Systematic relationships in *Juniperus* based on random amplified polymorphic DNAs (RAPDs).

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Summary

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44 taxa of *Juniperus* were analysed using RAPDs, similarity measures and principal coordinate analyses (PCO). The three sections of *Juniperus* (sect. *Caryocedrus*, sect. *Juniperus*, sect. *Sabina*) were found to be distinguished by RAPDs. The genus appears to be naturally divided into three major sections with two series (serrate and smooth leaf margins) in *J.* sect. *Sabina*. Additional examinations of specific and infraspecific taxa confirms earlier taxonomic work based on leaf terpenoids. Computer software (PCO3D from R.P.A.) for PCO is introduced for the analysis of RAPDs. RAPDs were found to be of taxonomic use ranging from sectional to varietal levels.

Introduction

The genus *Juniperus* L. is composed of approximately 75-80 taxa, of which 41 are found in the western hemisphere. The genus is extremely diverse, with taxa found above timberline *(J. monticola)*, to sea level *(J. lucayana)*, forming prostrate mats of plants *(J. horizontalis)* to large timber trees up to 50-60 m *(J. deppeana* var. *robusta)*. *Juniperus* is the second largest genus of the conifers, with only *Pinus* containing more species.

The evolution of the female cone with fused scales has led to co-evolution with birds, so that the seeds are often carried over long distances. The species are widely distributed and generally increase their range when habitat is disturbed. The taxa are allied in three definite groups and have been so recognized as three genera: *Arceuthos* Antoine & Kotschy with woody female cones and decurrent, acicular leaves, a monotypic group with only a single species found in Greece, Turkey, Syria and Lebanon *(Juniperus drupacea)*; *Juniperus* s. str. with acicular leaves, articulated at the base, and fleshy to obscurely woody female cones (approximately 7 to 9 species, all of which are in the eastern hemisphere except *J. communis*, which is circumboreal in distribution); and *Sabina* Mill. with scale-like leaves on mature trees and acicular leaves (decurrent, not jointed at the base) on juvenile plants or at the tips of fast growing branchlets (occasionally only juvenile leaves as in the case of *J. saxicola* of Cuba; see Adams & al., 1987), female cones fleshy to obscurely woody (with 28 species in the western hemisphere and about 25 species in the eastern hemisphere). Taxonomists of Europe and the Western Hemisphere later reduced the three aforementioned genera to sections within *Juniperus*: sect. *Caryocedrus* Endl.; sect. *Juniperus* (sect. *Oxycedrus* Gaussen); and sect. *Sabina* (Mill.) Gaussen, and so did Zanoni (1978) in a review of the junipers of North America. However, the most recent taxonomic treatment of the junipers of China (Cheng & Fu, 1978) still recognized the genera *Juniperus* and *Sabina* as distinct (*Arceuthos* is not found in China).

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The most primitive species of the juniperoid complex is *Juniperus drupacea* (= *Arceuthos drupacea* (Labill.) Ant. & Kotschy) which has spheroidal, woody female cones, 2-2.5 cm in diameter, much as found in *Cupressus* and *Taxodium*. In addition, the acicular leaves are very wide and flat (similar to the leaves of *Taxodium*). *J. drupacea* is distributed in Greece, Turkey, Syria and Lebanon at 300-2000 m. The junipers of *J.* sect. *Juniperus* are thought to be closely allied with *J. drupacea* and derived from a common ancestor. The junipers of *J.* sect. *Sabina* appear to be the most advanced taxa, a judged by leaf morphology and female cone characteristics (relative to outgroups such as *Cupressus* and *Chamaecyparis*). Based on morphology, it would appear that the sabinoid junipers were derived from an ancestor of the "oxycedroid" junipers.

There are three centers of diversity within the genus: the central highlands of Mexico; the western highlands of China (particularly the Sichuan and Gansu provinces) and the Mediterranean-Oriental region (in particular from Greece to Pakistan), where several taxa of *J.* sect. *Juniperus* co-occur. All junipers of central Mexico and most of those of western China belong to *J.* sect. *Sabina*.

