens	ACON	1	1/1, 1/2	6
	IDH	1	1/1, 1/2	6
	MDH'	1	-/-	7
	PGI	1	1/1, 1/2	6
	6-PGD	1	1/1, 1/2	6
cerasus (SOUR CHERRY)	ACON	4	1/1, 3/2	6, 18
	ADH	2	2/1	12, 18
	IDH	2	1/1, 2/2	6, 12, 18
	LAP	2	2/1	12, 18
	MDH	1	-/-	7
	PGI	3	1/1, 3/2	6, 12, 18
	PGM	4	1/1, 3/2	12, 18
	6-PGD	5-6	3/1, 2/2	6, 8, 12, 18
fruticosa (GROUND CHERRY)	ACON	1	1/1, 1/2	6
	MDH	1	-/-	7
	PGI	1	1/1, 1/2	6
	6-PGD	1	1/1, 2/2	6
incisa	MDH'	2	-/-	7

Table 5 (cont'd).

Species	Enzyme	Number of Polymorphisms	Number of Alleles / Locus	References
mahaleb	MDH[7]	1	-/-	7
subhirtella	ACON	1	1/1, 2/2	6
	MDH[7]	2	-/-	7
	PGI	1	1/1, 1/2	6
	6-PGD	1	1/1, 1/2	6
Subgenus *Prunus*				
armeniaca	LAP	1	1/1, 1/2	5, 13, 22
mandshurica (APRICOT)	MDH	5	2/1, 2/2	5, 13, 16, 22
	PX	1	1/2[4]	5, 13
	PGI	1	-/1[5], 1/2	5, 13, 22
	PGM	5	2/1, 3/2[6]	5, 13, 16, 22
	6-PGD	2	1/1, 2/2	5, 13, 16, 22
	TPI	1	1/1, 1/2	5, 13
americana	LAP	4	3/1, 1/2	11, 13, 22
angustifolia *cerasifera*	MDH	3	3/1, 1/2	11, 13, 22
hortulana *munsoniana*	PX	2	-/1[5], 2/2[4]	11, 13
salicina *simonii* (PLUM)	PGI	4	1/1, 3/2	11, 13, 21, 22
	PGM	6-8	2/1, 2/2	11, 13, 21, 22
	6-PGD	1	1/1, 1/2	11, 13, 22
	TPI	1	1/1, 1/2	11, 13

Table 5 (cont'd).

[1] Key to references follows.
[2] Synonymous
[3] Activity was evaluated from pollen.
[4] Cathodal activity
[5] Inconsistent resolution
[6] One of the alleles is a null allele.
[7] Activity was evaluated from open-pollinated progeny.

Key to References

1) Cerezo et al. (1989)
2) Arulsekar, Parfitt, & Kester (1986)
3) Hauagge, Kester, Arulsekar, Parfitt, & Liu (1987)
4) Hauagge, Kester, & Asay (1987)
5) Byrne & Littleton (1989a)
6) Kaurisch et al. (1988)
7) Hancock & Iezzoni (1988)
8) Fernqvist & Huntrieser (1988)
9) Durham et al. (1987)
10) Arulsekar, Parfitt, Beres, & Hansche (1986)
11) Byrne & Littleton (1988)
12) Chapter I
13) Byrne (1989a)
14) Werner (1992)
15) Mowrey et al. (1990a)
16) Byrne (1989b)
17) Messeguer et al. (1987)
18) Kaurisch et al. (1991)
19) Mowrey et al. (1990b)
20) Chaparro et al. (1987)
21) Parfitt et al. (1985)
22) Byrne & Littleton (1989b)

for PX, SKDH, and TPI to eight for PGM. Two loci were reported for each enzyme in leaf tissue except for IDH and SKDH which only exhibited one. The number of alleles per enzyme in leaf tissue varied from one in SKDH to five for LAP and PGM. One locus was expressed by pollen for APS, AAT, CAT, and LAP, while two loci were expressed for IDH, PGI, and PGM, and three for ADH. *P. argentea*, *P. bucharica*, and *P. tangutica* are all homozygous at two loci for AAT and PGI for alleles also found in *P. amygdalus*.

Eight out of the 14 enzyme systems studied in leaf tissue of peach clones were monomorphic (Table 5). All loci were homozygous in leaf tissue except *Cat-1*, *Idh-1*, *Px-2*, and *Skdh-1* and *Dia-1* and *Mdh-1* which exhibit two and three alleles each, respectively. *Px-2* shows cathodal activity. MDH was expressed at four loci in pollen as compared to only two in leaf tissue.

Mowrey et al. (1990b) studied isozyme polymorphisms in 'Redhaven' peach and several other species also in subgenus *Amygdalus*. Leaf tissue from *P. persica* ssp. *ferganensis* expressed the same results as 'Redhaven' peach leaf tissue. *P. davidiana* exhibited one different putative allele at *Aat-1*, *Pgi-2*, *Idh-1*, *Lap-1*, *Lap-2*, *6-Pgd-2*, *Pgm-2*, and *Skdh-1* and two different putative alleles at *Px-1* and *Pgm-1* as compared to 'Redhaven' peach. *P. kansuensis* expressed one different putative allele at *Aat-1*, *Aat-2*, *Lap-1*, *6-Pgd-2*, and *Pgm-1* in comparison with 'Redhaven' peach. *P. mira* exhibited one different putative allele at *Aat-1*, *Aat-2*, *Idh-1*, *Lap-2*, *6-Pgd-2*, *Pgm-2*, and *Skdh-1* as compared

to 'Redhaven' peach.

In sour cherry two alleles were identified at *Adh-1*, *Idh-2*, *Lap-1*, and *6-Pgd-2* and three alleles at *Acon-2*, *Pgm-2*, *Pgi-2*, and *6-Pgd-1* (Table 5). MDH bands of sweet and ground cherry, sour cherry's presumed progenitors, were codominantly expressed in sour cherry (Hancock and Iezzoni, 1988). No genetic description for MDH is presented for cherry species due to the complex nature of MDH inheritance and the lack of segregation data. Data from open-pollinated progeny of *P. canescens*, *P. incisa*, *P. mahaleb*, and *P. subhirtella* suggests, however, that MDH is dimeric in cherry and that segregation is occurring for two alleles at one cytosolic locus for *P. incisa* and *P. subhirtella*. *P. canescens* exhibits two homozygous loci per enzyme studied, not considering MDH. *P. subhirtella* and ground cherry are monomorphic for all enzyme systems except for MDH in *P. subhirtella;* however, they exhibit at least one heterozygous locus. One-half of the sweet cherry loci studied exhibit only one allele.

Santi et al. (1990) studied inheritance and linkage of isozyme loci in 286 wild sweet cherries. Santi and Lemoine (1990) found diversity at eight isozyme loci in 198 wild sweet cherries which allowed them to devise an identification key for the sweet cherries. Santi and Lemoine (1990) also studied 33 sour and duke cherries and found only three to ten additional isozyme bands which might characterize the ground cherry genome from that of sweet cherry. Santi et al. (1990) and Santi and Lemoine (1990)

are not included in Table 5 because they used PAGE and isoelectric focusing techniques to collect isozyme data. These data do not resemble and are not comparable to that from the other studies.

Apricot clones exhibited activity at two loci for each enzyme system except PX (Table 5). PX cathodal activity encoded by one locus was resolvable. LAP, PX, PGI, *6-Pgd-1*, and TPI loci were monomorphic. Two alleles were reported at *Mdh-1*, *Mdh-2*, *Pgm-1*, and *6-Pgd-2* and three at *Pgm-2*.

The plum clones studied generally involve several species in their pedigrees. The species involved in the clonal parentage precede the heading "Plum" (Table 5). Plums exhibit from one polymorphism for TPI and 6-PGD up to a minimum of six polymorphisms for PGM. All enzyme systems exhibit two loci. Two alleles were observed at *Px-2* and both PGM loci and three at *Lap-1*, *Mdh-1*, and *Pgi-2*. All other loci studied were monomorphic for one allele.

The *Prunus* species that exhibit the most isozyme polymorphism are almond, sour cherry, and plum. Almond's extensive isozyme variability has been attributed to outcrossing due to high levels of self-incompatibility (Arulsekar, Parfitt, and Kester, 1986). Plums also are self-incompatible and have complex pedigrees involving numerous species and ploidy levels (Byrne and Littleton, 1988). Sour cherry is also a polyploid with self-incompatibility prevalent in the species.

The high degree of isozyme homozygosity in peach could have resulted from inbreeding (self-incompatibility is rare)

and a limited gene pool. The peach cultivars in the United States trace back to just a few introductions (Arulsekar, Parfitt, and Kester, 1986). Apricots exhibit intermediate isozyme variability compared to other *Prunus* species and consist of inbreeding and outcrossing populations (Byrne and Littleton, 1989a).

METHODS

Plant Material

A total of 26 sweet, 67 sour, six ground, and 12 interspecific hybrid cherry genotypes and 'Redhaven' peach were evaluated for their isozyme patterns. All of the plant material was from the MSU collection at the Clarksville Horticultural Experiment Station, Clarksville, Michigan or the Horticultural Research Center, East Lansing, Michigan.

Two principal coordinate analyses were performed. Thirty-six selections were analyzed for all enzyme systems except PX. These 36 sweet, sour, ground, and interspecific hybrid cherries made up the first comparison (PCO1) based on a total of 44 isozyme bands. Fifty-seven tetraploid selections with sour cherry in their pedigrees were analyzed for all enzyme systems except PX and PGM. These were compared in the second analysis (PCO2) using 41 isozyme bands.

Isozyme Procedures

Starch gel electrophoresis was performed on extracts from young leaves and dormant vegetative buds. Enzyme systems studied include PGI, IDH, PGM, 6-PGD, and LAP as well as shikimate dehydrogenase (SKDH, E.C.1.1.1.25), malate dehydrogenase (MDH, E.C.1.1.1.37), and peroxidase (PX,

E.C.1.11.1.7). Electrophoresis and staining procedures were the same as for Chapter I. ADH did not exhibit sufficient activity from leaves and buds and so was not used in the diversity study. SKDH and MDH were resolved on morphiline citrate pH 6.1 gels (Clayton and Tretiak, 1972). PX was resolved on the cathodal (bottom) section of tris-citrate/lithium-borate pH 8.3 gels (Scandalios, 1969). SKDH, MDH, and PX were assayed according to Arulsekar and Parfitt (1986), Vallejos (1983), and Soltis et al. (1983), respectively. Hydrogen peroxide was added just prior to PX activity staining.

Isozymes were named based on their mobilities relative to the *100* band as in Chapter I. However, a few cherry genotypes exhibited rare isozymes anodal to *100* for PGI (*110* and *105*) and SKDH (*120*). The most anodal of these rare isozymes were not named *100* because of their scarcity and they were discovered after this nomenclature was all ready in use. 'Montmorency' or 'Meteor' controls were run on most gels during this germplasm diversity study to aid in band identification. Both cultivars possess the *100* band for all enzyme systems except SKDH.

Statistical Procedures

A '0' or a '1' was entered for each isozyme band using the Similarity Computer Program (Appendix D) indicating its absence or presence for every genotype analyzed. The matrix of 36 genotypes x 44 isozymes utilized in the first principal coordinate analysis (PCO) is presented in Appendix

A. The second analysis used a raw data matrix of 57 genotypes x 41 isozymes. The isozyme bands utilized are defined in Figures 3 and 4.

Data were analyzed by calculating similarity matrices (Appendix B) again using the Similarity Computer Program. The similarity statistic used by this program is the Marczewski and Steinhaus Similarity statistic: $S = w / (a + b - w)$, where 'a' and 'b' are the number of isozyme bands observed in the two individuals being compared and 'w' is the number of isozyme bands the two individuals share in common (Angus et al., 1988). The similarity matrices were subjected to PCO. SAS (SAS Institute, Inc., Cary, N.C.) was used on the IBM® mainframe at Michigan State University to run the PCO Program written by Dr. Carl Ramm (Appendix E).

Scores for the first two principal coordinates were imported into 123® version 2.01 for editing and for compatibility with PlotIt®. PlotIt® version 1.5 was then used to graph the data in two dimensions. Individual points on the graphs were labelled with the corresponding genotype's identity. The graphs were then scrutinized for possible ordinations.

RESULTS

Isozyme Polymorphisms

The cherry genotypes studied exhibited three polymorphisms for *Pgi-2* (Tables 6 and 7, Figures 2 and 3) encoded by three alleles at one locus. Results from progeny testing reported in Chapter I indicate that the isozyme bands *82* and *100* represent alleles while *91* is heteromeric. All of the cherry species and species hybrids exhibited polymorphisms 1 and 2. Putative allele *110* and the heteromeric band *105* were rare among the germplasm studied, being only exhibited by a NR3F2 open-pollinated mutant (c). 'Redhaven' peach exhibited the *100* allele.

Three polymorphisms were discovered for *Idh-2*, two of which were exhibited by cherry clones while polymorphism 3 was exhibited by 'Redhaven' peach (Tables 6 and 7, Figure 3). Bands *100* and *64* were diagnosed as alleles and *82* as their intralocus heterodimer (Chapter I). Nearly all sweet cherries were homozygous for the *100* allele, while all but one of the ground cherries possessed the heterozygous polymorphism 2. Sour cherry and the species hybrids exhibited polymorphisms 1 and 2.

Five polymorphisms were diagnosed for *Lap-1* (Tables 6 and 7, Figure 3). Polymorphism 3 was unique to 'Redhaven' peach. Bands *100* and *95* represent alleles (Chapter I).

Table 6. A list of isozyme phenotypes for each of 111 cherry selections and 'Redhaven' peach.

Clone	FIG. 6	FIG. 7	SPECIES	PGI	Idh-2	LAP	MDH	PX	PGM	6-PGD	SKDH
I 57(30): Englaise timpurii op		1	(cxa)op	1	2	1	2			2	8
I 24(41)	1		(f)op	1	1	1	2		1	4	13
I 23(18)			(f)op				5		1	1	4
Emperor Francis	2		a	2	1	4	3		2	3	7
Windsor	3		a	1	1	4	3		2	7	9
Hedelfingen	4		a	2	1	4	3		2	3	7
Angela	5		a	1	1	4	3		2	3	7
III 17(20): Germersdorf 41/20	6		a	2	2	4	3	3	2	3	9
III 17(19): Italia (6)	7		a	1	2	4	4	3	2	3	9
Schmidt			a	1		4	3		2	3	7
Black Tartarian			a				3		2	7	9
Governor Wood			a				3		2	7	9
Yellow Glass			a				3		2	7	7
Napoleon			a				3		2	3	7
Lambert			a				3		2	10	7
Ulster	8		a	1	1	4	3		2	10	7
V690616	9		a	2	1	4	3		2	3	7
Bing	10		a	2	1	4	3		2	7	7
V9062	11		a	1	1	4	3		2	7	7
Stark Crimson	12		a	2	1	4	3		2	7	7
Kristin	13		a	1	1	4	3		2	10	7
Lapins	14		a	2	1	4	3		2	3	9
Van	15		a	1	1	4	3		2	7	9
V690618	16		a	2	1	4	3		2	3	7
Ranier	17		a	1	1	4	3		2	7	9
Spur Lambert	18		a	2	1	4	3		2	10	7
Merpet	19		a	2	1	4	3		2	7	7
Stella	20		a	2	1	4	3		2	3	7
V69061	21		a	1	1	4	3		2	7	7
Coronation	22	2	c	2	1	1	1		1	1	1
George Glass	23	3	c	2	2	1	2		1	2	1
Meteor	24	4	c	2	2	1	4	2	1	4	1
Fruchtbare von Michurin		8	c	1	1	1	2	6		2	1
Montmorency	25	5	c	2	2	1	2	1	1	2	1
II 15(4): Mont x M 63		9	c	1	2	1	2			6	5
II 13(10): Cigany Meggy op			c	1	2		2		1	2	5
II 13(1): Csengodi csokras from Erd op		10	c	1	2	1	2	1		1	4

Table 6 (cont'd).

