Theor Appl Genet (1990) 80:129–133

THEORETICAL AND APPLIED GENETICS

Phylogenetic relationships among species of *Prunus* as inferred by isozyme markers*

B. D. Mowrey and D. J. Werner

Department of Horticultural Science, Box 7609, North Carolina State University, Raleigh, NC 27695-7609, USA

Received February 6, 1990; Accepted March 23, 1990
Communicated by G. Wenzel

Summary. An isozyme survey of 34 species of *Prunus* representing subgenera *Prunus*, *Amygdalus*, *Cerasus*, and *Lithocerasus* detected 110 presumptive alleles at 11 isozyme loci. Principal component analysis was conducted on the covariance matrix derived from allelic frequencies calculated for each species. Cluster analysis was performed on the first 30 principal components. Results generally support traditional classification of *Prunus* at the subgeneric level, except for members of subgenus *Lithocerasus* and two members of subgenus *Amygdalus*. *Prunus glandulosa* Thunb., *P. japonica* Thunb., and *P. tomentosa* Thunb. of subgenus *Lithocerasus* and *P. triloba* Lindl. of subgenus *Amygdalus* appear to represent primitive species. *P. besseyi* Bailey and *P. pumila* L. of subgenus *Lithocerasus* and *P. andersonii* of subgenus *Amygdalus* should be assigned to subgenus *Prunus*. Placement of its members indicates that subgenus *Lithocerasus* is an artificial grouping of species that are very different genetically although similar phenotypically.

Key words: Taxonomy – Peach – Almond – Cherry – Plum

Introduction

Prunus is a large diverse genus of woody plants endemic to much of the northern hemisphere (Krussmann 1986) and extending into the southern hemisphere in both the old and new world (Robertson 1974). Rehder (1940) divided the genus into five subgenera: *Prunus* [=*Prunophora* (Neck.) Focke], *Amygdalus* (L.) Benth. & Hook., *Cerasus* (Adans.) Focke, *Padus* (Moench) Focke, and *Laurocerasus* (Ser.) Rehd. The subgenus *Cerasus* as defined by Rehder was composed of a large, diverse group of species and was divided later by Ingram (1948) into the subgenera *Cerasus* and *Lithocerasus* Ingram. Mason (1913) proposed subgenus *Emplectocladus* (Torrey) Mason and sections *Piloprunus* Mason and *Penarmeniaca* Mason of the subgenus *Prunus* for the pubescent fruited *Prunus* species native to the Southwestern United States.

Evidence from interspecific crosses supports classification of subgenus *Lithocerasus* as distinct from subgenus *Cerasus*, and suggests a closer affinity to subgenus *Prunus*. Members of subgenus *Lithocerasus* sect. *Microcerasus* (Spach) Schneid. hybridize readily with species from subgenus *Prunus* and to some degree with members of subgenus *Amygdalus*, while generally failing to hybridize with species from subgenus *Cerasus* (Kataoka et al. 1988; Garley 1980; Hansen 1904). *Prunus andersonii* Gray generally is classified in the subgenus *Amygdalus*. However, Mason (1913) classified it in subgenus *Prunus* sect. *Penarmeniaca* Mason along with *P. eriogyna* Mason (syn. *P. fremontii* Watson).

Traditional taxonomy of *Prunus* has used outward phenotype to estimate genetic differences among species in question. Chemotaxonomic approaches, such as isozyme surveys, allow a more accurate estimate of genetic differences between two species. The objective of this investigation was to determine the isozyme profile of 33 species (Table 1) representing subgenera *Prunus*, *Amygdalus*, *Cerasus*, and *Lithocerasus* to clarify taxonomic relationships within *Prunus*.

Materials and methods

Electrophoretic techniques were as previously described (Mowrey et al. 1990). Isozyme systems were surveyed using standard pro-

* Paper No. 12529 of the Journal Series of the North Carolina Agricultural Research Service, Raleigh, NC 27695-7643, USA

Table 1. Species of *Prunus* examined and their classification according to Krussmann (1986)

