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PRINCIPAL COMPONENT ANALYSIS AND ISOZYME ANALYSIS OF SWEET, SOUR, AND GROUND CHERRY GERMPLASM

by

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ABSTRACT

PRINCIPAL COMPONENT ANALYSIS AND ISOZYME ANALYSIS OF SWEET, SOUR, AND GROUND CHERRY GERMPLASM

By

Kimberly Howell Krahl

Principal component analysis (PCA) was employed to examine variation between sweet cherry (*Prunus avium*), sour cherry (*P. cerasus*), and ground cherry (*P. fruticosa*) and within twelve sour cherry families. Sweet and ground cherry, the putative progenitors of sour cherry, occupy extreme opposite positions on the PC scattergram. Sour cherry families occupy various intermediate positions. However, ground cherry shares the extreme negative position on PC1 with a sour cherry family. Thus, using the characters in this study, it was not possible to distinguish ground cherry from sour cherry. Continued gene flow between these two tetraploid species may account for this intergradation. The PCA reveals that a great range of morphological variants are available for selection within the MSU sour cherry germplasm collection.

An isozyme analysis was conducted to determine protocol and to investigate possible polymorphisms between and within sweet, sour, and ground cherry. Sufficient polymorphisms were revealed in several enzyme systems to be used as genetic markers in future studies.

For Christie: friend, therapist, mentor. The literal truth is that this would not have been possible without you. Thank you for being on my side all the times I couldn't be there. Thank you for loving me. I love you.

For little Fessie. My little buddy who came with me to graduate school. I miss you and I love you. See you sometime.

And for Frank, whose love and laughter have seen me through on a daily basis. I love you, G.

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CHAPTER ONE

Principal Component Analysis of Sweet, Sour and Ground Cherry

INTRODUCTION

Sour cherry is considered to have originated in Asia Minor and to have spread west to Europe and east into what is now the Soviet Union. Sour cherry has been grown for many centuries in Europe and Asia. The French cultivar 'Montmorency' is one of the oldest of cultivated varieties and was grown in Europe hundreds of years before there was any commercial fruit production in the United States. It was brought to this country during colonial times. Although Hedrick (1915) reported that 270 sour cherry cultivars were once available in this country, by 1915 'Montmorency' was well on its way to becoming essentially the only commercial variety grown. For many years U.S. sour cherry production has been a virtual monoculture of 'Montmorency', and breeding programs have produced only a few cultivars ('Northstar', 'Meteor') which are primarily of interest to homeowners rather than commercial growers. While 'Montmorency' has generally been a dependable producer, it still has quite a few shortcomings. In Michigan, the state producing approximately 80% of the U.S. sour cherry crop, 'Montmorency' yields are dramatically reduced one year out of three due to spring frost injury. The cultivar is susceptible to several diseases, requiring many protective sprays, and only about 30% of the flowers set fruit.

Sour cherry landraces and native cherry populations in Europe and the Soviet Union have provided a great deal of diversity for European breeding programs, and the resultant varieties exhibit some of this diversity. The sour cherry germplasm collection at Michigan State University, containing material collected in Yugoslavia, Bulgaria, Hungary, and Poland plus germplasm from western Europe and the Soviet Union, is much more diverse than collections previously available in this country, and our breeding program therefore has high potential to develop superior cultivars for commercial sour cherry production in the United States.

A previous study using multivariate analysis to examine diversity in this germplasm collection suggested that existing variations reflected gradations between supposed progenitors of sour cherry -- sweet cherry and ground cherry. In an attempt to test this hypothesis, the present study included representatives of sweet and ground cherry in addition to sour cherry families exhibiting a range of variation. The most obvious difference between sweet and ground cherry is gross morphology. From leaf size to ultimate height, sweet cherry is consistently larger than ground cherry. My purpose was to examine several characters affecting habit and size to describe the range of variability encompassed by the collection.

LITERATURE REVIEW

Cytology and Origin of Sour Cherry

Prunus cerasus L., sour cherry, is a member of the family Rosaceae, subgenus Cerasus, section Eucerasus. All *Prunus* species have a basic chromosome number of $x=8$. Sour cherry is a tetraploid with $2n=4x=32$ (Darlington, 1928; Kobel, 1927; Okabe, 1928). Kobel (1927) suggested that sweet cherry (*P. avium* L.), a diploid species with $2n=2x=16$, was one of the progenitors of sour cherry. He tentatively suggested either ground cherry, _P_. _fruticosa_ Pall., a tetraploid species (2n=4x=32), or _P_. _frutescens_ Schneid. as the other progenitor. Darlington (1928) stated, "it is not impossible" that sour cherry arose due to hybridization between sweet cherry and a tetraploid species such as ground cherry.

Although Raptopoulos (1941) concluded that sour cherry was an autotetraploid, cytologists Kobel (1927), Prywer (1936), Hruby (1950), Blasse (1957), and Barg (1958) independently reported it to be an allotetraploid. Hruby (1950) cited the low percentage of quadrivalents observed during meiosis as evidence of allotetraploidy. Examination of 250 pollen mother cells during meiosis revealed that 90% of the chromosomes paired as bivalents, while only 1.2% grouped as quadrivalents (Hruby, 1950). Kobel (1927), Prywer (1936), and Hruby (1950) concluded that sour cherry originated by hybridization of two ancestral diploid species which subsequently doubled. Hruby (1962) concluded from his cytological work on sweet and sour cherry and hybrids between the two that both species were of hybrid origin and that they shared one ancestor but not the second. He maintained that while sweet cherry arose as a result of hybridization between two similar genomes, sour cherry arose from sexual polyploidization between two dissimilar genomes.

Olden and Nybom (1968) attempted to test experimentally the hypothesis that sour cherry resulted from hybridization between sweet and ground cherry by crossing these two species. Both diploid and tetraploid sweet cherries were used, the tetraploid being produced through colchicine treatment of a wild-type sweet cherry. Only triploid seedlings were obtained using the diploid sweet cherry. These resembled ground cherry in growth habit and leaf shape. The hybrid tetraploid seedlings obtained by crossing ground cherry with the tetraploid sweet cherry were morphologically very similar to wild and cultivated sour cherries. They closely resembled sour cherry in such characters as leaf shape and size, and leaf margin serrations and were sexually compatible with sour cherry. Olden and Nybom (1968) reported that leaf phenolics in the "resynthesized" hybrid and naturally occurring sour cherry were quite similar in composition. They concluded that the morphological and chemical evidence, along with the fact that the natural distribution of sour cherry corresponds with the area where the natural ranges of sweet and ground cherry overlap in southeast Europe and southwest Asia, strongly supports the hypothesis that sour cherry did in fact originate as a hybrid between the diploid sweet cherry and the tetraploid ground cherry.

This hypothesis is also supported by evidence that unreduced pollen grains occur in sweet cherry (Hruby, 1939; Galetta, 1959; Iezzoni and Hancock, 1984). In addition, sour cherry exhibits codominant expression of the MDH isozyme banding patterns of sweet and ground cherry (Hancock and Iezzoni, 1988) and chloroplast RFLPs of several sour cherry cultivars are similar to those of ground cherry (A. Iezzoni, pers. comm.). Thus sour cherry probably resulted from hybridization between the ancestors of ground cherry as the maternal parent (contributing a normal, reduced gamete) and sweet cherry as the paternal parent (contributing an unreduced gamete).

de Candolle (1882) hypothesized that sweet and sour cherry had both originated in the area from the Caspian Sea to western Turkey. Both species subsequently spread westward to Europe, but not to the same extent. Sweet cherry "extended further and at an earlier epoch and has become better naturalized". de Candolle concluded that sour cherry probably arose from sweet cherry in prehistoric times. Present day evolutionary theory supports de Candolle's conclusion; i.e., the species with lower chromosome number is assumed to predate the species with higher chromosome number. Vavilov (1951) hypothesized that sweet cherry originated in the Near East center of origin (including the Near East and Asia Minor, Transcaucasia, Iran and the highlands of Turkmenistan). He suggested that the center of origin of sour cherry might be Asia Minor. Watkins (1976) reasoned that if ground cherry was an ancestor of sour cherry, then the sour cherry must have evolved before sweet and sour cherry spread from western Asia to Europe, because western and central Asia is the center of origin for ground cherry.

Kolesnikova (1975) divided sour cherry into two ecogeographic groups, Western European and Middle Russian, based on morphology and winter hardiness. The Western European group, which included some Duke cherries (sweet cherry x sour cherry), had better eating quality and lower winter-hardiness. The majority of these varieties resembled sweet cherry in growth habit (single-trunk tree form with larger leaves). The Middle Russian ecotype had poorer eating quality but improved winter hardiness. Many of these varieties resembled ground cherry in having a multiple-stemmed shrub form with smaller leaves.

Yushev (1975,1977) used various morphological characters of leaves and fruit to study 119 cultivars of sour cherry. He concluded that the most significant traits for classifying cultivars were leaf form and size, serrations, pubescence, and glands. He divided the sour cherry cultivars studied into two groups, Western European and Eastern European. The Western European group exhibited leaf characteristics typical of sweet cherry; seventy percent of the cultivars had large, obovate leaves with large double serrations, large glandules and persistent pubescence -- all characteristics typical of sweet cherry. Also, 46% of the large fruited cultivars were of the Western European type. Typically the fruit was heart-shaped with light colored juice and medium to large pits. The Western European group generally exhibited lower winter hardiness. The majority (56.5%) of the Eastern European group of cultivars had medium-sized ovate leaves with small double crenations -- characteristics typical of ground cherry. This group contained many cutivars with small to medium-sized pits, and exhibited higher levels of winter-hardiness. Yushev hypothesized that the similarity of leaf morphology of the Western European cultivars to sweet cherry indicated that sweet cherry played a large part in the origin of these varieties, whereas the similarity of leaf morphology of the Eastern European cultivars to ground cherry indicated that ground cherry participated widely in the origin of these varieties.

Applications of Multivariate Analysis

Multivariate analysis encompasses the statistical methods used to analyze data when multiple variables or characters are scored for each unit studied (Afifi and Clark, 1984). Various types of multivariate analysis have been used in recent decades for taxonomic purposes. Numerical analysis is used in an attempt to create taxonomic classification that is quantifiable, repeatable, and objective. Principal components analysis (PCA) and cluster analysis are types of multivariate analysis that are employed to reveal underlying structure in a multiple-variable data set. These methods require no prior knowledge of the origins of the units. Both analyses can be used to detect groups within the units. PCA tends to display the general pattern of phenetic diversity at the expense of detail, and is best employed to reveal relationships between groups. Conversely, cluster analysis most clearly illustrates detailed patterns of phenetic similarity at the expense of the overall pattern of diversity, and is best employed to examine relationships within groups or between units in different groups.

PCA was first used by Hotelling (1933) in educational testing. Since then PCA has been used in psychological, medical, and biological applications (Seal,1964; Morrison,1976). Using PCA, every unit to be studied is scored for a chosen number (n) of variables or characters. Each variable or character can be imagined as representing an axis in space. Each unit has a value on each variable axis. Since each unit represents a unique combination of variable "scores", every unit can be represented by a point in a multi-dimensional space which is defined by n coordinate axes. The original data create an ellipsoidal cloud of points (representing units) in n-dimensional space. PCA is used to simplify the description of this set of correlated variables. It is a method of transforming the original variables into new, uncorrelated variables. These new variables are called principal components. The major axis of the ellipsoid becomes principal component 1 (PC1). The second axis (PC2) is at right angles to the first. Each successive axis is orthogonal to all others. The number of original axes eqhals the number of principal components. Each PC is a linear function of unit scores for the original n variables which have been transformed such that the mean of each transformed variable is zero and the standard deviation is unity. The total variance represented by the original axes is preserved by the principal components. With standardization of the data and rotation of the original axes, the first axis becomes the major axis of the ellipsoidal cloud. This axis accounts for the greatest amount of total variance and is called PC1. Each successive PC is at right angles to all others and accounts for a decreasing amount of the total variance. Thus PCA simplifies the examination of a data set with multiple variables. Since the first few PCs account for a large proportion of the total variance, interpretation of the relationships between units usually can be based on just the first few PCs. Principal components are interpreted by examining the variables with high coefficients for that PC. A high coefficient for a variable on a PC is an indication of high correlation between that variable and the PC. In other words, a variable with a high coefficient on a particular PC makes a high contribution to the variance of that PC. Given a high coefficient of a variable on a PC, examination of that PC alone can provide a picture of a high proportion of the total variance for that particular variable.

PCA is best employed to depict overall diversity, phenetic diversity in particular. Phenetics is the classification of populations of organisms based on their relative phenotypic similarity without regard to evolutionary relationships. This similarity or non-similarity is assessed on the basis of many equally-weighted variables. In its strictest sense a PCA scattergram simply provides a picture of phenetic dispersion. The extent to which the PCA scattergram can also be interpreted as a measure of genetic dispersion is still open to debate. Jardine (1971) states, "phenetic classification does have an evolutionary basis because the observed phenetic dissimilarities of populations have been established by evolutionary divergence". Sneath and Sokal (1973) state, "constant evolutionary divergence leads to overall phenetic divergence, though at different rates in different lineages. Hence forms that are distantly related phylogenetically will in general be phenetically distant and *vice versa*".

Adams and Wiersma (1978) proposed a method of using the phenetic distance revealed in a PCA scattergram to calculate genetic similarity between units. The "genetic distance" between units is determined from the geometric (Euclidean) distance between the units. The more closely related two genotypes are genetically, the closer they should be on the scattergram. Adams (1977) applied this method to dry bean (Phaseolus vulgaris) cultivars of known pedigree. He was able to demonstrate that the geometric distances between variables varied with genetic distances. In typical understated fashion Adams noted, "the outcome suggests, but of course does not 'prove', that 'distances' based upon PC scores of cultivars have a substantial genetic implication".

Isleib and Wynne (1983) employed this method of estimating genetic distance to evaluate heterosis in crosses between a high yielding, adapted peanut breeding line and exotic peanut cultivars of diverse origin. Due to the uncertain subspecific botanical classification of some of the exotic parents, PCA was used to cluster the exotic parents into five distinct morphologically similar groups. The phenetic grouping largely agreed with accepted botanical classification. Isleib and Wynne used the geometric distance from the adapted peanut to each exotic parent as an estimate of genetic divergence between the two parents. For characters exhibiting dominance, heterosis in offspring increased with morphological divergence between the parents.

Multivariate analysis has often been employed to correlate phenetic variation with geographic and ecotypic origin. Hussaini et al. (1977) used PCA to study 640 genotypes from the world germplasm collection of finger millet (Eleusine coracana(L.) Gaertn.). The material separated into 12 broad groups basically identifiable by country of origin. The groups containing Indian genotypes exhibited a clinal variation, with southern material at one extreme and eastern material at the opposite extreme of the scattergram. Morishima (1969) utilized PC and cluster analysis to study variation within Oryza perennis Moench. The Asian form of O. perennis may be the progenitor of cultivated rice, O. sativa L. The strains of O. perennis are commonly classified into Asian, African, and American forms. Whether or not any of these forms should be classified as a separate species has been a matter of controversy. All of the 24 morphological characters measured varied continuously among strains. None of the geographical groups could be distinguished on the basis of a single character. PCA based on the 24 morphological characters separated the entries into three groups. The African group was distinct and separate, whereas Oceanian and Asian material clustered together and was overlapped by the American group. The Asian group displayed a clinal variation, with annual types at one extreme and perennial types at the other. In the American group the strains were arranged according to latitudinal distribution from Cuba at one extreme to the Amazon at the other. Adding data for interspecific F_1 sterility to the morphological data differentiated the Oceanian material from all other groups. The Asian, African, and American groups were distinct and separate, with no overlap.

Nevo et al. (1979) used PCA to study genetic diversity in wild barley (Hordeum spontaneum), the recognized progenitor of cultivated barley. Twenty-eight natural populations representing the entire ecological range in Israel were sampled to study genetic variation in these populations and its association with ecological variables. An electrophoretic survey of 28 gene loci (in 15 enzyme systems) revealed isozyme variation at 25 (=89%) of the loci. These data, along with measurements for 4 morphological characters of the barley spikelet, were used in the PCA. Nevo et al. found that the geographic patterns of variation displayed for isozymes and spikelet morphology were correlated with temperature and humidity. However, the variation for isozyme loci and spikelet morphology were largely uncorrelated. Nevo et al. point out that the low level of correlation between isozymes and spikelet morphology may indicate that the two have developed along different lines. The wide divergence in land races for spikelet morphology was not paralleled by similar isozyme diversity.

Martin and Adams (1987) measured 26 characters (including 5 phenological, 11 morphological, 5 agronomic, and 5 qualitative traits) for 375 lines from 15 landraces of dry bean (P.vulgaris) from northern Malawi. A PC scattergram revealed great variation in quantitative and qualitative traits. A clinal pattern was observed, with lines from northern areas clustering at one extreme and those from southern areas at the other. Whether this variation was due to environmental factors or to consumer preference could not be determined. Estimated "genetic distance" indicated greater between-area than within-area variability.

Rhodes et al. (1971) utilized PC and cluster analysis based on 67 quantitative and qualitative characters to study 38 cultivars representing the three races (Mexican, Guatemalan, West Indian) of avocado (*Persea americana* Mill.) and their interracial hybrids. The PC scattergram revealed that the three races formed separate and distinct groups with interracial hybrids intermediate between the two parental racial groups. Rhodes et al. concluded that PCA revealed a clearer picture of overall diversity while cluster analysis was more useful in defining relationships between individual units.

Challice and Westwood (1973) employed PCA to analyze relationships between 17 *Pyrus* species. They generated three analyses: one based solely on 29 chemical characters (leaf phenolics), another based solely on 22 botanical characters, and a third using all 51 characters. The scattergram based upon both the chemical and botanical characters best agreed with the known geographic distribution of the species. When either the chemical or botanical characters were used alone, the PC scattergram contained seriously misplaced species.

