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Phylogenetic relationships of cultivated *Prunus* species from an analysis of chloroplast DNA variation

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Abstract Chloroplast DNA (cpDNA) restriction-site mutations in seven cultivated *Prunus* species were compared to establish the phylogenetic relationships among them. Mutations were detected in 3.2-kb and 2.1-kb amplified regions of variable cpDNA, cut with 21 and 10 restriction endonucleases, respectively, to reveal polymorphisms. Parsimony and cluster analyses were performed. The species pairs *P. persica-P. dulcis*, *P. domestica-P. salicina*, and *P. cerasus-P. fruticosa* were completely monophyletic. All of the species were grouped with conventional subgenus classifications. The subgenus *Cerasus* was the most diverged. *Cerasus* ancestors separated from the remainder of *Prunus* relatively early in the development of the genus. *P. persica-P. dulcis*, *P. domestica-P. salicina* and *P. armeniaca* formed a second monophyletic group. *Prunophora* species were less diverged than *Amygdalus* species. The results also suggest that the rate of mutation in *Cerasus* spp. chloroplast genomes is significantly greater than for the other subgenera sampled.

Key words Chloroplast · Phylogeny · DNA · PCR · *Prunus*

Introduction

The genus *Prunus* (Rosaceae, subfamily Prunoideae) comprises five subgenera, *Prunophora*, *Amygdalus*, *Cerasus*, *Padus*, and *Laurocerasus*. Rehder (1954) recognized 77 species, although Sauer (1993) suggests that as many as 150 species may exist. Classifications are generally based on morphology, although chromosome counts are available for many of the species. Several isozyme studies have been conducted (Arulsekar et al. 1986; Hauagge et al. 1987; Byrne and Littleton 1989; Byrne 1990; Mowrey and Werner 1990; Mowrey et al. 1990); however, they have been used primarily for the study of intraspecific variation. Crossing relationships among *Prunus* species, especially cultivated *Prunus*, have been important tools for ascertaining relationships within the genus.

The basic chromosome number for Prunus is x=8. Almond (*P. dulcis* D. A. Webb, formerly *P. amygdalus* Batsch.), peach (*P. persica* Batsch.), apricot (*P. armeniaca* L.), and sweet cherry (*P. avium* L.) are diploids with 2n=16. Diploid, tetraploid, and hexaploid plum species are present within the subgenus *Prunophora* (Bailey 1975; Hesse 1975; Kester and Asay 1975; Weinberger 1975). *P. domestica* L., the European plum, is a hexaploid, probably resulting from the tetraploid *P. spinosa* L. and diploid *P. cerasifera* Ehrh. (Sauer 1993). Japanese plum, *P. salicina* Lindl., and hybrids with American species (which are also called Japanese plums) derived from them, are both diploid. Sour cherry, *P. cerasus* L., is a tetraploid, probably derived from the tetraploid *P. fruticosa* Pall. and 2n gametes of *P. avium* L. (Sauer 1993). Fogle (1975) considered *P. fruticosa* to be a parent for both *P. avium* and *P. cerasus*.

Watkins (1976) postulated a center of origin for *Prunus* in central Asia. Cherries (subgenus *Cerasus*) are considered to be the most distantly related species group. Almonds and peaches, members of subgenus *Amygdalus*, are apparently closely related (they hybridize easily) but are relatively distant from plum and apricot of the species subgenus *Prunophora*. Watkins (1976) considered *Amygdalus* and *Prunophora* to be genetically monophyletic, with the caveat, based on rather limited evidence, that almond and Damson plums were somewhat genetically isolated from the rest of the group. He did not believe that genetic transfer was likely to occur directly between the *Amygdalus-Prunophora* group and the subgenus *Cerasus*. He postulated any gene transfer between the subgenera as occurring via the *Microcerasus* section of *Cerasus*, with *Prunophora* being more closely associated with the section *Microcerasus*. Although he suggested that the *Prunus* ancestors were located in *Cerasus*, Watkins (1976) concluded

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that plum species in the *Prunophora* are the central species for *Prunus* evolution at the present time, due to their ability to hybridize with species from the other subgenera.