II 13(9): Cigany Meggy op			c	1	2		2		1	2	5
Ukrainische Griotte		11	c	1	2	1	5			1	3
II 13(4): Csengodi csokras from Erd op		12	c	1	2	1	3	4		1	7
I 61(59): Mont x Oblacinska		13	c	1	2	1	2			5	1
I 61(26): H 8/121 op		14	c	1	2	1	2			6	4
I 52(9): H 15/10 op		15	c	1	2	1	5			6	4
II 12(19): Cigany Meggy op		16	c	1	2	1	2	7		14	5
II 7(11): Wolynska x Oblacinska	26	6	c	1	2	1	2	1	1	4	1
II 13(36): Cigany Meggy op		17	c	1	2	1	6	1		2	7
II 10(23): Kantorjanosi		18	c	1	2	1	2	4		3	4
II 5(24): English Morello x R5		19	c	2	2	1	2	1		2	4
II 28(40): AI4 (Korai Pipac Meggy)		20	c	1	2	1	2			2	6
II 16(20): Cigany Meggy op		21	c	1	2	1	2			2	9
II 30(39): AI3 (Csengodi Csokins)		22	c	1	2	1	2			1	4
II 30(40): V2103 Ujfehertoi Furtos		23	c	2	2	1	2			2	3
II 30(29): AI3 (Csengodi Csokins)		24	c	1	2	1	2			1	4
II 12(33): Dobraia op		25	c	1	1	1	5			5	1
IV 8(34): NR3F2 mutant op		26	c	1	1	1	2			2	5
IV 8(33): NR3F2 mutant op		27	c	3	2	1	9			11	2
Maksimoskaja op		45	c	1	1	2	1			13	1
Altaiskaja op		28	c	1	1	1	5			4	3
Mustila op		29	c	1	2	1	2			9	4
IV 7(51): Zuckovskaja op			c	1	1		1			3	1
IV 7(52): Zuckovskaja op		30	c	1	2	1	5			3	1
III 17(2): Mont x Sumadinka		31	c	2	2	1	4	2		3	5
III 10(6): Mocanesti 16		32	c	1	2	1	6	7		4	4
II 34(3): HY8/121		33	c	2	2	1	2	1		4	4
II 35(32): Dropia		34	c	2	1	1	5	3		5	4
II 32(26): HY60/29	27	7	c	2	2	1	9	4	1	1	4
II 24(32): Mont x Meteor Korai		35	c	2	2	1	2	7		2	1
II 19(12): Erdi Jubileum op		36	c	2	2	1	2	7		2	4
II 38(26): Erdi Botermo / Mah		37	c	1	2	1	2			3	5
II 17(20): Pandy 114		38	c	2	2	1	2			2	5
IV 7(1): Smudencheskaja op		39	c	1	1	1	2			6	7
IV 2(53): Koros Meggy		40	c	2	2	1	2			2	3
II 5(25): English Morello x R11			c	1	1	1	5		1		5
II 7(12): B5(12) x M112			c	1	1		5			6	4
IV 7(3): Smudencheskaja op			c	2	1		2			3	5
IV 7(4): Smudencheskaja op			c	2	1		2			4	5

Table 6 (cont'd).

IV 7(5): Smudencheskaja op			c	2	1		2			2	5
IV 7(7): Smudencheskaja op			c	1	2		2			3	1
IV 7(8): Smudencheskaja op			c	2	1		2			3	4
IV 7(9): Smudencheskaja op			c	2	2		2			15	10
IV 7(10): Smudencheskaja op			c	2	2		2			2	8
IV 8(8): NR3F2 mutant op			c	1	2		2			12	1
IV 8(10): NR3F2 mutant op		41	c	1	1	1	1			4	5
IV 1(46): II 16(3) CC		42	c	1	2	1	2			1	4
IV 1(55): Kirsa		43	c	2	1	1	2			5	1
IV 8(24): NR3F2 mutant op			c	2	1		2			2	5
IV 8(26): NR3F2 mutant op			c	1	1		2			1	1
IV 9(47): Pernella		44	c	1	2	1	4			6	1
IV 7(29): Smudencheskaja op			c	2	2		7			15	11
IV 8(15): NR3F2 mutant op			c	2	1		2			2	4
IV 7(23): Smudencheskaja op			c	2	2		7			12	11
IV 7(13): Smudencheskaja op			c	2	1		2			3	7
IV 7(17): Smudencheskaja op			c	1	1		5			4	1
IV 7(12): Smudencheskaja op			c	2	1		2			4	4
Nefris	28	46	c	1	2	1	2		1	2	5
II 34(25): Oblacinska op		47	c	1	2	1	5			1	4
I 51(17): Nana x Mari Timpurii	29	48	cx(cxa)	1	2	1	2		1	6	1
II 13(11): Mont x R 5 (H 18/21)		49	cx(cxa)	2	2	1	4			2	5
II 15(5): Mont x Fruchtbare von Michurin		50	cx(fxc)op	1	1	1	4	1		2	1
II 12(29): Mont x Fruchtbare von Michurin		51	cx(fxc)op	1	1	1	2	5		2	1
I 1(44): Mont x Angela	30	52	cxa	2	1	1	3		1	10	5
I 1(58): Mont x Angela	31	53	cxa	2	1	4	2		1	8	5
II 18(2): Pitic de Iasi op	32	54	cxf	1	1	1	2	2	1	2	1
IR 323-2	36		f	2	2	5	1		3	4	4
IR 587-1	34		f	2	2	2	1		3	1	14
IR 883-1	33		f	1	2	1	2		1	1	13
Dwarf Rich	35		f	2	2	2	1		3	17	1
Redhaven (peach)			p	1	3	3	8		4	16	12
II 17(1): Pitic de Iasi op		55	[(fxc)op]op	2	2	1	5			2	4
II 13(40): Pitic de Iasi op		56	[(fxc)op]op	1	1	1	2	5		8	1
II 16(24): Pitic de Iasi op			[(fxc)op]op	1	1		2			2	1
II 19(39): Pitic de Iasi op		57	[(fxc)op]op	1	1	1	2			3	1

NOTE: a = sweet cherry; c = sour cherry; f, f(op) = ground cherry; p = peach
other = interspecific hybrid cherry

Polymorphisms are defined in Figures 3, 4, and 5.

Table 7. Number of sweet, sour, ground, and hybrid cherry selections which possessed polymorphisms for eight enzyme systems.

Enzyme system	Polymorphism	Species				
		Prunus avium	*Prunus cerasus*	*Prunus fruticosa*	*P. cerasus* x *P. avium*	*P. cerasus* x *P. fruticosa*
Pgi-2	1[1]	10	39	2	2	6
	2	11	27	3	3	1
	3	-	1	-	-	-
Idh-2	1	18	23	1	2	6
	2	2	44	4	3	1
	3[1]	-	-	-	-	-
Lap-1	1	-	46	2	4	6
	2	-	1	2	-	-
	3[1]	-	-	-	-	-
	4	21	-	-	1	-
	5	-	-	1	-	-
MDH	1	-	4	3	-	-
	2	-	43	2	3	5
	3	25	1	-	1	-
	4	1	3	-	1	1
	5	-	10	1	-	1
	6	-	2	-	-	-
	7	-	2	-	-	-
	8[1]	-	-	-	-	-
	9	-	2	-	-	-
Pgm-2	1	-	10	3	3	1
	2	26	-	-	-	-
	3	-	-	3	-	-
	4[1]	-	-	-	-	-
6-PGD	1	-	10	3	-	-
	2	-	20	-	2	5
	3	11	9	-	-	1
	4	-	9	2	-	-
	5	-	4	-	-	-
	6	-	6	-	1	-
	7	11	-	-	-	-
	8	-	-	-	1	1
	9	-	1	-	-	-
	10	4	-	-	1	-
	11	-	1	-	-	-
	12	-	2	-	-	-
	13	-	1	-	-	-
	14	-	1	-	-	-
	15	-	2	-	-	-
	16[1]	-	-	-	-	-
	17	-	-	1	-	-

Table 7 (cont'd).

Enzyme system	Polymorphism	Species				
		Prunus avium	*Prunus cerasus*	*Prunus fruticosa*	*P. cerasus* x *P. avium*	*P. cerasus* x *P. fruticosa*
Skdh-1	1	-	18	1	1	6
	2	-	1	-	-	-
	3	-	4	-	-	-
	4	-	19	2	-	1
	5	-	15	-	3	-
	6	-	1	-	-	-
	7	18	4	-	-	-
	8	-	1	-	1	-
	9	8	1	-	-	-
	10	-	1	-	-	-
	11	-	2	-	-	-
	12[1]	-	-	-	-	-
	13	-	-	2	-	-
	14	-	-	1	-	-
PX	1	-	6	-	-	1
	2	-	2	-	-	1
	3	2	1	-	-	-
	4	-	3	-	-	-
	5	-	-	-	-	2
	6	-	1	-	-	-
	7	-	4	-	-	-

[1]Polymorphism exhibited by 'Redhaven' peach

Figure 2. (A) PGI, (B) MDH, (C) PGM, (D) 6-PGD, (E) SKDH, and (F) PX isozyme patterns in cherry. The origin is at the bottom of each photograph. The bottom zone of activity in A was due to *6-Pgd-2*. Anodal activity was observed in A - E and cathodal activity in F. Mobilities are given for bands which represent true and putative alleles in A and C - E. Mobilities are presented for all isozymes in B and F because genetic control of these enzymes is not understood in cherry species. Band *63* in B is the mitochondrial form of MDH in cherry. In B and C, lanes 1 - 6 are sweet cherries, 7 - 9 and 11 are ground cherries, 10 is a sour x sweet cherry hybrid, and 12 - 16 are sour cherries.


110
100
A
100
88
63
95
75
51
35
B
100
75
C
100
82
72
D
120
100
95
77
71
E
100
92
81
64
30
15
F

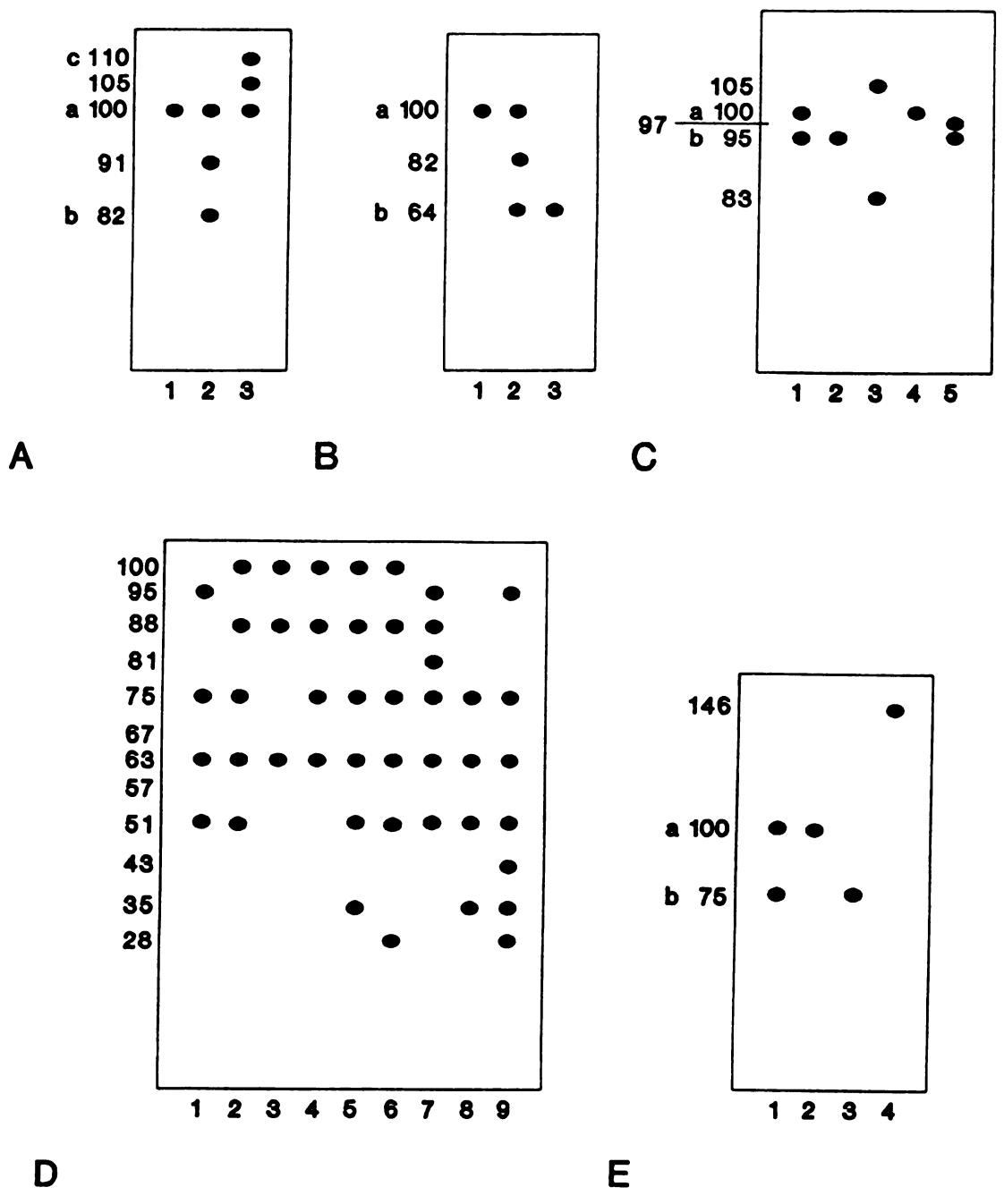

Figure 3. Isozyme phenotypes exhibited by cherry selections and 'Redhaven' peach for (A) *Pgi-2*, (B) *Idh-2*, (C) *Lap-1*, (D) MDH, and (E) *Pgm-2*. The origin is at the bottom of each diagram.

Putative allele *97* was only diagnosed in one ground cherry clone. All ground cherries possessed the *95* allele. All sweet cherries that were studied for LAP were homozygous for the *100* allele. Sour cherry genotypes predominantly exhibited polymorphism 1, which is comprised of both the *100* and *95* alleles. Four out of the five genotypes studied with sour and sweet cherry in their pedigrees also exhibit polymorphism 1. All six of the genotypes studied for LAP that contain sour and ground cherry in their pedigrees and the one open-pollinated ground cherry studied exhibited polymorphism 1.

A total of nine polymorphisms were discovered for MDH (Tables 6 and 7, Figures 2 and 3). Polymorphism 8 was only recorded for 'Redhaven' peach. All but one of the sweet cherries possessed polymorphism 3, while the other exhibited polymorphism 4. Both polymorphisms have bands *100*, *88*, and *63*. Band *63* was the largest and most intense of the MDH bands. Sometimes additional bands were resolved at *67* and *57* when gels exhibited excellent resolution, suggesting possible comigration with *63*. Polymorphism 4 also has band *75*. The six ground cherries possessed polymorphisms 1, 2, and 5 which contain the bands possessed by the sweet cherry as well as three unique bands: *95*, *51*, and *35*. Sour cherries possess all of the polymorphisms except number 8. Bands *81*, *43*, and *28* were unique to sour cherry, although 'Redhaven' peach also possessed band *35*. Progeny testing (data not presented) using the parental sour cherries from Chapter I was unsuccessful at determining a genetic model

for MDH, although a general lack of segregation indicated possible fixed heterozygosity in these genotypes. MDH may be dimeric in cherry due to the large number of bands comprising many of the polymorphisms and the spatial symmetries among the bands.

Four polymorphisms were diagnosed for *Pgm-2* (Tables 6 and 7, Figures 2 and 3). Polymorphism 4 was only found in 'Redhaven' peach. Bands *100* and *75* were found to be alleles (Chapter I). Sweet cherries were all homozygous for the *100* allele, while three out of four ground cherries (f) were homozygous for the *75* allele. All other genotypes tested for PGM were heterozygous.