During the past 20 years there has been considerable study of *Juniperus* sect. *Sabina* in the western hemisphere (see Adams, 1991 for a review of the terpenoid data; Adams, 1989, 1993 for recent treatments using morphological data). In general, the morphology and leaf volatile oils have been congruent in the junipers, although the latter have been more useful than the morphological characters at the infra-specific level and for analysing hybridization. The leaf terpenoids have also proved useful in analysing closely related species (Adams, 1989; Zanoni & Adams, 1976). However, at the higher levels of classification, the terpenoids are not as useful, due to convergence and reticulate evolution.

The emergence of techniques for the rapid, efficient, and routine analyses of DNA offers a new suite of characters that promises to enable one to examine accumulated genetic differences without the interferences of environmental and seasonal variations. In addition, conserved DNA sequences might be useful to examine relationships among more distantly related taxa.

Recently, DNA polymorphisms have been detected by Random Amplified Polymorphic DNA (RAPDs): Welsh & McClelland, 1990; Williams & al., 1990; Caetano-Anolles & al., 1991; Hu & Quiros, 1991; Martin & al., 1991; Michelmore & al., 1991; Rafalski & al., 1991; Carlson, & al., 1991; Demeke & al., 1992; Klein-Lankhorst & al., 1991). RAPDs are generated by the amplification of genomic DNA with a single primer of arbitrary nucleotide sequence. The polymorphisms generated function as genetic markers and can be used to construct genetic maps (Williams & al., 1990). Quiros & al. (1991) have identified genome specific markers in *Brassica* using RAPDs.

Demeke & al. (1992) used RAPDs to analyse the classical *Brassica* U triangle relationships. They found that using about 100 RAPD bands gave very good agreement with the classical *Brassica* relationships. In addition, RAPDs clearly resolved the three diploid ancestral species (*B. campestris*, *B. nigra*, *B. oleracea*) as a triangular relationship, with the amphidiploids (*B. carinata*, *B. juncea*, *B. napus*) intermediate in the triangle (Demeke & al., 1992). Chloroplast restriction site mutations revealed the maternal parent of the amphidiploids but were unable to group accessions of *B. nigra* and *B. napus* by species, nor did the DNA chloroplast phylogeny give any correlation to the classical U triangle of relationships (Palmer & al., 1983). This

presents a major limitation in the use of chloroplast DNA data in that the uniparental inheritance effectively eliminates the systematic analysis of taxa derived from hybridization.

In addition to analyses of inter-specific taxa, Demeke & Adams (1992) used 13 primers to generate 69 RAPD bands from 6 individuals of *Brassica carinata* cv. 'dodola'. 63 (91.7 %) of the bands were monomorphic and 6 (8.7 %) were polymorphic among the individuals. The bands were reproducible as were the bands in individuals of *Juniperus excelsa* and *J. procera* (Adams & Demeke, 1993).

Other taxonomic studies using RAPDs include Heusden & Bachmann (1992, *Microseris*), Kaemmer & al. (1992, *Musa*), and Arnold & al. (1991, Irises).

RAPDs appear ideal for taxonomic use because analyses are very quick and inexpensive and RAPDs are randomly distributed over the entire genome. However standardization of reaction conditions is generally critical to obtain reproducible bands (see conclusions).

This study examines the utility of RAPDs in *Juniperus* taxonomy, ranging in application from sectional to infraspecific levels. Due to the considerable amount of taxonomic research amassed over the past 2 decades, *Juniperus* is ideally suited to assess the taxonomic utility of RAPDs at various taxonomic levels.