An extensive phylogenetic analysis of 34 *Prunus* species based on isozyme polymorphism was conducted by Mowrey and Werner (1990). The results from this study are quite detailed but somewhat confusing because the taxonomy of Krussman (1986), rather than the earlier and more traditional treatments (Rehder 1954; Watkins 1976), was used as the basis for defining subgenera and sections. Other than re-naming *Prunophora* as the subgenus *Prunus*, the major differences in classification are in the sections within *Cerasus*. In addition, methodological problems with the combined principal component-cluster analyses employed could result in different species arrangements if alternative statistical procedures were to be used. Preliminary DNA analysis was conducted on several groups of *Prunus* species (Kaneko et al. 1986; Nybom et al. 1990; Uematsu et al. 1991). Kaneko et al. (1986) used RFLP analysis of chloroplast DNA (cpDNA) to classify Japanese flowering cherries. Uematsu et al. (1991) used cpDNA RFLPs from isolated cpDNA to classify wild and cultivated peach and apricot species, as well as *P. domestica* and several wild species. However, this study had several major problems, including the use of questionable band-pattern data and reliance on cluster analysis for establishing species relationships. The resulting dendrogram associated apricot species most closely with the *P. davidiana* peach relative, then with *P. domestica*, and most distantly with *P. persica*, which is inconsistent with any conventional interpretation. Uematsu et al. (1991) concluded that apricot species are at the center of stone fruit diversity, although their dendrogram does not directly support this conclusion.

Variation at the DNA level in the chloroplast genome has been used since the early 1980s to study species relationships (Palmer 1987; Dowling et al. 1990; Clegg and Zurawski 1992). cpDNA is especially useful for phylogenetic analysis due to its highly conserved nature and relative abundance in the cell as compared to nuclear DNA. Wilson et al. (1990) reported that the mutation rate for palms (Arecaceae) is 5–13 times less than for annuals. Frascaria et al. (1993) reported a five-fold reduction in the mutation rate for chestnut (*Castanea sativa* Mill.) compared to Solanaceae and Poaceae family members. Parfitt and Badenes (unpublished) have found similar results for *Juglans* and *Pistacia*. Conventional cpDNA Southern analysis did not provide sufficient polymorphic sites to develop meaningful phylogenies for these genera. Therefore, more variable non-coding cpDNA regions, described by Ogihara et al. (1991), were used to identify additional polymorphisms. Arnold et al. (1991), Liston et al. (1992) and Rieseberg et al. (1992) have used restriction-site analysis of a 3.2-kb region of the chloroplast genome for phylogenetic analysis. An additional non-coding region with conserved flanking PCR primer sites was described for rice, tobacco, and liverwort (Taberlet et al. 1991). The flanking primer sites were postulated to be conserved across a wide taxonomic range. The fragment was successfully amplified from 19 species representing a variety of diverse families by Taberlet et al. (1991). The limited variability and wide use of cpDNA in phylogenetic studies made it an appropriate genome for the development of a *Prunus* phylogeny. The present study was undertaken to determine the relationships among the seven cultivated *Prunus* species. The procedure of Arnold et al. (1991) was used because of its simplicity and our previous experience with low mutation rates from cpDNA Southern analysis in other genera. We therefore examined restriction-site variation in these two fragments from eight species of *Prunus* to ascertain their phylogenetic relationships.

Materials and methods

Total DNA was isolated from fresh leaves of 2–5 accessions of *P. dulcis*, *P. persica*, *P. armeniaca*, *P. domestica*, *P. salicina*, *P. avium*, *P. fruticosa* and *P. cerasus* (Table 1). Genotypes were chosen to represent germplasm diversity from Europe and the United States. The CTAB method of Doyle and Doyle (1987) was modified by the use of three chloroform extractions to eliminate, or mitigate, the high level of polysaccharide present in some the species, especially members of the subgenera *Cerasus* and *Prunophora*. Leaves collected near the end of the growing season had significantly less polysaccharide than those collected earlier. *Fragaria vesca* L., family Rosaceae, was used as an outgroup taxon for statistical analyses. Five grams of fresh leaves in 20 ml of CTAB buffer yielded 75–500 µg of DNA.