Seventeen polymorphisms encoded by nine alleles at two loci were discovered for 6-PGD (Tables 6 and 7, Figures 2 and 4). Progeny testing (Chapter I) indicated that bands *100*, *88*, and *76* at *6-Pgd-1* and bands *60* and *48* at *6-Pgd-2* are alleles and bands *94* and *82* at *6-Pgd-1* and band *54* at *6-Pgd-2* are heteromeric. Band *88* is an intralocus heterodimer for polymorphisms 1, 5, and 17. Band *82* at *6-Pgd-1* is an intralocus heterodimer for polymorphisms 4 and 6, while it is a putative allele at *6-Pgd-2* for polymorphisms 12 and 15. Band *72* at *6-Pgd-2* is a putative allele for polymorphisms 11 and 16 and an intralocus heterodimer for polymorphism 15. Bands *66* and *43* at *6-Pgd-2* are intralocus heterodimers. Band *38* at *6-Pgd-2* is a putative allele for only polymorphism 14, and an intralocus heterodimer for polymorphisms 9, 13, and 17. Band *28* is a putative allele at *6-Pgd-2*. The middle band of

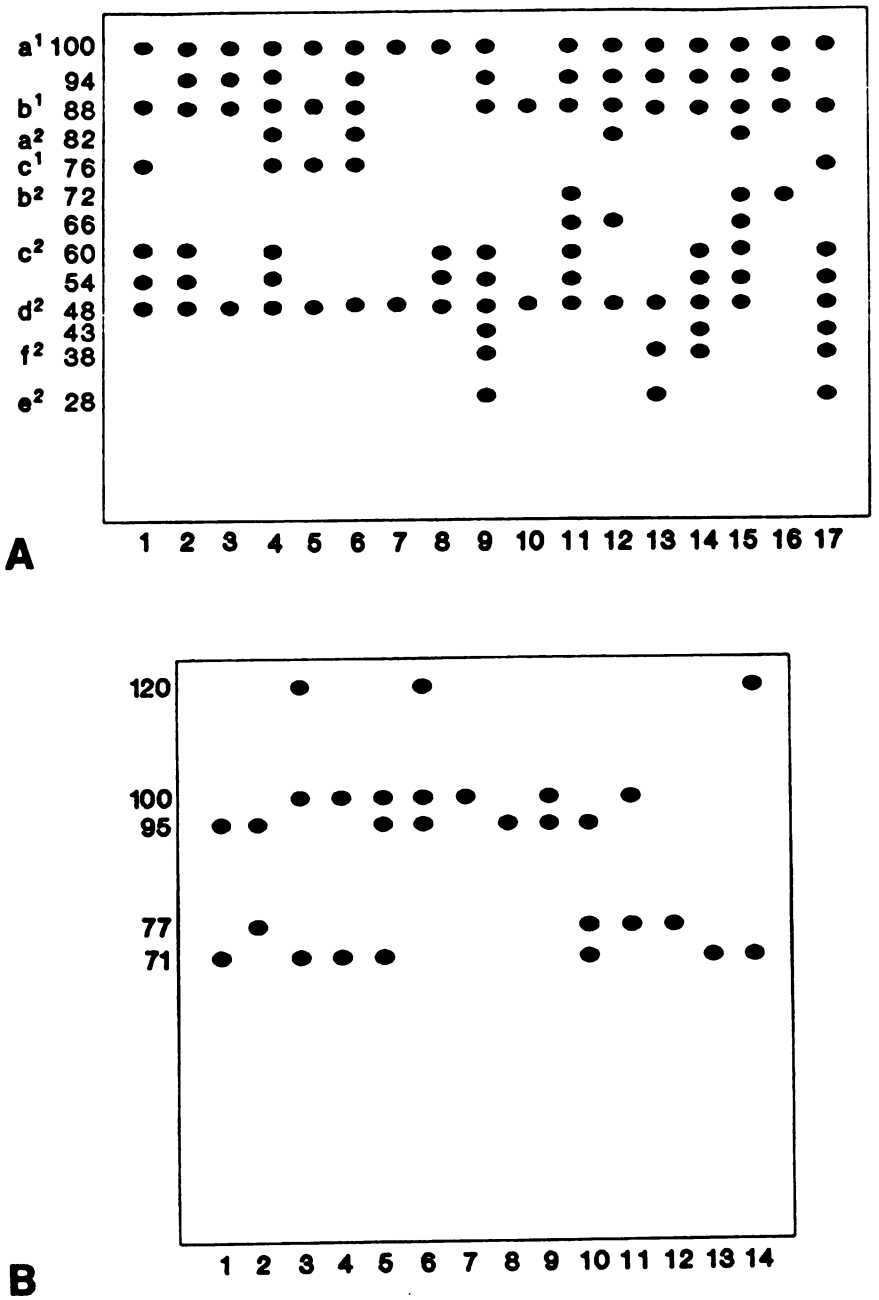

Figure 4. Isozyme phenotypes exhibited by cherry selections and 'Redhaven' peach for (A) *6-Pgd-1* and *6-Pgd-2* and (B) *Skdh-1*. The origin is at the bottom of each diagram.

five-banded patterns at both 6-PGD loci represents comigration between an intralocus heterodimer and a homodimer representing an allele.

Sweet cherries possess one or both of alleles *100* and *88* at *6-Pgd-1* and were all homozygous for *6-Pgd-2*^{48}. All of the ground cherries possess *6-Pgd-1*^{76} in addition to one or both of the sweet cherry alleles at this locus. In addition to *6-Pgd-2*^{48}, the ground cherry clones possess allele *60* and one also possesses putative allele *28* at this locus. Sour cherry germplasm exhibited all of the polymorphisms for 6-PGD except 7, 8, 10, 16, and 17. Sour cherry possesses all of the 6-PGD alleles and putative alleles found in sweet and ground cherry. Only 'Redhaven' exhibited pattern 16.

Fourteen polymorphisms encoded by five putative alleles were found for *Skdh-1* (Tables 6 and 7, Figures 2 and 4). Polymorphism 12 was found only in 'Redhaven' peach. Sweet cherries exhibit either polymorphism 7 or 9 consisting of the putative *100* or *100* and *95* alleles, respectively. The ground cherries exhibit polymorphisms 1, 4, 13, and 14 and all of the putative alleles except *Skdh-1*^{77}. Sour cherries exhibit the first eleven polymorphisms and have all of the putative alleles found in sweet and ground cherry and also putative allele *77*. The structure of SKDH appears to be monomeric in cherry.

Seven polymorphisms were found for PX (Tables 6 and 7, Figures 2 and 5). PX was difficult to resolve consistently; therefore, data exists for only a few genotypes and it was not used in the principal coordinate analyses. PX appears

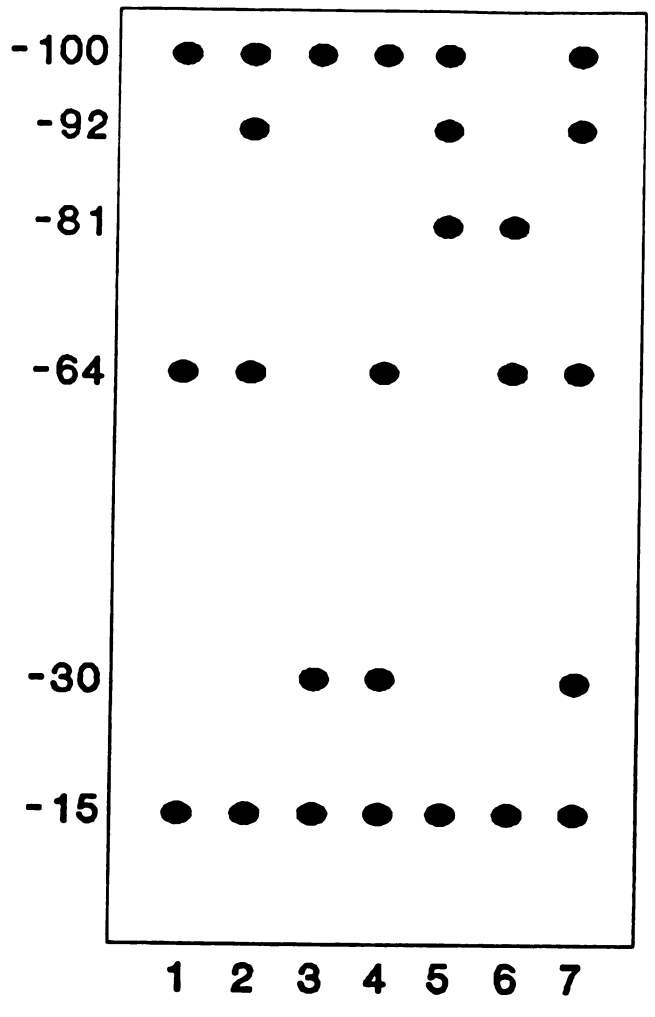

Figure 5. Isozyme phenotypes exhibited by cherry selections for cathodal PX. The origin is at the bottom of the figure.

to be monomeric in structure and encoded by a minimum of two cathodal loci in cherry. Polymorphism 7 exhibits five bands which is one too many for one locus in tetraploid sour cherry assuming each band was encoded by a unique allele. Polymorphism 3 has three bands which is one too many for one locus in diploid sweet cherry. The only two sweet cherries for which data are available exhibit polymorphism 3. Data for ground cherries are unavailable. Sour cherry exhibits all of the PX polymorphisms except number 5 which was expressed by two species hybrids.

None of the diploid sweet cherry genotypes were heterozygous for *Lap-1*, *6-Pgd-2*, or *Pgm-2* (Table 8). Only ten percent were heterozygous for *Idh-2* and 42% and 52% were heterozygous for *6-Pgd-1* and *Pgi-2*, respectively. Tetraploid cherry genotypes exhibited heterozygosity for all of the isozyme loci presented. Total heterozygosity ranged from 42 percent for *Pgi-2* to 96 percent for *6-Pgd-1*. Most of the heterozygous tetraploid genotypes were di-allelic, some were tri-allelic, and none were tetra-allelic. A much greater percentage of tetraploid genotypes were heterozygous than were diploid genotypes for all isozyme loci except *Pgi-2*. Average heterozygosity over the loci was 78% for the tetraploids compared to 19% for the diploids.

PCO1

The first two principal coordinates from the PCO of 36 clones and seven enzyme systems, PCO1, accounted for 53.8% of the isozyme variation (Table 9). A two-dimensional plot

Table 8. Percent of diploid and tetraploid genotypes heterozygous for each of the enzyme loci listed.

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

[1]*Prunus avium*
[2]*Prunus cerasus*, *P. cerasus* x *P. avium*, *P. cerasus* x *P. fruticosa*, and *P. fruticosa*

Table 9. Percent variation accounted for by the first five principal coordinates for PCO1 (Figure 6).

Principal coordinate	Percent variation
1	41.1
2	12.7
3	9.7
4	6.2
5	5.5
Total	75.2

separated the diploid sweet cherry selections from the sour cherry, ground cherry, and sour x ground cherry tetraploids along the first axis (Figure 6). *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* are primarily responsible for this separation. These alleles and bands were generally present in the tetraploids on the positive end of axis 1 and were generally absent in the sweet cherry diploids on the negative end of axis 1. Most other alleles and bands were present in diploids and tetraploids.

Sweet cherry genotypes 6 and 7, 'Germersdorf' and 'Italia,' respectively, lie closer to the tetraploids on the first axis than the other diploids. 'Germersdorf' and 'Italia,' unlike the other sweet cherries, exhibit *Idh-2* polymorphism 2 consisting of alleles *100* and *64* as do the tetraploids except 30 and 31. 'Italia,' again unlike the other sweet cherries, also has MDH band *75* as do all of the tetraploids excluding genotype 30.

The 'Montmorency' x 'Angela' (sour x sweet) tetraploids 30 and 31 resembled the other tetraploids for *Pgm-2* and *Skdh-1* and the sweet cherries for *Idh-2*. Genotype 30 resembled the sweet cherries for MDH and 6-PGD and the tetraploids for *Lap-1*, while genotype 31 was just the opposite. Therefore, both 30 and 31 lie near the center of axis 1 in between the diploid sweet cherries and the tetraploids.

The presence or absence of *Pgi-2*^{82} separated both the sweet cherry diploids and the sour cherry, ground cherry,

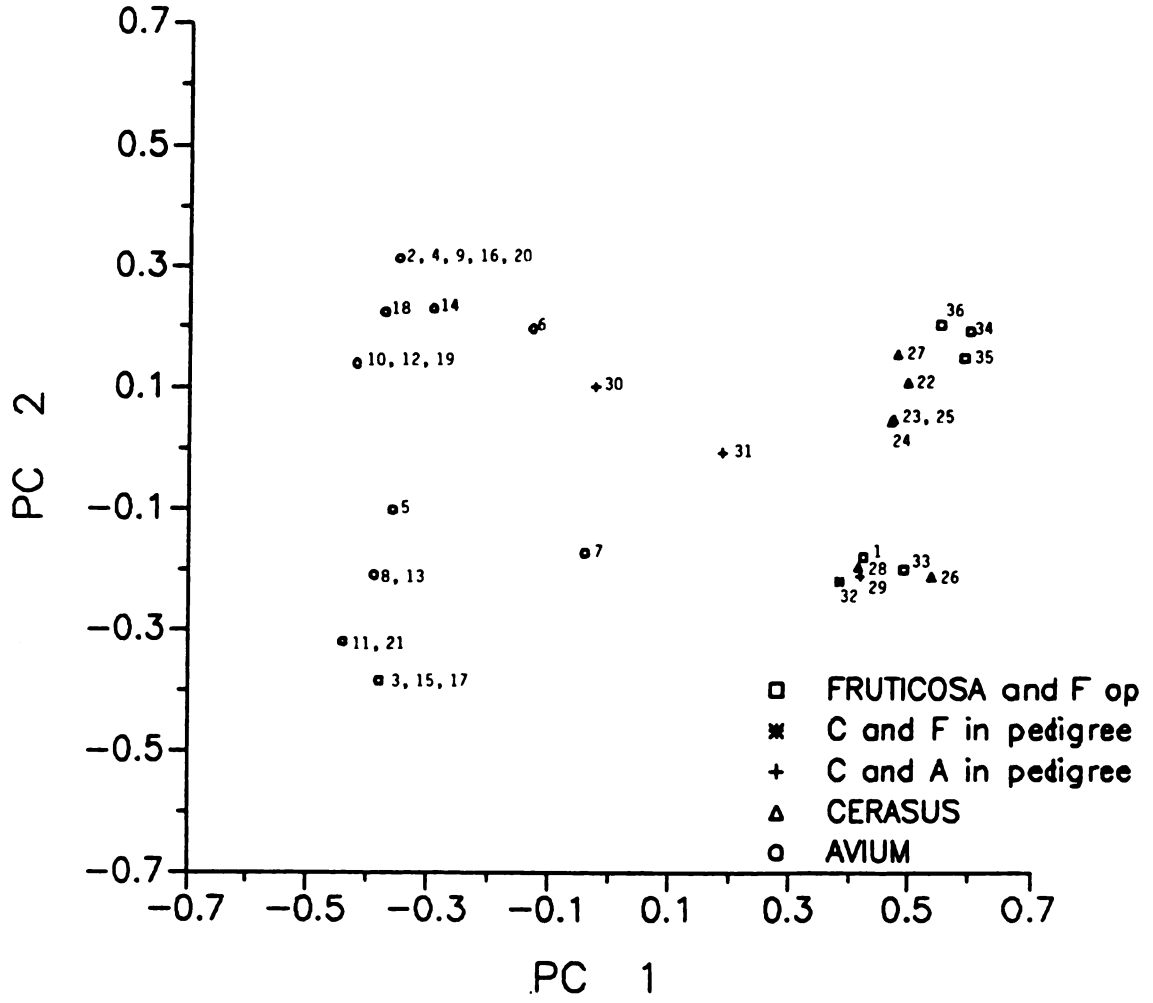

Figure 6. Principal coordinate analysis for PC1 vs. PC2 for selected cherry genotypes (PCO1). Identities of clones are presented in Table 6.

and sour x ground cherry tetraploids into two groups along axis 2. Individuals of both groups at the positive end of axis 2 exhibited polymorphism 2 for PGI consisting of alleles *100* and *82*. Those at the negative end of axis 2 were homozygous for *Pgi-2*^{100}.

PCO2

The first two principal coordinates from the PCO of 57 sour cherries and six enzyme systems, PCO2, accounted for only 30.2% of the isozyme variation (Table 10). A two-dimensional plot (Figure 7) did not reveal any natural clusters in the data. Additionally, the sour cherry, sour cherry x sweet cherry, and sour cherry x ground cherry clones did not aggregate.

The three isozyme loci contributing to the separation in the first two dimensions were *Idh-2*, *Pgi-2*, and *6-Pgd-1*. *Idh-2*^{64} was more frequent at the positive end of the first axis. *Pgi-2*^{82} was very frequent at the negative end of axis 2 and rare at the positive end of axis 2. *6-Pgd-1*^{76} was more prevalent towards the positive end of axis 2. None of these alleles were found exclusively at one end of an axis.

Table 10. Percent variation accounted for by the first five principal coordinates for PCO2 (Figure 7).

Principal coordinate	Percent variation
1	15.8
2	14.4
3	11.0
4	10.1
5	7.3
Total	58.6

DISCUSSION

Isozyme Genetics

SKDH polymorphisms are probably encoded by genetic variation at one locus in cherry. No more than three bands were reported for tetraploid cherries and two bands for diploid sweet cherries (Figure 4) which lends support for a one locus hypothesis. Asymmetrical polymorphisms and the apparent lack of heterodimeric bands indicate that SKDH is most likely a monomeric enzyme in cherry. Segregation data for peach indicated that SKDH is encoded by one locus and that SKDH is a monomer in peach (Mowrey et al., 1990a). Data for almond and other species in subgenus *Amygdalus* (Table 5) also suggest that SKDH is encoded by one locus in these species.