Materials and methods

When possible three to five samples from each taxon were used but in several cases only 1 or 2 plants were available (Table 1). Although most samples were taken from natural sites, some had to be collected from botanical gardens (Table 1). All samples are vouchered at the Baylor University Herbarium (BAYLU). Leaves were either transported fresh and frozen upon arrival or desiccated in silica gel (Pyle & Adams, 1989; Adams & al., 1992). DNA was extracted from juniper leaves by the SDS protocol (Dellaporta & al., 1983) with 1 % (w/v) PVP added to the extraction buffer (CTAB extraction was not as effective as SDS in obtaining DNA that could be amplified). PCR was performed in a volume of 25 l containing 50 mM Tris-HCl (PH 9), 1.5 mM $MgCl_2$, 0.01 % gelatin and 0.1 % Triton X-100, 0.2 mM of each DNTPs, 0.36 M primers, 0.5 ng genomic DNA, and 1 unit of Taq DNA polymerase (Promega). A control PCR tube containing all components, but no genomic DNA, was run with each primer to check for contamination. Inhibition of PCR amplification was observed for some taxa when the amount of genomic DNA exceeded 1.0 ng per PCR tube. Reducing the amount of DNA to 0.5 or 0.2 ng per PCR tube resulted in amplification of all DNA samples. Reduction of the amount of genomic DNA extract apparently diluted the PCR inhibitors (polysaccharides; see Demeke & Adams, 1992) present in the DNA extract. Ten-mer primers (Table 2) that gave several bright bands, did not have any false bands (in the controls) and found to be reproducible in replicated analyses were used. DNA amplification was performed in an MJ Programmable Thermal Cycler (MJ Research, Inc.). The thermal cycle was: 94C (1.5 min) for initial strand separation, then 40 cycles of 37°C (2 min), 72°C (2 min), 94°C (1 min). Two additional steps were used: 37°C (2 min) and 72°C (5 min) for final extension. Amplification products were analysed by electrophoresis on 1.5 % agarose gels and detected by staining with ethidium bromide. The gels were photographed under UV light with Polaroid film 667. pGEM DNA (Promega) was used as a molecular weight marker. The RAPD bands were scored for molecular weight and

Table 1. Symbols, names, origin and voucher No. (R. P. Adams) for *Juniperus* taxa used in this study. Unless the contrary is stated, all samples were collected growing in the wild.

Symbol	Name	Origin	Voucher No.
Juniperus sect. *Caryocedrus*			
DP	*J. drupacea* Labill.	Greece	4940[a], 5650, 5651
Juniperus sect. *Juniperus*			
CE	*J. cedrus* Webb & Berthel.	Canary Islands	5629[a]
CC	*J. communis* L. var. *communis*	Scotland	4933[b]
CD	var. *depressa* Pursh	Utah, U.S.A.	5685
CH	var. *hemispherica* (J. Presl & C. Presl) Parl.	Greece	5644
CM	var. *montana* Aiton (= *J. siberica* Burgsd.)	Russia	6120-6122
CF	*J. conferta* Parl.	Japan	5626[a]
FR	*J. formosana* Hayata	China	6772, 6774, 6792
OB	*J. oblonga* M. Bieb.	Russia	5510[c],5640[d],6128[e]
OX	*J. oxycedrus* L.	Greece	5650, 5988, 5989
RG	*J. rigida* Miq.	China	6797-6799[f]
Juniperus sect. *Sabina*			
	A. Taxa with serrate leaf margins		
AS	*J. ashei* J. Buchholz	Texas, U.S.A.	6746, 6751, 6752
CT	*J. comitana* Mart.	Mexico	6858-6862
DD	*J. deppeana* Steud. var. *deppeana*	Texas, U.S.A.	4974-4983
DP	var. *patoniana* (Mart.) Zanoni	Mexico	6836-6839
DR	var. *robusta* Mart.	Mexico	6826-6828
DZ	var. *zacatacensis* Mart.	Mexico	6840-6844
EN	*J. erythrocarpa* Cory	New Mexico, U.S.A.	2204
ET	*J. erythrocarpa* Cory	Texas, U.S.A.	4987
FF	*J. flaccida* Schldl. var. *flaccida*	Mexico	6892-6896
FM	var. *martinezii* (Pérez de la Rosa) Silba	Mexico	5950-5953
FP	var. *poblana* Mart.	Mexico	6868-6873
GB	*J. gamboana* Mart.	Mexico	6863-6867
MN	*J. monosperma* (Engelm.) Sarg. var. *monosperma*	New Mexico, U.S.A.	5027-5036
MG	var. *gracilis* Mart.	Mexico	6881-6885
	J. monticola Mart. f. *monticola*	Mexico	6874-6878
OC	*J. occidentalis* Hook. var. *occidentalis*	Oregon, U.S.A.	5077-5086
PN	*J. pinchotii* Sudw.	Texas, U.S.A.	4997
ST	*J. standleyi* Steyerm.	Guatemala	6852-6856