Subgenus *Prunus*	Subgenus *Cerasus*
Section *Prunus*	(Adans.) Focke
Benth & Hook.	*avium* L.
cerasifera L.	*cerasus* Ehrh.
domestica L.	*fruiticosa* Pall.
salacina Lindl.	*gonduinii* (Poit. & Turp.) Rehd.
simonii Carr.	Section *Sargentiella*
spinosa L.	*campanulata* Maxim.
Section *Prunocerasus* Koehne	Section *Microcalymma* Koehne
americana Marsh.	*subhirtella* Mig.
angustifolia Marsh	Section *Magniculpula* Ingram
hortulana Bailey	*serrula* Franch.
maritima Marsh.	Section *Phyllomahaleb*
munsoniana	(Koehne) Rehd.
Wright & Hedr.	*mahaleb* L.
sucordata Benth.	Subgenus *Lithocerasus* Ingram
umbellata Ell.	Section *Microcerasus*
Section *Armeniaca*	(Spach) Schneid.
(Mill.) K. Koch	*besseyi* Bailey
armeniaca L.	*glandulosa* Thunb.
mume S. & Z.	*japonica* Thunb.
Subgenus *Amygdalus* (L.)	*pulmila* L.
Benth. & Hook.	Section *Armeniacocerasus*
andersonii Gray	Ingram
davidiani (Carr.) Franch.	*tomentosa* Thunb.
dulcis Webb.	
kansuensis Rehd.	
mira Koehne.	
persica (L.) Batsch.	
triloba Lindl.	

cedures and included aspartate aminotransferase (AAT), malate dehydrogenase (MDH), glucosephosphate isomerase (GPI), phosphoglucomutase (PGM), 6-phosphogluconate dehydrogenase (PGD), and shikimate dehydrogenase (SKDH) (Stuber et al. 1988). MDH, PGD, and SKDH were stained on morpholine-citrate pH 6.1 gels (Conkle et al. 1982); AAT and GPI were stained on lithium-borate/TRIS-citrate pH 8.3 gels (Stuber et al. 1988); PGM was stained on histidine-citrate pH 6.5 gels (Stuber et al. 1988).

Two to 11 clones of each of the species were examined, except for *P. spinosa*, *P. simonii*, and *P. subcordata*, where only one clone was available. Sample sizes were relatively small, however, genetic distance estimates are fairly independent of sample size (Gorman and Renzi 1979). Eleven isozyme loci were characterized in most clones. *Pgd-1* could not be characterized in *P. triloba* and *P. serrula*. Based upon migrational differences, 110 presumptive alleles were detected among the 11 loci. The average number of alleles per locus was high due to the diversity of species examined; however, it is likely that some alleles were not detected due to use of only one gel buffer system per enzyme system.

Statistical analyses, similar to those of Goodman and Stuber (1983), were performed on isozyme data using SAS (1982). Principal component analysis was conducted on the covariance matrix derived from allelic frequencies calculated for each species. Two separate principal component analyses were performed, one based on 10 loci, including all species, the other based on 11 loci, including all species except *P. triloba* and *P. serrula*. The first 30 principal components from each analysis, which accounted for nearly 100% of the variation present, were subjected to cluster analysis using the average linkage method to obtain a phylogenetic tree.

Results and discussion

The first four principal components accounted for 27%, 15%, 12%, and 7% of the total variation in the analysis of 11 loci and 26%, 14%, 10%, and 7% of the total variation in the analysis of 10 loci. These values are similar in magnitude to those observed in maize (Goodman and Stuber 1983; Doebley et al. 1985; Smith et al. 1985). Principal component analysis (Fig. 1) and cluster analysis (Figs. 2 and 3) produced groupings consistent with traditional subgeneric classification for most species. However, members of subgenus *Lithocerasus* were grouped with members of several subgenera and two members of subgenus *Amygdalus* were grouped with members of subgenus *Prunus*. Although the groupings obtained were in close agreement at the subgenus level, they did not agree with traditional classification as to section.

Members of subgenus *Prunus* were grouped together, along with *Prunus besseyi* and *P. pumila* of subgenus *Lithocerasus* sect. *Microcerasus*, *P. tomentosa* of *Lithocerasus* sect. *Armeniacocerasus*, and *P. andersonii* and *P. triloba* of subgenus *Amygdalus*. This grouping is in agreement with crossing behavior of *P. besseyi* and *P. pumila*, which both cross freely with plums producing fertile F_1 hybrids (Garley 1980; Hansen 1904). Crossing behavior of *P. andersonii* and *P. triloba* has not been documented. *Prunus tomentosa* and *P. triloba* were grouped together (Fig. 3), however, they diverge from other species in this grouping far back on the dendrogram. *Prunus tomentosa* crosses freely with *P. besseyi*, but it does not cross readily with other species (Kataoka et al. 1988). The relative positions of *P. tomentosa* and *P. triloba* in Fig. 3 suggest that these species probably are primitive.

Members of subgenus *Prunus* sect. *Prunus* were grouped together and subgrouped according to origin in the cluster analyses (Figs. 2 and 3). European species *P. cerasifera*, *P. domestica*, and *P. spinosa* were grouped, while Asian species *P. salacina* and *P. simonii* were grouped. The grouping of European species supports the proposed origin of *P. domestica* as an allohexaploid originating from hybridization of diploid *P. cerasifera* and tetraploid *P. spinosa*.