No other PX studies utilizing horizontal starch gel electrophoresis exist for cherry species. In peach, inheritance data has confirmed the existence of two alleles at one cathodal locus (Durham et al., 1987). However, in my study, three bands and up to five bands were observed on the cathodal section of gels for sweet and sour cherry, respectively. Assuming that PX is monomeric and that each band is encoded by a unique allele as was the case at the peach cathodal locus (Durham et al., 1987), a minimum of two cathodal loci is needed to genetically explain the observed

PX polymorphisms in sweet and sour cherry.

The genetic basis for the banding patterns observed for MDH in the three cherry species studied is not known. However, the polymorphisms presented in my diversity study and data on the genetic control of MDH in other *Prunus* species do provide some clues. MDH polymorphisms observed in almond, peach, apricot, and plum leaf tissue are encoded by alleles at two different loci (Table 5). In almond, apricot, and plum, both loci are polymorphic exhibiting typical banding patterns for a dimeric enzyme (one-banded homozygotes and symmetrical three-banded heterozygotes). In peach, the polymorphisms at *Mdh-1* are often complex due to single alleles producing two protein products which can interact to produce homodimers and heterodimers for a homozygous genotype. *Mdh-2* encodes the mitochondrial form of MDH and is monomorphic for one band in peach (Mowrey et al., 1990a). Likewise in cherry, MDH polymorphisms are quite complex, but may also be accounted for by genetic variation at a minimum of two loci. Hancock and Iezzoni (1988) diagnosed the mitochondrial form of MDH in sour cherry which corresponds to the *63* band in my study. This MDH band is the only one found in every genotype analyzed in my diversity study. Therefore, two loci can be hypothesized. Putative *Mdh-2* is probably monomorphic for the mitochondrial band *63*, and the other isozyme bands may be accounted for by alleles at *Mdh-1*.

Comparisons with Other Cherry Studies

The MDH polymorphisms presented by Hancock and Iezzoni (1988) for sour and sweet cherry compare well with those diagnosed in my diversity study. Even though different gel and electrode buffers were used, their patterns for sweet and sour cherries correspond to polymorphisms 3 and 2, respectively (Figure 3) in my study. In addition to these polymorphisms, one out of 26 sweet cherries was found to exhibit polymorphism 4 and sour cherries were found to exhibit polymorphisms 1 through 7 and 9. Fifty-one out of 79 genotypes with sour cherry in their pedigrees exhibited MDH polymorphism 2. Hancock and Iezzoni (1988) studied 19 sour cherries and six sweet cherries and so subsampled the set of diversity found in my larger study for sour and sweet cherry.

The MDH polymorphism presented by Hancock and Iezzoni (1988) for ground cherry was similar to two of the three that my study diagnosed with two exceptions. Polymorphism 1 has the most anodal band at *95* rather than theirs which corresponds to band *88* in my study and polymorphism 2 has band *100* in addition to *88* which they did not find. Both studies may have analyzed the same ground cherry clones as such germplasm is limited in the Michigan State University collection. They analyzed four ground cherries and my study analyzed six.

Kaurisch et al. (1988) assayed a ground cherry genotype for 6-PGD and PGI. The six ground cherries assayed in my study were more heterozygous than the genotype they studied.

Their clone was homozygous for my *6-Pgd-1*^{100} and *Pgi-2*^{92} alleles and heterozygous for my *60* and *48* alleles at *6-Pgd-2*. The ground cherries in my study exhibited the *88* and *76* alleles at *6-Pgd-1*, the *28* allele at *6-Pgd-2*, and the *100* allele for *Pgi-2* as well as those present in the genotype studied by Kaurisch et al. (1988).

Fernqvist and Huntrieser (1988) presented 6-PGD polymorphisms for cultivated and wild sweet cherry. These polymorphisms generally agreed with my results in sweet cherry except that some of the patterns seem to be missing bands. Fernqvist and Huntrieser (1988) present some two-banded polymorphisms corresponding to my *100* and *94* and *54* and *48* bands for *6-Pgd-1* and *6-Pgd-2*, respectively, which are unlikely due to the dimeric nature of 6-PGD. Another pattern that they present for 'Fanal' sweet cherry is suspect due to its highly asymmetric pattern at *6-Pgd-1*.

Isozyme inheritance and diversity data presented by Santi et al. (1990) and Santi and Lemoine (1990) is not directly comparable to my study due to different electrophoretic methods. They used PAGE and isoelectric focusing. Also, expression of SKDH isozymes in their study was dependent on the physiological state of the clones. Banding patterns did not phenotypically vary with date of sampling in my study.

Kaurisch et al. (1991) studied IDH, LAP, 6-PGD, PGI, and PGM polymorphisms in 65 sweet cherry cultivars and 45 sour cherry cultivars. Their results were similar to those of my study with a few exceptions. The sour cherry

collection analyzed by me exhibited more diversity for *6-Pgd-1* and *6-Pgd-2*. This is probably due to two reasons: 1) the Michigan State University sour cherry germplasm collection is very diverse (Hillig and Iezzoni, 1988) and includes specimens collected throughout sour cherry's center of diversity, and 2) a large number of sour cherry genotypes, 66, were analyzed for 6-PGD in my study.

Kaurisch et al. (1991) presented a two-banded pattern for *6-Pgd-1* in sour cherry which corresponds to my *100* and *88* bands. Band *94* should be present also since inheritance data in Chapter I demonstrate that 6-PGD is dimeric at both loci in cherry. 6-PGD is also dimeric in other *Prunus* species.

For *Idh-2*, Kaurisch et al. (1991) found sweet cherry homozygotes for my *64* allele. I did not observe the $Idh\text{-}2^{64/64}$ genotype in sweet cherry. For *Lap-1*, they found sour cherries homozygous for my *100* allele, while I did not. I did find one sour cherry that was homozygous for $Lap\text{-}1^{95}$, while they did not observe this polymorphism. For *Pgm-2*, Kaurisch et al. (1991) found more polymorphisms for sweet and sour cherry probably because they assayed many more specimens. Their *Pgm-2* polymorphism 3 in sweet cherry appears to be the same as polymorphism 1 in my study. My polymorphism 1 was only found in sour and ground cherry genotypes.

PGI exhibited only one locus of activity in my study, while Kaurisch et al. (1988, 1991) identified two PGI loci for cherry. My diversity data for *Pgi-2* appears to be the

same as that presented by Kaurisch et al. (1988, 1991) for sweet and sour cherry. Cherry genotypes assayed by Kaurisch et al. (1988, 1991) were found to be monomorphic for one band at *Pgi-1*. Assuming only one-banded activity for *Pgi-1*, I may not have observed *Pgi-1* activity due to its comigration with the $Pgi\text{-}2^{100}$ band present in all cherry selections in my study. I used a different buffer system for PGI electrophoresis than did Kaurisch et al. (1988, 1991). Another possibility for the lack of *Pgi-1* activity in my study is that *Pgi-1* isozymes were inactive under my assay conditions.

Usually, the minimum number of loci encoding an enzyme and the subcellular compartmentalization of its isozymes are conserved in plants (Gottlieb, 1982). Studies involving other *Prunus* species (Table 5) also report two loci of activity for PGI.

Comparisons with Other *Prunus* Species

Isozyme polymorphisms were studied for 'Redhaven' peach for all enzyme systems except PX in order to make the cherry diversity data more easily comparable with studies in other *Prunus* species. Mowrey et al. (1990b) studied various species in the subgenus *Amygdalus* and included 'Redhaven' also. They studied all of the enzyme systems that I did and used the same gel and electrode buffers for IDH, LAP, 6-PGD, and SKDH.

'Redhaven' is homozygous for $Pgi\text{-}2^{100}$ and $Idh\text{-}2^{44}$ in my study which most likely correspond to the $Pgi\text{-}2^{1}$ and $Idh\text{-}1^{2}$

alleles of Mowrey et al. (1990b). In my study, 'Redhaven' exhibits LAP bands *105* and *83* which are not present in the cherry clones examined. These two bands correspond to their *Lap-1*^{1} and *Lap-2*^{3} alleles.

For MDH, 'Redhaven' was found to possess the *75*, *63*, *51*, and *35* bands, polymorphism 8, in my study. The first three bands correspond to the *Mdh-1*^{2} allele of Mowrey et al. (1990b) and the last band is from the mitochondria and corresponds to their monomorphic locus *Mdh-2*. In peach, homozygous genotypes for *Mdh-1* produce two homodimers and one heterodimer (Mowrey et al., 1990a).

'Redhaven' possesses PGM band *146* in my study. It most likely corresponds to *Pgm-2*^{2} in Mowrey et al. (1990b) as their *Pgm-1* alleles produce two-banded phenotypes. In my study 'Redhaven' exhibits *6-Pgd-1*$^{100/88}$ and putative allele *6-Pgd-2*^{72}. In their study 'Redhaven' exhibits *6-Pgd-1*^{2} and *6-Pgd-2*^{1}. *6-Pgd-1* is problematic because 'Redhaven' is heterozygous in my study and homozygous in theirs. Results for *6-Pgd-2* correspond in these two studies. 'Redhaven' exhibits polymorphism 12 and is homozygous for *Skdh-1*^{77} in my study. This corresponds to *Skdh-1*^{2} in Mowrey et al. (1990b). A standard such as 'Redhaven' peach should be included in all *Prunus* isozyme work to help eliminate uncertainties when comparing data from different studies.

Comparisons Between Sweet, Ground, and Sour Cherry

Sour cherry has been shown to exhibit fixed heterozygosity or segregation also consistent with digenic-

disomic inheritance at several isozyme marker loci (Chapter I). This confirms an allotetraploid origin for sour cherry. Olden and Nybom (1968) hypothesized that ground and sweet cherry are the parents of sour cherry. The diversity data for sour, sweet, and ground cherry do suggest that ground cherry is a parent of sour cherry but are inconclusive as to whether sweet cherry is the other parent. All alleles or bands found in ground cherry are expressed in sour cherry except *Lap-1*". Many of these ground cherry alleles and bands are not expressed in sweet cherry. However, all sweet cherry alleles or bands are not only expressed in sour cherry, but are also expressed in ground cherry. Because all discovered sweet cherry alleles or bands were also found in ground cherry, the diversity data does not clearly demonstrate that sweet cherry is a parent of sour cherry. The data does not discredit this hypothesis either.

Hancock and Iezzoni (1988) concluded that their data for MDH supported both sweet and ground cherry as parents of sour cherry. The unique bands they found for sweet and ground cherry were codominantly expressed in sour cherry. My diversity study surveyed a larger number of sweet and sour cherry genotypes than did Hancock and Iezzoni (1988) and at least as many ground cherry genotypes. My study found additional MDH polymorphism for sweet and ground cherry resulting in a lack of unique MDH bands for sweet cherry.

My diversity data may even suggest that some of the sweet cherry gene pool has been introgressed into ground

cherry. Interspecific hybridization could have occurred in sympatric ground cherry and sweet cherry populations resulting in allotetraploid sour cherry. Sour cherry could have then backcrossed with ground cherry, introgressing sweet cherry alleles into the ground cherry gene pool. Any of the alleles or bands that sweet and ground cherry share in common may be evidence for such a process. However, these shared alleles may have also existed in both species before sour cherry evolved.

Whichever the case, whether sweet cherry genes have been introgressed into ground cherry via sour cherry or the shared diversity existed in both species before the evolution of sour cherry, hybridizing surely and frequently occurs between sour and ground cherry. They have the same ploidy levels. There is no evidence of any reproductive isolation between sour and ground cherry; they can be crossed easily and this probably occurs frequently in nature. Both of the species share much of the diversity found in the enzyme systems studied. Multivariate statistics using morphological characteristics (Hillig and Iezzoni, 1988) and these enzyme systems were unable to separate sour and ground cherry graphically (Figure 6).

The reverse example of introgression, ground cherry alleles into sweet cherry, probably would occur much less frequently. Many of the progeny resulting from a sweet cherry (2n=2x) x sour cherry (2n=4x) cross would be expected to be triploid and therefore infertile due to abnormal segregation of chromosomes to their gametes. This is called

segregational hybrid sterility (Stebbins, 1977) and is one form of postzygotic reproductive isolation. Also, sweet cherry is monomorphic for *Lap-1*, *Pgm-2*, and *6-Pgd-2* and lacks many of the tetraploid alleles and bands for MDH, 6-PGD, SKDH, and PX demonstrating that such introgression into sweet cherry is probably limited. Santi and Lemoine (1990) do propose a possible slight introgression of sour cherry into three out of 286 wild sweet cherry clones.

Due to their system of self-incompatibility, diploid sweet cherries were expected to be highly heterozygous. However, the 26 diploid cherries exhibited no heterozygosity or polymorphism at three out of seven isozyme loci and only a small percentage exhibited heterozygosity at another locus. A much higher percentage of tetraploid sour and ground cherries were heterozygous at each locus except one, keeping in mind the low number of diploid cherries studied. My study, like many others (Lack and Kay, 1986; Roose and Gottlieb, 1976; Soltis and Rieseberg, 1986; Soltis and Soltis, 1989), demonstrates that polyploids can maintain higher levels of heterozygosity than their diploid relatives.

Soltis and Rieseberg (1986) state that unlike autopolyploids, allopolyploids maintain heterozygosity through fixed heterozygosity. While examples of fixed heterozygosity for most isozyme loci studied in allotetraploid sour cherry were found (Chapter I), other sour cherry selections did not exhibit fixed heterozygosity at these same loci. Therefore, fixed heterozygosity and

outcrossing together most likely maintain heterozygosity in allotetraploid sour cherry. Self-incompatibility commonly occurs in sour cherry (Lansari and Iezzoni, 1990) which would encourage outcrossing.

Tetraploid cherries exhibited more enzyme multiplicity than diploid cherries for all enzyme systems studied except PGI and IDH. Many studies have suggested that enzyme multiplicity and increased heterozygosity (Adams and Allard, 1977; Lack and Kay, 1986; Roose and Gottlieb, 1976; Soltis and Rieseberg, 1986; Soltis and Soltis, 1989) may provide polyploids with an adaptive advantage over diploid ancestors. In the case of sour cherry, interspecific hybridization with ground cherry, in addition to enzyme multiplicity and increased heterozygosity, may also confer such an advantage over diploid sweet cherry.

Comparisons Within Sour Cherry

PCO did not identify different clusters of sour cherry clones within the collection. The germplasm evaluated was sufficiently diverse by origin to be considered representative of the species. In contrast, Hillig and Iezzoni (1988) also using multivariate statistics, found that sour cherry is morphologically very diverse representing gradations between morphologies exhibited by sweet and ground cherry.

This lack of ordination indicates that sour cherry populations from which selections were made are relatively homogeneous for the diversity presented in my study. Gene

flow may exist between these sour cherry populations and also ground cherry populations resulting in the presence of this diversity throughout the center of diversity for sour cherry. Also, certain alleles or isozyme bands may be selectively neutral over environments and therefore are not more frequent in germplasm from different environments.

Examples of tri-allelism were found in sour cherry for *6-Pgd-1* and *6-Pgd-2* and possibly *Skdh-1*, PX, and MDH. No sour cherries were tetra-allelic for the enzyme loci studied. Self-compatible sour cherries do tolerate some inbreeding and may be more inbred than expected. Also, sweet cherry, an obligate outcrosser, frequently exhibited only one allele per locus. Therefore, the sweet cherry genome contributed to sour cherry may not be highly heterozygous, setting an upper limit of three alleles per locus.

APPENDICES

APPENDIX A

RAW DIVERSITY DATA

The raw data matrix of 36 genotypes x 44 isozymes presented in this appendix is a printout from the Similarity Computer Program (Appendix D). This is the raw binary data used to calculate the similarity matrix presented in Appendix B, and subsequently the PCO which is graphed in Figure 6.

The row of numbers across the top of each section represent individual genotypes for which binary data is presented in the columns directly beneath. The left-hand column of each section is the abbreviated enzyme band names. The first letter or number represents the enzyme system and the following numbers represent the particular band's relative mobility. The absence of a particular band for each genotype is represented by a '0,' while its presence is indicated by a '1.'