Table 1. continued

Symbol	Name	Origin	Voucher No.
	B. Taxa with entire leaf margine		
	1. Mature foliage: scale leaves (decurrent leaves only on juvenile branches or apically)		
EX	*J. excelsa* M. Bieb.	Greece	5984-5986
FT	*J. foetidissima* Willd.	Greece	5645, 5982, 5986
LC	*J. lucayana* Britton	Cuba	5282[h]
PH	*J. phoenica* Pall.	Greece	5630, 5653, 5654
PR	*J. procera* Hochst. ex Endl.	Ethiopia	6184-6186
PZ	*J. przewalskii* Kom.	China	6775-6777
PS	*J. pseudosabina* Fisch.	Kazahkstan	6716-6718[g]
RC	*J. recurva* var. *coxii* (A. B. Jacks.) Melville	Nepal	4930[b]
SB	*J. sabina* L.	Kazahkstan	6719-6721[g]
SC	*J. scopulorum* Sarg.	New Mexico, U.S.A.	5037-5046
TK	*J. turkestanica* Kom.	Kazahkstan	6729,6730[g]
	2. Mature foliage: mostly decurrent, plus a few scale leaves		
CN	*J. chinensis* L.	China	6764-6766
DV	*J. davurica* Pall.	Russia	6724[f]
	3. Mature foliage: decurrent leaves only		
SQ	*J. squamata* D. Don	China	6769, 6778, 6787
SX	*J. saxicola* Britton & P. Wilson	Cuba	5284, 5285

[a] Royal Botanical Garden, Edinburgh, U.K.
[b] Royal Botanical Garden, Kew, U.K.
[c] Arnold Arboretum, U.S.A.
[d] Berlin Botanical Garden, Germany.
[e] Moscow Botanical Garden, Russia.
[f] Beijing Botanical Garden, China.
[g] Alma Ata Botanical Garden, Kazakhstan.
[h] National Botanical Garden, Havana, Cuba.

assigned a code based on primer number (or name) prefix and molecular weight. In addition, the RAPD band intensity was scored as: 0 = no band; 1 = very, very faint; 2 = very faint; 3 = faint ; 4 = medium; 5 = bright; 6 = very bright band, in reference to a gray tone standard. It may be that the bright bands are due to repeated DNA and the faint bands due to a lower copy number or perhaps to partial mismatching of

Table 2. List of the primers used in this study for the random amplification of polymorphic DNA (RAPDs) by PCR.

Code[a]	Sequence (5'- 3')	Code[a]	Sequence (5'- 3')
116	TAC GAT GAC G[b,c,d,e,f]	218	CTC AGC CCA G[b,c,d,f]
123	GTC TTT CAG G[b,c,]	223	GAT CCA TTG C[b,e]
131	GAA ACA GCG T[b,c,d,f]	227	CTA GAG GTC C[b,c,d,e,f]
134	AAC ACA CGA G[b,c,d,f]	232	CGG TGA CAT C[b,c,e,f]
138	GCT TCC CCT T[b]	234	TCC ACG GAC G[b,c,d,e,f]
143	TCG CAG AAC G[b,c,e,f]	237	CGA CCA GAG C[b,c,d,e,f]
153	GAG TCA CGA G[b,c,d,e,f]	239	CTG AAG CGG A[b,c,d,e,f]
172	ACC GTC GTA G[b]	244	CAG CCA ACC G[b,c,d,e,f]
184	CAA ACG GAC C[c,d,f]	247	TAC CGA CGG A[b]
204	TTC GGG CCG T[b,c,d,e]	RC42	GCA AGT AGC T[b,c,d,e,f]
212	GCT GCG TGA C[b,c,e,f]		

[a] The RC42 primer was synthesized at the Plant Biotechnology Institute, NRC, all other primers were purchased from the University of British Colombia.

[b] Primers used in the study of *Juniperus* sect. *Juniperus*.