Jensen and Hancock (1982) performed multivariate analyses of three species of California strawberry (*Fragaria*) from 32 wild populations, and used PCA to classify the collection sites into six community types based on ten environmental variables. PC and cluster analysis based on 19 morphological characters suggested that the three *Fragaria* taxa were morphologically distinct. The differentiation between the three taxa was further verified by discriminant analysis. Discriminant analysis of all 254 individual plants resulted in 77% correct classification of individuals as to population of origin. However, 99.6% correct classification was attained in assigning individuals to the correct taxon. Discriminant analysis was also used to examine correlations between Fragaria populations and community types. The two Fragaria taxa studied revealed morphological variation that corresponded to population and community of origin.

Numerical taxonomic methods have also been employed to examine taxa above the species level. Sneath (1976) reviewed these studies, outlined difficulties and suggested certain stipulations to enhance the use of numerical taxonomic techniques in classifying higher ranking taxa. Prance et al. (1969) utilized PC and cluster analysis to examine genera in the family Chrysobalanaceae. They conducted a preliminary study of herbarium material of 254 species in the Hirtelleae tribe. This preliminary study prompted them to question previously accepted generic concepts. The diversity found in the genus Parinari, in particular, suggested splitting into several more clearly defined taxa. PC and cluster analysis conducted with data (21 quantitative and qualitative morphological characters) from 140 species in the Hirtelleae confirmed the heterogeneous nature of the genus. Further, most of the tentative groupings indicated by the herbarium study were confirmed by the taximetric analyses. Indecisive results were obtained for only two of the 140 species studied. As a result of the study five new genera were described by Prance.

Jensen (1977) utilized PC and cluster analysis to examine seven oak species representing three series of oaks. The study included five species in the Coccineae (scarlet oak) series plus two oak species whose relationships to the scarlet oaks has been debated for many years. The analysis, based on 36 quantitative and qualitative characters, indicated little basis for recognizing the three traditional series in the red and black oak subgenus Erythrobalanus. The phenetic differences between the species within the scarlet oak series were often greater than the differences between these species and the two species representing different series. Jensen pointed out that while these analyses are purely phenetic, the fact that various multivariate methods repeat basic patterns indicates that these patterns do indeed reflect evolutionary relationships.

To determine whether Cannabis is a monotypic genus or if more than one species should be recognized, Small et al. (1976) analyzed plants from 232 populations of wild and domesticated Cannabis for 47 characters using cluster and discriminant analysis. (Discriminant analysis is used to classify units into one of two or more previously defined and distinct groups.) Cluster analysis, which weights all characters equally without assuming any groupings, revealed no evidence of distinct groupings that might warrant consideration as separate species. Discriminant analysis was then used to group nonintoxicant and semi-intoxicant populations together for comparison with the intoxicant populations. Ninety-four percent correct classification of populations was achieved based solely on morphological characters. Further, the two groupings, the nonintoxicants and the semi-intoxicants, within the less intoxicant category could be distinguished with 75% accuracy. "Wild" plants could not be consistently distinguished from domesticated plants; rather variation appeared to continuous. Small et al. found that intoxicant plants could be distinguished from less intoxicant plants, when grown under standard conditions, by numerical analysis of many morphological characters of plants. However, these characters were much too affected by the environment for consistent identification of plants from various sources.

PCA has been employed to examine accepted phylogenetic relationships. It has also been utilized to examine interspecific hybrids of known and unknown parentage. Rhodes et al. (1968) employed several different methods of cluster analysis to examine the genus *Cucurbita*. The results were compared with accepted phylogenetic relationships within the genus. This genus displays a high degree of intraspecific uniformity and a large amount of interspecific variability. Twenty-one species of *Cucurbita* were scored for 93 morphological characters. Rhodes et al. decided that the cluster analysis derived from product-moment correlation coefficients most closely agreed with known cross compatibility, geographical distribution, and ecological adaption for the species studied. Bemus et al. (1970), in a follow-up study, augmented the original 21 *Cucurbita* species with 29 representatives of F_1 hybrids, cultivars, and unclassified accessions. Data from 93 quantitative and qualitative characters were used in a cluster analysis. The additional 29 units only slightly altered the original cluster patterns established with the *Cucurbita* species alone. The F_1 interspecific hybrids generally clustered with one of the parent species. However, two interspecific hybrids whose parents were widely divergent morphologically exhibited little phenetic similarity with either parent and thus did not cluster near either parent. F_1 hybrids between wild and cultivated species clustered near the wild parent. The cluster analysis resulted in a grouping of species that closely agreed with known genetic compatibility relationships.

Jensen and Estbaugh (1976a) studied three populations of red oak with narrow geographic distributions and low taxonomic diversity utilizing PC and cluster analysis to determine probable parentage of putative oak hybrids. Sixty-three quantitative and qualitative characters were scored. Each test population consisted of all the oaks found in a small area, or three to five oak species plus their putative hybrids. PCA was useful in determining probable parentage of naturally occurring putative hybrids. In another study, Jensen and Estbaugh (1976b) examined two oak populations with wide geographic distribution and high taxonomic diversity. One population consisted of ten taxa of red oak, the other of seven taxa, plus the putative hybrids in each population. They concluded that in populations with many potential parental taxa, especially when many are highly divergent, an estimation of the parentage of hybrids relying solely on multivariate techniques is difficult.

Heiser et al. (1965) employed cluster analysis to study Solanum species, artificial hybrids, autoploids and alloploids to test the efficacy of numerical taxonomic techniques with plants of known relationship. The 75 units studied represented 17 species, 4 autoploids, 6 alloploids, 19 hybrids, and one plant of unknown origin. Fifty-eight quantitative and qualitative characters were measured. The cluster analysis disagreed in several cases with orthodox taxonomic opinion about the position of some species. The interspecific hybrids tended to cluster close to one of their parent species. The autoploids clustered close to their diploid parent. The artificial alloploids were morphologically distinct from both parental species and therefore did not consistently cluster with either parent.

To date, only two PCAs have been done with the genus *Prunus*. Carter et al. (1983) used multivariate analysis to examine geographic variation and identify desirable seed sources for the important lumber species, *Prunus serotina* (black cherry), after Pitcher (1982) had concluded that phenotypic selection was ineffective. Seed was collected from 33 stands of black cherry throughout its natural range in the eastern United States. The resulting trees were evaluated at ten years of age for phenological and morphological traits. Cluster analysis based on leaf morphology clearly separated the southern from the central and northern provenance trees. The seven provenances identified as southern on the basis of leaf measurements included most of the provenances that had been placed in the southern grouping on the basis of leaf and flower phenology (Cech and Carter, 1979).

Hillig (1988) used PC and cluster analysis to examine a sour cherry germplasm collection. Morphological traits of leaves and fruits were employed to study 16 sour cherry cultivars plus hybrids and open-pollinated seedlings of Eastern European varieties. Russian cultivars were found to be morphologically diverse. The other cultivars plus the hybrids and open-pollinated seedlings tended to cluster by area of origin and known genetic relationship. PCA revealed that character states typical of sweet cherry and ground cherry (the putative progenitors of sour cherry) tended to fall at opposite ends of the PCs. Thus, the PCs were interpreted as representing gradations between sweet and ground cherry-like morphology.

MATERIALS AND METHODS

Plant Material

Pollen was collected in Spring 1983 from the following locations in eastern Europe: Fruit Research Institute, Cacak, Yugoslavia; Fruit Growing Research Institute, Plovdiv, Bulgaria; Research Institute for Pomology, Pitesti, Romania; Enterprise in Extension in Fruit Growing and Ornamentals, Budapest, Hungary; and the Research Institute of Pomology, Skierniewice, Poland. The pollen was brought back to Michigan and used in crosses with the sour cherry cultivars 'Montmorency', 'English Morello', and 'Wolynska', generating three half-sib families (Tables 1 and 2).

Additionally, two representatives of the presumed progenitor species of sour cherry were included in the study; *P. avium* 'Angela' and *P. fruticosa* (ground cherry) o.p. Self-pollinated 'Montmorency' seed was collected from a Michigan orchard.

All trees were planted in a completely randomized design at the Clarksville Horticultural Experiment Station, Clarksville, Michigan. The trees were three years old when evaluated in 1987.

Characters Measured

Data for sixteen vegetative characters were collected in August, 1987, after vegetative growth had ceased. Five leaves were collected at random from the mid-length of the current year's growth from each tree and evaluated for the following characters: leaf blade length and width, petiole length and width, (widths measured at mid-lengths), the number of swollen glands on the petiole and on the basal leaf edge, and vein angle (measured between the mid-vein and the secondary vein closest to the leaf blade mid-length). Pubescence on the lower leaf

Table 1. Pedigrees of parents of the sour cherry families.

Cultivar	Pedigree	Comments	References[1]
English Morello	unknown	an old cultivar, presumably German	3
Fructbare von Michurin	ground cherry x sour cherry (Stepnaya Samarskaya x Michurina Karlikovaya)	syn. Plodorodnaya Michurina	6,7,8
H 17/39	Vladimirskaya x Polevka	Romanian hybrid Polevka is an o.p. progeny of Ideal. Ideal is ground cherry (Stepnaya Dikaya) x *P. pennsylvanica*.	2,6,7
H 18/21	English Timpurii x Visin Tufa	Romanian hybrid English Timpurii is a natural hybrid between sweet and sour cherry.	2,5
M63	Pandy x Nagy Gobet	Hungarian hybrid Nagy Gobet syn. of Gros Gobet	4
Meteor Korai	Pandy 29 x Nagy Angol	Hungarian cultivar	1

Table 1 (cont'd.).

Cultivar	Pedigree	Comments	References[1]
Montmorency	unknown possibly a progeny of Cerise Hative or Cerise Commune	old French cultivar	3
Nefris	unknown	presumably Polish	4
Oblacinska	———	Carpathian landrace, mostly in Yugoslavia	4
Sumadinka	maternal parent is Fanal	Yugoslavian cultivar. Fanal (syn. Heimann's Conserve) is very similar to Nefris.	4
Wolynska	unknown	Polish cultivar	4

[1] Numerical code for references:

1. Apostol, J. (pers. comm. to A. Iezzoni)
2. Cociu and Gozob, 1985
3. Hedrick, 1915
4. Iezzoni, A. (pers. comm.)
5. Iezzoni, 1985
6. Michurin, 1949
7. Michurin, 1955
8. Trajkovski, V. (pers. comm.)

Table 2. Names, abbreviations, and progenies evaluated for the clone and each family.

Name	Abbreviation	No. of trees evaluated
P. avium Angela	A	3
P. fruticosa o.p.	F	3
Montmorency		
x H 18/21	R1	10
x H 17/39	R2	7
x Meteor Korai	MK	9
x M 63	H4	10
x Nefris	Nf	9
x Fructbare von Michurin	VM	4
x mixture	AV	6
x self	M	10
Wolynska		
x Sumadinka	L1	10
x Oblacinska	L2	8
English Morello		
x Sumadinka	E1	10
x Oblacinska	E2	10

surface on the midvein and secondary veins was subjectively rated from 0 to 4, with 0 = maximum of three trichomes per leaf, to 4 = very pubescent. Leaf area was measured utilizing a leaf area meter. Trunk diameter was measured with a caliper 8 centimeters from the soil line, once in May and again in September, and trunk diameter increase was calculated. Branch angle was measured for the first 5 lateral branches from the base of the tree. Current year's terminal growth was measured on 3 randomly chosen lateral branches. Internode length of the lateral branches was measured at mid-shoot of the current year's growth, and the number of leaves per lateral branch was recorded.

Data Analysis

Principal component analysis was performed using the PRINCOMP procedure of the SAS statistical package (1985a). Family means were used to create a correlation matrix from which standardized principal component scores were extracted. Scatter plots of the first 3 PCs were created with SAS/GRAPH (1985b). To determine which of the PCs accounted for the greatest amount of variation for each trait, the eigenvectors of the 3 PCs were compared for each trait. The trait being considered was ascribed to the PC having the largest value. The progressive increase or decrease of the clone or family means for each trait along a given PC was followed to assign a trend to the PC for the trait being considered.

RESULTS

The first 3 PCs account for, respectively, 62.9%, 12.6%, and 9.4% of the variance between means or 84.9% of the total variation (Table 3). The contribution of the various traits to the eigenvectors is

Table 3. Eigenvalues, proportions of variance, and cumulative variances from the principal component analysis of the cherry clones and families.

Principal component	Eigenvalue	Proportion of variance	Cumulative variance
1	10.079	0.629	0.629
2	2.018	0.126	0.756
3	1.496	0.094	0.849
4	0.987	0.062	0.911
5	0.489	0.030	0.942
6	0.365	0.022	0.965

listed in Table 4. Almost two-thirds of the total variance is accounted for by PC1. From plus to minus on PC1 the family means generally decrease for leaf length, leaf width, leaf area, petiole length, lateral length, internode length, and trunk diameter increase. From plus to minus along PC2 number of leaves per lateral, number of petiole glands tend to decrease while pubescence increases. On PC3 from plus to minus there is a general decrease in branch angle, serrations per centimeter, number of glands on the leaf blade basal edge, and trunk diameter increase.

P. avium 'Angela' is located at the positive extreme of PC1 and distant from the ground cherry and the sour cherry families (Figure 1). R1, the 'Montmorency' family with a Romanian cherry [sour cherry x (sour x sweet cherry)] as the paternal parent does not cluster with the other sour cherry families and is situated closer to 'Angela' than any other family. Ground cherry (P. fruticosa) is not separate from the sour cherry families, sharing the extreme negative position of PC1 with 'Wolynska' x 'Oblacinska' progeny. The 'Montmorency' family (VM) with the Russian cultivar Fructbare von Michurin (ground cherry x sour cherry) as the paternal parent is situated outside the sour cherry cluster at the extreme of PC2. However, its position on PC1 is within the sour cherry cluster.

'Angela' had the longest internode and lateral lengths and the largest leaf area, the greatest leaf length, leaf width, petiole length, vein angle, pubescence on both mid and secondary veins, and the lowest number of serrations per cm (Table 5). It was second to only one other family (VM) for the greatest number of swollen petiole glands and it had the lowest number of glands on the leaf blade edge. The

Table 4. Eigenvectors of the first 3 principal component axes from principal component analysis of the cherry clones and families.[z]

	Eigenvectors		
Character	PC1	PC2	PC3
Petiole length	0.304	-0.003	-0.041
Leaf length	0.309	-0.049	0.059
Leaf width	0.306	0.008	-0.028
Petiole width	0.252	0.210	-0.097
Vein angle	0.261	0.216	-0.186
Serrations per cm	-0.234	0.311	0.298
Petiole glands	0.167	0.487	0.166
Blade glands	-0.118	0.254	0.276
Pubescence (Mid-rib)	0.209	-0.405	0.327
Pubescence (Side-ribs)	0.240	-0.376	0.235
Branch angle	-0.120	-0.053	0.699
Leaf area	0.300	-0.127	-0.029
Trunk diameter increase	0.273	0.064	0.285
Lateral length	0.297	0.189	0.010
Leaves per lateral	0.204	0.374	0.149
Internode length	0.279	-0.060	0.025

[z] PC1, PC2, and PC3 account for 62.9%, 12.6% and 9.4% of variance between means, respectively.

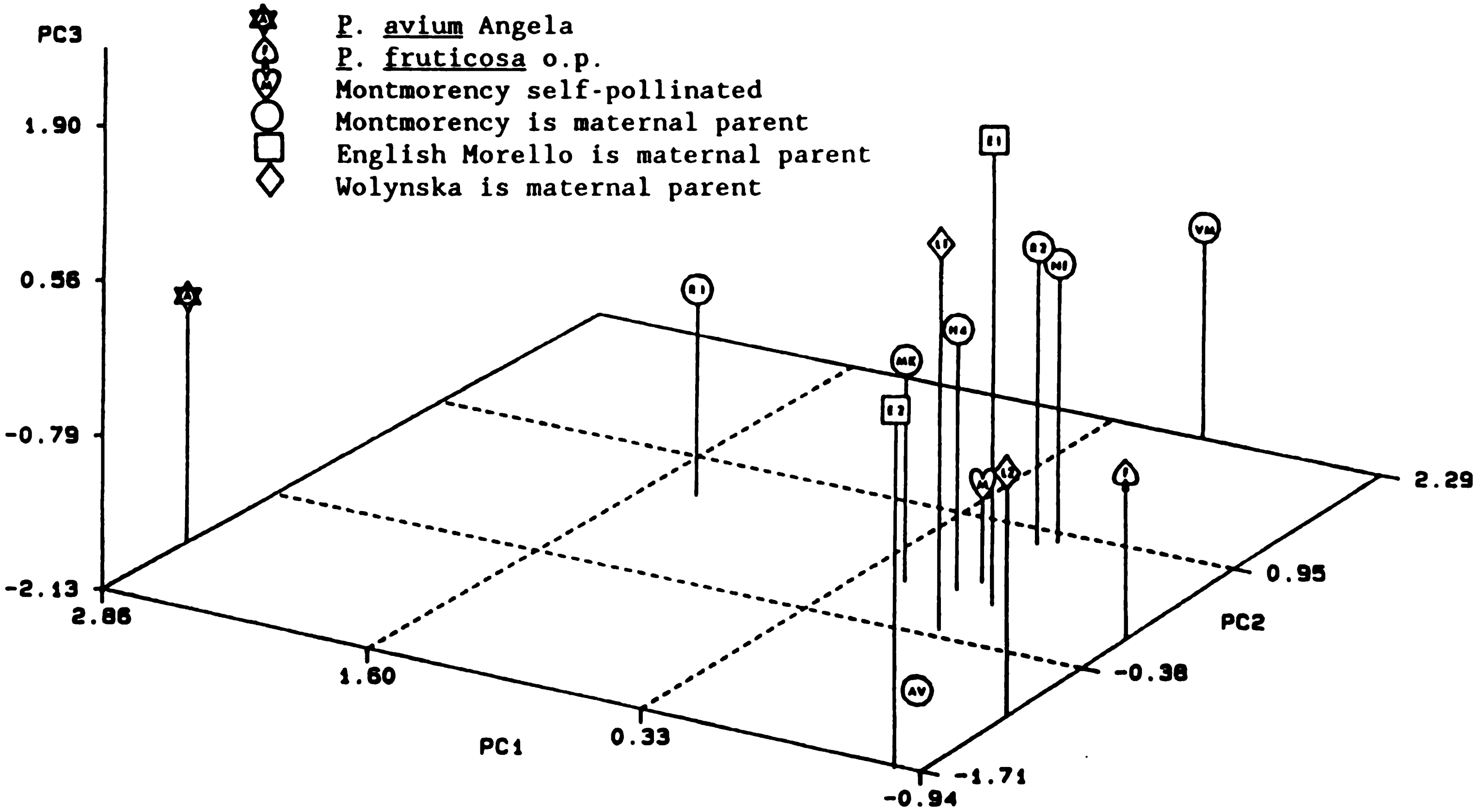

Figure 1. Positions of PC scores of sweet, sour and ground cherry family means on the first three PC axes. Abbreviations as in Table 2.