Although grouped with other members of subgenus *Prunus*, members of sect. *Prunocerasus* were not consistently grouped with each other. Native North American species *Prunus umbellata* and *P. maritima* were more closely associated with members of sect. *Prunus* than with other species of sect. *Prunocerasus*. This is unexpected since members of sect. *Prunocerasus* are endemic to North America, while members of sect. *Prunus* are en-

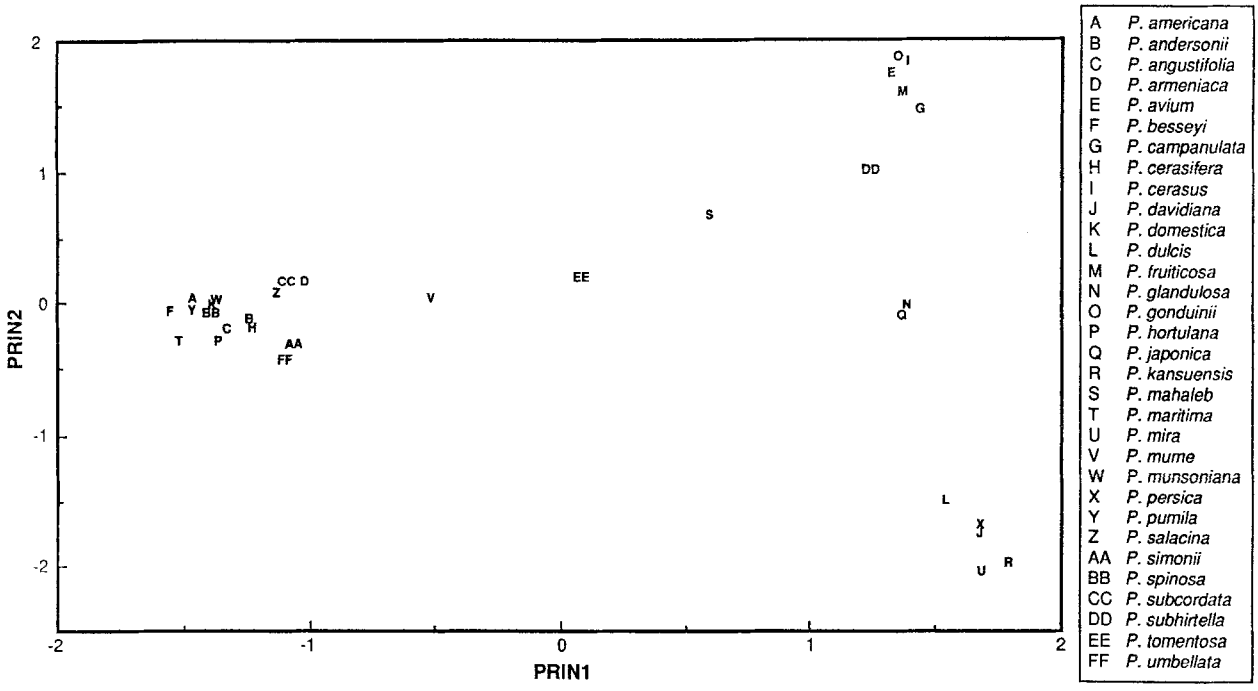

Fig. 1. Plot of the first two principal components derived from covariance matrix of allelic frequencies among 32 species of *Prunus*

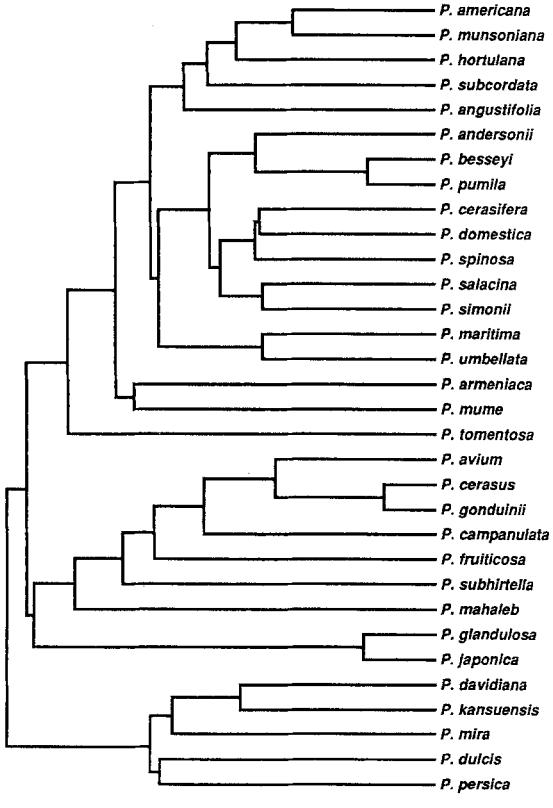

Fig. 2. Average linkage cluster analysis of 32 *Prunus* species performed using first 30 principal components derived from covariance matrix of allelic frequencies at ten isozyme loci