RAW DATA MATRIX

	1	2	3	4	5	6	7	8	9	10
G110	0	0	0	0	0	0	0	0	0	0
G105	0	0	0	0	0	0	0	0	0	0
G100	1	1	1	1	1	1	1	1	1	1
G91	0	1	0	1	0	1	0	0	1	1
G82	0	1	0	1	0	1	0	0	1	1
I100	1	1	1	1	1	1	1	1	1	1
I82	0	0	0	0	0	1	1	0	0	0
I64	0	0	0	0	0	1	1	0	0	0
M100	1	1	1	1	1	1	1	1	1	1
M95	0	0	0	0	0	0	0	0	0	0
M88	1	1	1	1	1	1	1	1	1	1
M81	0	0	0	0	0	0	0	0	0	0
M75	1	0	0	0	0	0	1	0	0	0
M637	1	1	1	1	1	1	1	1	1	1
M51	1	0	0	0	0	0	0	0	0	0
M43	0	0	0	0	0	0	0	0	0	0
M35	0	0	0	0	0	0	0	0	0	0
M28	0	0	0	0	0	0	0	0	0	0
6100	1	1	1	1	1	1	1	0	1	1
694	1	1	0	1	1	1	1	0	1	0
688	1	1	0	1	1	1	1	1	1	0
682	1	0	0	0	0	0	0	0	0	0
676	1	0	0	0	0	0	0	0	0	0
672	0	0	0	0	0	0	0	0	0	0
666	0	0	0	0	0	0	0	0	0	0
660	1	0	0	0	0	0	0	0	0	0
654	1	0	0	0	0	0	0	0	0	0
648	1	1	1	1	1	1	1	1	1	1
643	0	0	0	0	0	0	0	0	0	0
638	0	0	0	0	0	0	0	0	0	0
628	0	0	0	0	0	0	0	0	0	0
S120	0	0	0	0	0	0	0	0	0	0
S100	0	1	1	1	1	1	1	1	1	1
S95	0	0	1	0	0	1	1	0	0	0
S77	0	0	0	0	0	0	0	0	0	0
S71	1	0	0	0	0	0	0	0	0	0
L105	0	0	0	0	0	0	0	0	0	0
L100	1	1	1	1	1	1	1	1	1	1
L97	0	0	0	0	0	0	0	0	0	0
L95	1	0	0	0	0	0	0	0	0	0
L83	0	0	0	0	0	0	0	0	0	0
P146	0	0	0	0	0	0	0	0	0	0
P100	1	1	1	1	1	1	1	1	1	1
P75	1	0	0	0	0	0	0	0	0	0

	11	12	13	14	15	16	17	18	19	20
G110	0	0	0	0	0	0	0	0	0	0
G105	0	0	0	0	0	0	0	0	0	0
G100	1	1	1	1	1	1	1	1	1	1
G91	0	1	0	1	0	1	0	1	1	1
G82	0	1	0	1	0	1	0	1	1	1

I100	1	1	1	1	1	1	1	1	1	1
I82	0	0	0	0	0	0	0	0	0	0
I64	0	0	0	0	0	0	0	0	0	0
M100	1	1	1	1	1	1	1	1	1	1
M95	0	0	0	0	0	0	0	0	0	0
M88	1	1	1	1	1	1	1	1	1	1
M81	0	0	0	0	0	0	0	0	0	0
M75	0	0	0	0	0	0	0	0	0	0
M637	1	1	1	1	1	1	1	1	1	1
M51	0	0	0	0	0	0	0	0	0	0
M43	0	0	0	0	0	0	0	0	0	0
M35	0	0	0	0	0	0	0	0	0	0
M28	0	0	0	0	0	0	0	0	0	0
6100	1	1	0	1	1	1	1	0	1	1
694	0	0	0	1	0	1	0	0	0	1
688	0	0	1	1	0	1	0	1	0	1
682	0	0	0	0	0	0	0	0	0	0
676	0	0	0	0	0	0	0	0	0	0
672	0	0	0	0	0	0	0	0	0	0
666	0	0	0	0	0	0	0	0	0	0
660	0	0	0	0	0	0	0	0	0	0
654	0	0	0	0	0	0	0	0	0	0
648	1	1	1	1	1	1	1	1	1	1
643	0	0	0	0	0	0	0	0	0	0
638	0	0	0	0	0	0	0	0	0	0
628	0	0	0	0	0	0	0	0	0	0
S120	0	0	0	0	0	0	0	0	0	0
S100	1	1	1	1	1	1	1	1	1	1
S95	0	0	0	1	1	0	1	0	0	0
S77	0	0	0	0	0	0	0	0	0	0
S71	0	0	0	0	0	0	0	0	0	0
L105	0	0	0	0	0	0	0	0	0	0
L100	1	1	1	1	1	1	1	1	1	1
L97	0	0	0	0	0	0	0	0	0	0
L95	0	0	0	0	0	0	0	0	0	0
L83	0	0	0	0	0	0	0	0	0	0
P146	0	0	0	0	0	0	0	0	0	0
P100	1	1	1	1	1	1	1	1	1	1
P75	0	0	0	0	0	0	0	0	0	0

	21	22	23	24	25	26	27	28	29	30
G110	0	0	0	0	0	0	0	0	0	0
G105	0	0	0	0	0	0	0	0	0	0
G100	1	1	1	1	1	1	1	1	1	1
G91	0	1	1	1	1	0	1	0	0	1
G82	0	1	1	1	1	0	1	0	0	1
I100	1	1	1	1	1	1	1	1	1	1
I82	0	0	1	1	1	1	1	1	1	0
I64	0	0	1	1	1	1	1	1	1	0
M100	1	0	1	1	1	1	0	1	1	1
M95	0	1	0	0	0	0	1	0	0	0
M88	1	0	1	1	1	1	0	1	1	1
M81	0	0	0	0	0	0	0	0	0	0
M75	0	1	1	1	1	1	1	1	1	0
M637	1	1	1	1	1	1	1	1	1	1
M51	0	1	1	0	1	1	1	1	1	0
M43	0	0	0	0	0	0	1	0	0	0
M35	0	0	0	0	0	0	1	0	0	0

M28	0	0	0	0	0	0	1	0	0	0
6100	1	1	1	1	1	1	1	1	1	0
694	0	0	1	1	1	1	0	1	1	0
688	0	1	1	1	1	1	1	1	1	1
682	0	0	0	1	0	1	0	0	1	0
676	0	1	0	1	0	1	1	0	1	0
672	0	0	0	0	0	0	0	0	0	0
666	0	0	0	0	0	0	0	0	0	0
660	0	1	1	1	1	1	1	1	0	0
654	0	1	1	1	1	1	1	1	0	0
648	1	1	1	1	1	1	1	1	1	1
643	0	0	0	0	0	0	0	0	0	0
638	0	0	0	0	0	0	0	0	0	0
628	0	0	0	0	0	0	0	0	0	0
S120	0	0	0	0	0	0	0	0	0	0
S100	1	0	0	0	0	0	1	1	0	1
S95	0	1	1	1	1	1	0	1	1	1
S77	0	0	0	0	0	0	0	0	0	0
S71	0	1	1	1	1	1	1	1	1	1
L105	0	0	0	0	0	0	0	0	0	0
L100	1	1	1	1	1	1	1	1	1	1
L97	0	0	0	0	0	0	0	0	0	0
L95	0	1	1	1	1	1	1	1	1	1
L83	0	0	0	0	0	0	0	0	0	0
P146	0	0	0	0	0	0	0	0	0	0
P100	1	1	1	1	1	1	1	1	1	1
P75	0	1	1	1	1	1	1	1	1	1

	31	32	33	34	35	36
G110	0	0	0	0	0	0
G105	0	0	0	0	0	0
G100	1	1	1	1	1	1
G91	1	0	0	1	1	1
G82	1	0	0	1	1	1
I100	1	1	1	1	1	1
I82	0	0	1	1	1	1
I64	0	0	1	1	1	1
M100	1	1	1	0	0	0
M95	0	0	0	1	1	1
M88	1	1	1	0	0	0
M81	0	0	0	0	0	0
M75	1	1	1	1	1	1
M637	1	1	1	1	1	1
M51	1	1	1	1	1	1
M43	0	0	0	0	0	0
M35	0	0	0	0	0	0
M28	0	0	0	0	0	0
6100	1	1	1	1	1	1
694	0	1	0	0	0	1
688	0	1	1	1	1	1
682	0	0	0	0	0	1
676	0	0	1	1	1	1
672	0	0	0	0	0	0
666	0	0	0	0	0	0
660	1	1	1	1	1	1
654	1	1	1	1	1	1
648	1	1	1	1	1	1
643	0	0	0	0	1	0

638	0	0	0	0	1	0
628	0	0	0	0	1	0
S120	0	0	0	1	0	0
S100	1	0	0	0	0	1
S95	1	1	0	0	1	0
S77	0	0	0	0	0	0
S71	1	1	1	1	1	1
L105	0	0	0	0	0	0
L100	1	1	1	0	0	0
L97	0	0	0	0	0	1
L95	0	1	1	1	1	1
L83	0	0	0	0	0	0
P146	0	0	0	0	0	0
P100	1	1	1	0	0	0
P75	1	1	1	1	1	1

APPENDIX B

SIMILARITY MATRIX

The 36 x 36 similarity matrix presented in this appendix was calculated from the raw data matrix presented in Appendix A by the Similarity Computer Program (Appendix D). This similarity matrix was subjected to PCO and graphed (Figure 6).

The numbers in the top row and left column of each section refer to the individual genotypes being compared. A '0' in the matrix body means that the two genotypes being compared are completely different for the enzyme systems studied, a '1' means that they are the same, while numbers in between indicate increasing similarity with increasing size.

MARCZEWSKI AND STEINHAUS SIMILARITY MATRIX

	1	2	3	4	5	6	7	8	9	10
1	1									
2	.478	1								
3	.409	.667	1							
4	.478	1	.667	1						
5	.524	.857	.769	.857	1					
6	.423	.824	.647	.824	.706	1				
7	.5	.667	.688	.667	.75	.833	1			
8	.429	.714	.75	.714	.833	.588	.625	1		
9	.478	1	.667	1	.857	.824	.667	.714	1	
10	.391	.857	.769	.857	.714	.706	.556	.692	.857	1
11	.429	.714	.909	.714	.833	.588	.625	.818	.714	.833
12	.391	.857	.769	.857	.714	.706	.556	.692	.857	1
13	.429	.714	.75	.714	.833	.588	.625	1	.714	.692
14	.458	.933	.733	.933	.8	.882	.722	.667	.933	.8
15	.409	.667	1	.667	.769	.647	.688	.75	.667	.769
16	.478	1	.667	1	.857	.824	.667	.714	1	.857
17	.409	.667	1	.667	.769	.647	.688	.75	.667	.769
18	.391	.857	.643	.857	.714	.706	.556	.833	.857	.846
19	.391	.857	.769	.857	.714	.706	.556	.692	.857	1
20	.478	1	.667	1	.857	.824	.667	.714	1	.857
21	.429	.714	.909	.714	.833	.588	.625	.818	.714	.833
22	.667	.417	.348	.417	.333	.423	.385	.304	.417	.391
23	.72	.542	.417	.542	.458	.667	.625	.375	.542	.458
24	.76	.52	.4	.52	.44	.64	.6	.36	.52	.44
25	.72	.542	.417	.542	.458	.667	.625	.375	.542	.458

26	.87	.423	.417	.423	.458	.538	.625	.375	.423	.346
27	.552	.393	.286	.393	.321	.448	.414	.296	.393	.37
28	.75	.5	.5	.5	.545	.625	.727	.455	.5	.417
29	.783	.458	.455	.458	.5	.583	.682	.409	.458	.375
30	.5	.667	.588	.667	.556	.65	.524	.625	.667	.647
31	.625	.571	.579	.571	.476	.565	.522	.45	.571	.632
32	.857	.5	.5	.5	.55	.5	.591	.45	.5	.409
33	.818	.417	.409	.417	.455	.48	.565	.429	.417	.391
34	.538	.308	.192	.308	.231	.37	.333	.2	.308	.28
35	.483	.276	.214	.276	.207	.379	.345	.179	.276	.25
36	.593	.37	.214	.37	.296	.429	.393	.222	.37	.296

	11	12	13	14	15	16	17	18	19	20
1										
2										
3										
4										
5										
6										
7										
8										
9										
10										
11	1									
12	.833	1								
13	.818	.692	1							
14	.667	.8	.667	1						
15	.909	.769	.75	.733	1					
16	.714	.857	.714	.933	.667	1				
17	.909	.769	.75	.733	1	.667	1			

18	.692	.846	.833	.8	.643	.857	.643	1		
19	.833	1	.692	.8	.769	.857	.769	.846	1	
20	.714	.857	.714	.933	.667	1	.667	.857	.857	1
21	1	.833	.818	.667	.909	.714	.909	.692	.833	.714
22	.304	.391	.304	.458	.348	.417	.348	.391	.391	.417
23	.375	.458	.375	.583	.417	.542	.417	.458	.458	.542
24	.36	.44	.36	.56	.4	.52	.4	.44	.44	.52
25	.375	.458	.375	.583	.417	.542	.417	.458	.458	.542
26	.375	.346	.375	.462	.417	.423	.417	.346	.346	.423
27	.296	.37	.296	.379	.286	.393	.286	.37	.37	.393
28	.455	.417	.455	.542	.5	.5	.5	.417	.417	.5
29	.409	.375	.409	.5	.455	.458	.455	.375	.375	.458
30	.529	.647	.625	.722	.588	.667	.588	.75	.647	.667
31	.526	.632	.45	.619	.579	.571	.579	.55	.632	.571
32	.45	.409	.45	.545	.5	.5	.5	.409	.409	.5
33	.429	.391	.429	.4	.409	.417	.409	.391	.391	.417
34	.2	.28	.2	.296	.192	.308	.192	.28	.28	.308
35	.179	.25	.179	.31	.214	.276	.214	.25	.25	.276
36	.222	.296	.222	.357	.214	.37	.214	.296	.296	.37

	21	22	23	24	25	26	27	28	29	30
1										
2										
3										
4										
5										
6										
7										
8										

9										
10										
11										
12										
13										
14										
15										
16										
17										
18										
19										
20										
21	1									
22	.304	1								
23	.375	.72	1							
24	.36	.692	.88	1						
25	.375	.72	1	.88	1					
26	.375	.654	.84	.88	.84	1				
27	.296	.731	.655	.633	.655	.6	1			
28	.455	.615	.875	.769	.875	.875	.621	1		
29	.409	.577	.76	.8	.76	.913	.533	.792	1	
30	.529	.565	.625	.6	.625	.5	.464	.583	.542	1
31	.526	.696	.75	.654	.75	.615	.571	.708	.538	.667
32	.45	.696	.826	.72	.826	.826	.517	.864	.739	.591
33	.429	.667	.792	.76	.792	.87	.667	.826	.783	.5
34	.2	.739	.654	.63	.654	.593	.731	.556	.519	.385
35	.179	.72	.643	.621	.643	.586	.655	.552	.517	.393
36	.222	.654	.643	.679	.643	.643	.714	.607	.571	.393

	31	32	33	34	35	36

31	1					
32	.727	1				
33	.625	.773	1			
34	.5	.5	.667	1		
35	.5	.5	.593	.792	1	
36	.5	.5	.593	.792	.704	1

APPENDIX C

INHERITANCE COMPUTER PROGRAM

This computer program was written by the author using QuickBasic on an IBM® compatible 486 computer with a SVGA monitor. It can be easily modified to accommodate slower computers and those with other monitor types. The program was written to aid with calculating tetraploid disomic and tetrasomic genetic models, testing these models using the chi-square statistic, and double checking these calculations. The program will print a hard copy of the calculations if desired. Use the subprograms in the following order: Disomic or Tetrasomic Inheritance, Chi-Square Analysis, Print Data, and Erase Data.