A 3.2-kb region of variable cpDNA (Ogihara et al. 1991) bounded by the conserved sequences, 5′-ATGTCACCACAAACAGAAACTAAAGCAAGT-3′ (*rbcL*) and 5′-ACTACAGATCTCATACTACCCC-3′ (*ORF 106*), was amplified via PCR as described by Arnold et al. (1991) with the following modifications: 0.1–0.5 µg of total genomic DNA per 100-µl reaction (50 mM KCl, 10 mM TRIS-HCl pH 8.3, 2 mM $MgCl_2$, 1 µM of primer, 200 µM of each dNTP and 0.5 units of *Taq* polymerase) were amplified during 35 cycles of 1 min at 94°C, 1 min at 50°C, and 3 min at 72°C. Primers for the flanking sequences were synthesized by Operon Technologies, Inc. Complete amplification was obtained for all samples. Twenty restriction endonucleases (*Alu*I, *Ase*I, *Bam*HI, *Cla*I, *Dde*I, *Dra*I, *Eco*RI, *Eco*RV, *Hha*I, *Hin*dIII, *Hinf*I, *Mbo*I, *Msp*I, *Pal*I, *Pst*I, *Rsa*I, *Scr*FI, *Sty*I, *Taq*I, and *Xba*I) were chosen for their ability to restrict

Table 1 Species and genotypes used for DNA isolation and amplification

Species	Genotypes
Prunus dulcis	'Nonpareil', 'Cristomorto', 'Ferraduel', 'Padre', 'Marcona'
Prunus persica	'Spring Crest', 'Halford', 'Tebana', 'Catherine'
Prunus domestica	'French prune', 'Reina claudia', 'Claudia tolosa'
Prunus salicina	'Golden Japan', 'Satsuma'
Prunus armeniaca	'Tilton', 'Goldrich', 'Velazquez','Bulida' 'Canino'
Prunus avium	'Bing', 'Van', 'Burlat'
Prunus cerasus	'Montmorency', #2534-5, #2534-6
Prunus fruticosa	#323-2, #586-3
Fragaria vesca	'Douglas'

Table 2 Character states for mutation sites in the 3.2-kb and 2.1-kb amplified cpDNA regions for eight *Prunus* spp. and a *Fragaria vesca* outgroup

Mutation sites[a]	*P. dulcis*	*P. persica*	*P. avium*	*P. fruticosa*	*P. cerasus*	*P. armeniaca*	*P. domestica*	*P. salicina*	*F. vesca*
3.2-kb									
*Hinf*I-1	1	1	0	0	0	1	1	1	0
*Sty*I-2	0	0	1	1	1	0	0	0	0
*Sty*I-3	0	0	0	0	0	0	1	1	0
*Mbo*I-4	1	1	0	0	0	0	0	0	0
*Mbo*I-5	0	0	1	1	1	1	1	1	0
*Msp*I-6	0	0	1	1	1	0	0	0	0
*Taq*I-7	0	0	0	0	0	1	0	0	0
*Msp*I-8	0	0	0	0	0	0	0	0	1
*Scr*FI-9	0	0	0	0	0	0	0	0	1
*Mbo*I-10	0	0	0	0	0	0	0	0	1
*Hinf*I-11	0	0	0	0	0	0	0	0	1
*Sty*I-12	0	0	0	0	0	0	0	0	1
*Xba*I-13	0	0	0	0	0	0	0	0	1
2.1-kb									
*Pal*I-14	0	0	1	1	1	0	0	0	0
*Hinf*I-15	0	0	1	1	1	0	0	0	0
*Mbo*I-16	1	1	0	0	0	0	0	0	0
*Mbo*I-17	0	0	0	0	0	0	0	0	1
*Mbo*I-18	0	0	0	0	0	0	0	0	1
*Bst*I-19	0	0	0	0	0	0	0	0	1
*Sty*I-20	0	0	0	0	0	0	0	0	1
*Scr*FI-21	0	0	0	0	0	0	0	0	1
*Taq*I-22	0	0	0	0	0	0	0	0	1
Length mutation[b]	0	0	1	3	3	0	2	2	0

[a] 0=loss of mutation site, 1=gain of mutation site
[b] Coded from short to long; 2000, 2050, 2100, and 2150, respectively

cpDNA within the amplified fragment based on previous studies in tobacco (Dowling et al. 1990) and *Pistacia* (Parfitt and Badenes, unpublished). Approximately 1 µg of amplified digested DNA was electrophoresed in 2% agarose gels at 50 V for 4 h. As a result of our previous experience with *Pistacia*, all digestions with four-base restriction enzymes were run on 5% FMC 'Metaphor' agarose as well as 2% conventional agarose, which permitted the resolution of fragments as small as 40 bp. Thirteen mutations were coded as character states 0 and 1, with 0 indicating site loss (see Table 2).