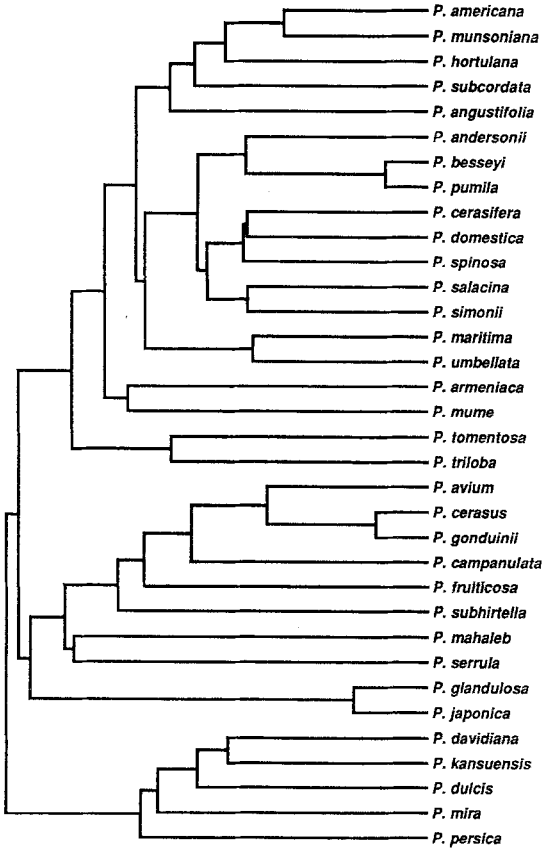

Fig. 3. Average linkage cluster analysis of 34 *Prunus* species performed using first 30 principal components derived from covariance matrix of allelic frequencies at 11 isozyme loci

demic to Europe and Asia. Other members of sect. *Prunocerasus* were grouped together. The results suggest that *P. umbellata* and *P. maritima* are related more closely to European and Asian species, possibly representing more recent introductions from Europe.

Prunus mume and *P. armeniaca* of subgenus *Prunus* sect. *Armeniaca* were grouped together, but diverge from each other far back on the dendrogram (Fig. 3). Placement of *P. mume* in Fig. 1 suggests that it may be a progenitor to *P. armeniaca*, as proposed previously based upon peroxidase isozymes (Sokolova and Andreev 1983).

Members of subgenus *Cerasus*, as defined by Ingram, also were closely associated, although there were some discrepancies at the sectional level. *Prunus campanulata* of sect. *Sargentiella* was placed with members of sect. *Cerasus* in both cluster analyses (Figs. 2 and 3) and was placed more closely to *P. cerasus* and *P. gonduinii* than *P. fruiticosa*. This would seem to contradict the proposed origins of *P. cerasus* and *P. gonduinii*, however, isozyme genotypes at most loci supported their proposed origins. *Prunus cerasus* reportedly arose as an allotetraploid from hybridization between *P. avium* ($2n=2x=16$) and *P. fruiticosa* ($2n=4x=32$) (Olden and Nybom 1968), while *P. gonduinii* reportedly originated thorough hybridization between *P. cerasus* and *P. avium* (Fogle 1975). The allelic frequency array of *Prunus companulata* was more similar to that of *P. avium* than the array of *P. fruiticosa*, therefore it was placed more closely to *P. avium* than was *P. fruticosa*.

Prunus glandulosa and *P. japonica* of *Lithocerasus* sect. *Microcerasus* also were grouped with subgenus *Cerasus*. This grouping is not supported by interspecific hybridization studies. *Prunus japonica* crosses with *P. armeniaca*, *P. mume*, and *P. salacina* of subgenus *Prunus* and *P. persica* of subgenus *Amygdalus*, but it will not cross with *P. avium* of subgenus *Cerasus*. *Prunus japonica* and *P. glandulosa* diverge from members of subgenus *Cerasus* near the base of the dendrograms (Figs. 2 and 3). Their placement in an intermediate position in Fig. 1 may indicate that these species are primitive and not related closely to species in other subgenera. *Prunus japonica* has been classified as a subspecies of *P. glandulosa* (Komarov 1941). This classification was supported in this study, since *P. japonica* differed from *P. glandulosa* by only one allele.