When calculating disomic progeny classes and frequencies, the order of the four alleles is important. Segregation or fixed heterozygosity can be achieved with the Disomic Inheritance Subprogram based upon the allele order in the parental genotype. For example, the genotype *abab* will result in *aa*, *ab*, and *bb* gametes in a 1:2:1 ratio. However, if the order is *aabb*, the result will be only *ab* gametes (fixed heterozygosity).

```
DECLARE SUB title ()
DECLARE SUB cprint (mating$, diexp$(), ex!(), dichi!,
  obspro!, start!, para$, parb$, obs(), defree)
DECLARE SUB chi (diexp$(), difreq!(), start, obspro, dichi,
  ex(), obs(), defree)
DECLARE SUB tetragam (a$(), b$(), asort!(), bsort!(),
  atotgam$(), btotgam$(), atotfreq!(), btotfreq!(),
  acounter, bcounter, start, progeny$(), profreq!(),
  prosort!(), diexp$(), difreq(), mating$, para$, parb$,
  agam$(), bgam$(), afreq(), bfreq())
DECLARE SUB mate (atotgam$(), btotgam$(), atotfreq!(),
  btotfreq!(), asort!(), bsort!(), acounter!, bcounter!,
  progeny$(), profreq!(), prosort!(), diexp$(), difreq(),
  start)
DECLARE SUB digam (a$(), b$(), asort(), bsort(), atotgam$(),
  btotgam$(), atotfreq(), btotfreq(), acounter, bcounter,
  start, progeny$(), profreq!(), prosort!(), diexp$(),
  difreq(), mating$, para$, parb$, agam$(), bgam$(),
  afreq(), bfreq())
DECLARE SUB mogene (a$(), b$(), mating$, para$, parb$)
DECLARE SUB datagone (prosort(), agam$(), bgam$(),
  progeny$(), profreq(), diexp$(), difreq(), ex(), obs(),
  start, a$(), b$(), atotgam$(), btotgam$(), atotfreq(),
  btotfreq(), para$, parb$, asort(), bsort(), afreq(),
  bfreq())
DIM a$(10), b$(10), asort(10), bsort(10), atotgam$(10),
  btotgam$(10), afreq(10), bfreq(10), ex(36), obs(36)
DIM atotfreq(10), btotfreq(10), progeny$(100), profreq(100),
  prosort(100), agam$(10), bgam$(10), diexp$(100),
  difreq(100)
SCREEN 9
CLS 0
LOCATE 4, 1: PRINT "TETRAPLOID INHERITANCE"
LOCATE 7, 1: PRINT "By James A. Beaver"
LOCATE 24, 1
PRINT "Press 'enter' to continue.  Use this command"
  throughout the program."
LOCATE 10, 1: PRINT "This program calculates expected"
  progeny classes and frequencies from a cross,"
PRINT "compares these expected disomic and tetrasomic values"
  to your observed values,"
PRINT "and calculates chi-squared values for statistical"
  analysis of the results."
LOCATE 25, 1
INPUT cont$


12 CLS 0
PRINT : PRINT , "MAIN MENU"
LOCATE 5, 1
PRINT , "1", "Disomic Inheritance"
PRINT : PRINT , "2", "Tetrasomic Inheritance"
PRINT : PRINT , "3", "Chi-Square Analysis"
PRINT : PRINT , "4", "Print Data"
```

```
PRINT : PRINT , "5", "Erase Data"
PRINT : PRINT , "9", "Exit"
LOCATE 25, 5
INPUT "What is the number of your topic of interest"; topic
IF topic = 1 THEN CALL digam(a$(), b$(), asort(), bsort(),
  atotgam$(), btotgam$(), atotfreq(), btotfreq(), acounter,
  bcounter, start, progeny$(), profreq!(), prosort!(),
  diexp$(), difreq(), mating$, para$, parb$, agam$(),
  bgam$(), afreq(), bfreq())
IF topic = 2 THEN CALL tetragam(a$(), b$(), asort!(),
  bsort!(), atotgam$(), btotgam$(), atotfreq!(),
  btotfreq!(), acounter, bcounter, start, progeny$(),
  profreq!(), prosort!(), diexp$(), difreq(), mating$,
  para$, parb$, agam$(), bgam$(), afreq(), bfreq())
IF topic = 3 THEN CALL chi(diexp$(), difreq!(), start,
  obspro, dichi, ex(), obs(), defree)
IF topic = 4 THEN CALL cprint(mating$, diexp$(), ex!(),
  dichi!, obspro!, start!, para$, parb$, obs(), defree)
IF topic = 5 THEN CALL datagone(prosort(), agam$(), bgam$(),
  progeny$(), profreq(), diexp$(), difreq(), ex(), obs(),
  start, a$(), b$(), atotgam$(), btotgam$(), atotfreq(),
  btotfreq(), para$, parb$, asort(), bsort(), afreq(),
  bfreq())
IF topic = 9 THEN END
GOTO 12

END
```

```
SUB title

CLS 0
a = 1
b = 640
c = 350

FOR x = 1 TO 135
        LINE (1, a)-(640, a)
        LINE (b, 1)-(b, 350)
        LINE (640, c)-(1, c)
        LINE (a, 350)-(a, 1)
        a = a + 1
        b = b - 1
        c = c - 1
        mycolor = INT(x / 9)
        COLOR mycolor
        FOR y = 1 TO 50
        NEXT y
NEXT x

END SUB
```

```
SUB cprint (mating$, diexp$(), ex(), dichi, obspro, start,
  para$, parb$, obs(), defree)

CALL title
LOCATE 13, 35: PRINT "PRINT DATA"
LOCATE 25, 1
INPUT cont$
CLS 0
PRINT : IF mating$ = "s" THEN INPUT "Please enter the
  identification of the selfed parent.", id1$
IF mating$ <> "s" THEN PRINT "Please enter the
  identification of each of the two parents.  Press 'enter' after each."
IF mating$ <> "s" THEN INPUT id1$
IF mating$ <> "s" THEN INPUT id2$
IF mating$ = "" THEN PRINT "Please enter the genotype of
  each of the two parents.  Press 'enter' after each."
IF mating$ = "" THEN INPUT para$
IF mating$ = "" THEN INPUT parb$
PRINT : INPUT "What locus or enzyme system is being
  studied"; locus$
PRINT : PRINT : INPUT "Please be sure your printer is ready
  and then press enter.", pri$
LPRINT : LPRINT locus$
IF mating$ = "s" THEN LPRINT id1$, "selfed"
IF mating$ <> "s" THEN LPRINT id1$, "x", id2$
LPRINT para$, "x", parb$
LPRINT "Observations ="; dichi
LPRINT "_____
  _____"
LPRINT : LPRINT "Progeny classes and frequencies"
LPRINT : LPRINT : LPRINT "CLASS", , , "EXP", "OBS": LPRINT
x = start

DO UNTIL diexp$(x) = "0"
        IF diexp$(x) <> "1" AND diexp$(x) <> "0" THEN LPRINT
          diexp$(x)
        IF diexp$(x) <> "1" AND diexp$(x) <> "0" AND ex(x)
          <> 0 THEN LPRINT , , , ex(x), obs(x)
        x = x + 1
LOOP

LPRINT "_____
  _____"
IF start <> 1 AND start <> 5 THEN LPRINT : LPRINT
  "Chi-square ="; obspro
IF start = 1 THEN LPRINT "DISOMIC chi-square ="; obspro
IF start = 5 THEN LPRINT "TETRASOMIC chi-square ="; obspro
LPRINT "Degrees of freedom ="; defree
LPRINT : LPRINT : LPRINT
END SUB
```

```
SUB chi (diexp$(), difreq(), start, dichi, obspro, ex(),
  obs(), defree)

CALL title
LOCATE 13, 30: PRINT "CHI-SQUARE ANALYSIS"
LOCATE 25, 1
INPUT cont$
CLS 0
DIM term(36)
INPUT "How many progeny have you observed"; obspro
IF diexp$(start) <> "" THEN GOTO 180
INPUT "How many progeny classes do you expect"; exclass
PRINT "Enter each progeny class and its expected frequency
  in decimal."
PRINT "Press 'enter' after the progeny class and after its
  frequency."
PRINT

FOR x = 1 TO exclass
        INPUT "Class"; diexp$(x)
        INPUT "Frequency"; difreq(x)
        PRINT
NEXT x

defree = exclass - 1
exclass = exclass + 1
diexp$(exclass) = "0"
b = 1
CLS 0


180 PRINT : PRINT
IF b <> 1 THEN b = start
x = 1
y = 0

DO UNTIL diexp$(b) = "0"
        ex(b) = difreq(b) * obspro
        IF diexp$(b) <> "1" AND ex(b) <> 0 THEN PRINT ;
          TAB(x); b; SPC(2); diexp$(b); SPC(2); ex(b)
        x = x + 40
        IF diexp$(b) <> "" AND ex(b) <> 0 THEN y = y + 1
        b = b + 1
LOOP

defree = y - 1
VIEW PRINT 21 TO 25
INPUT "Would you like to sum any of the expected classes
  (y or n)"; m$
IF m$ = "n" THEN GOTO 251
```

```
232 INPUT "How many classes would you like to sum together";
  sum
defree = (defree - sum) + 1
PRINT : PRINT "Enter the numbers of the classes to be summed
  together.  Press enter after each class."
summer$ = ""
sumfreq = 0

FOR x = 1 TO sum
        INPUT sumclass
        IF x = 1 THEN y = sumclass
        summer$ = summer$ + diexp$(sumclass) + " "
        diexp$(sumclass) = "1"
        sumfreq = sumfreq + ex(sumclass)
        ex(sumclass) = 0
NEXT x

diexp$(y) = summer$
ex(y) = sumfreq
PRINT : INPUT "Would you like to sum another (y or n)"; m$
IF m$ = "y" THEN GOTO 232
CLS 0
x = start
PRINT : PRINT "EXPECTED PHENOTYPIC CLASSES AND FREQUENCIES"
PRINT : PRINT

DO UNTIL diexp$(x) = "0"
        IF diexp$(x) <> "1" AND ex(x) <> 0 THEN PRINT ;
          diexp$(x); TAB(65); ex(x)
        x = x + 1
LOOP


251 x = start
CLS 0
VIEW PRINT 21 TO 25
dichi = 0

DO UNTIL diexp$(x) = "0"
        IF diexp$(x) <> "1" AND ex(x) <> 0 THEN PRINT "Enter
          the observed frequency for progeny class", diexp$(x)
        IF diexp$(x) <> "1" AND ex(x) <> 0 THEN INPUT obs(x)
        IF diexp$(x) <> "1" AND ex(x) <> 0 AND defree <> 1
          THEN term(x) = CSNG(CSNG(obs(x) - ex!(x))) ^ 2 / ex!(x)
        IF diexp$(x) <> "1" AND ex(x) <> 0 AND defree = 1
          THEN term(x) = CSNG(CSNG(ABS(obs(x) - ex!(x)) - .5)) ^ 2 / ex!(x)
        IF diexp$(x) <> "1" AND ex(x) <> 0 THEN dichi = dichi + term(x)
        x = x + 1
        PRINT
LOOP

PRINT : PRINT
```

```
IF start = 1 THEN statement$ = " DISOMIC INHERITANCE"
IF start = 5 THEN statement$ = " TETRASOMIC INHERITANCE"
IF start <> 1 AND start <> 5 THEN statement$ = " the NULL
  HYPOTHESIS"
PRINT "The chi-square value for"; statement$; " is"; dichi
PRINT "Degrees of freedom ="; defree
LOCATE 25, 1
INPUT cont$
VIEW PRINT
CLS 0

END SUB
```

```
SUB tetragam (a$(), b$(), asort(), bsort(), atotgam$(),
  btotgam$(), atotfreq(), btotfreq(), acounter, bcounter,
  start, progeny$(), profreq!(), prosort!(), diexp$(),
  difreq(), mating$, para$, parb$, agam$(), bgam$(),
  afreq(), bfreq())

CALL title
LOCATE 13, 29: PRINT "TETRASOMIC INHERITANCE"
LOCATE 25, 1
INPUT cont$
CALL mogene(a$(), b$(), mating$, para$, parb$) 'Allows entry
  of genetic model for each parent.
CLS 0
PRINT : PRINT
agam$(5) = a$(1) + a$(2)
asort(5) = VAL(a$(1)) ^ 2 + VAL(a$(2)) ^ 2
afreq(5) = 1 / 6
bgam$(5) = b$(1) + b$(2)
bsort(5) = VAL(b$(1)) ^ 2 + VAL(b$(2)) ^ 2
bfreq(5) = 1 / 6
agam$(6) = a$(1) + a$(3)
asort(6) = VAL(a$(1)) ^ 2 + VAL(a$(3)) ^ 2
afreq(6) = 1 / 6
bgam$(6) = b$(1) + b$(3)
bsort(6) = VAL(b$(1)) ^ 2 + VAL(b$(3)) ^ 2
bfreq(6) = 1 / 6
agam$(7) = a$(1) + a$(4)
asort(7) = VAL(a$(1)) ^ 2 + VAL(a$(4)) ^ 2
afreq(7) = 1 / 6
bgam$(7) = b$(1) + b$(4)
bsort(7) = VAL(b$(1)) ^ 2 + VAL(b$(4)) ^ 2
bfreq(7) = 1 / 6
agam$(8) = a$(2) + a$(3)
asort(8) = VAL(a$(2)) ^ 2 + VAL(a$(3)) ^ 2
afreq(8) = 1 / 6
bgam$(8) = b$(2) + b$(3)
bsort(8) = VAL(b$(2)) ^ 2 + VAL(b$(3)) ^ 2
bfreq(8) = 1 / 6
agam$(9) = a$(2) + a$(4)
asort(9) = VAL(a$(2)) ^ 2 + VAL(a$(4)) ^ 2
afreq(9) = 1 / 6
bgam$(9) = b$(2) + b$(4)
bsort(9) = VAL(b$(2)) ^ 2 + VAL(b$(4)) ^ 2
bfreq(9) = 1 / 6
agam$(10) = a$(3) + a$(4)
asort(10) = VAL(a$(3)) ^ 2 + VAL(a$(4)) ^ 2
afreq(10) = 1 / 6
bgam$(10) = b$(3) + b$(4)
bsort(10) = VAL(b$(3)) ^ 2 + VAL(b$(4)) ^ 2
bfreq(10) = 1 / 6
n = 5
PRINT : PRINT "Tetrasomic gametic classes and frequencies of
  the first parent are"
```

```
FOR x = 5 TO 10
        atotgam$(x) = agam$(x)
        atotfreq(x) = afreq(x)
        btotgam$(x) = bgam$(x)
        btotfreq(x) = bfreq(x)
        FOR y = x + 1 TO 10
                IF asort(x) = asort(y) THEN agam$(y) = "0"
                IF asort(x) = asort(y) THEN atotfreq(x) =
                  atotfreq(x) + afreq(y)
                IF asort(x) = asort(y) THEN afreq(y) = 0
                IF bsort(x) = bsort(y) THEN bgam$(y) = "0"
                IF bsort(x) = bsort(y) THEN btotfreq(x) =
                  btotfreq(x) + bfreq(y)
                IF bsort(x) = bsort(y) THEN bfreq(y) = 0
        NEXT y
        IF atotgam$(x) <> "0" THEN PRINT
        IF atotgam$(x) <> "0" THEN PRINT atotgam$(x)
        IF atotfreq(x) <> 0 THEN PRINT atotfreq(x)
        IF atotgam$(x) <> "0" THEN acounter = x
NEXT x

LOCATE 25, 1
INPUT cont$
CLS 0
LOCATE 3, 1: PRINT "Tetrasomic gametic classes and
  frequencies of the second parent are"

FOR x = 5 TO 10
        IF btotgam$(x) <> "0" THEN PRINT
        IF btotgam$(x) <> "0" THEN PRINT btotgam$(x)
        IF btotfreq(x) <> 0 THEN PRINT btotfreq(x)
        IF btotgam$(x) <> "0" THEN bcounter = x
NEXT x

start = 5
LOCATE 25, 1
INPUT cont$
CLS 0
CALL mate(atotgam$(), btotgam$(), atotfreq!(), btotfreq!(),
  asort!(), bsort!(), acounter!, bcounter!, progeny$(),
  profreq!(), prosort!(), diexp$(), difreq(), start)

END SUB
```

```
SUB mate (atotgam$(), btotgam$(), atotfreq(), btotfreq(),
  asort(), bsort(), acounter, bcounter, progeny$(),
  profreq(), prosort(), diexp$(), difreq(), start)

REM Calculates progeny classes and frequencies.
z = start
PRINT : IF start = 1 THEN PRINT "The expected disomic
  progeny classes and frequencies are"
IF start = 5 THEN PRINT "The expected tetrasomic progeny
  classes and frequencies are"
PRINT : PRINT

FOR x = start TO acounter
        FOR y = start TO bcounter
                profreq(z) = atotfreq(x) * btotfreq(y)
                IF profreq(z) <> 0 THEN prosort(z) =
                  asort(x) + bsort(y)
                IF profreq(z) <> 0 THEN progeny$(z) =
                  atotgam$(x) + btotgam$(y)
                IF profreq(z) <> 0 THEN z = z + 1
        NEXT y
NEXT x

num = start

FOR x = start TO z
        FOR y = x + 1 TO z
                IF prosort(x) = prosort(y) THEN progeny$(y)
                  = "0"
                IF prosort(x) = prosort(y) THEN profreq(x) =
                  profreq(x) + profreq(y)
                IF prosort(x) = prosort(y) THEN profreq(y)
                  = 0
        NEXT y
        IF progeny$(x) <> "0" THEN diexp$(num) = progeny$(x)
        IF progeny$(x) <> "0" AND profreq(x) <> 0 THEN
          difreq(num) = profreq(x)
        PRINT diexp$(num): IF difreq(num) <> 0 THEN PRINT
          difreq(num): PRINT
        IF progeny$(x) <> "0" THEN num = num + 1
NEXT x

IF diexp$(num) <> "0" THEN num = num + 1
diexp$(num) = "0"
INPUT cont$

END SUB
```

```
SUB digam (a$(), b$(), asort(), bsort(), atotgam$(),
  btotgam$(), atotfreq(), btotfreq(), acounter,
  bcounter, start, progeny$(), profreq!(), prosort!(),
  diexp$(), difreq(), mating$, para$, parb$, agam$(),
  bgam$(), afreq(), bfreq())

CALL title
LOCATE 13, 30: PRINT "DISOMIC INHERITANCE"
LOCATE 25, 1
INPUT cont$
CALL mogene(a$(), b$(), mating$, para$, parb$)'Allows entry
  of genetic model for each parent.
CLS 0
LET agam$(1) = a$(1) + a$(3) 'Beginning of calculating
  parent 'a' gametes
LET asort(1) = VAL(a$(1)) ^ 2 + VAL(a$(3)) ^ 2
LET agam$(2) = a$(2) + a$(4)
LET asort(2) = VAL(a$(2)) ^ 2 + VAL(a$(4)) ^ 2
LET agam$(3) = a$(1) + a$(4)
LET asort(3) = VAL(a$(1)) ^ 2 + VAL(a$(4)) ^ 2
LET agam$(4) = a$(2) + a$(3)
LET asort(4) = VAL(a$(2)) ^ 2 + VAL(a$(3)) ^ 2

FOR w = 1 TO 4
        afreq(w) = .25
NEXT w

PRINT : PRINT "Disomic gametic classes and frequencies of
  the first parent are"
atotgam$(1) = agam$(1) 'Beginning of sorting 'a' gametes
atotfreq(1) = afreq(1)

FOR x = 2 TO 4
        IF asort(1) = asort(x) THEN atotfreq(1) = afreq(x) +
          atotfreq(1)
        IF asort(1) = asort(x) THEN agam$(x) = "0"
        IF asort(1) = asort(x) THEN afreq(x) = 0
NEXT x

PRINT : PRINT atotgam$(1): PRINT atotfreq(1): PRINT
acounter = 1
atotgam$(2) = agam$(2)
atotfreq(2) = afreq(2)

FOR y = 3 TO 4
        IF asort(2) = asort(y) THEN atotfreq(2) = afreq(y) +
          atotfreq(2)
        IF asort(2) = asort(y) THEN agam$(y) = "0"
        IF asort(2) = asort(y) THEN afreq(y) = 0
NEXT y

IF agam$(2) <> "0" THEN PRINT atotgam$(2)
IF agam$(2) <> "0" THEN PRINT atotfreq(2): PRINT
```

```
IF agam$(2) <> "0" THEN acounter = 2
atotgam$(3) = agam$(3)
atotfreq(3) = afreq(3)
IF asort(3) = asort(4) THEN atotfreq(3) = afreq(4) +
  atotfreq(3)
IF asort(3) = asort(4) THEN agam$(4) = "0"
IF asort(3) = asort(4) THEN afreq(4) = 0
IF agam$(3) <> "0" THEN PRINT atotgam$(3)
IF agam$(3) <> "0" THEN PRINT atotfreq(3): PRINT
IF agam$(3) <> "0" THEN acounter = 3
atotgam$(4) = agam$(4)
atotfreq(4) = afreq(4)
IF agam$(4) <> "0" THEN PRINT atotgam$(4)
IF agam$(4) <> "0" THEN PRINT atotfreq(4): PRINT
IF agam$(4) <> "0" THEN acounter = 4

LOCATE 25, 1
INPUT cont$
CLS 0

LET bgam$(1) = b$(1) + b$(3) 'Beginning of parent 'b' gamete
  calculation
LET bsort(1) = VAL(b$(1)) ^ 2 + VAL(b$(3)) ^ 2
LET bgam$(2) = b$(2) + b$(4)
LET bsort(2) = VAL(b$(2)) ^ 2 + VAL(b$(4)) ^ 2
LET bgam$(3) = b$(1) + b$(4)
LET bsort(3) = VAL(b$(1)) ^ 2 + VAL(b$(4)) ^ 2
LET bgam$(4) = b$(2) + b$(3)
LET bsort(4) = VAL(b$(2)) ^ 2 + VAL(b$(3)) ^ 2

FOR w = 1 TO 4
        bfreq(w) = .25
NEXT w

PRINT : PRINT
PRINT "Disomic gametic classes and frequencies of the second
  parent are"
btotgam$(1) = bgam$(1) 'Beginning of sorting b gametes
btotfreq(1) = bfreq(1)

FOR x = 2 TO 4
        IF bsort(1) = bsort(x) THEN btotfreq(1) = bfreq(x) +
          btotfreq(1)
        IF bsort(1) = bsort(x) THEN bgam$(x) = "0"
        IF bsort(1) = bsort(x) THEN bfreq(x) = 0
NEXT x

PRINT : PRINT btotgam$(1): PRINT btotfreq(1): PRINT
bcounter = 1
btotgam$(2) = bgam$(2)
btotfreq(2) = bfreq(2)
```

```
FOR y = 3 TO 4
        IF bsort(2) = bsort(y) THEN btotfreq(2) = bfreq(y) +
          btotfreq(2)
        IF bsort(2) = bsort(y) THEN bgam$(y) = "0"
        IF bsort(2) = bsort(y) THEN bfreq(y) = 0
NEXT y

IF bgam$(2) <> "0" THEN PRINT btotgam$(2)
IF bgam$(2) <> "0" THEN PRINT btotfreq(2): PRINT
IF bgam$(2) <> "0" THEN bcounter = 2
btotgam$(3) = bgam$(3)
btotfreq(3) = bfreq(3)
IF bsort(3) = bsort(4) THEN btotfreq(3) = bfreq(4) +
  btotfreq(3)
IF bsort(3) = bsort(4) THEN bgam$(4) = "0"
IF bsort(3) = bsort(4) THEN bfreq(4) = 0
IF bgam$(3) <> "0" THEN PRINT btotgam$(3)
IF bgam$(3) <> "0" THEN PRINT btotfreq(3): PRINT
IF bgam$(3) <> "0" THEN bcounter = 3
btotgam$(4) = bgam$(4)
btotfreq(4) = bfreq(4)
IF bgam$(4) <> "0" THEN PRINT btotgam$(4)
IF bgam$(4) <> "0" THEN PRINT btotfreq(4): PRINT
IF bgam$(4) <> "0" THEN bcounter = 4
start = 1
LOCATE 25, 1
INPUT cont$
CLS 0
CALL mate(atotgam$(), btotgam$(), atotfreq!(), btotfreq!(),
  asort!(), bsort!(), acounter!, bcounter!, progeny$(),
  profreq!(), prosort!(), diexp$(), difreq(), start)

END SUB
```

```
SUB mogene (a$(), b$(), mating$, para$, parb$) 'Allows entry
  of genetic model for each parent.

CLS 0
PRINT "Define the genetic model."
PRINT : INPUT "Did the progeny result from a self or a cross
  (s or c)"; mating$
PRINT : PRINT "Enter the genotype of the first parent.  Type"
  a number first and"
PRINT "then a letter to indicate an allele.  Always use the"
  same number with the"
PRINT "same letter to designate a unique allele.  Note the"
  following examples:"
PRINT "1A, 2B, 3C, and so on.  Press return after entering"
  each allele."
PRINT
para$ = ""

FOR x = 1 TO 4
        INPUT a$(x)
        IF mating$ = "s" THEN b$(x) = a$(x)
        para$ = para$ + a$(x)
NEXT x

IF mating$ = "s" THEN parb$ = para$
IF mating$ = "s" THEN GOTO 100
PRINT : PRINT "Enter the genotype of the second parent."
parb$ = ""

FOR x = 1 TO 4
        INPUT b$(x)
        parb$ = parb$ + b$(x)
NEXT x


100 PRINT "The genotype of the first parent is "; para$
PRINT
PRINT "The genotype of the second parent is "; parb$
LOCATE 25, 1
INPUT cont$
CLS 0

END SUB
```

```
SUB datagone (prosort(), agam$(), bgam$(), progeny$(),
  profreq(), diexp$(), difreq(), ex(), obs(), start, a$(),
  b$(), atotgam$(), btotgam$(), atotfreq(), btotfreq(),
  para$, parb$, asort(), bsort(), afreq(), bfreq())

CALL title
LOCATE 13, 35: PRINT "ERASE DATA"
LOCATE 25, 1
INPUT cont$
CLS 0
PRINT : PRINT :

FOR x = 1 TO 3
        LOCATE 3, 1
        SOUND 100, 12
        SOUND 250, 3
        SOUND 50, 6
        PRINT "WARNING:  THIS SUBPROGRAM ERASES YOUR DATA!"
        FOR y = 1 TO 100000
        NEXT y
        CLS 0
        FOR y = 1 TO 30
        NEXT y
NEXT x

PRINT : PRINT : PRINT "Would you like to erase your data
  (y or n)?  If you have not made"
PRINT "a hard copy of your data and would like to do so,
  press 'n'."
INPUT gone$
IF gone$ <> "y" THEN PRINT : PRINT "Your data is safe."
IF gone$ <> "y" THEN GOTO 559
ERASE a$, b$, asort, bsort, atotgam$, btotgam$, ex, obs,
  atotfreq, btotfreq, progeny$, profreq, prosort, agam$,
  bgam$, diexp$, difreq, afreq, bfreq
start = 0
para$ = "": parb$ = ""
PRINT : PRINT
PRINT "Your data has been erased."


559  LOCATE 25, 1
INPUT cont$
CLS 0

END SUB
```