Table 5. Clone and family means and ranges of morphological characters. The range values are of the progeny means averaged over samples.

Clone/Family	Trunk diameter increase (mm)		Leaf area (cm^2)		Lateral length (cm)		Leaves per lateral		Internode length (cm)	
	$\bar{x}$	min-max	$\bar{x}$	min-max	$\bar{x}$	min-max	$\bar{x}$	min-max	$\bar{x}$	min-max
P. *avium* 'Angela'	20.4	17.2-23.9	68.2	66.1-71.8	57.9	40.6-86.1	25	19-33	3.6	3.3-3.8
P. *fruticosa* o.p.	8.2	6.0-11.1	23.5	16.1-30.4	19.8	13.7-24.6	15	12-20	2.3	2.0-2.5
Montmorency										
x H 18/21	50.0	7.1-69.9	40.7	12.0-60.1	50.0	7.1-69.9	26	7-40	2.3	2.0-2.5
x Meteor Korai	10.0	3.6-20.3	25.2	9.6-34.5	27.9	11.2-38.9	19	10-28	2.3	1.0-2.8
x M63	11.1	4.3-31.6	23.4	14.6-41.1	28.7	4.6-67.8	20	5-32	2.0	1.0-2.8
x H 17/39	11.0	1.1-20.2	23.1	6.8-37.1	31.2	3.6-49.0	23	5-37	1.8	1.3-2.0
x Nefris	8.5	0.9-22.2	22.1	9.5-33.2	30.5	7.6-65.3	24	10-40	1.8	1.3-2.3
x Fructbare von Michurin	11.4	8.8-14.3	19.5	13.0-28.9	35.3	20.3-48.3	23	19-25	2.3	1.8-2.5
x mixture	6.9	1.2-19.5	22.3	6.5-53.6	19.3	3.8-48.3	14	6-22	1.8	0.8-2.8
x self	7.1	1.5-12.7	21.0	6.6-35.8	21.3	3.6-35.1	17	6-24	2.0	1.3-3.1
Wolynska										
x Sumadinka	11.3	5.4-18.5	20.3	10.6-31.5	23.4	7.9-50.6	18	9-34	2.0	1.3-3.1
x Oblacinska	3.9	0-10.2	15.0	7.6-29.3	17.3	5.1-38.9	20	9-38	1.8	1.0-2.5
English Morello										
x Sumadinka	14.0	7.3-22.9	23.0	15.8-35.6	22.4	5.8-36.6	18	8-30	2.0	1.0-2.8
x Oblacinska	8.8	3.9-13.6	18.8	13.1-28.4	13.5	4.6-24.6	13	9-19.7	1.8	1.0-2.5

Table 5 (cont'd.).

Clone/Family	Petiole length (cm)		Leaf length (cm)		Leaf width (cm)		Petiole width (cm)	
	$\bar{x}$	min-max	$\bar{x}$	min-max	$\bar{x}$	min-max	$\bar{x}$	min-max
P. avium 'Angela'	2.8	2.5-3.1	14.3	13.2-15.6	7.2	6.9-7.5	57.3	55.9-58.7
P. fruticosa o.p.	1.2	0.9-1.5	6.3	6.2-6.3	3.7	3.4-4.2	33.1	31.5-35.8
Montmorency								
x H 18/21	2.0	1.2-2.7	10.2	4.8-12.7	6.4	3.7-8.2	62.9	29.0-74.9
x Meteor Korai	1.3	0.8-1.6	7.8	5.2-9.7	4.8	2.9-6.1	52.6	35.3-62.5
x M63	1.3	1.0-2.2	7.8	5.9-10.0	5.0	3.6-6.4	50.5	39.9-65.0
x H 17/39	1.5	0.7-2.1	7.3	3.7-9.1	4.5	2.4-5.4	45.8	25.7-56.1
x Nefris	1.5	1.0-2.0	7.9	5.3-9.8	4.4	3.3-5.4	48.0	40.6-55.1
x Fructbare von Michurin	1.3	0.8-1.7	7.8	7.1-8.8	4.5	4.1-4.8	47.3	39.1-56.1
x mixture	1.3	0.7-2.1	6.9	3.8-11.3	4.6	2.3-7.9	43.9	29.0-65.8
x self	1.7	0.8-2.4	7.8	3.9-11.0	4.4	2.6-5.6	50.7	29.5-65.3
Wolynska								
x Sumadinka	1.4	0.9-1.7	7.7	6.2-9.7	4.3	3.0-5.5	40.5	31.2-53.3
x Oblacinska	0.9	0.5-1.2	5.9	4.3-7.5	3.6	2.9-4.3	34.2	27.4-46.2
English Morello								
x Sumadinka	1.3	0.9-1.8	8.0	6.4-9.4	4.7	4.0-5.7	44.3	38.6-53.1
x Oblacinska	1.1	0.9-1.5	6.9	5.3-8.7	3.9	3.1-4.9	38.2	31.2-48.8

Table 5 (cont'd.).

Clone/Family	Vein Angle (deg)		Serrations per centimeter		Petiole Glands		Blade Glands	
	$\bar{x}$	min-max	$\bar{x}$	min-max	$\bar{x}$	min-max	$\bar{x}$	min-max
P. avium 'Angela'	63.2	60.6-66.9	6.3	5.8-6.8	0.7	0.2-1.2	0.7	0.4-0.8
P. fruticosa o.p.	56.3	53.6-58.4	10.2	9.2-10.8	0.1	0.0-0.4	1.9	1.8-2.0
Montmorency								
x H 18/21	63.1	55.5-68.7	7.9	5.8-9.6	0.4	0.0-0.8	1.4	0.4-2.0
x Meteor Korai	60.4	53.2-67.1	8.6	5.6-11.0	0.4	0.0-1.2	1.6	0.2-3.0
x M63	54.8	51.9-62.8	9.7	6.4-13.2	0.4	0.0-1.8	1.3	0.4-2.0
x H 17/39	57.8	49.5-62.0	9.8	7.6-12.0	0.6	0.0-2.0	0.8	0.0-1.4
x Nefris	57.8	52.2-64.8	10.0	8.4-12.6	0.5	0.0-1.0	1.4	0.2-2.4
x Fructbare von Michurin	59.6	50.9-65.4	10.2	10.0-10.4	0.8	0.0-1.6	2.0	0.4-2.8
x mixture	56.2	50.2-67.3	8.1	6.0-10.8	0.1	0.0-0.6	0.9	0.0-1.8
x self	57.8	44.8-64.8	9.0	6.4-11.4	0.6	0.0-1.6	1.1	0.2-2.0
Wolynska								
x Sumadinka	56.7	51.4-62.6	9.0	5.4-12.4	0.5	0.0-1.6	1.6	1.0-2.2
x Oblacinska	55.9	52.3-59.4	8.4	1.2-12.6	0.1	0.0-0.4	0.9	0.2-2.0
English Morello								
x Sumadinka	54.8	51.0-62.9	11.1	8.4-14.0	0.5	0.0-1.6	1.8	1.2-3.4
x Oblacinska	53.6	43.7-62.8	8.9	6.4-12.2	0.0	0.0-0.2	1.7	1.2-2.0

Table 5 (cont'd.).

Clone/Family	Pubescence on midrib vein $\bar{x}$	Pubescence on midrib vein min-max	Pubescence on siderib veins $\bar{x}$	Pubescence on siderib veins min-max	Branch angle (deg) $\bar{x}$	Branch angle (deg) min-max
P.avium 'Angela'	2.6	2.6-2.6	2.8	2.6-3.0	44.4	40.2-47.2
P. fruticosa o.p.	0.3	0.0-0.8	0.2	0.0-0.6	47.3	39.2-52.8
Montmorency						
x H 18/21	1.8	1.0-2.8	1.1	1.0-1.6	47.4	38.8-70.8
x Meteor Korai	1.3	0.0-2.0	1.0	0.0-1.8	51.2	38.0-58.2
x M63	1.2	0.4-2.6	0.9	0.0-1.6	50.4	38.0-64.6
x H 17/39	0.8	0.4-1.8	0.6	0.0-1.0	59.0	49.9-71.0
x Nefris	0.8	0.2-1.6	0.7	0.0-1.0	55.3	31.8-79.4
x Fructbare von Michurin	0.1	0.0-0.4	0.2	0.0-0.8	47.2	45.2-49.2
x mixture	0.7	0.0-2.6	0.5	0.0-1.2	42.2	33.2-49.0
x self	0.8	0.0-2.2	0.6	0.0-1.2	45.1	33.6-59.8
Wolynska						
x Sumadinka	1.6	0.8-2.4	0.9	0.6-1.2	60.3	37.8-76.2
x Oblacinska	1.3	0.0-2.4	1.0	0.0-2.2	54.8	44.1-63.0
English Morello						
x Sumadinka	1.5	0.8-2.0	1.1	0.6-1.6	59.6	47.6-78.7
x Oblacinska	1.6	1.0-2.4	1.3	0.6-2.0	58.7	41.8-70.8

family 'Montmorency' x H18/21 (R1) had the largest trunk diameter increase and number of leaves per lateral, and was second only to 'Angela' for leaf area, lateral length (Table 4), leaf length and width, and the least number of serrations per cm.

Ground cherry occupies the most extreme negative position on PC1 (Figure 1), sharing this position with one other family, 'Wolynska' x 'Oblacinska' (L2). Ground cherry is not the most extreme for most of the characters measured. Two sour cherry families have fewer leaves per lateral, 3 have a smaller trunk diameter increase and lateral length, 1 family has a shorter leaf length and width, and 3 have a much smaller leaf area. VM, the sour cherry family with a ground cherry grandparent, is positioned outside the general sour cherry cluster, at the extreme positive end of PC2. VM (with a ground cherry grandparent) and R2 (with a ground cherry great-grandparent) are located very close together on PC1.

The 'Wolynska' and 'English Morello' half-sib families both were crossed to the same paternal parents, 'Sumadinka' and 'Oblacinska'. Virtually all characters measured were smaller for the individuals in half-sib families with 'Oblacinska' as the paternal parent than for those in half-sib families with 'Sumadinka' as the paternal parent. In fact, the 'Wolynska' x 'Oblacinska' family represents the extreme low value among all the families studied for trunk diameter increase, leaf area, leaf length, leaf width, and petiole length. The 'English Morello' x 'Oblacinska' family represents the extreme low value among all families for lateral length, number of leaves per lateral, and leaf vein angle. 'Oblacinska' is a small statured selection of a Yugoslavian landrace.

Two 'Montmorency' half-sib families are worthy of comparison -- 'Montmorency' self-pollinated and 'Montmorency' cross-pollinated with a mixture of cherry pollen from Romania (AV). For many of the characters in Table 5, the means are similar; however, the 'Montmorency' selfed progeny exhibited a smaller range of values and is often missing the high or 'vigorous' end of the maximum values shown by the 'Montmorency' outcrossed family.

DISCUSSION

Previous PCA of a diverse sour cherry seedling collection (Hillig, 1988) suggested that the values of the individual PCs might represent gradations between character states typifying the putative progenitors of sour cherry -- ground cherry and sweet cherry. This study, with the inclusion of a sweet cherry cultivar and open-pollinated ground cherry, is an attempt to more clearly define the wide variation exhibited by sour cherry varieties. Additionally, this study includes measurements of characters affecting growth habit and size which vary greatly within the sour cherry collection.

Many morphological characters of sour cherry varieties are intermediate between ground cherry and sweet cherry. The most obvious difference between ground and sweet cherry is tree size. Sweet cherry forms a large, upright-pyramidal tree with a height reaching to 18 meters, with large, thin, coarsely serrate obovate-elliptic leaves (Bailey et al., 1976). The leaf is glabrous above and persistently pubescent below, with a long petiole and prominent glands. Fruit of cultivated varieties is large and sweet. Sweet cherry is the least winter-hardy of the three species.

Ground cherry forms a spreading, multi-stemmed shrub about one meter tall, with small, thick, finely serrate, elliptic leaves (Bailey et al., 1976). The leaf is glabrous above and below, and has a short petiole with few glands. The fruit is small to medium sized and acidic. It is the most winter hardy and drought resistant of the three species (Kolesnikova, 1975).

Sour cherry forms a shrub or small tree with medium-sized doubly serrate ovate to obovate leaves. The fruit is usually medium sized and tartly sweet (Bailey et al., 1976).

The sweet cherry 'Angela' was distinctly separate from the sour cherry families, occupying the extreme positive position on PC1, largely due to the high contributions of the following characters on PC1: leaf length and width, leaf area, petiole length, lateral length, internode length, and trunk diameter increase.

Although ground cherry occupies the extreme negative position on PC1, it shares this position with one sour cherry family ('Wolynska' x 'Oblacinska'), and is not distinct and separate from the other sour cherry families. In the Eastern European and Russian literature there is no clear delineation between sour cherry and ground cherry. Cultivars which are hybrids between sweet and sour cherry or sour and ground cherry, and cultivars which are large fruited selections of ground cherry are all considered to be "sour cherries". From the characters measured in this study a clear distinction cannot be made between ground cherry and sour cherry. Continued gene flow between these two tetraploid species might account for the overlap between the two. Kolesnikova (1975) has reported the occurrence of natural hybrids between sour and ground cherry in the middle Volga region of Russia.

C.D.Darlington (1928) stated, "if we endeavour, following the older systematists, to distinguish among the cherries three species, P. avium, P. cerasus, and P. fruticosa, we find that the last two merge into one another by imperceptible gradations".

A basic limitation in this study is that only one sweet cherry cultivar and open-pollinated progeny from three clones of ground cherry were used in the experiment. Obviously these few selections cannot exemplify the range of variation within either sweet or ground cherry. If a more diverse sweet cherry population were used some individuals might resemble sour cherry more closely than does 'Angela'. Similarly, the ground cherries used in this study may represent cultivated ground cherry clones rather than wild ground cherry types. Kolesnikova (1975) describes several different groupings of both sweet cherry and ground cherry based on morphological characteristics.

Although this study cannot begin to address the question of the variability to be found within either sweet cherry or ground cherry, it does indicate the potential within the sour cherry germplasm collection at MSU.

The sour cherry family (R1) with a sweet cherry great-grandparent is separate from the other sour cherry families and toward the "sweet cherry side" of the PC scattergram. R1 is second only to the sweet cherry 'Angela' for greatest leaf area, leaf length, leaf width, lateral length, petiole length, and vein angle. It slightly exceeds 'Angela' for the greatest number of leaves per lateral. This family's exceptionally high trunk diameter increase suggests that sour cherry clones may be selected that approach sweet cherry in size. Several of the sour cherry varieties in Hungary (e.g. Pandy, 'Ujfehertoi Furtos', and 'Erdi Nagygyumolcsu') approximate sweet cherry in tree size and are planted at distances of 8 x 5 meters (Kollar, 1987).

In contrast, numerous "low vigor" sour cherry cultivars exist, such as 'Oblacinska' from Yugoslavia and 'Ilva' from Romania. They are planted at distances of 4 x 2 m and 4 x 2.5 m, respectively (Iezzoni, 1984). Also, the dwarf sour cherry cultivar 'Kirsa' from Sweden attains a mature height of only one meter and is spaced one meter within the row (Iezzoni, pers. comm.). The two sour cherry families with 'Oblacinska' as a paternal parent indicate that sour cherry clones may be selected that approach the diminutive size of ground cherry. In Sweden, meter-high dwarf sour cherry clones are presently being tested in high density plantings. These dwarf sour cherries could be harvested with an over-the-row harvester. Pruning and spraying procedures would also be simplified. Such dwarf clones might also be used in the United States. The dwarf clones could also prove to be valuable as dwarfing rootstocks for sour cherry. Pick-your-own operations and homeowners would undoubtedly welcome bush-type sour cherries. The homeowner could grow a hedge of sour cherries which, in addition to providing ornamental value (cherry blossoms in spring, red fruit in summer, and glossy green leaves all season) and edible fruit, could be much more easily protected from bird predation than the standard size sour cherry tree.

Because sour cherry is a naturally outcrossing species, inbreeding would be expected to reduce seedling vigor. The similarity between the progeny means from 'Montmorency' self-pollinated and 'Montmorency' outcrossed suggests that inbreeding depression does not have a major effect in the S1 generation for the vigor characters measured in our study. However, individuals in the 'Montmorency' outcrossed progeny, were more vigorous than those in the 'Montmorency' self-pollinated progeny.