An additional 2.1-kb region of non-coding variable DNA was amplified, as described by Taberlet et al. (1991), using the flanking primers 5′-CATTACAAATGCGATGCTCT-3′ and 5′-ATTTGAACTGGTGACACGAG-3′ synthesized by Operon Technologies. DNA was cut with 11 restriction endonucleases (*Alu*I, *Bst*NI, *Dde*I, *Hin*FI, *Mbo*I, *Msp*I, *Pal*I, *Rsa*I, *Scr*FI, *Sty*I, and *Taq*I) selected as the most frequent cutters of cpDNA from the results of the 3.2-kb digests. Nine additional mutations were coded 0 and 1 and length mutations in this region were scored from 0 to 3, shortest to longest.

Unweighted pair group mean cluster analyses (UPGMA) with the average distance option using similarity matrices derived from the simple matching coefficient was performed with NTSYS-pc ver. 1.80 (Rohlf 1993). The neighbor-joining algorithm from NTSYS was also performed with this matrix. UPGMA with average linkage was performed with BIOSYS-1 ver. 1.7 (Swofford and Selander 1981, 1989) software using Nei's unbiased genetic distance and Roger's genetic distance. A Wagner distance tree was also computed from the Rogers distance matrix with BIOSYS-1. Site gain and loss data were recoded as allelic data (homozygotes) for these analyses. Unordered (Fitch) and Dollo parsimony analyses were performed with PAUP ver. 3.0 (Swofford 1990) using a complete exhaustive search of 135135 trees to find the most parsimonious unrooted trees. Bootstrap searches (1000 replications) for both unordered and Dollo parsimony, with a 50% majority rule consensus, were done to establish arm-length confidence levels. We also tested the restriction-site weighting procedure of Albert et al. (1992) with PAUP.

Results

Amplification was obtained for all species with primers for both the 3.2-kb region described by Ogihara et al. (1991) and the 2.1-kb region [analogous to the 1.5-kb region described by Taberlet et al. (1991)]. The ability to amplify these DNA regions for subsequent analysis was highly significant, because good yields of *Prunus* DNA for Southern (1975) transfers were difficult to obtain, especially for *Cerasus* and *Prunophora* species, which tend to have excessive levels of interfering polysaccharides. Fortunately, the PCR-based procedure described above required only 0.1–0.5 µg quantities of DNA. Other studies with *Pistacia* and *Juglans* in our laboratory (unpublished) also suggested that reliance on Southern analysis of the whole chloroplast genome would probably not provide a useful number of site changes.

Of the nucleotides in the 3.2-kb fragment 11.8% were sampled with 20 restriction enzymes while 8.7% of the nucleotides in the 2.1-kb fragment were sampled with 11 selected restriction endonucleases. Seven mutations were identified in *Prunus* from the 21 fragments representing site polymorphisms in the 3.2-kb region (Table 2, Fig. 1).

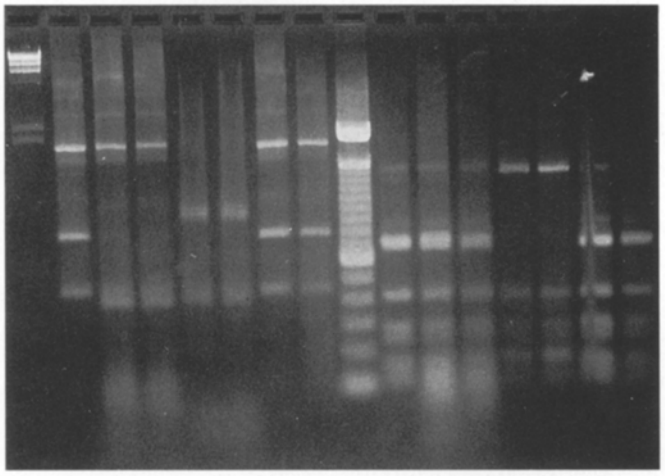

Fig. 1 Polymorphic cpDNA restriction fragments from a 3.2-kb fragment of amplified chloroplast genome of seven *Prunus* species, run in a 2% agarose gel. *Lane 1, γ, lanes 2–8* restricted with *Sty*I, *P. armeniaca, P. salicina, P. domestica, P. cerasus, P. avium, P. dulcis, P. persica*; *lane 9*, 100-bp ladder; *lanes 10–16*, restricted with HinfI, *P. armeniaca, P. salicina, P. domestica, P. cerasus, P. avium, P. dulcis, P. persica*