Most members of subgenus *Amygdalus* were grouped together. Subgenus *Amygdalus* is not divided into sections, although its members often are classified as either almond-like or peach-like. *Prunus dulcis* was the only almond species examined. None of the cluster analyses divided *P. dulcis* from the peach species, however, it does diverge somewhat from the peaches (Fig. 1). *Prunus triloba* and *P. andersonii* are distinct phenotypically from other members of the subgenus, except for their pubescent fruit, and were grouped with subgenus *Prunus* in the analyses (Figs. 1, 2, and 3).

The results of this study suggest that *P. glandulosa*, *P. japonica*, *P. triloba*, and *P. tomentosa* represent more primitive species and that *P. besseyi*, *P. andersonii*, and *P. pumila* should be assigned to subgenus *Prunus*. The grouping of *P. andersonii* with members of subgenus *Prunus* supports the classification proposed by Mason (1913). Placement of members of subgenus *Lithocerasus* in both cluster analyses (Figs. 2 and 3) and in the principal component analysis (Fig. 1) indicates that this subgenus is an artificial grouping of genetically distinct although phenotypically similar species. Results of this study are far from conclusive, although they provide evidence for reclassification of *Prunus* at the subgeneric level.

Acknowledgements. The authors thank Drs. D. W. Cain, W. R. Okie, D. W. Ramming, and R. C. Rom for providing budwood of the clones used in this study. Special thanks to Dr. Major M. Goodman for his assistance in the statistical analysis.

References

Conkle MT, Hodgskiss PD, Nunnally LB, Hunter SC (1982) Starch gel electrophoresis of conifer seeds: a laboratory manual. USDA For Serv Gen Tech Rep PSW-64

Doebley JF, Goodman MM, Stuber CW (1985) Isozyme variation in the races of maize from Mexico. Am J Bot 72:629–639

Fogle HW (1975) Cherries. In: Janick J, Moore JN (eds) Advances in fruit breeding. Purdue University Press, West Lafayette/IN, pp 348–366

Garley B (1980) Ah-oon-ye-ya-pa, the sand cherry: its origin, improvement, and nomenclature. Fruit Var J 34:13–17

Goodman MM, Stuber CW (1983) Races of maize. VI. Isozyme variation among races of maize in Bolivia. Maydica 28:169–187

Gorman GC, Renzi JR Jr (1979) Genetic distance and heterozygosity estimates in electrophoretic studies: effects of sample size. Copia 2:242–249

Hansen NE (1904) New hybrid fruit. SD Agric Exp Stn Bull 108

Ingram C (1948) Ornamental cherries. London

Kataoka I, Sugiura A, Tomana T (1988) Interspecific hybridization between *Microcerasus* and other *Prunus* spp. J Jpn Soc Hortic Sci 56:398–407

Komarov VL (ed) (1941) Flora of the U.S.S.R., vol X. Rosaceae – Rosoideae, Prunoideae. Israel Prog Sci Trans, Jerusalem, pp 381–448

Krussman G (1986) Manual of cultivated broad-leaved trees and shrubs vol III. Timber Press, Portland/OR, pp 18–85

Mason SC (1913) The pubescent-fruited species of *Prunus* of the southwestern states. J Agric Res 1:147–179

Mowrey BD, Werner DJ, Byrne DH (1990) Inheritance of isocitrate dehydrogenase, malate dehydrogenase, and shikimate dehydrogenase in peach and peach × almond hybrids. J Am Soc Hortic Sci 115:312–319

Olden EJ, Nybom N (1968) On the origin of *Prunus cerasus* L. Hereditas 59:327–345

Rehder A (1940) Manual of cultivated trees and shrubs, 2nd edn. McMillan, New York, pp 452–481

Robertson KR (1974) The genera of Rosaceae in the southeastern United States. J Arnold Arbor Harv Univ 55:303–662

SAS Institute, Inc. (1982) SAS User's guide: statistics. SAS Institute, Cary/NC

Smith JSC, Goodman MM, Stuber CW (1985) Relationship between maize and teosinte of Mexico and Guatemala: numerical analysis of allozyme data. Econ Bot 39:12–24

Sokolova EA, Andreev VI (1983) Forms of peroxidase in the leaves of apricot and their utilization for the systematics of the genus *Armeniaca* Scop. Trudy Prikl. Bot Genet 76:61–68

Stuber CW, Wendel JF, Goodman MM, Smith JSC (1988) Techniques and scoring procedures for starch gel electrophoresis of enzymes from maize (*Zea mays* L.). NC Agric Res Serv Tech Bull 286