CHAPTER TWO

Isozyme Analysis of Sweet, Sour and Ground Cherry

INTRODUCTION

Isozymes have been widely employed to detect variability in plant populations. The use of isozyme analysis enables the plant breeder to screen plants at the seedling stage, thus conserving limited resources, including time, money, land, and labor. A previous isozyme study of cherry (Hancock and Iezzoni, 1988) revealed that MDH exhibited enough polymorphisms to be used to identify interspecific cherry hybrids. Codominant expression of MDH banding patterns of sweet cherry and ground cherry in sour cherry provided additional evidence that sweet and ground cherry (or, more accurately, the ancestral forms of these two species) were the progenitors of sour cherry. The present study was initiated to determine if other enzyme systems could be utilized for the study of diversity and inheritance within and between the three species, sweet cherry, ground cherry, and sour cherry.

LITERATURE REVIEW

Applications of Isozyme Analysis

Isozymes, or isoenzymes, are the multiple molecular forms of an enzyme occurring within a single organism and having similar or identical catalytic activities (Scandlios, 1969). Isozyme heterogeneity was first reported for esterase and lactate dehydrogenase in 1957 (Hunter and Markert).

Starch gel electrophoresis is proven an efficient method to separate enzymes into their different molecular forms. Electrophoresis is fundamentally forced diffusion through an electrical field. Proteins such as isozymes migrate at rates proportional to their net charges and molecular weights, resulting in separation into bands which can be revealed through staining.

Isozymes have proven to be extremely valuable biochemical markers. The genetic control of isozyme polymorphism is chiefly monogenic (Peirce and Brewbaker, 1973) and isozymes are often codominant in inheritance (Moore and Collins, 1983).

Isozymes are being used in all areas of plant biology,including identification of cultivars (Weeden and Lamb, 1985; Byrne and Littleton, 1988; Hauagge et al., 1987; 1988; Bassiri, 1976; Blogg and Imrie, 1982; Salinas et al., 1982; Weeden, 1984), species (Arulsekar et al., 1985; Hancock and Iezzoni, 1988; Moore and Litz, 1984; Arulsekar et al., 1986b), and interspecific hybrids (Byrne and Littleton, 1989; Chaparro et al., 1989; Parfitt et al., 1985; Chaparro et al., 1987). Isozymes have been used to estimate the degree of outbreeding or inbreeding in populations (Allard et al., 1971; Brown and Allard, 1970), as well as genetic, taxonomic, and evolutionary relationships between and within plant populations (Gottlieb, 1981; Hamrick, 1983; Ladizinsky and Hymowitz, 1979; Crawford, 1983; Hancock and Bringhurst, 1981).

In Prunus several isozyme analyses have been conducted. Arulsekar et al. (1986a) analyzed 290 peach cultivars for ten enzyme systems, including aspartate amino transferase (AAT), acid phosphatase (AcP), esterase (Est), malate dehydrogenase (MDH), phosphoglucomutase (PGM), leucine aminopeptidase (LAP), alcohol dehydrogenase (ADH), shikimate dehydrogenase (SkDH), and 6-phosphogluconate dehydrogenase (6-PGD).

Malate dehydrogenase (MDH) was the only enzyme that showed polymorphisms.

MDH displayed three distinct banding patterns in the 290 cultivars analyzed. The F_2 seedlings segregated for the three banding patterns in a 1:2:1 ratio. This is consistent with a codominant, single locus, Mendelian segregation. Segregation was not observed for four of the bands. Therefore, the exact number of loci involved could not be determined. Haploid plants derived from the heterozygous parents exhibited only homodimeric banding patterns, suggesting that heterodimers are formed between the products of the two alleles. MDH functions as a dimeric enzyme in many plant species (Yang et al., 1977; Sari Gorla et al., 1986; Orton, 1983; Morgan and Bell, 1983; Torres and Bergh, 1980). The formation of two interaction bands in the heterozygote in peach indicates that MDH has a polymeric subunit structure in peach. Arulsekar et al. (1986a) concluded that MDH is a useful biochemical marker in peach because the multiple banded types show simple Mendelian inheritance.

In contrast to peach, 87 almond cultivars exhibited polymorphisms in the enzyme systems AAT, LAP, PGM, MDH, 6-PGD, and PGI (Arulsekar et al., 1986b). Although only 1/3 as many almond cultivars were analyzed, they exhibited 11 times the variability found within the 290 peach cultivars. Because the peach cultivars chosen for the study exhibited much morphological variability, Arulsekar et al. (1986b) concluded that morphological variability does not necessarily ensure isozyme variability. Arulsekar et al. (1986b) point out that while peach is predominantly self-fertile, almond is a self-incompatible, outcrossing species. This could account for the vast difference in isozyme variability between the two species.

Durham et al. (1987) examined 38 enzyme systems in 59 peach cultivars. Twelve of the enzyme systems produced well-resolved banding patterns. Of these twelve, nine were monomorphic and only three [diaphorase (DIA), malate dehydrogenase (MDH), and peroxidase (PX)] were polymorphic.

Diaphorase (DIA) displayed three banding patterns in peach, two single-banded and one double-banded. F_2 progeny of the cultivars with double-banded patterns segregated in a 1:2:1 ratio (single:double: single types) indicating that only one locus is involved and that the two bands are allelic. No intermediate bands were found in heterozygous plants so diaphorase appears to function as a monomer in peach. Diaphorase functions as a monomer in soybean (Kiang and Gorman, 1983) and Camellia (Wendel and Parks, 1982).

Durham et al. (1987) found three banding patterns for MDH in peach. Although the patterns were similar to those found by Arulsekar et al. (1986) they were not identical. Durham et al. (1987) noted that this discrepancy could easily reflect the use of different buffer systems in the two studies. Durham and Arulsekar agreed as to the genetic interpretation of MDH in peach. In addition to a four-banded pattern like that found by Arulsekar et al. (1986), Durham also found a widely spaced, triple-banded type which Arulsekar et al. (1986) did not find. Upon selfing, both these types gave rise to progeny exhibiting only the parental banding pattern. Durham concluded that these types were homozygous at this MDH locus. The third banding pattern was a seven-banded pattern, including all the bands represented in the two homozygotes plus two intermediate bands. When Durham crossed the two different homozygotes, all the resultant progeny exhibited the seven-banded pattern, as expected when using parents homozygous for different alleles at a single locus. When Durham backcrossed the heterozygous type to one of the homozygous types, he recovered progeny in the expected 1:1 ratio of parental patterns. Durham et al. (1987) concluded that the variability of MDH in peach is explained by two alleles interacting at a single locus.

Peroxidase (PX) exhibited three well-resolved banding patterns in the cathodal region of the gel for peach (Durham et al., 1987). These patterns included two single-banded patterns and one double-banded pattern which was a combination of the two single-banded patterns. Peroxidase functions as a monomeric enzyme in other plant species (Wijsman, 1983; Torres, 1983). In peach, selfed single-banded types produced progeny with the parental banding pattern. When the two single-banded types were crossed, all progeny exhibited the double-banded pattern. Selfed double-banded types produced the expected 1:2:1 ratio (single:double:single types) in the progeny. Durham et al. (1987) concluded that the variability for peroxidase in peach could be explained by the presence of a single locus with two alleles.

Chaparro et al. (1987) used isozyme analysis to identify peach x almond hybrids. They tested nine different enzyme systems, including isocitrate dehydrogenase (IDH), esterase (Est), phosphohexose isomerase (PHI), superoxide dismutase (SOD), acid phosphatase (AcP), PGM, MDH, LAP, and 6-PGD. SOD, LAP, and AcP were difficult to resolve and were not evaluated. IDH, MDH and PHI were also not included in the study because peach and almond were monomorphic for these three enzymes. PGM and 6-PGD proved to be useful in distinguishing hybrids. These enzymes produced well-resolved banding patterns, and the patterns differed between peach and almond.

Chaparro et al. (1987) found two zones of activity for PGM, as described by Parfitt et al. (1985). (PGM-1 indicates the more anodal zone, while PGM-2 indicates the more cathodal zone.) Peach and almond exhibited the same double-banded pattern for PGM-1. For PGM-2, almond exhibited a double-banded pattern, whereas peach exhibited a single band at the same position as the faster band in almond. Peach x almond progeny exhibited both parental banding patterns. Segregating F_2 almond x peach progeny displayed the expected 1:2:1 ratio (single:double:single). PGM is a monomer in many other plants (Cheliak and Pitel, 1985; Gottlieb, 1981). Based on this study PGM also functions as a monomer in almond and peach.

6-PGD functions as a dimeric enzyme in corn (Goodman and Stuber, 1983), barley (Brown, 1983), and soybean (Kiang and German, 1983). Peach and almond both displayed two zones of activity for 6-PGD, but the bands had differing mobilities in both regions. Both species exhibited single-banded patterns at each locus. All peach x almond progeny exhibited both parental bands and an intermediate band in both the 6-PGD-1 and 6-PGD-2 regions. This finding is consistent with the hypothesis that 6-PGD is a dimeric enzyme, displaying intermediate bands that are heterodimers. F_2 peach x almond progeny produced the expected segregation at both the 6-PGD-1 and the 6-PGD-2 loci. Chaparro et al. (1987) concluded that PGM could be used to distinguish only 50% of the peach x almond hybrids, whereas 6-PGD could distinguish all hybrids since peach and almond share no common alleles for 6-PGD.

Hauagge et al. (1987a) studied the inheritance of five polymorphic enzyme systems in almond x almond and in F_1 and F_2 peach x almond hybrids. For the enzyme systems AcP, glyceraldehyde-phosphate dehydrogenase (GAP), and 6-PGD Hauagge et al. (1987a) found two zones of activity in almond but all loci were monomorphic. The enzyme systems AAT, PGI, LAP, and PGM each exhibited two zones of activity. Polymorphisms occurred at five of the eight loci.

In almond AAT-2 was monomorphic, exhibiting a single-banded pattern. AAT-1 was polymorphic, exhibiting three banding patterns, two single-banded patterns with either a "fast" or a "slow" band and a triple-banded pattern with bands at the "fast" and "slow" migration positions plus an intermediate band. The peach cultivar used exhibited the same AAT-2 band found in almond, and the same AAT-1 band found in the almond "slow" banded homozygote. When triple-banded almond cultivars were crossed with single-banded cultivars the progeny exhibited the parental banding patterns in a 1:1 ratio. Crossing parents with the triple-banded pattern resulted in the expected Mendelian segregation of 1:2:1. Almond, peach, and segregating hybrids within almond and between almond and peach all exhibited two zones of activity for AAT in identical positions. F_1 and F_2 segregating progenies indicated that the AAT-1 and AAT-2 loci were homologous between almond and peach. Hauagge et al. (1987a) concluded that, in almond and peach, AAT-1 functions as a dimeric enzyme composed of two subunits with two alleles.

PGI also exhibited two zones of activity in almond (Hauagge et al., 1987a). PGI-1 was monomorphic with a single-banded pattern, whereas PGI-2 was polymorphic, exhibiting two single-banded patterns with either a "fast" or a "slow" band and a triple-banded pattern with bands at the "fast" and "slow" migration locations plus an intermediate band. Just as with AAT-1, when a homozygous single-banded parent was crossed with a heterozygous triple-banded parent the progeny closely fit the expected 1:1 ratio for parental banding patterns. Similarly, crossed heterozygotes produced the expected 1:2:1 segregation ratio. The peach cultivar used in the study exhibited the same banding pattern at PGI-1 as that found in almond. At PGI-2 the peach cultivar exhibited the "fast" homozygous pattern found in almond. The F_1 and F_2 progenies of almond x peach hybrids indicate that peach and almond have homologous PGI loci.

LAP exhibited two zones of activity in almond (Hauagge et al., 1987a). LAP-2 was monomorphic with a single-banded pattern and LAP-1 was polymorphic with two single-banded patterns and a double-banded pattern (with bands at the same migration locations as those in the single-banded patterns). Many of the almond crosses produced the expected segregation ratios for a monomeric enzyme with two alleles. However, several of the crosses involving either of two particular almond cultivars deviated from expected ratios. Hauagge et al. (1987a), proposed that in these two cultivars a null allele existed at the LAP-1 locus. Null alleles for LAP have been reported in several other plant species (Neale and Adams, 1981; Quiros and Morgan, 1981; Wilson, 1976; Guries and Ledig, 1978; Adams and Joly, 1980). Peach also exhibited two zones of activity for LAP, with a single band at LAP-1 and LAP-2, both at migration locations different from those found in almond. Peach x almond hybrids combined the isozyme patterns of both parental species, producing quadruple-banded patterns. The F_2

peach x almond progenies did not exhibit segregation ratios for the LAP loci consistent with independent assortment. The segregation ratio exhibited closely matched a 1:2:1 segregation of the parental peach, hybrid, and parental almond genotypes, with only a low percentage of recombinants. This suggests that peach and almond both have linkage between LAP-1 and LAP-2. Hauagge et al. (1987a) concluded that since almond and peach exhibit different migration distances for bands at LAP-1 and LAP-2, LAP provides a unique biochemical marker for tracing gene introgression between the two species.

In almond PGM exhibited two zones of activity (Hauagge et al., 1987a). Both loci were polymorphic, exhibiting single- and double-banded patterns. Crosses between single- and double-banded parents produced two classes of progeny with the parental banding patterns in a 1:1 ratio. Crosses between double-banded parents produced the expected 1:2:1 segregation ratio. Hauagge concluded that PGM behaves as a monomeric enzyme in almond.

In a further study, Hauagge et al. (1987b) used the five polymorphic loci, AAT-1, PGI-2, LAP-1, PGM-1 and PGM-2, to characterize and group 76 almond cultivars and accessions. These loci permitted exclusion of certain cultivars as parents. Hauagge found that the isozyme analysis, combined with historical analysis, phenotypic comparison, genetic studies, and pollen incompatibility studies, provided additional evidence that the almond gene pool in California is dominated by the descendants of the two major almond cultivars grown in California. Additionally, isozyme differences showed that two cultivars could not be bud mutations of one of the cultivars as assumed.

Byrne and Littleton (1988) tested eight enzyme systems for polymorphisms in 29 cultivars of Japanese plum. The enzymes tested were, glutamate dehydrogenase (GDH), PGI, LAP, MDH, PGM, peroxidase (PX), 6-PGD, and triosephosphate isomerase (TPI). The first six enzymes are polymorphic in plum. 6-PGD and TPI were not useful since both systems exhibited monomorphic banding patterns. GDH, although variable, could not be resolved consistently, and was not used in this study.

PGI exhibited two zones of activity in plum. PGI-1, although variable, was not well resolved. PGI-2 was polymorphic and behaved as a dimeric enzyme. Four PGI-2 banding patterns were observed in the plums studied, two single-banded patterns and two triple-banded patterns. Byrne and Littleton (1988) proposed three alleles at PGI-2, with the intermediate bands in the triple-banded genotypes representing interaction products.

LAP exhibited two zones of activity, while LAP-2 displayed a single invariate band. LAP-1 was polymorphic and behaved like a monomeric enzyme as in other plant species. Four banding patterns were observed in the plum cultivars studied, two single-banded patterns and two double-banded patterns. Byrne and Littleton (1988) proposed the occurrence of three alleles at LAP-1 in plum.

MDH in plum exhibited two zones of activity, as reported in other *Prunus* species. MDH-2, found to be mitochondrial in origin in peach (Arulsekar et al., 1986) and cherry (Hancock and Iezzoni, 1988), was monomorphic in plum. MDH-1, having cytosolic activity (Arulsekar et al., 1986), was polymorphic in plum. MDH-1 exhibited three banding patterns, a single-banded pattern and two triple-banded patterns. As in other crops (Cheliak and Pitel, 1985), MDH functions as a dimeric enzyme in plum.

PGM exhibited two zones of activity, both polymorphic. PGM-1 displayed four banding patterns, three double-banded and one triple-banded. Byrne and Littleton (1988) proposed that the double-banded patterns represent homozygous individuals, whereas the triple-banded patterns represent heterozygous individuals. PGM exhibits double-banded homozygotes in other species (Arus and Shields, 1983; Goodman et al., 1980; Neal and Adams, 1981; Wendel and Parks, 1982). PGM-2 exhibited two banding patterns in plum, a single-banded pattern and a double-banded pattern. Byrne and Littleton (1988) concluded that in plum, as in other plant species (Cheliak and Pitel, 1985; Gottlieb, 1981), PGM functions as a monomeric enzyme.

Peroxidase (PX) produced anodal and cathodal banding patterns in plum. Only the cathodal bands were consistently resolved. The cathodal bands exhibited two patterns, a single-banded pattern and a double-banded pattern. PX is monomeric in other plant species (Torres, 1983; Wijsman, 1983). Byrne and Littleton (1988) proposed that in plum, as in peach (Durham, 1986) PX functions as a monomer with the two bands representing alleles at a single locus, and that the PX bands are consistent through the season. Therefore, PX was useful for plum cultivar identification.

Using the five polymorphic enzyme systems, Byrne and Littleton (1988) were able to resolve the original 29 plum cultivars into 19 groups, eleven of which consisted of a single cultivar. The other cultivars were divided into eight groups of two or three cultivars each. The cultivars within these groups were then readily characterized by vegetative characteristics. Byrne and Littleton (1988) also used the presumptive genotypes generated by the isozyme analysis to examine nine of the 29 plum cultivars and their reported parents. For three of the cultivars neither parent was possible and for two more the reported male parent was not possible. Four of the nine cultivars exhibited genotypes consistent with that of their reported parents. For the 29 plum cultivars examined, there was approximately 50% inconsistency between the isozyme genotype and the presumed parentage.

In another study Byrne and Littleton (1989) used five enzyme systems: PGI, LAP, MDH, PGM, 6-PGD, and PX, to identify plum x apricot hybrids. They analyzed 30 plum cultivars, 40 apricot cultivars and six plum x apricot (plumcot) hybrids. PGI-2, a polymorphic dimeric enzyme in plum (Byrne and Littleton, 1988), was monomorphic in the apricots examined and exhibited a single-banded pattern at the same migration distance as one of the bands found in some of the plum cultivars. Therefore, Byrne and Littleton concluded that PGI was only useful in uniquely identifying some plum x apricot hybrids, depending upon the genotype of the plum parent.