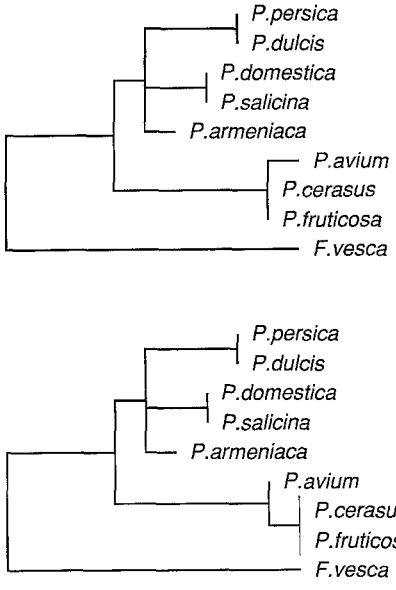

Fig. 2 Phylograms obtained from unordered parsimony analysis with PAUP of eight *Prunus* spp. with a *Fragaria vesca* outgroup. Arm lengths represent relative extent of divergence from the nodes

Six additional mutations were present in *F. vesca*. Three mutations were identified from nine fragments among *Prunus* species in the analysis of the 2.1-kb region and six more were identified for *F. vesca*. The per nucleotide mutation frequency for the 3.2- and 2.1-kb fragments was 0.9±0.35% and 0.8±0.5% (Nei 1987), respectively, with a combined frequency for both fragments of 0.9±0.3%. Three different length mutations (total of four states) were also identified. *F. vesca* was monophyletic with *P. dulcis*, *P. persica* and *P. armeniaca* for this character.

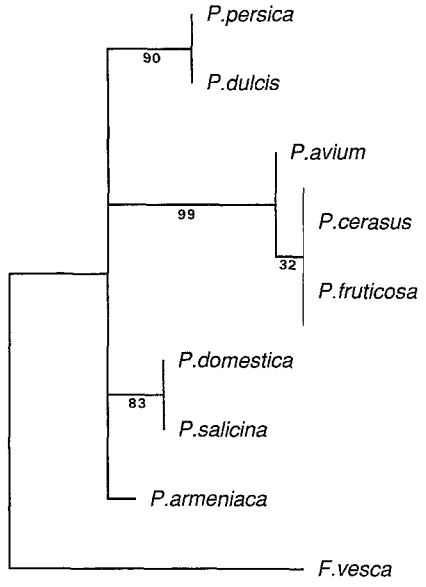

Fig. 3 50% majority rule consensus tree for eight *Prunus* spp. with *Fragaria vesca*, derived from bootstrap analysis (1000 replications) with confidence levels for arms

Ten minimum-length trees from the unordered parsimony analysis (Sneath and Sokal 1973), 26 units long and with a consistency index (CI) of 0.96, were obtained from both branch and bound and exhaustive searches. Four of these had F values of 4 with the remainder having an F value of 6. All of the trees had appropriate monophyletic groups consistent with the subgenera *Amygdalus*, *Cerasus* and *Prunophora*. In addition to being separable by 'F' the two groups of trees also differed in the placement of subgenera within the trees, the major difference being whether *Amygdalus* or *Cerasus* bifurcated first. The four equally parsimonious trees are presented as two similar phylograms (Fig. 2) differing only in whether *P. avium* or *P. fruticosa-P.cerasus* are considered to be the more-derived species. (The other two trees had zero arm-length polytomies and were indistinguishable from those in Fig.2.) They are separated by a 100-bp length difference in the 2.1-kb region, so the interpretation of the correct representation depends on whether length gains or losses are more likely. Unlike point mutations, there is no a priori reason to assume that either is more likely. Conventional Dollo parsimony, where constraints of repeated site loss but only one site gain is allowed (Swofford and Olsen 1990), produced four equally parsimonious trees 26 units long with a CI of 0.89 and F=24–38. These trees were less parsimonious than the unordered trees as indicated by CI and F indices. The weighting method of Albert et al. (1992) yielded long trees with large Fs (>38) for all weighting from 1.2 to 2 forward: reverse mutation rates. The unordered bootstrap analysis was not especially informative, since it basically averaged the two general tree forms obtained previously. Consequently, all of the subgenera (*Amygdalus*, *Cerasus*, and *Prunophora*) were represented as having a common ancestral divergence point. The connection of the subgenus *Cerasus* is most strongly supported (99%), followed by *Amyg-*