APPENDIX D

SIMILARITY COMPUTER PROGRAM

I created this program by using QuickBasic to greatly modify code written by Angus et al. (1988). The program will run on IBM® and other compatible computers. It can be used to input binary data indicating the presence or absence of enzyme bands for each genotype; edit, append, save, and print raw data files; and calculate similarity matrices from the binary data. Similarity matrices can also be saved and printed.

Before entering data, the program asks for the number of enzyme bands studied, the four-character alphanumeric names of the enzyme bands, and the number of genotypes being studied. After data entry is complete, the program will create a matrix of similarity values calculated using the Marczewski and Steinhaus Similarity statistic (Angus et al., 1988).

```
DIM bandname$(1 TO 45), rawdata(1 TO 115, 1 TO 45)
DIM similar(1 TO 115, 1 TO 115)
REM Major modifications by James Beaver, January 12, 1991
CLS 0
LOCATE 10, 1: PRINT "Similarity program"
LOCATE 12, 1: PRINT "This program calculates Marczewski and"
  Steinhaus Similarity between genotypes."


100 LOCATE 19, 1: INPUT "Would you like to enter new data or
  recall data from a disk file (e or r)"; cont$
  IF cont$ = "r" THEN GOTO 300
LOCATE 21, 5: INPUT "How many individuals are you studying";
  indiv
LOCATE 22, 5: PRINT "What is the total number of different"
  enzyme bands that you have observed"
INPUT ; pronum
LOCATE 25, 1: INPUT "Please press 'enter' to continue.  Use
  this command throughout the program."; cont$
CLS 0
PRINT "Please label the bands.  Use four characters or less"
  for a name."
LOCATE 6, 1
VIEW PRINT 6 TO 23

FOR x = 1 TO pronum
       PRINT : PRINT x: INPUT bandname$(x)
NEXT x

VIEW PRINT
LOCATE 25, 1: INPUT cont$
counter = 1


249 CLS 0
PRINT "Please enter the protein band data for each"
  individual. Use '0' for absence of a band and '1' for
  presence of a band."

FOR x = counter TO indiv
        SOUND 300, 4: SOUND 150, 2
        LOCATE 4, 1: PRINT "Group "; x: PRINT : PRINT
        VIEW PRINT 6 TO 23
        FOR y = 1 TO pronum
                PRINT bandname$(y)
275             INPUT rawdata(x, y): PRINT
                IF rawdata(x, y) <> 0 AND rawdata(x, y) <> 1
                  THEN BEEP: GOTO 275
        NEXT y
        CLS
        VIEW PRINT
NEXT x
```

```
LOCATE 25, 1: INPUT ; cont$


299 CLS 0
INPUT "Would you like to create a disk file for your raw"
  "data (y or n)"; cont$
IF cont$ = "n" THEN GOTO 350
PRINT "Name of the raw data file to create or append"
  (x:xxx.bas)"; file$
var = FREEFILE
OPEN file$ FOR OUTPUT AS #var

FOR x = 1 TO indiv
        FOR y = 1 TO pronum
                WRITE #var, x, y, rawdata(x, y),
                  bandname$(y)
        NEXT y
NEXT x

GOTO 350


300 INPUT "Name of disk file of raw data to recall
  (x:xxx.bas)"; file$
var = FREEFILE
OPEN file$ FOR INPUT AS #var

DO UNTIL EOF(var)
        INPUT #var, x, y, rawdata(x, y), bandname$(y)
LOOP

CLOSE #var
counter = x + 1
pronum = y
INPUT "Would you like to add data on new individuals to the
  recalled file (y or n)"; cont$
IF cont$ <> "y" THEN indiv = x
IF cont$ <> "y" THEN GOTO 340
INPUT "How many more individuals are you studying"; add
indiv = x + add
GOTO 249


340 INPUT "Would you like to edit your data in the recalled
  file (y or n)"; ask$
IF ask$ <> "y" THEN GOTO 350

DO UNTIL stop$ = "n"
        INPUT "What is the location of the datum to be
          edited (x,y)"; x, y
        PRINT "Current value is ", rawdata(x, y)
        INPUT "Change value to "; new
        rawdata(x, y) = new
```

```
        INPUT "Would you like to edit another datum
          (y or n)"; stop$
LOOP

GOTO 299


350 CLS 0
INPUT "Would you like to make a hard copy of your raw data
  (y or n)"; cont$
IF cont$ = "n" THEN GOTO 400
j = 1
IF indiv < 10 THEN k = indiv
IF indiv > 9 THEN k = 10
w = 1
LPRINT : LPRINT : LPRINT "RAW DATA MATRIX"

DO
        top$ = "       "
        FOR z = j TO k
                IF z < 10 THEN top$ = top$ + "  " + STR$(z)
                  + "   "
                IF z > 9 AND z < 100 THEN top$ = top$ + " "
                  + STR$(z) + "   "
                IF z > 99 THEN top$ = top$ + STR$(z) + "   "
        NEXT z
        LPRINT : LPRINT
        LPRINT top$
        LPRINT
        FOR x = 1 TO pronum
                IF LEN(bandname$(x)) = 3 THEN bandname$(x) =
                  bandname$(x) + " "
                IF LEN(bandname$(x)) = 2 THEN bandname$(x) =
                  bandname$(x) + "  "
                IF LEN(bandname$(x)) = 1 THEN bandname$(x) =
                  bandname$(x) + "   "
                enter$ = bandname$(x) + "   "
                FOR y = j TO k
                        enter$ = enter$ + "  " +
                          STR$(rawdata(y, x)) + "   "
                NEXT y
                LPRINT enter$
        NEXT x
        j = j + 10
        m = k
        IF m + 10 <= indiv THEN k = k + 10
        IF m + 10 > indiv THEN k = indiv
        w = w + 1
LOOP UNTIL w > indiv / 10 + 1


400 CLS 0
PRINT : INPUT "Would you like to calculate Marczewski and"
  Steinhaus Similarity between individuals (y or n)"; cont$
```

```
IF cont$ = "n" THEN END
PRINT : PRINT "Calculating Marczewski and Steinhaus"
  Similarity between individuals"

FOR x = 1 TO indiv
        FOR y = 1 TO indiv
                IF x = y THEN similar(x, y) = 1: GOTO 500
                w = 0: a = 0: b = 0
                FOR m = 1 TO pronum
                        IF rawdata(x, m) <> 0 THEN a = a + 1
                        IF rawdata(y, m) <> 0 THEN b = b + 1
                        IF rawdata(x, m) <> 0 AND
                          rawdata(y, m) <> 0 THEN w = w + 1
                NEXT m
                IF w = 0 THEN similar(x, y) = 0: GOTO 500
                similar(x, y) = w / (a + b - w)
                similar(x, y) = INT((similar(x, y) + .0005)
                  * 1000) / 1000
500     NEXT y
NEXT x


CLS 0
INPUT "Would you like to create a similarity matrix disk
  file (y or n)"; disk$
IF disk$ = "n" THEN GOTO 600
INPUT "Name of similarity matrix file to create
  (x:xxx.bas)"; myfile$
var = FREEFILE
OPEN myfile$ FOR OUTPUT AS #var

FOR x = 1 TO indiv
        data$ = ""
        FOR y = 1 TO indiv
                data$ = data$ + STR$(similar(x, y))
        NEXT y
        WRITE #var, data$
NEXT x


600 PRINT
INPUT "Would you like to make a hard copy of your similarity
  matrix. "; cont$"
IF cont$ = "n" THEN END
x = 1
IF indiv < 10 THEN y = indiv
IF indiv > 9 THEN y = 10
z = 1
LPRINT : LPRINT : LPRINT : LPRINT "MARCZEWSKI AND STEINHAUS"
  SIMILARITY MATRIX"

DO
        head$ = "       "
        FOR I = x TO y - 1
```

```
                IF I < 10 THEN head$ = head$ + STR$(I) +
                  "    "
                IF I > 9 AND I < 100 THEN head$ = head$ +
                  STR$(I) + "    "
                IF I > 99 THEN head$ = head$ + STR$(I) +
                  "   "
        NEXT I
        head$ = head$ + STR$(y)
        LPRINT : LPRINT : LPRINT
        LPRINT head$: LPRINT
        FOR k = 1 TO indiv
                data$ = ""
                FOR j = x TO y
                        w$ = STR$(similar(j, k))
                        sp = 7 - LEN(w$)
                        in$ = ""
                        FOR q = 1 TO sp
                                in$ = in$ + " "
                        NEXT q
                        IF k >= j THEN data$ = data$ +
                          STR$(similar(j, k)) + in$
                NEXT j
                LPRINT
                IF k < 10 THEN LPRINT "  "; k; "  "; data$
                IF k > 9 AND k < 100 THEN LPRINT " "; k;
                  "  "; data$
                IF k > 99 THEN LPRINT k; "  "; data$
        NEXT k
        x = x + 10
        p = y
        IF p + 10 <= indiv THEN y = y + 10
        IF p + 10 > indiv THEN y = indiv
        z = z + 1
LOOP UNTIL z > indiv / 10 + 1

LPRINT : LPRINT : LPRINT
CLOSE
END
```