LAP-1, a polymorphic monomeric enzyme in plum (Byrne and Littleton, 1988), was monomorphic in apricot. In apricot, LAP-1 exhibited a single-banded pattern at the same migration distance as one of the bands found in the plum cultivars. As in the case of PGI, LAP-1 can only identify some plumcots, depending on the genotype of the plum parent.

MDH-1 was polymorphic in plum, while MDH-2 was monomorphic (Byrne and Littleton, 1988). In apricot, both loci were polymorphic.

Although plum and apricot exhibited alleles in common, apricot also possessed an allele at each locus not found in plum. If one or both of the unique alleles were present in the apricot parent, then MDH could be used to identify plum x apricot hybrids.

PGM, a monomeric enzyme in Prunus (Chaparro et al., 1987; Hauagge et al., 1987), was polymorphic at both PGM-1 and PGM-2 in plum (Byrne and Littleton, 1988). Apricot was also found to be polymorphic at both PGM loci. Although both species exhibit alleles in common at PGM-1 and PGM-2, plum and apricot also have species-specific alleles at each locus. Hybrid plumcots could be identified using PGM if either the plum or the apricot parent used exhibited a species-specific allele. 6-PGD, a dimeric enzyme with two loci in Prunus (Byrne and Littleton, 1988; Chaparro et al., 1987; Hauagge et al., 1987), was monomorphic in plum, exhibiting a single-banded pattern. Apricot was monomorphic at 6-PGD-1, exhibiting a single band at the same migration distance as that for 6-PGD-1 in plum. 6-PGD-2 was polymorphic in apricot, exhibiting two banding patterns, a single-banded pattern at the same migration location as exhibited in plum and a triple-banded pattern. If the apricot parent was heterozygous at 6-PGD-2, then this enzyme system could be used to identify hybrid plumcot progeny.

Byrne and Littleton (1988) concluded that while the five enzyme systems, PGI, LAP, MDH, PGM, and 6-PGD, could be useful in identifying plumcots, depending on the genotype of the plum and/or apricot parent, only peroxidase could be used to uniquely identify plum x apricot hybrids. Peroxidase was the only system in which plum and apricot exhibited no alleles in common. Parfitt et al. (1985) reported on the use of two enzyme systems, phosphoglucose isomerase (PGI) and phosphoglucomutase (PGM), to identify plum x peach (*Prunus salicina* x *Prunus persica*) interspecific hybrids. Malate dehydrogenase (MDH), leucine aminopeptidase (LAP), and esterase (Est) were also tested but were not useful in identifying such hybrids.

Both PGI and PGM produced two zones of activity in plum and peach. In other plant species PGM and PGI produce two zones of activity which have been associated with individual loci (Gottlieb, 1981).

Parfitt et al. (1985) analyzed eighteen plum cultivars, 62 peach cultivars, and nine plum x peach interspecific hybrids for PGI and PGM. Peach was found to be monomorphic for PGI-1 and PGI-2, producing a single band for each locus. The peach PGI-2 band was at the same migration distance as one of the plum PGI-2 bands. Plum was monomorphic for PGI-1, producing a single band with a greater mobility than the peach PGI-1 band. Plum displayed three banding patterns for PGI-2 -- a "fast" single-band pattern, a "slow" single-band pattern, and a triple-banded pattern with the "fast" band, the "slow" band, and an intermediate band. The triple-banded hybrid pattern indicates that PGI-2 functions like a dimeric enzyme in plum, as in other plant species (Arulsekar and Bringhurst, 1981; Gottlieb, 1977). Since the peach and plum isozymes represented by the PGI-1 band had different migration rates, PGI-1 was useful to uniquely characterize plum x peach interspecific hybrids. Peach was monomorphic for PGM-1, showing a double-banded pattern and for PGM-2, with a single-banded pattern. All three peach bands had faster migration rates than those found in plum PGM-1 and PGM-2 zones.

Plum was polymorphic for PGM-1 (two patterns, a double- and a triple-banded pattern) and for PGM-2 (two patterns, a single- and a double-banded pattern). Since plum and peach did not share any common bands at either PGM-1 or PGM-2, both loci could be used to characterize plum x peach hybrids. The interspecific hybrids all displayed banding patterns that combined both parental types for PGM-1 and PGM-2.

In cherry, only three isozyme studies have been reported. Hancock and Iezzoni (1988) analyzed 19 sour cherry (*P. cerasus* L.) cultivars, six sweet cherry (*P. avium* L.) cultivars, four ground cherry (*P. fruticosa* Pall.) clones, the open-pollinated progeny of two sour cherry cultivars, plus the open-pollinated progeny of *P. incisa*, *P. subhirtella*, *P. canescens*, and *P. mahaleb* for MDH variability. MDH functions as a dimeric enzyme in many species (Orton, 1983; Sari Gorla et al., 1986; Cheliak and Pitel, 1985) and as a multimeric enzyme in peach (Arulsekar et al., 1986). *P. avium* and *P. fruticosa* share some alleles for MDH. In addition, each also exhibited unique alleles. *P. cerasus* has a banding pattern that exhibits codominant expression of both the *P. avium* and the *P. fruticosa* bands. Hancock and Iezzoni's findings (1988) supported the hypothesis that *P. avium* and *P. fruticosa* are the progenitor species of *P. cerasus* (Olden and Nybom, 1968). Open-pollinated progeny of *P. mahaleb* and *P. canescens* exhibited invariate multi-banded patterns. The open-pollinated progeny of *P. incisa*, *P. subhirtella*, and the two sour cherry cultivars each exhibited two banding patterns. Hancock and Iezzoni (1988) concluded that MDH exhibits enough polymorphism to be used to identify interspecific hybrids in cherry.

Fernquist and Huntrieser (1986-1987) used isozyme analysis to characterize five sour cherry cultivars and six wild sweet cherry genotypes. 6-PGD exhibited two zones of activity in both the sour and sweet cherries studied. In sour cherry 6-PGD-2 was invariate, exhibiting a double-banded pattern. 6-PGD-1 in sour cherry exhibited three triple-banded patterns. The sweet cherry genotypes were polymorphic for both loci. In sweet cherry 6-PGD-2 exhibited three banding patterns, a single-, a double- and a triple-banded pattern. 6-PGD-1 exhibited three single-banded patterns, plus a double- and a triple-banded pattern. Fernquist and Huntrieser (1986-87) state that they found sufficient polymorphism in 6-PGD, PGI, MDH, shikimate dehydrogenase (SkDH), hexokinase (Hk), and PGM to uniquely characterize sour cherry cultivars and sweet cherry genotypes using cross examination of these enzyme systems (details not disclosed).

Kaurisch et al. (1988) employed isozyme analysis to examine five *Prunus* species; *P. avium*, *P. cerasus*, *P. fruticosa*, *P. canescens*, and *P. subhirtella*. The enzyme systems tested were aconitase (Acon), isocitrate dehydrogenase (IDH), MDH, 6-PGD, SkDH, PGI, LAP, and PGM. Useful polymorphisms were found within the following loci; Acon-2, 6-PGD-1, 6-PGD-2, IDH-2, and PGI-2. The six sweet cherry cultivars in the study were monomorphic at 6-PGD-2, displaying a single "slow" band. The four sour cherry cultivars were also monomorphic at 6-PGD-2, displaying the single "slow" band. *P. canescens* and *P. subhirtella* were monomorphic at 6-PGD-2, displaying a single "fast" band. *P. fruticosa* displayed a triple-banded hybrid pattern, including the "slow" and "fast" bands and an intermediate band. Using this locus alone, *P. fruticosa* could be distinguished from the other four *Prunus* species. *P. avium* and *P. cerasus* could not be distinguished from each other but they could be distinguished from *P. canescens* and *P. subhirtella*.

Sweet cherry was polymorphic at 6-PGD-1 and displayed two patterns, a single "fast" banded pattern and a triple-banded hybrid pattern. P. cerasus exhibited the same patterns at 6-PGD-1. P. canescens, P. subhirtella, and P. fruticosa all exhibited a monomorphic single "fast" banded pattern at 6-PGD-1. While these patterns suggest a typical Mendelian distribution for a dimeric enzyme, Kaurisch et al. (1988) noted that they found variants at 6-PGD-1 in the tetraploid cherry species (P. cerasus and P. fruticosa) for which they could offer no genetic explanation.

Sweet cherry exhibited three banding patterns at Acon-2, two single-banded patterns with either a "fast" or a "slow" band and a double-banded pattern with both bands. Aconitase exhibited banding patterns typical of a monomeric enzyme. Sour cherry exhibited two banding patterns at Acon-2, a single "slow" banded pattern and the hybrid double-banded pattern. P. canescens and P. fruticosa were both monomorphic at Acon-2, exhibiting the single "fast" banded pattern. P. subhirtella exhibited the hybrid double-banded pattern.

Sweet cherry and P. canescens were monomorphic for IDH-2, with a single "fast" banded pattern. P. cerasus displayed two patterns, a single "fast" banded pattern and a triple-banded pattern. P. fruticosa and P. subhirtella were not tested for IDH.

Sweet cherry was polymorphic for PGI-2, with two banding patterns, a single-banded pattern and a triple-banded pattern. P. fruticosa and P. subhirtella were found to be monomorphic at PGI-2, with the same single band found in sweet cherry. Sour cherry also exhibited this single-banded pattern in addition to two hybrid triple-banded patterns. P. canescens was monomorphic for PGI-2, with a single-banded pattern at a slower migration rate than that found in the other species. Kaurisch et al. concluded that PGI functions as a dimeric enzyme in cherry, as in other *Prunus* species (Hauagge et al., 1987; Parfitt et al., 1985). Between the five species, Kaurisch et al. (1988) propose the occurrence of four alleles at PGI-2.

Kaurisch et al. (1988) observed polymorphisms in LAP, PGM, and SkDH in the five *Prunus* species studied. However, the banding patterns were not consistent, so these enzyme systems were not used in the study.

For MDH, Kaurisch et al. (1988) found that both sweet and sour cherry exhibited the same four-banded pattern. *P. canescens* displayed a distinctive double-banded pattern. *P. subhirtella* also displayed a distinctive five-banded pattern. *P. fruticosa* was not tested for MDH.

MATERIALS AND METHODS

Seeds and leaves for isozyme analysis were collected from trees growing at the Clarksville Horticultural Experiment Station, Clarksville, Michigan, and the Horticultural Research Center, East Lansing, Michigan. Four sweet cherry cultivars, two ground cherry selections, and 'Montmorency' sour cherry were analyzed for isozyme variation. Mature fruit was collected during the summer of 1988. The fleshy pericarp was removed and the endocarps were dipped in a Thiram (fungicide) solution, dried overnight, and stored in polyethylene bags at approximately 5C. Young, newly expanded leaves were collected during the spring and summer of 1988. They were placed in polyethylene bags and stored on ice at approximately 3C. Also, one-year-old branches were collected in February, forced in the laboratory at ambient temperature for approximately one week, and the newly expanded leaves used for isozyme analysis.

Seeds and leaves were ground by hand in porcelain mortars and pestles which were cooled to 0C. The extraction buffer consisted of 0.05 M Tris base, 0.007 M citric acid (monohydrate), 0.1% cysteine hydrochloride, 0.1% ascorbic acid, 1.0% polyethylene glycol (Mr 3500) and 1mM 2-mercaptoethanol, final pH approximately 8.0 (Arulsekar et al., 1986). Approximately 0.3 ml extraction buffer was used to grind each seed. One gram of insoluble polyvinyl polypyrrolidone (PVPP, Sigma) was added to each 12 ml extraction buffer for use with leaf tissue. Approximately 0.3 ml extraction buffer was used to grind 15 mg of minced leaf tissue. After grinding, the plant tissue was held in the mortars on ice prior to electrophoresis. Small squares of fine nylon mesh were placed over each sample. 3 x 13 mm. paper wicks were placed on top of the nylon mesh to absorb supernatant from the samples.

Four kinds of buffer systems were used depending on the enzymes studied. A histidine gel buffer system (Arulsekar et al., 1986) was used for MDH, ADH, SkDH, and PGM. The gel buffer consisted of 1.2 g/liter DL-histidine-HCL (monohydrate), pH adjusted to 7.0 with sodium hydrochloride. The electrode buffer consisted of 18.2 g/liter Tris base and 9.0 g/liter citric acid (monohydrate), pH appproximately 7.0. A histidine-citric acid buffer system (Durham et al., 1987) was used for 6-PGD, IDH, PGI, and Acon. The gel buffer consisted of 0.009 M histidine and 0.003 M citric acid, pH adjusted to 5.7 with citric acid. The electrode buffer consisted of 0.065 M histidine and 0.02 M citric acid, pH adjusted to 5.7 with citric acid. A morpholine-citric acid buffer system (Clayton and Tretiak, 1972) was used for 6-PGD, IDH, and

MDH. The gel and electrode buffer consisted of 0.04 M citric acid (monohydrate), pH adjusted to 6.1 with N-(3-amino propyl) morpholine. For gels the buffer solution was diluted 1:20 with deionized water. A Tris base-citric acid gel buffer system (Kaurisch et al., 1988) was used for LAP and PX. The gel buffer consisted of 0.015 M Tris base and 0.003 M citric acid, pH adjusted to 7.7 with citric acid. The electrode buffer consisted of 0.031 M NaOH and 0.3 M boric acid, pH adjusted to 7.5 with 1 N NaOH.

Eleven percent starch gels were prepared by placing 33 g of potato starch (Sigma) and 300 ml of gel buffer in a dry 1-liter vacuum flask. The solution was vigorously swirled over a bunsen burner flame until it began to boil. Vacuum was applied to the flask until large bubbles formed. The gel was then poured onto the gel plate (length 22 cm., width 21 cm., depth 10 mm.) and any bubbles or undissolved starch flakes were removed with a pipette. The gel was allowed to cool for approximately 45 minutes and covered with polyethylene film. Next morning the gel was cooled at approximately 5C for twenty minutes prior to electrophoresis.

Just prior to electrophoresis the gels were removed from the refrigerator. The gel was separated on all 4 sides from the gel plate frame with a scalpel. A slot was cut in the gel at a 90 degree angle to the direction of electrophoresis, approximately 5 cm. from one end (referred to as the lower or cathodal end). The lower gel plate frame piece was removed. The cut gel was pulled back slightly to allow insertion of the paper wicks. The wicks were picked up with a forceps, blotted on a paper towel, and placed in the slot. After all samples were inserted, the shorter piece of gel was pushed against the larger to ensure contact with the sample wicks. The lower gel frame piece was replaced. All gel buffer systems were run at 40 mA for approximately 5 hours, being removed after the first 30 minutes. At the end of the run, gels were sliced transversely. Slices were immersed in the various staining solutions (Table 1) and incubated at approximately 35C in the dark until the isozyme bands became visible. The excess staining solution was decanted. Gel slices were rinsed with 1.0% acetic acid, and the rinse decanted. The gel slices were either rinsed several times with water or immersed in 50% glycerol or 50% ethanol for approximately 30 minutes and then stored in polyethylene bags.

RESULTS AND DISCUSSION

Preliminary isozyme gels in the early spring of 1988 revealed that resolution for several enzyme systems was possible. However, as the season advanced and summer temperatures regularly reached 32C activity was almost completely lost. Despite using many different recipes for stains, grinding buffers, electrode and gel systems it is apparent that young, partially expanded cherry leaves are virtually mandatory for electrophoretic success with cherries. More mature cherry leaves accumulate phenolics which interact with proteins and inhibit enzyme activity (Wendel and Parks, 1982). Therefore, in midsummer of 1988 the isozyme study was continued with cherry seeds. Although the pollinations were not controlled and a formal inheritance study could not be conducted, the seed zymograms were useful in revealing the range of polymorphisms for several enzyme systems.

MDH functions as a dimer in many crops (Yang et al., 1977; Orton, 1983; Morgan and Bell, 1983) and as a multimer in peach (Arulsekar et

Table 1. Staining solutions for enzymes

Ingredients	Amount
Glucose phosphate isomerase (PGI)^z	
NADP	10 mg
PMS	5 mg
MTT	15 mg
1 M Tris-HCl (pH 8.0)	10 ml
0.1 M $MgCl_2$ $6H_2O$	10 ml
DI water	80 ml
0.18 M fructose-6-phosphate	2 ml
Glucose-6-phosphate dehydrogenase	40 units
Alcohol dehydrogenase (ADH)^z	
NAD	50 mg
PMS	5 mg
MTT	15 mg
0.1 M Tris-HCl (pH 7.5)	100 ml
Ethanol (100% absolute)	3 ml
Malate dehydrogenase (MDH)^z	
NAD	30 mg
MTT	15 mg
PMS	5 mg
1 M Tris-HCl (pH 8.0)	20 ml
1 M sodium L-malate	10 ml
DI water	70 ml

^{z}Arulsekar and Parfitt (1986)

Table 1 (cont'd.).

Ingredients	Amount
6-phosphogluconate dehydrogenase (6-PGD)[z]	
NADP	10 mg
MTT	15 mg
PMS	5 mg
6-phosphogluconic acid (sodium salt)	50 mg
1 M Tris-HCl (pH 8.0)	5 ml
DI water	95 ml
Phosphoglucomutase (PGM)[z]	
NADP	10 mg
PMS	5 mg
MTT	15 mg
alpha-D-glucose-1-phosphate	75 mg
1 M Tris-HCl (pH 8.0)	2 ml
DI water	93 ml
0.1 M $MgCl_2$	5 ml
Glucose-6-phosphate dehydrogenase	40 units
Isocitrate dehydrogenase (IDH)[y]	
NADP	10 mg
MTT	15 mg
PMS	2 mg
Isocitric acid, trisodium salt	100 mg
1.0 M Tris-HCl (pH 8.0)	10 ml
DI water	85 ml
1.0 M $MgCl_2$	5 ml

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[z] Arulsekar and Parfitt (1986)

[y] Soltis et al. (1983)

Table 1 (cont'd.).