dalus (90%), and *Prunophora* (83%). The resulting consensus tree (Fig. 3) had a length of 27, an F of 10, and a CI=0.93, suggesting that this tree provided a less optimal fit to the data than the unordered trees.

The UPGMA tree from the NTSYS analysis using the simple matching coefficient (Fig. 4) correctly placed *F. vesca* outside of the *Prunus* species. No mutation-site or length differences were shown for the species pairs *P. dulcis-P. persica*, *P. domestica-P. salicina*, and *P. fruticosa-P. cerasus*. *P. avium* was joined to the *P. fruticosa-P.cerasus* cluster, and *P. armeniaca* was joined to the *P. domestica-P. salicina* cluster at the next level. The *P. dulcis-P. persica* cluster next joined the *Prunophora* cluster and this cluster was finally joined by the subgenus *Cerasus* cluster. The Nei's distance cluster analysis (F=2.6, standard deviation=13.3%) was not as precise as the Roger's distance cluster analysis (F=0.9, standard deviation=7.3%). Both Nei's and Roger's distance cluster analyses produced a similar arrangement of species clustering and were generally consistent with the UPGMA tree from the NTSYS analysis, except that a *tie* was obtained where *P. armeniaca* and *Prunophora* joined the *Amygdalus* cluster. The subgenus *Cerasus* bifurcated first in all of the trees and consequently had the longest arm-length, suggesting that *Cerasus* was most distant from the other subgenera. However, as seen from the best-fit unordered phylograms (Fig. 2) this distance is due in part to the considerably derived state of *Cerasus*.

The Wagner distance tree generated with BIOSYS-1 (F=0.3 and 3.3% standard deviation) differed from the unordered (PAUP) trees with respect to the divergence order for *Cerasus*, which is placed as the last bifurcating group rather than the first. This may be an artifact of the data transformation into genetic distance matrices, which is required by BIOSYS-1 but is probably not the most appropriate treatment for +/– character-state data.

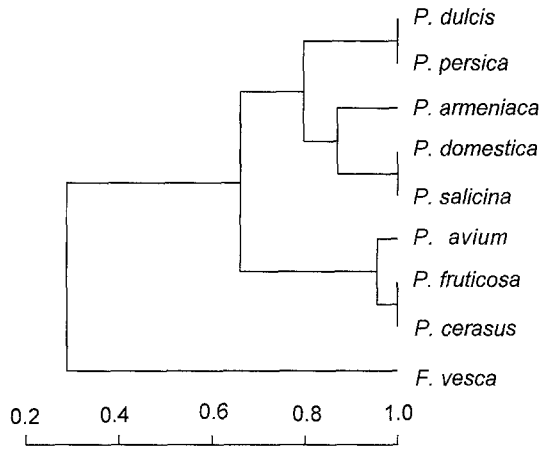

Fig. 4 Unweighted pair group mean cluster analysis (average linkage clustering) of a simple matching coefficient data matrix for eight *Prunus* spp. and *Fragaria vesca* from NTSYS

Discussion

PCR amplification and restriction of the 3.2- and 2.1-kb cpDNA sequences permitted the analysis of 558 sites which is about one-third of the approximately 1430 nucleotides sampled in a typical *rbcL* sequence analysis. However, the analysis is easier to do, less costly, and samples a larger subset of the chloroplast genome including noncoding regions reported to have a higher level of variation than found in coding sequences (Ogihara 1991).

Several of the analytical procedures produced unsatisfactory results, most notably the neighbor-joining procedure, which produced a tree that had no relationship to any known *Prunus* taxonomic alignment. *Fragaria vesca* was misplaced in the tree and *P. persica* and *P. dulcis*, which were identical for all polymorphisms, were split into different terminal groups. The Wagner distance analysis also produced a tree which could not be reconciled with either the cluster or parsimony analyses. Therefore, the remainder of the discussion will be based upon the parsimony analyses which were consistent with the UPGMA cluster analyses.