[c] Primers used in the study of *J.* sect. *Sabina*.

[d] Primers used in the study of varieties of *J. deppeana* and *J. flaccida*.

[e] Primers used in the study of *J.* sect. *Caryocedrus*, *Juniperus* and *Sabina*.

[f] Primers used in the study of the *J. monosperma* complex.

primers for the faint bands or some other factor. In any case, Demeke & al. (1992) demonstrated, at least in *Brassica*, that utilization of only the faint bands gave essentially the same classification as using only the bright bands. Using both the bright and faint bands gave a taxonomic representation closer to the classical relationships in *Brassica* than using either only the bright or only the faint bands. This is an area where additional research is needed. Data were coded into a matrix of taxa by character values.

Similarity measures were computed using absolute character state differences (Manhattan metric), divided by the maximum observed value for that character over all taxa (= Gower metric; Gower, 1971; Adams, 1975). Division by the character state range was tried and found to be less informative than using the maximum observed character value (i.e., including zero in the range). Principal coordinate analysis (PCO) of the similarity matrix follows Gower (1966).

Results and discussion

Although we have demonstrated the taxonomic utility of RAPDs at the species level in *Brassica* (Demeke & al., 1992), it is important to continue to examine the utility of RAPDs at different taxonomic levels in different groups before this method can be universally accepted. So we will begin at the sectional level in *Juniperus* and proceed to examine taxonomic problems down to the variety level.

Fig. 1 shows a typical gel of RAPD bands (RAPDs) for *Juniperus* sect. *Caryocedrus*, sect. *Juniperus,* and sect. *Sabina*. Note the conserved band (c. 250 bp) present in *J.* sect. *Juniperus* and the 350 bp band delineating the monotypic *J.* sect. *Caryocedrus*. Also notice the several bands distinguishing *J.* sect. *Sabina*.

However, the presence of a few bands may not necessarily be consistent in separating taxa. In order to examine the overall pattern a more powerful multivariate approach is needed. Principal coordinate analysis (PCO) is ideally suited for use with large numbers of characters and numerous taxa. Any measure of similarity can be used. This computer software (PCO3D) for RAPDs analyses is now available (from R.P.A.). PCO3D is available for MS DOS IBM compatibles with a hard disk and math co-processor (correspond with R.P.A. for distribution details). PCO using 186 RAPDs gave 5 eigenroots that accounted for 77 % of the variance (% variance: 23, 17, 15, 13, 9) among the taxa (OTUs). The eigenroots began to asymptote after five roots. PCO of taxa in all three sections of *Juniperus* is shown in Fig. 2. Although the biological basis for the coordinate axes is sometimes difficult to ascertain (Pimentel, 1979), in this case it is clear. The first axis separates *J.* sect. *Sabina* and sect. *Caryocedrus* plus sect. *Juniperus*. The second axis separates the serrate and smooth leaf margined junipers of *J.* sect. *Sabina*, and the third axis separates *J. excelsa* and *J. procera*. The fourth axis (Fig. 3) separates *J.* sect. *Caryocedrus* from sect. *Oxycedrus* and sect. *Sabina*, whereas the fifth axis distinguishes *J. communis* from *J. oxycedrus* (both in *J.* sect. *Juniperus*). These data give support to maintaining the three sections in *Juniperus* rather than recognizing separate genera.