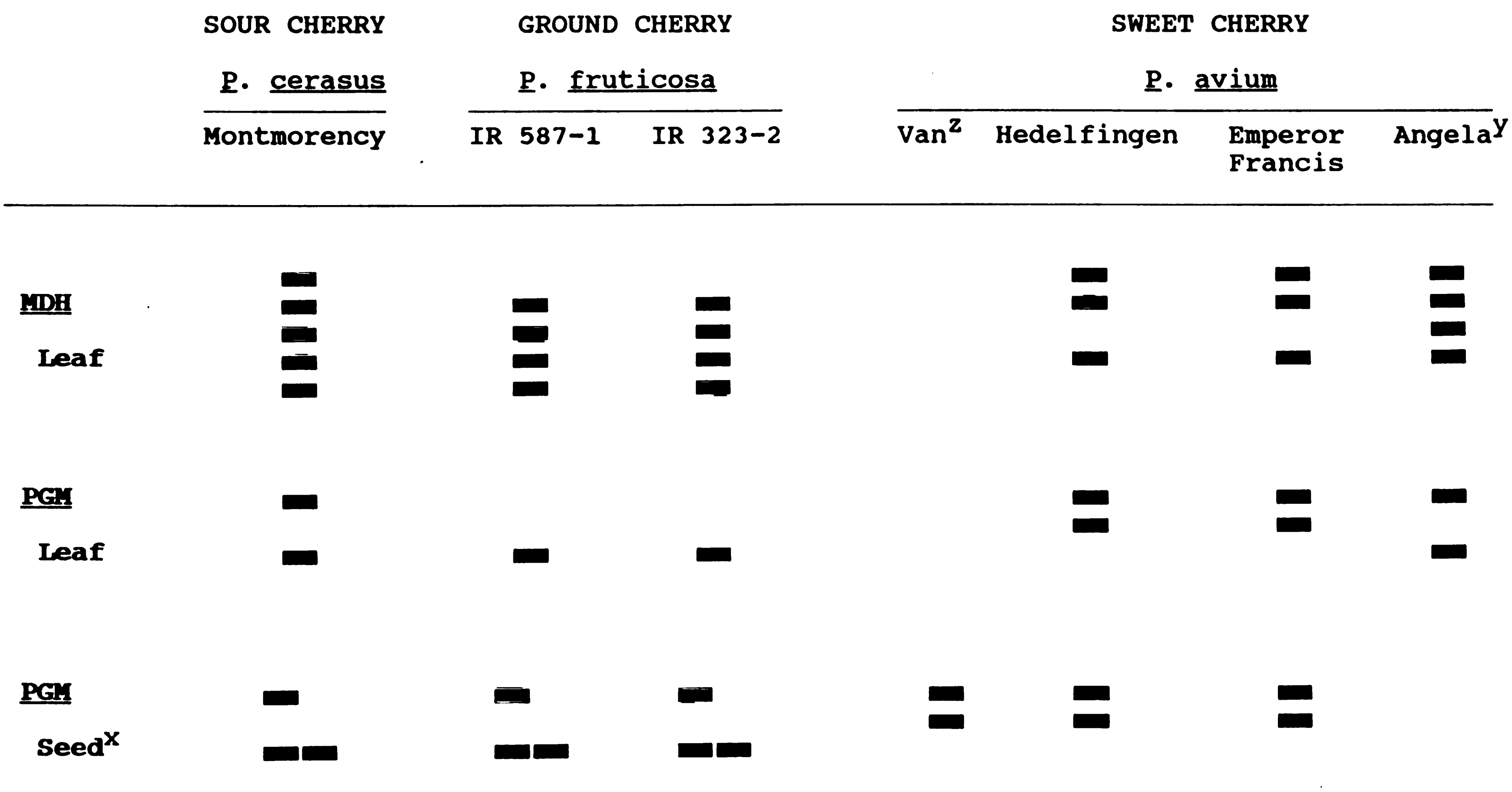
Ingredients	Amount
Aconitase (Acon)[y]	
NADP	15 mg
MTT	10 mg
PMS	2 mg
cis-aconitic acid	100 mg
1.0 M Tris-HCl (pH 8.5)	10 ml
DI water	90 ml
1.0 M $MgCl_2$	1 ml
Isocitrate dehydrogenase	7 units
Leucine aminopeptidase (LAP)[y]	
L-leucine-beta-naphthylamide	20 mg
dissolved in dimethyl formamide	5 ml
1.0 M phosphate buffer, pH 6.0	10 ml
DI water	90 ml
Black K salt or fast black K salt	50 mg
Peroxidase (PX)[y]	
3-amino-9-ethyl carbazole	65 mg
dissolved in dimethyl formamide	5 ml
0.05 M sodium acetate buffer, pH 5.0	95 ml
0.1 M $CaCl_2$	2 ml
3% H_2O_2	2 ml
Shikimate dehydrogenase (SkDH)[y]	
NADP	10 mg
MTT	10 mg
PMS	2 mg
1.0 M Tris-HCl buffer, pH 8.5	10 ml
DI water	90 ml
Shikimic acid	100 mg

[y] Soltis et al. (1983)

al., 1986). As previously reported (Hancock and Iezzoni, 1988), our results confirm that sour cherry leaves exhibited a five-banded pattern for MDH (Figure 1). Leaf tissue of sweet cherries 'Hedelfingen' and 'Emperor Francis' had a triple-banded pattern, while the sweet cherry 'Angela' displayed a four-banded pattern. Both clones of ground cherry exhibited four-banded patterns. Sweet and ground cherry, while sharing some bands, also each exhibited unique bands. Sour cherry exhibited codominant expression of the sweet and ground cherry bands. Cherry seeds revealed the same banding patterns for each species plus often an indefinite number of lower bands. Hancock and Iezzoni (1988) found that sweet and sour cherry pollen displayed an additional four lower bands. This indicates that cherry pollen and seed have at least one additional MDH locus.

PGM functions as a monomer in many crops (Cheliak and Pitel, 1985), including plum and peach (Byrne and Littleton, 1988; Chapparo et al., 1987). The two clones of ground cherry exhibited a single "slow"-banded pattern for leaf tissue (Figure 1). The sweet cherries 'Hedelfingen', and 'Emperor Francis' (plus sweet cherry 'Napoleon') exhibited a double-banded pattern for leaf and seed tissue. The sweet cherry 'Angela' and the sour cherry 'Montmorency' (plus sour cherries 'Northstar', 'Meteor', and 'Montearly') exhibited a double-banded pattern with a "slow" band at the same mobility as in ground cherry and a "fast" band at the same mobility as in sweet cherry. Seed tissue of ground cherry also exhibited this double-banded pattern, indicating cross-pollination had occurred. Sweet cherry seed tissue of 'Van', 'Hedelfingen', and 'Emperor Francis' revealed the double-banded pattern found in 'Hedelfingen' and 'Emperor Francis' leaf tissue.

Figure 1. Zymograms of sour cherry, ground cherry, and sweet cherry leaf and seed tissue.



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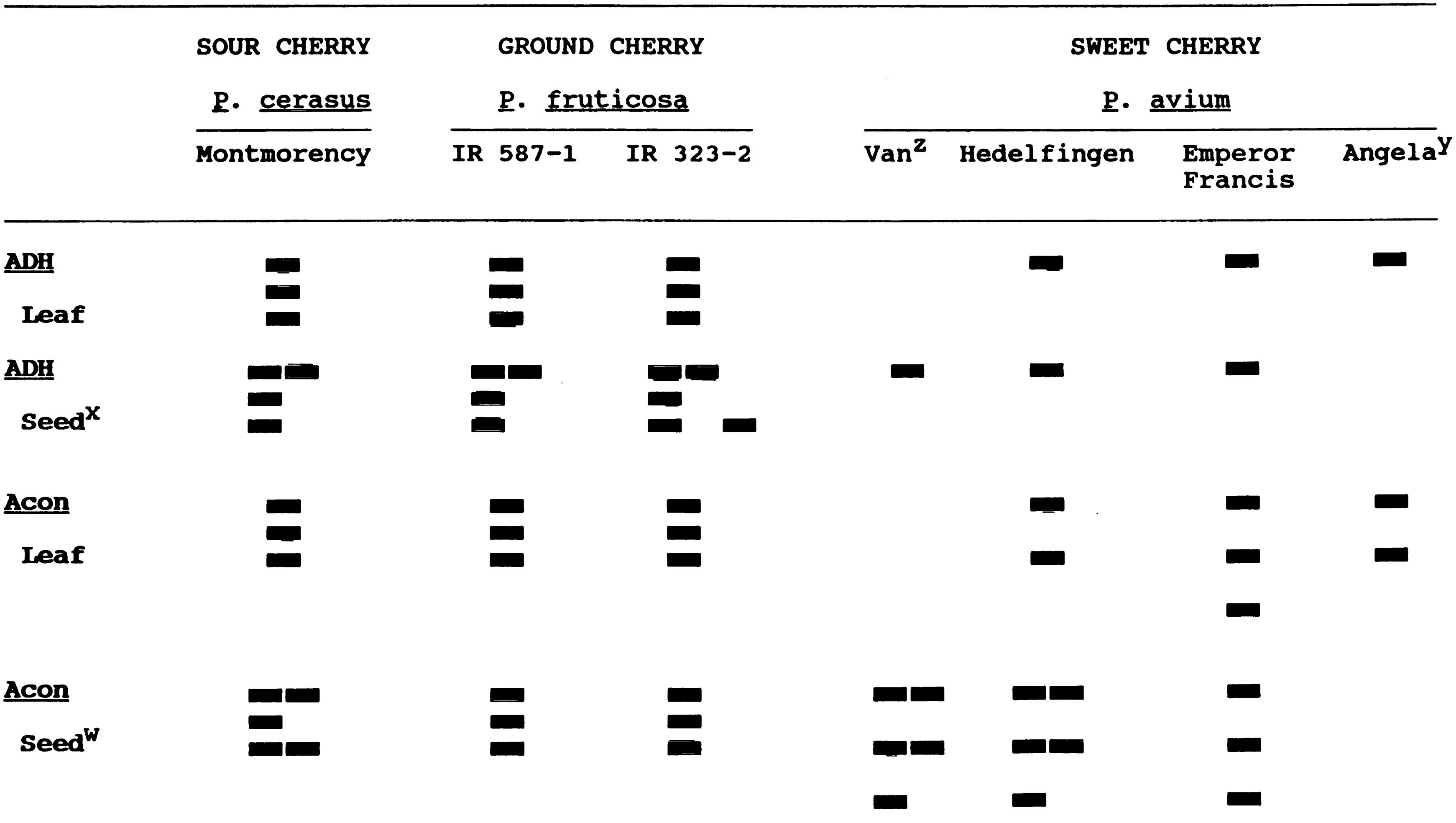
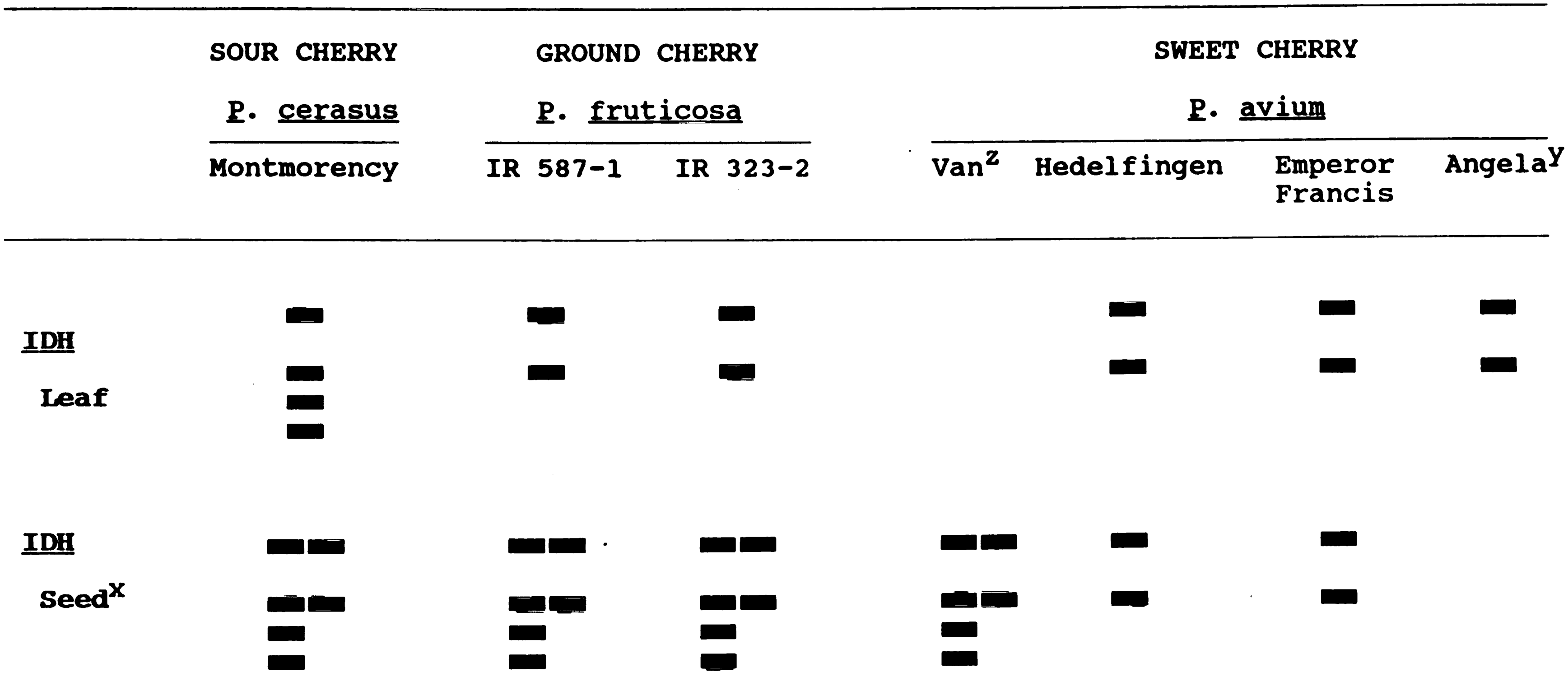
[z] Leaf tissue of Van was not tested.
[y] Seed tissue of Angela was not tested.
[x] Seed tissue of Montmorency, IR 587-1 and IR 323-2 segregated for isozyme banding patterns.

Figure 1 (cont'd.).



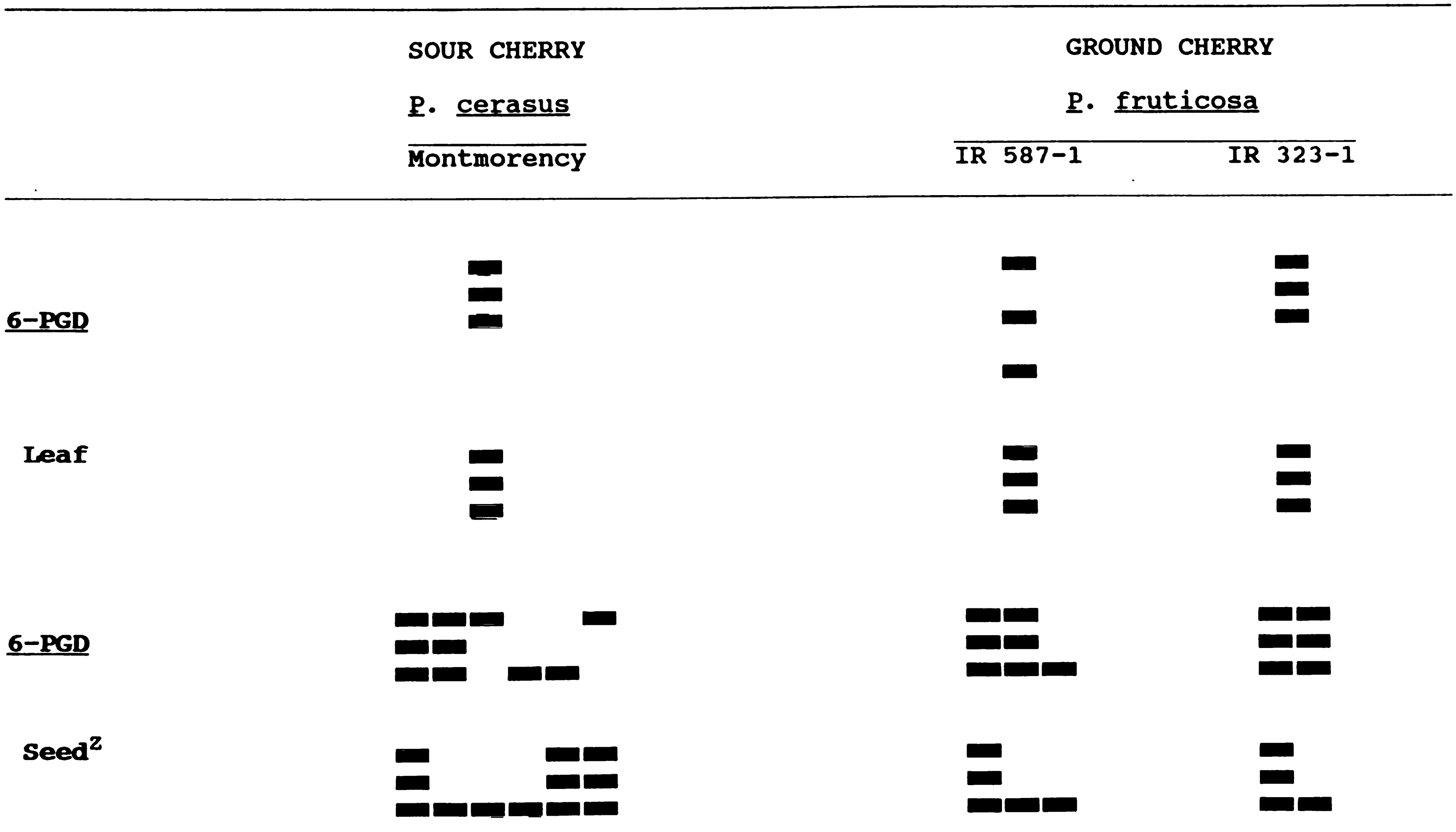
[z] Leaf tissue of Van was not tested.
[y] Seed tissue of Angela was not tested.
[x] Seed tissue ofMontmorency, IR 587-1 and IR 323-2 segregated for isozyme banding patterns.
[w] Seed tissue of Montmorency, Van and Hedelfingen segregated for isozyme banding patterns.