A single most parsimonious tree was not obtained in this study. Therefore, three criteria, tree length, consistency index and F (Farris 1972), were used to select trees that would best explain the observed data in terms of taxonomic significance. These trees suggest an early divergence of *Cerasus* from the rest of *Prunus*, which is consistent with the conventional taxonomic interpretation of Watkins (1976). The bootstrap consensus tree does not permit conclusions concerning the relative placement of the subgenera since they are all connected at a single node. This is not surprising since it is the average of two best trees derived from the bootstrap analysis. However, the subgenus *Cerasus* has the strongest connection (99% probability) while *Prunophora* species had the weakest connection (83%), so a case could be made for an early separation of *Cerasus* ancestors. The F value and CI for this tree were inferior to the trees in Fig. 2.

The most parsimonious trees (Fig. 2), the bootstrap consensus tree (Fig. 3), and the cluster analyses all support the hypothesis that the subgenus *Cerasus* has evolved more extensively than either *Prunophora* or *Amygdalus*. This could be due to a more ancient origin for the subgenus or else to a more rapid rate of evolution either at some point in the past or continuously. It is interesting to note that extant cherry species, especially *P. avium*, are recognized for their ability to develop clonal sports. The trees in Figs. 2 and 3 also suggest that the ancestors of *P. armeniaca* may have diverged at approximately the same time as the other *Prunophora* and *Amygdalus* species and could support its placement in a different subgenus rather than in the section *Armeniaca* within the subgenus *Prunophora* (Rehder 1954). The suggestion by Uematsu et al. (1991) that apricots occupy a central position in *Prunus* could not be confirmed since the ancestors of apricot species either separated synonymously with *Cerasus* and the other *Prunophora*species or else diverged as part of the *Prunophora* group after the separation of the ancestors of the subgenus *Cerasus*.

P. cerasus was correctly placed with respect to its tetraploid genome, which is considered to be derived from female gametes of 4x *P. fruticosa* and 2n pollen of 2x *P. avium* (Sauer 1993). Our data also suggest that the chloroplast genome of *P. cerasus* is derived from *P. fruticosa*. However, Iezzoni et al. (1989) reported that she had found two chloroplast genotypes in a survey of *P. cerasus* species, probably explained by the occasional occurrence of 2n eggs in *P. avium*. Most *P. cerasus* genotypes were synonymous with *P. fruticosa* in this regard. This is an example of reticulate evolution, which may be relatively common within *Prunus* subgenera. If proven, her results would suggest that *P. cerasus* is of very recent origin.

The species examined in the present study grouped appropriately into their respective subgenera as described by Watkins (1976) and Rehder (1954). The theory of a section *Microcerasus* bridge (Watkins 1976) is difficult to support given the considerable divergence of *Cerasus* from the rest of *Prunus*. Species that are conventionally classified in the *Prunophora* (*P. domestica*, *P. salicina*, and *P. armeniaca*) were less diverged than the remaining species in all of the phylograms, with the implication that less genetic change is occurring in this subgenus than in *Amygdalus* and (especially) *Cerasus*. While analysis of additional species within the *Prunus* subgenera are needed to confirm the placement of subgenera from the present study, the results suggest that the subgenus *Prunophora* cannot be identified as the central evolutionary group for *Prunus* as suggested by Watkins (1976).

The phylogeny presented here is similar to that of Mowrey and Werner (1990) which placed cherries in a more ancestral position to plums if constant evolution rates are assumed, i.e., greater difference equals earlier divergence. The distinct clusters developed in their study were nearly equally distant, similar to the result in Fig. 3. However, their dendrogram also separated the subgenus *Amygdalus* cluster from all of the remaining species, a result which we observed only for the Wagner distance tree and which we consider to be less likely than the alternative arrangements presented in this paper. Mowrey and Werner (1990) used isozyme data for their analysis, which correspond to nuclear genome variation. The results from the present study suggest that molecular genetic information from chloroplast and nuclear genomes will permit generally similar conclusions to be formulated concerning species relationships within *Prunus*.

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