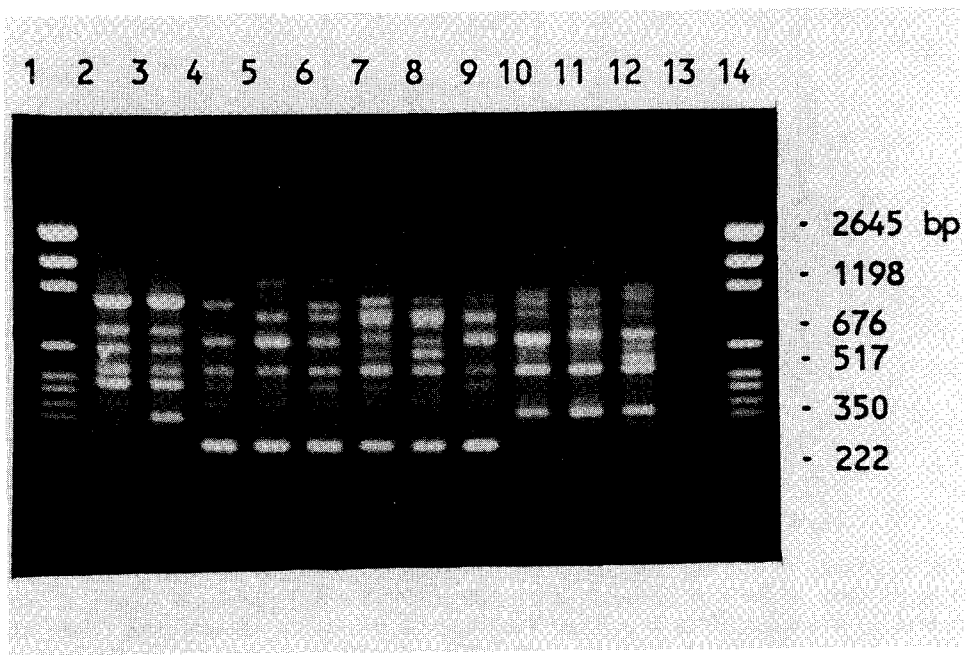

Fig. 1. RAPDs for junipers in all three sections of *Juniperus*. Lane 1 = PGEM marker DNA; Lanes 2-3 = *J.* sect. *Sabina: J. excelsa*; Lanes 4-6 = *J.* sect. *Juniperus: J. communis* var. *montana*; Lanes 7-9 = *J.* sect. *Juniperus: J. oxycedrus*; Lanes 10-12 = *J.* sect. *Caryocedrus: J. drupacea*; Lane 13 = control (no DNA); Lane 14, PGEM marker DNA.