Figure 1 (cont'd.).



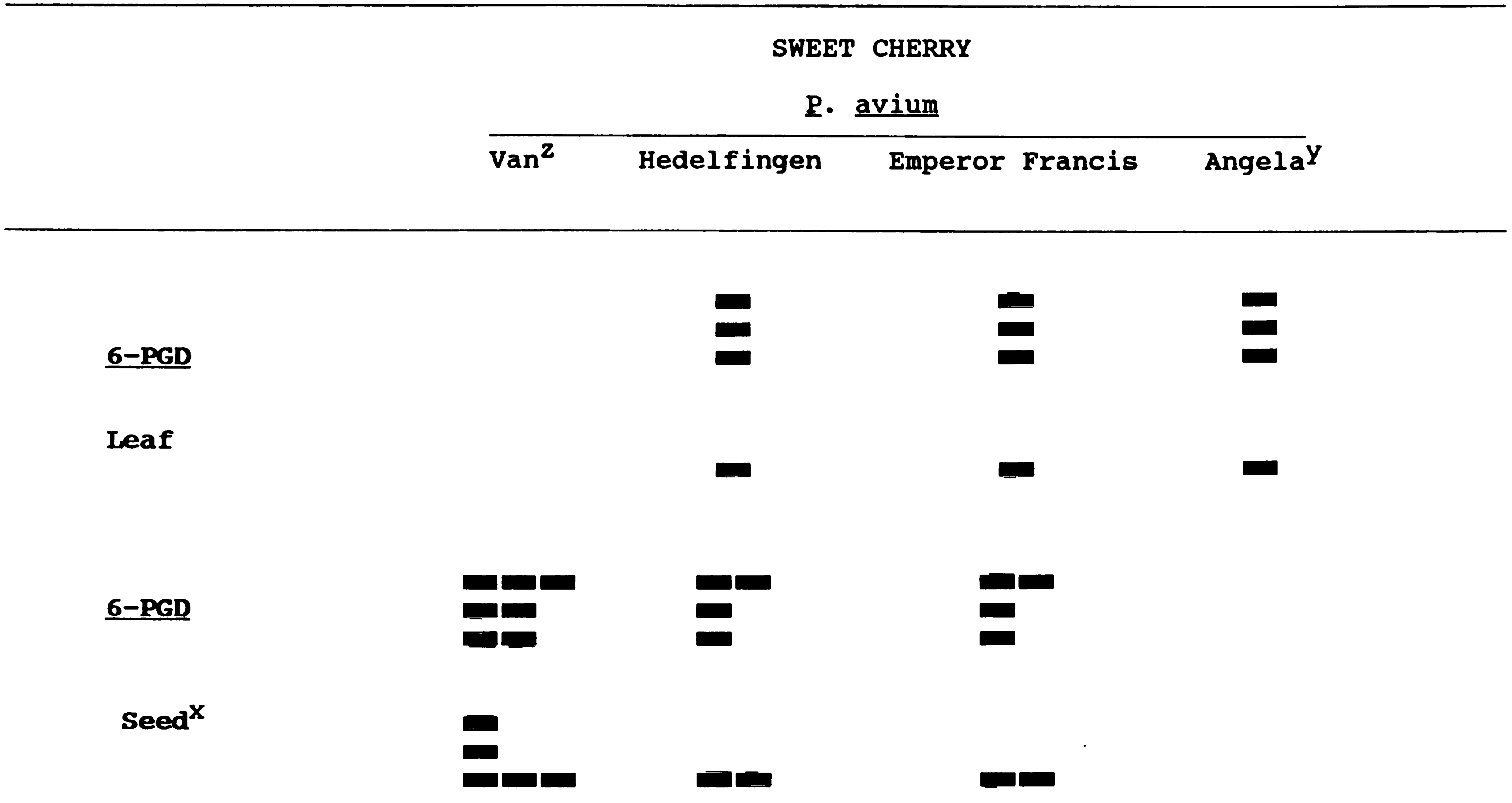
[z] Leaf tissue of Van was not tested.
[Y] Seed tissue of Angela was not tested.
[X] Seed tissue of Montmorency, IR 587-1, IR 323-2, and Van segregated for isozyme banding patterns.

Figure 1 (cont'd.).



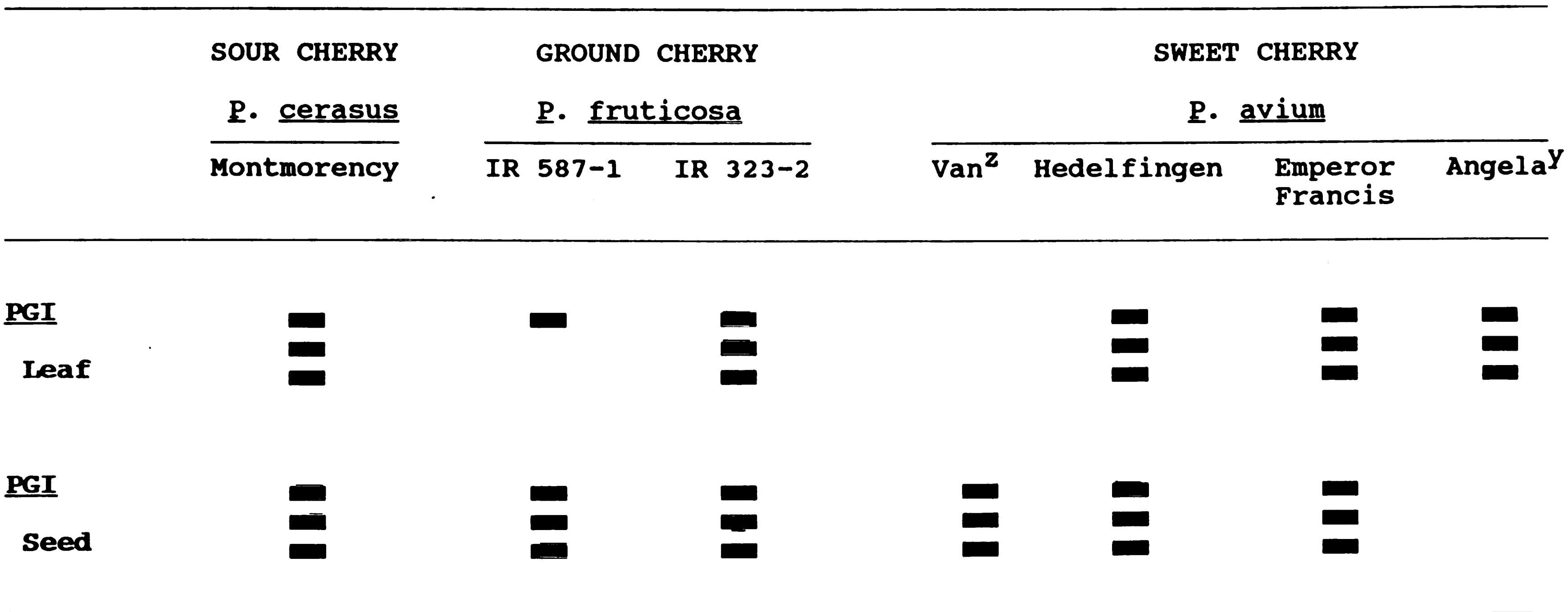
[z] Seed tissue of Montmorency, IR 587-1 and IR 323-2 segregated for isozyme banding patterns.

Figure 1 (cont'd.).



[z] Leaf tissue of Van was not tested.
[y] Seed tissue of Angela was not tested.
[x] Seed tissue of Van, Hedelfingen and Emperor Francis segregated for isozyme banding patterns.

Figure 1 (cont'd.).



[z] Leaf tissue of Van was not tested.
[y] Seed tissue of Angela was not tested.

'Montmorency' seed exhibited the double-banded pattern found in leaf tissue plus a single "slow"-banded pattern.

ADH functions as a dimer in other crops (Hart, 1970; Scandalios, 1974). It is known to increase in activity under anaerobic conditions. Unlike most enzymes whose activity increases after germination, ADH activity declines rapidly with germination. Thus, ADH zymograms from seed tissue proved to be excellently resolved, while those from leaf tissue were extremely faint. The leaf tissue for sour and ground cherry appeared to exhibit an invariate triple-banded pattern (Figure 1). Leaf tissue of sweet cherries 'Hedelfingen', 'Emperor Francis', and 'Angela' (plus sweet cherry 'Napoleon') exhibited a monomorphic single "fast"-banded pattern. In addition, all the sweet cherry cultivars exhibited the same single "fast"-banded pattern for seed tissue. The sour cherry 'Montmorency' and ground cherry IR 587-1 exhibited the single "fast"-banded homodimeric pattern plus a triple-banded heterodimeric pattern. Ground cherry IR 323-2 proved to be most interesting because it displayed both homodimeric patterns, the single "slow" and "fast"-banded patterns plus the triple-banded heterodimeric pattern. This segregation into three patterns was observed in a seed population size of 70 (Appendix A). In addition it appeared that dosage effects could be detected in many of the triple-banded zymograms.

Acon functions as a monomer in several crops, including cherry (Kaurisch et al., 1988). 'Montmorency' (plus sour cherries 'Meteor' and 'Montearly') and the ground cherries exhibited a similar triple-banded pattern for leaf tissue (Figure 1). Sweet cherries 'Hedelfingen' and 'Angela' exhibited a double-banded pattern for leaf tissue. The bands were at the same migration distance as the "fast" and "slow" bands in the sour and ground cherry zymograms. Sweet cherry 'Emperor Francis' exhibited a triple-banded pattern sharing the "slow" and "fast" bands found in the other cultivars and clones, plus a unique "slow" band at a more cathodal position. (Sour cherry 'English Morello' and sweet cherry 'Napoleon' also exhibited this pattern.) Kaurisch et al. (1988) interpreted the consistent "fast" band found in all cherry genotypes as representing one locus, and the variable lower bands as representing a second locus. Seed tissue of the ground cherry clones exhibited the same triple-banded pattern found in leaf tissue. 'Montmorency' seed exhibited the triple-banded pattern plus a double-banded pattern. Seed of sweet cherries 'Van' and 'Hedelfingen' also exhibited this double-banded pattern. Additionally, 'Van', 'Hedelfingen', and 'Emperor Francis' seed exhibited a triple-banded pattern containing bands at the "fast" and "slow" positions exhibited in the sour and ground cherry zymograms plus a lower band at the same position as the lowest band in the 'Emperor Francis' leaf tissue zymogram.

IDH functions as a dimer in several crops, including cherry (Kaurisch et al., 1988). Sweet cherry and ground cherry leaf tissue exhibited a similar double-banded pattern (as did sour cherry 'Meteor') (Figure 1). 'Montmorency' exhibited a four-banded pattern, sharing the two bands exhibited in sweet and ground cherry plus two lower bands unique to 'Montmorency'. As with aconitase, Kaurisch et al. (1988) interpreted the invariate "fast" band as representing a separate locus. Seed tissue of sweet cherries 'Hedelfingen' and 'Emperor Francis' had the same banding pattern as in the leaf tissue. However, the sweet cherry 'Van', while sharing this banding pattern, also exhibited the four-banded pattern as found in 'Montmorency'. Seed tissue of 'Montmorency' exhibited the four-banded pattern found in leaf tissue plus a double-banded pattern. Conversely, seed tissue of both ground cherry clones exhibited the double-banded pattern found in leaf tissue plus the four-banded pattern.

6-PGD functions as a dimer in many plant species (Goodman and Stuber, 1983; Brown, 1983; Kiang and German, 1983). The three cherry species studied revealed two zones of activity for 6-PGD, as found by Kaurisch et al. (1988) (Figure 1). 'Montmorency' leaf tissue exhibited triple-banded patterns in both zones (as did sour cherries 'Montearly' and 'Meteor'). Ground cherry IR 323-2 leaf tissue also had this pattern. Leaf tissue of ground cherry IR 587-1 exhibited the triple-banded pattern common to 'Montmorency' and the other ground cherry clone for the zone of slower mobility. In the zone of faster mobility IR 587-1 exhibited a unique triple-banded pattern. The bands in this zone shared the fastest and slowest bands of the other triple-banded pattern. In addition, ground cherry IR 587-1 had a unique third band at a slower mobility in the upper zone of activity. Leaf tissue of all the sweet cherry cultivars (plus sweet cherry 'Napoleon') exhibited the triple-banded pattern in the zone of faster mobility and a single-banded pattern in the zone of slower mobility. 'Montmorency' seed tissue exhibited a range of zymograms (Figure 1) including the homodimeric "slow"-banded pattern in the zone of slower mobility and both homodimeric ("slow" and "fast"-banded) patterns in the zone of faster mobility plus the triple-banded pattern in both zones of mobility and various combinations of the heterodimeric and homodimeric banding patterns. The three banding patterns (both homodimeric patterns and the heterodimeric pattern) in the zone of faster mobility were observed in a seed population size of 73 (Appendix A). Ground cherry IR 587-1 seed tissue exhibited triple-banded patterns in both zones, as found in ground cherry IR 323-2 leaf tissue. In addition, IR 587-1 exhibited the "slow"-banded homodimeric pattern in both zones of slower and faster mobility. Ground cherry IR 323-2 seed tissue exhibited the triple-banded pattern in both zones, as found in leaf tissue, plus the "slow"-banded homodimeric pattern in the zone of slower mobility. Sweet cherry 'Van' seed tissue exhibited the same pattern as found in the other sweet cherry cultivars' leaf tissue, plus the triple-banded heterodimeric pattern in the zone of slower mobility and the "fast"-banded homodimeric band found in the zone of faster mobility. 'Hedelfingen' and 'Emperor Francis' seed tissue exhibited the same pattern as found in leaf tissue, plus the "fast"-banded homodimeric pattern in the zone of faster mobility. In addition it appeared that dosage effects could be detected in many of the triple-banded zymograms.

PGI functions as a dimer in many crops (Arulsekar and Bringhurst, 1981; Gottlieb, 1977). All the sweet cherry cultivars (plus sweet cherry 'Napoleon') exhibited a monomorphic triple-banded pattern for leaf and seed tissue (Figure 1). 'Montmorency' leaf and seed tissue exhibited this same pattern as did ground cherry IR 323-2. (Leaf tissue of sour cherries 'Meteor' and 'Montearly' also exhibited this pattern.) Ground cherry IR 587-1 revealed a single-banded pattern for leaf tissue. (This pattern was also found in sour cherry cultivars 'English Morello' and 'Northstar'.) Seed tissue of IR 587-1 exhibited the monomorphic triple-banded pattern as found in the other chery genotypes.

Other enzyme systems in cherry with apparent polymorphisms but inconsistent resolution include, LAP, SkDH, and PX.

It is apparent from the zymograms for the three species, sweet, sour, and ground cherry, that a great deal of isozyme homology does exist between the three species. This finding concurs with Raptopoulos' hypothesis (1941) that all three species may have arisen from a common diploid ancestor. This study also demonstrates that abundant polymorphisms exist in several enzyme systems both within and between the three cherry species. This is in sharp contrast to the situation in another *Prunus* species, peach. Arulsekar et al. (1986) had to test ten and Durham (1987) thirty-eight enzyme systems to find, respectively, one and three enzyme systems that revealed polymorphisms. The wealth of polymorphisms found in cherry promises to provide valuable genetic markers for future research.

LIST OF REFERENCES

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Adams, M. W. 1977. An estimation of homogeneity in crop plants with special reference to genetic vulnerability in the dry bean, Phaseolus vulgaris L. Euphytica 26(3):665-679.

Adams, W. T. and R. J. Joly. 1980. Linkage relationships among twelve allozyme loci in loblolly pine. J. Hered. 71:199-202.

Adams, W. T. and J. V. Wiersma. 1978. An adaptation of principal components analysis to an assessment of genetic distance. Mich. Agric. Exp. Stn. Res. Rep. 347.

Afifi, A. A. and V. Clark. 1984. Computer-aided multivariate analysis. Lifetime Learning, Belmont, CA.

Allard, R. W., A. L. Kahler, and B. S. Weir. 1971. Isozyme polymorphisms in barley populations. Barley Genetics Symp. Proc. 2D:1-13.

Arulsekar, S. and D. E. Parfitt. 1986. Isozyme analysis procedures for stone fruits, almond, grape, walnut, pistachio, and fig. HortSci. 21(4):928-933.

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

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

Arulsekar, S., D. E. Parfitt, and G. H. McGranahan. 1985. Isozyme gene markers in Juglans species. J. Hered. 76:103-106.

Arus, P., and C. R. Shields. 1983. Cole crops (Brassica oleracea L.), p. 339-350. In: S. D. Tanksley and T. J. Orton (eds.). Isozymes in plant genetics and breeding, part B. Elsevier, Amsterdam.

Bailey, L. H., et al. 1976. Hortus third: a concise dictionary of plants cultivated in the United States and Canada. Macmillan, New York.

Barg, T. 1958. Cytological investigations on sour cherries (in German). Gartenbauwiss. 23:200-208. [Hort. Abstr. 29:1175c; 1959].