APPENDIX E

PRINCIPAL COORDINATE ANALYSIS COMPUTER PROGRAM

This program subjects values in similarity matrices to principal coordinate analysis (PCO). It was written by Dr. Carl Ramm of the Department of Forestry at Michigan State University. The program was run using SAS (SAS Institute, Inc., Cary, N.C.) on the IBM® mainframe at Michigan State University. Print out from the program includes the percent of genetic variation accounted for by each principal coordinate, PCO scores in the first eight dimensions (coordinates used for graphing), and two-dimensional graphs of all combinations of the first three dimensions as axes. However, the graphs should be recreated using other software, such as PlotIt®, that can produce graph axes of equal length.

```
CMS FILEDEF DATA1 DISK NSALL1 DATA A1;
DATA D1;
INFILE DATA1;
    INPUT PLOTID $ S1-S12;
RUN;
CMS FILEDEF DATA2 DISK NSALL2 DATA A1;
DATA D2;
INFILE DATA2;
    INPUT PLOTID $  S13-S24;
RUN;
CMS FILEDEF DATA3 DISK NSALL3 DATA A1;
DATA D3;
INFILE DATA3;
    INPUT PLOTID $ S25-S36;
RUN;
DATA FULLSET;
   MERGE D1 D2 D3;
   BY PLOTID;
PROC IML;
USE FULLSET;
READ ALL INTO A (|ROWNAME=PLOTID COLNAME=COLS|);
PRINT A [FORMAT=10.4];
NN = NROW(A);
 * ---------------  CONVERSION OPTION 1 -----------------------;
 * -------CHATFIELD & COLLINS PAGE 201 CHANGE SIMILARITY TO DISTANCE ;
 *      D = 1(1') - A SO HAVE ZERO DIAGONAL MATRIX                 ;
 *      THEN SQUARES DISTANCES (DIJ^2) AND RUN ANALYSIS AS USUAL   ;
 *        SONE = J(NN,NN,1);
 *        DIST = SONE - A;
 *        S = (-1/2) * (DIST # DIST);
 * ------------------------------------------------------------ ;
 * -------------- CONVERSION OPTION 2 ----------------------- ;
 * ------- DIGBY  & KEMPTION PAGE 83 ------------------------ ;
 *  IF AIJ = SIMILARITY 0<=AIJ<=1, USE A                       ;
        S = A;
 * ------------------------------------------------------------  ;
PRINT S [FORMAT=8.4] ;
RESET NONAME;
ONEN=J(NN,1,1);
I = DIAG(ONEN);
H = I - (1/NN)*ONEN*(ONEN');
*   ;
*               USE H TO DOUBLE-CENTER THE MATRIX  AT ORIGIN ;
*               RESULTS IN LOSS OF ONE DIMENSION             ;
*   ;
E = H * S * (H');
*   ;
  CALL EIGEN(D,L,E);
*   ;
*      SAS/IML PRODUCES ORTHONORMAL EIGEN VECTORS !!!         ;
*            L'L = I                                          ;
*      FORM THE MATRIX X = L *+ SQRT(D) WHERE L IS THE        ;
*      MATRIX OF EIGEN VECTORS, D THE DIAGONAL MATRIX         ;
*      OF EIGEN VALUES.  THE NEW COORDINATES FOR THE N        ;
*      POINTS ARE THE ROWS - REPEAT ROWS - OF X               ;
```

```
*            SEE KRZANOWSKI, PAGE 106 +                        ;
*      ( IF L'L = D THEN ROWS OF L ARE THE NEW COORDINATES     ;
*   ;
DP = 100 * D/(SUM(D));
RESET NAME;
PRINT "EIGEN VALUES  &  PERCENT VARIATION";
HVAR = D || DP;
HH = {"EIGEN VALUES"  "% VARIATION"};
PRINT HVAR (|COLNAME=HH    FORMAT=20.4|);
L = L[,1:4];
D = D[1:4,];
DD = DIAG(D);
DD = SQRT(DD);
PTSCORE = L*DD;
RESET NONAME;
PRINT "PCO SCORES IN FIRST FOUR DIMENSIONS";
PRINT PLOTID [FORMAT=10.4] PTSCORE [FORMAT=10.4];
RNAME = PLOTID;
R1=PTSCORE[,1];   R2=PTSCORE[,2];
R3=PTSCORE[,3];   R4=PTSCORE[,4];
RSCORE1=R1||R2;
RSCORE2=R2||R3;
 CALL PGRAF(RSCORE1,RNAME,"PCO1","PCO2","PRINCIPAL COORDINATE ANALYSIS");
 CALL PGRAF(RSCORE2,RNAME,"PCO2","PCO3","PRINCIPAL COORDINATE ANALYSIS");
RSCORE3=R1||R3;
 CALL PGRAF(RSCORE3,RNAME,"PCO1","PCO3","PRINCIPAL COORDINATE ANALYSIS");
```

LITERATURE CITED

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Adams, W. T., & Allard, R. W. (1977). Effect of polyploidy on phosphoglucose isomerase diversity in *Festuca microstachys*. Proc. Natl. Acad. Sci. USA, 74(4), 1652-1656.

Angus, R. A., Hardwick, Jr., S. W., & Cline, G. B. (1988). A computer program that calculates various similarity coefficients between electropherograms. J. Hered., 79(1), 74-75.

Arulsekar, S., & Parfitt, D.E. (1986). Isozyme analysis procedures for stone fruits, almond, grape, walnut, pistachio, and fig. HortScience, 21, 928-933.

Arulsekar, S., Parfitt, D. E., Beres, W., & Hansche, P. E. (1986). Genetics of malate dehydrogenase isozymes in the peach. J. Hered., 77, 49-51.

Arulsekar, S., Parfitt, D. E., & Kester, D. E. (1986). Comparison of isozyme variability in peach and almond cultivars. J. Hered., 77, 272-274.

Bailey, L. H., & Bailey, E. Z. (1976). Hortus third: A concise dictionary of plants cultivated in the United States and Canada. New York: Macmillan.

Byrne, D. H. (1989a). Electrophoretic variability in four diploid stone fruits. Acta Horticulturae, 254, 29-34.

Byrne, D. H. (1989b). Inheritance of five isozyme loci in apricot. HortScience, 24(6), 1015-1016.

Byrne, D. H., & Littleton, T. G. (1988). Electrophoretic characterization of diploid plums of the southeastern United States. J. Amer. Soc. Hort. Sci., 113(6), 918-924.

Byrne, D. H., & Littleton, T. G. (1989a). Characterization of isozyme variability in apricots. J. Amer. Soc. Hort. Sci., 114(4), 674-678.

Byrne, D. H., & Littleton, T. G. (1989b). Interspecific hybrid verification of plum x apricot hybrids via isozyme analyses. HortScience, 24(1), 132-134.

Cerezo, M., Socias i Company, R., & Arus, P. (1989). Identification of almond cultivars by pollen isoenzymes. J. Amer. Soc. Hort. Sci., 114(1), 164-169.

Chaparro, J. X., Durham, R. E., Moore, G. A., & Sherman, W. B. (1987). Use of isozyme techniques to identify peach x 'Nonpareil' almond hybrids. HortScience, 22(2), 300-302.

Clayton, J. W., & Tretiak, D. N. (1972). Amine-citrate buffers for pH control in starch gel electrophoresis. J. Fisheries Res. Board Can., 29, 1169-1172.

Durham, R. E., Moore, G. A., & Sherman, W. B. (1987). Isozyme banding patterns and their usefulness as genetic markers in peach. J. Amer. Soc. Hort. Sci., 112(6), 1013-1018.

Fernqvist, I., & Huntrieser. (1988). Sortidentifiering. In Verksamhetsberattelse 1986-1987 Report. Balsgard: Alnarp.

Fogle, H. W. (1975). Cherries. In J. Janick & J.N. Moore (Eds.), Advances in fruit breeding (pp. 348-366). West Lafayette, Indiana: Purdue University Press.

Galletta, G. J. (1959). Comparative breeding, morphological and cytological studies of polyploid sweet cherries (*Prunus avium* L.). Doctoral dissertation, University of California, Davis.

Gottlieb, L. D. (1982). Conservation and duplication of isozymes in plants. Science, 216, 373-380.

Hancock, A. M., & Iezzoni, A. F. (1988). Malate dehydrogenase isozyme patterns in seven *Prunus* species. HortScience, 23(2), 381-383.

Hauagge, R., Kester, D. E., Arulsekar, S., Parfitt, D. E., & Liu, L. (1987). Isozyme variation among California almond cultivars: II. Cultivar characterization and origins. J. Amer. Soc. Hort. Sci., 112(4), 693-698.

Hauagge, R., Kester, D. E., & Asay, R. A. (1987). Isozyme variation among California almond cultivars: I. Inheritance. J. Amer. Soc. Hort. Sci., 112(4), 687-693.

Hedrick, U. P. (1915). The cherries of New York. Albany, New York: J.B. Lyon.

Hillig, K. W., & Iezzoni, A. F. (1988). Multivariate analysis of a sour cherry germplasm collection. J. Amer. Soc. Hort. Sci., 113(6), 928-934.

Hruby, K. (1939). The cytology of the Duke cherries and their derivatives. J. Genet., 38, 125-131.

Iezzoni, A. F., & Hancock, A. M. (1984). A comparison of pollen size in sweet and sour cherry. HortScience, 19, 560-562.

Iezzoni, A. F., Hancock, A. M., & Krahl, K. (1989). Evolution of sour cherry. Clues from isozyme and chloroplast DNA polymorphisms. ASHS 1989 Annu. Mtg., Tulsa, OK, Prog. & Abstr., p. 132.

Kaurisch, P., Gruppe, W., & Kohler, W. (1988). Enzympolymorphismen bei kirschen (*Prunus* spp.) und Arthybriden (P. x spp.). Methode, ausgewahlte arten/sorten und unterlagen-reis-wechselwirkungen. Angew. Bot., 62, 41-52.

Kaurisch, P., Throll, M., Gruppe, W., & Kohler, W. (1991). Enzympolymorphismen bei suB-(*Prunus avium*) und sauerkirschen (*P. cerasus*): Kultursortendifferenzierung [Enzyme polymorphisms in sweet and sour cherries (*Prunus avium, P. cerasus*): Cultivar differentiation]. Angew. Bot., 65, 35-43.

Kolesnikova, A. F. (1975). Breeding and some biological characterisitcs of sour cherry in central Russia. Orel, USSR: Priokstoc izdatel'stvo.

Krebs, S. L., & Hancock, J. F. (1989). Tetrasomic inheritance of isoenzyme markers in the highbush blueberry, *Vaccinium corymbosum* L. Heredity, 63, 11-18.

Lack, A. J., & Kay, Q. O. N. (1986). Phosphoglucose isomerase (EC 5.3.1.9.) isozymes in diploid and tetraploid *Polygala* species: evidence for gene duplication and diversification. Heredity, 55, 111-118.

Lansari, A., & Iezzoni, A. (1990). A preliminary analysis of self-incompatibility in sour cherry. HortScience, 25, 1636-1638.

Messeguer, R., Arus, P., & Carrera, M. (1987). Identification of peach cultivars with pollen isozymes. Scientia Hort., 31, 107-117.

Mowrey, B. D., & Werner, D. J. (1990). Developmental specific isozyme expression in peach. HortScience, 25(2), 219-222.

Mowrey, B. D., Werner, D. J., & Byrne, D. H. (1990a). Inheritance of isocitrate dehydrogenase, malate dehydrogenase, and shikimate dehydrogenase in peach and peach x almond hybrids. J. Amer. Soc. Hort. Sci., 115(2), 312-319.

Mowrey, B. D., Werner, D. J., & Byrne, D. H. (1990b). Isozyme survey of various species of *Prunus* in the subgenus *Amygdalus*. Scientia Hort., 44, 251-260.

Olden E. J., & Nybom, N. (1968). On the origin of *Prunus cerasus* L. Hereditas, 59(18), 327-345.

Parfitt, D. E., Arulsekar, S., & Ramming, D. W. (1985). Identification of plum x peach hybrids by isoenzyme analysis. HortScience, 20(2), 246-248.

Raptopoulus, T. (1941). Chromosomes and fertility of cherries and their hybrids. J. Genet., 42, 91-113.

Roose, M. L., & Gottlieb, L. D. (1976). Genetic and biochemical consequences of polyploidy in *Tragopogon*. Evolution, 30, 818-830.

Santi, F., Lemoine, M., & Bruant, B. (1990). Genetic markers for *Prunus avium* L.: Inheritance and linkage of isozyme loci. Ann. Sci. For., 47, 131-139.

Santi, F., & Lemoine, M. (1990). Genetic markers for *Prunus avium* L.: 2. Clonal identifications and discrimination from *P. cerasus* and *P. cerasus* x *P. avium*. Ann. Sci. For., 47, 219-227.

Scandalios, J. G. (1969). Genetic control of multiple molecular forms of enzymes in plants: A review. Biochem. Genet., 3, 37-79.

Soltis, D. E., Haufler, C. H., Darrow, D. C., & Gastony, G. J. (1983). Starch gel electrophoresis of ferns: A compilation of grinding buffers, gel and electrode buffers, and staining schedules. Amer. Fern J., 73, 9-27.

Soltis, D. E., & Rieseberg, L. H. (1986). Autopolyploidy in *Tolmiea menziesii* (Saxifragaceae): Genetic insights from enzyme electrophoresis. Amer. J. Bot., 73, 310-318.

Soltis, D. E., & Soltis, P. S. (1989). Genetic consequences of autopolyploidy in *Tolmiea* (Saxifragaceae). Evolution, 43(3), 586-594.

Stebbins, G. L. (1977). Processes of organic evolution (3rd ed.). Englewood Cliffs, New Jersey: Prentice-Hall.

Stebbins, G. L. (1947). Types of polyploids: Their classification and significance. Adv. Genet., 1, 403-429.

Vallejos, E. (1983). Enzyme activity staining. In S. D. Tanksley & T. J. Orton (Eds.), Isozymes in plant genetics and breeding: Part A (pp. 469-516). New York: Elsevier.

Werner, D. J. (1992). Catalase polymorphism and inheritance in peach. HortScience, 27(1), 41-43.