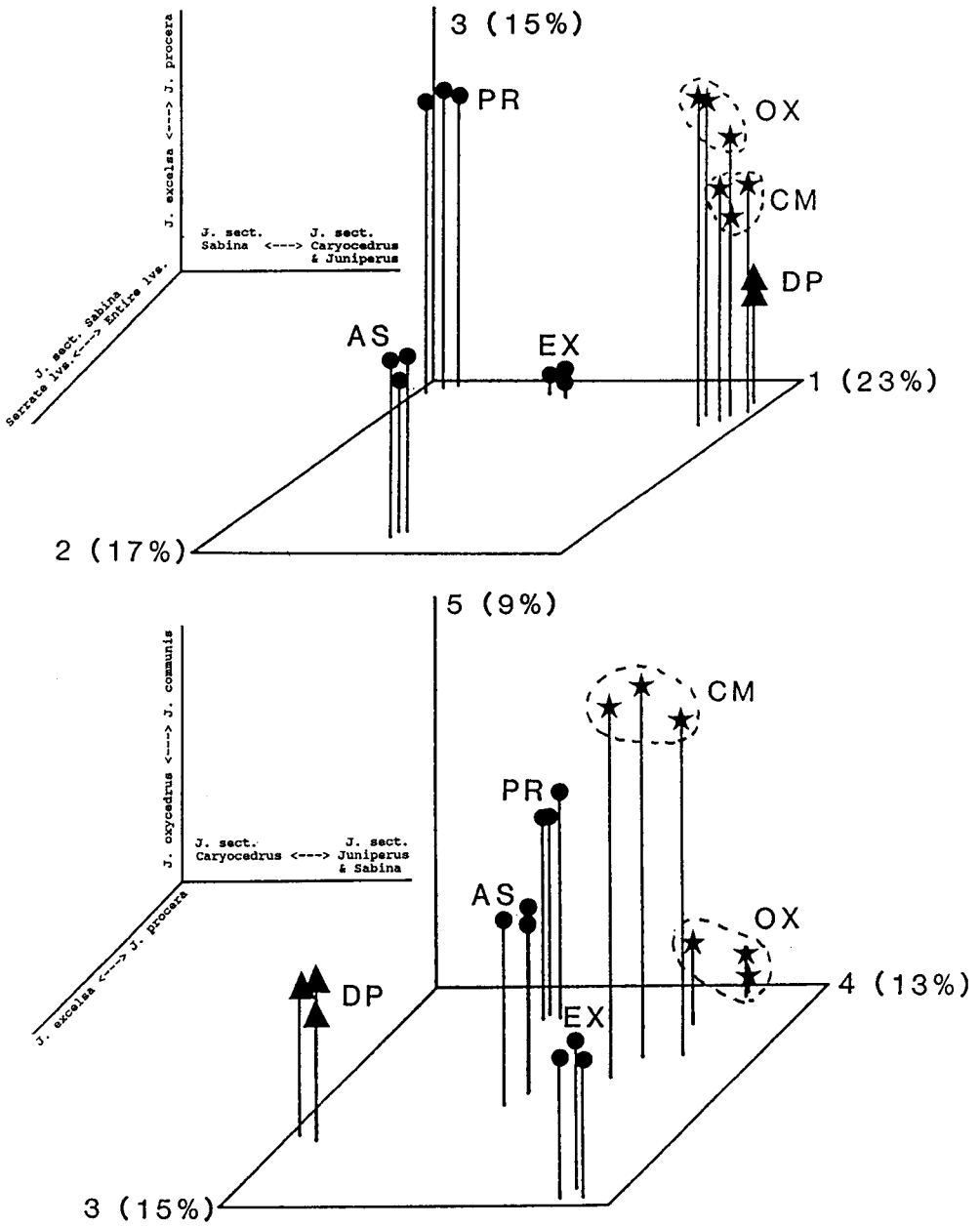

Fig. 2 (above). Plot of coordinates 1-3 for the principal coordinate analyses (PCO) of taxa from all three sections of *Juniperus* using 186 RAPD bands. Triangles = *J.* sect. *Caryocedrus: J. drupacea*; Stars = *J.* sect. *Juniperus: J. communis* var. *montana* (CM) and *J. oxycedrus* (OX); Circles = *J.* sect. *Sabina: J. ashei* (AS), *J. excelsa* (EX), and *J. procera* (PR). *J.* sect. *Sabina* is clearly separated from *J.* sect. *Caryocedrus* and sect. *Juniperus*.

Fig. 3 (below). Plot of coordinates 3-5 for PCO of taxa from all three sections of *Juniperus* using 186 RAPD bands. *J.* sect. *Caryocedrus* is clearly separated from *J.* sect. *Juniperus* and sect. *Sabina*.

The marked separation of *J.* sect. *Sabina* from sect. *Caryocedrus* and sect. *Juniperus* (axis one, 23 %) gives some credence to advocates of the elevation of *J.* sect. *Sabina* to generic rank. Note also that *J. excelsa* and *J. procera* are quite distinct, confirming their maintenance as distinct species (Adams, 1990).

Clearly RAPDs are taxonomically useful at the sectional level in *Juniperus*. It should be noted that some RAPDs show considerable variation among individuals (see Fig. 3, CM and OX individuals). If those bands that vary within species were eliminated, the more conserved bands would result in greater discrimination (computer algorithm being developed; Adams, in prep.).

PCO using 312 RAPDs of taxa in *Juniperus* sect. *Juniperus* resulted in eigenroots that generally levelled off with no obvious asymptote. Veldman (1967) suggests that one may attach significance to eigenroots that are larger than the average value of the diagonal elements in the factor matrix. Applying that criterion, the first five eigenroots would be judged significant (% variance: 18, 13, 12, 10, 10; total 63 %). Of course, one would do well to heed the advice of Pimental (1979) that the final test of significance is "does it make biological sense?". Fig. 4 shows the taxa of *J.* sect. *Juniperus* plotted onto the first three coordinate axes. Five groups are apparent. Individual species: *J. cedrus* (endemic to the Canary Islands); *J. oxycedrus* (a distinct Mediterranean species); *J. oblonga* (a species often lumped into *J. communis*, from