Bassiri A. 1976. Barley cultivar identification by use of isozyme electrophoretic patterns. Can. J. Plant Sci. 56:1-6.

Bemis W. P., A. M. Rhodes, T. W. Whitaker and S. G. Carmer. 1970. Numerical taxonomy applied to Cucurbita relationships. Amer. J. Bot. 57(4):404-412.

Blasse, W. 1957. Heredity analysis of interspecific hybrids between Prunus cerasus L. and P. avium L. (in German). Arch. Gartenb. 5:104-172. [Plant Breed. Abstr. 29:668; 1959].

Blogg, D. and B. C. Imrie. 1982. Starch gel electrophoresis for soybean cultivar identification. Seed Sci. and Technol. 10:19-24.

Brown A. H. D. 1983. Barley. p. 55-78. In: S. D. Tanksley and T. J. Orton (eds.). Isozymes in plant genetics and breeding, part B. Elsevier, Amsterdam.

Brown A. H. D. and R. W. Allard. 1970. Estimation of the mating system in open-pollinated maize populations using isozyme polymorphisms. Genetics 66:133-145.

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

Byrne D. H. and T. G. Littleton. 1989. Interspecific hybrid verification of plum x apricot hybrids via isozyme analysis. HortSci. 24(1):132-134.

Carter, K. K., F. C. Cech, and D. H. DeHayes. 1983. Geographic variation in Prunus serotina. Can. J. For. Res. 13(6):1025-1029.

Cech, F. C. and K. K. Carter. 1979. Geographic variation in black cherry - ten year results of a West Virginia provenance test. Proceedings of the First North Central Tree Improvement Conference. Madison, WI. pp. 21-27.

Challice, J. S. and M. N. Westwood. 1973. Numerical taxonomic studies of the genus Pyrus using both chemical and botanical characters. Bot. J. Linn. Soc. 67:121-148.

Chaparro, J. X., R. E. Durham, G. A. Moore, and W. B. Sherman. 1987. Use of isozyme techniques to identify peach x 'Nonpareil' almond hybrids. HortSci. 22:300-302.

Chaparro, J. X., R. G. Goldy, B. D. Mowrey, and D. J. Werner. 1989. Identification of Vitis vinifera L. x Muscadinia rotundifolia small hybrids by starch gel electrophoresis. HortSci. 24(1):128-130.

Cheliak, W. M. and J. A. Pitel. 1985. Inheritance and linkage of allozymes in Larix laricina. Silvae Genet. 34:142-148.

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

Cociu, V. and T. Gozob. 1985. The sour cherry breeding program in Romania. Acta Horticulturae 169:91-96.

Conkle, M. T., P. D. Hodgskiss, L. B. Hunnally, and S. C. Hunter. 1982. Starch gel electrophoresis of conifer seeds: a laboratory manual. USDA For. Ser. Tech. Rep. PSW-64, Pacific Southwest Forest and Range Exp. Sta., Berkeley.

Crawford, D. J. 1983. Phylogenetic and systemic inferences from electrophoretic studies, p. 257-288. In: S. D. Tanksley and T. J. Orton (eds.). Isozymes in plant genetics and breeding, part A. Elsevier, Amsterdam.

Darlington, C. D. 1928. Studies in Prunus I and II. J. Genet. 19: 213-256.

de Candolle, A. 1882. Origin of cultivated plants. Hafner, New York.

Durham, R. E. 1986. Isozyme banding patterns and inheritance studies in peach (Prunus persica L.). MS thesis, Univ. of Florida, Gainesville.

Fernquist, I. and I. Huntrieser. 1986-1987. Use of isozyme analysis for the identification of fruit cultivars and genotypes. In: Breeding of Fruit and Small Fruit at Balsgard. Annual Report for 1986-1987, the Swedish University of Agricultural Sciences, Division of Fruit Breeding. Balsgard, Sweden.

Galletta, G. J. 1959. Comparative breeding, morphological and cytological studies of polyploid sweet cherries (Prunus avium L.). PhD Diss., Univ. of California, Davis.

Goodman, M. M., C. W. Stalser, K. Newton, and H. H. Weissinger. 1980. Linkage relationships of 19 enzyme loci in maize. Genetics 96:697-710.

Goodman, M. M. and C. W. Stuber. 1983. Maize, p. 1-34. In: S. D. Tanksley and T. J. Orton (eds.). Isozymes in plant genetics and breeding, part B. Elsevier, Amsterdam.

Gottlieb, L. D. 1981. Electrophoretic evidence and plant populations. Prog. Phytochem. 7:1-46.

Guries, R. P. and F. T. Ledig. 1978. Inheritance of some polymorphic isoenzymes in pitch pine (Pinus rigida Mill.). Heredity 40:27-32.

Hamrick, J. L. 1983. The distribution of genetic variation within and among natural plant populations, p. 335-348. In: C. M. Shonewald-Cox, S. M. Chambers, B. MacBryde, and L. Thomas (eds.). Genetics and Conservations. Benjamin/Cummings, Menlo Park, N.J.

Hancock A. M. and A. F. Iezzoni. 1988. Malate dehydrogenase isozyme patterns in seven Prunus species. HortSci. 23(2):381-383.

Hancock, J. F. and R. S. Bringhurst. 1978. Inter-populational differentiation and adaptation in the perennial, diploid species Fragaria vesca L. Amer. J. Bot. 65(7):795-803.

Hancock, J. F. and R. S. Bringhurst. 1981. Evolution in California populations of diploid and octoploid Fragaria (Rosaceae): a comparison. Amer. J. Bot. 68(1):1-5.

Hart,G. E. 1970. Evidence for triplicate genes for alcohol dehydrogenase in hexaploid wheat. Proc. Nat'l. Acad. Sci. 66(4):1136-1141.

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

Hauagge, R., D. E. Kester, and R. A. Asay. 1987b. Isozyme variation among California almond cultivars: II. Cultivar characterization and origins. J. Amer. Soc. Hort. Sci. 112(4):687-693.

Hedrick, U. P. 1915. The cherries of New York. J. B. Lyon, Albany, N.Y.

Heiser, C. B. Jr., J. Soria, and D. L. Burton. 1965. A numerical taxonomic study of Solanum species and hybrids. Amer. Naturalist 99(909):471-488.

Hillig, K. W. 1988. A multivariate analysis of a sour cherry germplasm collection. MS Thesis, Mich. State Univ., East Lansing.

Hotelling, H. 1933. Analysis of a complex of statistical variables into principal components. Jour. Educ. Psych. 24:417-441.

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

Hruby, K. 1950. The cytology of tetraploid cherries. Studia Botan. Cechosl. 11:87-97. [Plant Breed. Abstr. 26:3705; 1956].

Hruby, K. 1962. Chromosome behaviour and phylogeny of cultivated Cerasus. Preslia 34:85-97.

Hunter, R. L. and C. L. Markert. 1957. Histochemical demonstration of enzymes separated by zone electrophoresis in starch gels. Science 125:1294-1295.

Hussaini, S. H., M. M. Goodman and D. H. Timothy. 1977. Multivariate analysis and the geographic distribution of the world collection of finger millet. Crop Sci. 17:257-263.

Iezzoni, A. F. 1984. Sour cherry breeding in Eastern Europe. Fruit Var. J. 38:121-125.

Iezzoni, A. F. 1985. Sour cherry germplasm and cultivar development in Romania. Rept. Nat. Acad. Sci. 14 pgs.

Iezzoni, A. F. and R. L. Hamilton. 1985. Differences in spring floral bud development among sour cherry cultivars. Hortscience 20(5):915-916.

Isleib, T. G. and J. C. Wynne. 1983. Heterosis in testcrosses of 27 exotic peanut cultivars. Crop Sci. 23(5):832-841.

Jardine, S. and R. Sibson. 1971. Mathematical taxonomy. Wiley, New York.

Jensen, R. J. 1977. Numerical analysis of the scarlet oak complex (*Quercus* subgen. *Erythrobalanus*) in the eastern United States: relationships above the species level. Syst. Bot. 2(2):122-133.

Jensen, R. J. and W. H. Eshbaugh. 1976a. Numerical taxonomic studies of hybridization in *Quercus*, I. Populations of restricted areal distribution and low taxonomic diversity. Syst. Bot. 1(1):1-10.

Jensen, R. J. and W. H. Eshbaugh. 1976b. Numerical taxonomic studies of hybridization in *Quercus*, II. Populations with wide areal distributions and high taxonomic diversity. Syst. Bot. 1(1):11-19.

Jensen, R. J. and J. F. Hancock Jr.. 1982. Multivariate relationships among California strawberries. Bull. Torrey Bot. Club. 109(2):136-147.

Kobel, F. 1927. Cytological studies on Prunoideae and Pomoideae (in German). Arch. Jul. Klaus-Stift. 3:1-84.

Kolesnikova, A. F. 1975. Species and ecologo-geographical groups of the sorts of cherry used in selection. Priokskogo Izd-vo, Orel, U.S.S.R. [Plant Breed. Abstr. 47:10743; 1977].

Kollar, G. 1987. Cultivars, pruning systems, general cultural practices for sour cherry in Hungary. Proc. Intl. Cherry Res. Conf., Traverse City, Mich., pp. 19-26.

Kaurisch, P., W. Gruppe and W. Kohler. 1988. Enzyme polymorphisms in cherries (*Prunus* species) and species hybrids (*P.* x species). Method, selected species and rootstock-scion interactions. Angew. Botanik 62:41-52.

Kiang, Y. T. and M. B. Gorman. 1983. Soybeans, p. 295-328. In: S. D. Tanksley and T. J. Orton (eds.). Isozymes in plant genetics and breeding, part B. Elsevier, Amsterdam.

Ladizinsky, G. and T. Hymowitz. 1979. Seed protein electrophoresis in taxonomic and evolutionary studies. Theor. Applied Genet. 54:145-151.

Martin, G. B. and M. W. Adams. 1987. Landraces of Phaseolus vulgaris (Fabaceae) in Northern Malawi. I. Regional variation. Econ. Bot. 41(2):190-203.

Michurin, I. V. 1949. Selected works. Foreign Languages Publishing House, Moscow.

Michurin, I. V. 1955. Izbrannie sochineniya. Gosudarst-vennoe Izd-vo, Moscow.

Moore, G. A. and G. B. Collins, 1983. New challenges confronting plant breeders, p. 36. In: S. D. Tanksley and T. J. Orton (eds.). Isozymes in plant genetics and breeding, part A. Elsevier, Amsterdam.

Moore, G. A. and R. E. Litz. 1984. Biochemical markers for Carica papaya, C. cauliflora, and plants from somatic embryos of their hybrids. J. Amer. Soc. Hort. Sci. 109:213-218.

Morgan, G. F. and J. C. Bell. 1983. Eucalyptus, p. 423-441. In: S. D. Tanksley and T. J. Orton (eds.). Isozymes in plant genetics and breeding, part B. Elsevier, Amsterdam.

Morishima, H. 1969. Phenetic similarity and phylogenetic relationships among strains of Oryza perennis, estimated by methods of numerical taxonomy. Evol. 23(3):429-443.

Morrison, D. F. 1976. Multivariate statistical methods. McGraw-Hill:New York.

Neale, D. B. and W. T. Adams. 1981. Inheritance of isozyme variants in seed tissues of balsam fir (Abies balsamea). Can. J. Bot. 59:1285-1291.

Nevo E., D. Zohary, A. Brown and M Haber. 1979. Genetic diversity and environmental association of wild barley (Hordeum spontaneum) in Israel. Evol. 33(3):815-833.

Okabe, S. 1928. On the cytology of the genus Prunus (in German). Sci. Rep. Tohoku Imp. Univ. 4(3(:733-743. [Bio. Abstr. 3:4463; 1929].

Olden, E. J. and N. Nybom. 1968. On the origin of Prunus cerasus L. Hereditas 59:327-345.

Orton, T. J. 1983. Celery and celeriac (Apium graveolens L.), p. 351-367. In: S. D. Tanksley and T. J. Orton (eds.). Isozymes in plant genetics and breeding, part B. Elsevier, Amsterdam.

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

Pierce, L. C. and J. L. Brewbaker. 1973. Applications of isozyme analysis in horticultural science. HortSci. 8(1):17-22.

Pitcher, J. A. 1982. Phenotype selection and half-sib family performance in black cherry. For. Sci. 28:251-256.

Prance G. T., D. J. Rogers and F. White. 1969. A taximetric study of an angiosperm family: generic delimitation in the Chrysobalanaceae. New Phytol. 68:1203-1234.

Prywer, C. 1936. Cytological studies of some species of the genus *Prunus* (in Polish). Acta Soc. Bot. Polon. 13:51-83. [Plant Breed. Abstr. 8:634; 1937-1938].

Quiros, C. F. and K. Morgan. 1981. Peroxidase and leucine-aminopeptidase in diploid *Medicago* species closely related to alfalfa: multiple gene loci, multiple allelism, and linkage. Theor. Applied Genet. 60:221-228.

Raptopoulos, T. 1941. Chromosomes and fertility of cherries and their hybrids. J. Genet. 42:91-113.

Rhodes, A. M., W. P. Bemis, T. W. Whitaker, and S. G. Carmer. 1968. A numerical taxonomic study of *Cucurbita*. Brittonia 20:251-266.

Rhodes, A. M., S. E. Malo, C. W. Campbell and S. G. Carmer. 1971. A numerical taxonomic study of the avocado (*Persea americana* Mill.). J. Amer. Soc. Hort. Sci. 96(3):391-395.

Salinas, J., M. P. de la Vega, and C. Benito. 1982. Identification of hexaploid wheat cultivars based on isozyme patterns. J. Sci. Food Agr. 33:221-226.

Sari Gorla, M., C. Frova, G. Binelli and E. Ottaviano. 1986. The extent of gametophytic-sporophytic gene expression in maize. Theor. Applied Genet. 72:42-47.

SAS Institute, Inc. 1985a. SAS user's guide: Statistics, version 5 ed. SAS Institute, Inc., Cary, N.C.

SAS Institute, Inc. 1985b. SAS/GRAPH user's guide, version 5 ed. SAS Institute, Inc., Cary, N.C.

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

Scandalios, J. G. 1974. Isozymes in development and differentiation. Ann. Rev. Plant Physiol. 25:225-258.

Seal, H. 1964. Multivariate statistical analysis for biologists. Wiley:New York.

Simmonds, N. W. (ed.). 1976. Evolution of crop plants. Longman, New York.

Small E., P. Y. Jui and L. P. Lefkovitch. 1976. A numerical taxonomic analysis of Cannabis with special reference to species delimitation. Syst. Bot. 1(1):67-84.

Sneath, P. H. A. 1976. Phenetic taxonomy at the species level and above. Taxon 254:437-450.

Sneath, P. H. A. and R. R. Sokal. 1973. Numerical taxonomy. Freeman, San Francisco.

Soltis, D. E., C. H. Haufler, D. C. Darrow, and G. J. Gastony. 1983. Starch gel electrophoresis of ferns: a compilation of grinding buffers, gel and electrode buffers, and staining schedules. Amer. Fern J. 73(1):9-27.

Torres, A. M. 1983. Fruit trees, p. 401-421. In: S. D. Tanksley and T. J. Orton (eds.). Isozymes in plant genetics and breeding, part B. Elsevier, Amsterdam.

Torres, A. M. and B. O. Bergh. 1980. Fruit and leaf isozymes as genetic markers in avocado. J. Amer. Soc. Hort. Sci. 105:614-619.

Torres, A. M., R. K. Soost and T. Mau-Lastovicka. 1982. Citrus isozymes. J. Hered. 73:335-339.

Vavilov, N. I. 1951. The origin, variation, immunity and breeding of cultivated plants. Ronald Press, New York.

Weeden, N. F. 1984. Distinguishing among white seeded bean cultivars by means of allozyme genotypes. Euphytica 33:199-208.

Weeden, N. F. and R. C. Lamb. 1985. Identification of apple cultivars by isozyme phenotypes. J. Amer. Soc. Hort. Sci. 110(4):509-515.

Wendel, J. F. and C. R. Parks. 1982. Genetic control of isozyme variation in Camellia japonica L. J. Hered. 73:197-204.

Wijsman, H. J. W. 1983. Petunia, p. 229-252. In: S. D. Tanksley and T. J. Orton (eds.). Isozymes in plant genetics and breeding, part B. Elsevier, Amsterdam.

Wilson, H. D. 1976. Genetic control and distribution of leucine aminopeptidase in the cultivated chenopods (Chenopodium) and related weed taxa. Biochem. Genet. 14:913-919.

Yang, N. S., J. C. Sorenson, and J. G. Scandalios. 1977. Genetic control of mitochondrial malate dehydrogenases: Evidence for duplicated chromosome segments. Proc. Natl. Acad. Sci. USA. 74:310-314.

Yushev, A. A. 1975. Morphological characters of the leaf in sour cherry and their use in the classification of varieties (in Russian). Byull. Vses. Ordena Lenina 54:34-40. [Plant Breed. Abstr. 47:3633; 1977].

Yushev, A. A. 1977. Morphological characters of the fruit in sour cherry and their use in the classification of varieties (in Russian). Byull. Vses. Ordena Lenina 75:27-31. [Plant Breed. Abstr. 49:7429; 1979].

APPENDIX

APPENDIX A: Segregation numbers for seed tissue zymograms

Table A1. Segregation numbers for seed tissue zymograms of ADH for ground cherry 323-2 and for the zone of faster mobility of 6-PGD for 'Montmorency'.

Genotype	Enzyme	Banding pattern	Segregation numbers
Ground cherry 323-2	ADH	"fast"-banded homodimer	5
		triple-banded heterodimer	62
		"slow"-banded homodimer	3
'Montmorency'	6-PGD (zone of faster mobility)	"fast"-banded homodimer	3
		triple-banded heterodimer	48
		"slow"-banded homodimer	22