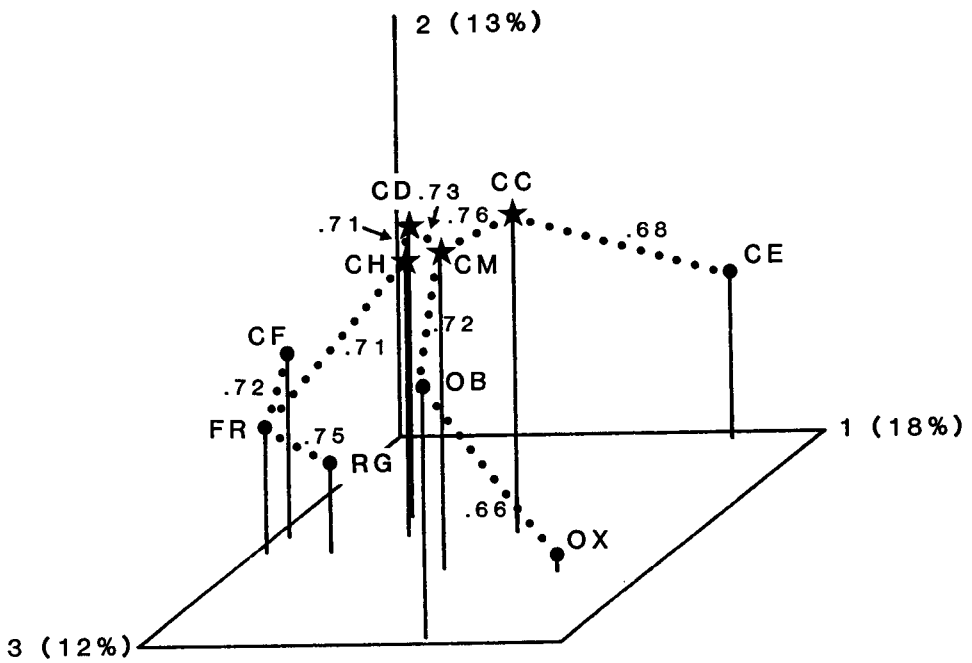

Fig. 4. PCO for taxa in *Juniperus* sect. *Oxycedrus* using 312 RAPDs, with a minimum spanning network (dotted lines) superimposed. The numbers next to the minimum spanning network (dotted lines) are the similarity values. CC = *J. communis* var. *communis*; CD = *J. communis* var. *depressa*; CH = *J. communis* var. *hemispherica*; CM = *J. communis* var. *montana*; CE = *J. cedrus*; CF = *J. conferta*; FR = *J. formosana*; OB = *J. oblonga*; OX = *J. oxycedrus*; RG = *J. rigida*.

the Caucasus region); *J. communis* taxa (*J. communis* var. *communis*, European; var. *depressa*, North American; var. *hemisphaerica*, Mediterranean; var. *montana*, circumboreal) and the junipers from the far east (*J. formosana*, mainland China and Taiwan; *J. conferta*, Japan and Sakhalin Island; and *J. rigida*, northern China, Korea, and Japan).

One of the most interesting aspects is the loose clustering of *Juniperus communis* var. *hemisphaerica* with other *J. communis* varieties (note the similarity of 0.71 to *J. communis* var. *depressa,* the same as its similarity to *J. formosana*). *J. communis* will require additional study to resolve this taxonomic problem. The taxa are rather loosely clustered, lending support to the concept that *J.* sect. *Juniperus* is ancient. *Juniperus* sect. *Sabina* contains the weedy junipers. Many have evolved female cones with fused cone scales that are soft and fleshy when mature, and sometimes sweet. The female cones ("fruits") are widely eaten by birds, opossums, raccoons, and other animals. Therefore, the seeds are widely distributed and these junipers invade overgrazed grasslands and disturbed habitats.

Juniperus sect. *Sabina* has been subdivided on the basis of leaf margins serrate (denticulate) or smooth (entire) (Adams, 1993; Gaussen, 1968; Vasek, 1966; Zanoni, 1978). Gaussen (1968) placed several species with allegedly serrate leaf margins in a series he called *J.* ser. *Phoenicioides* (including *J. phoenicea* and *J. pseudosabina*). The senior author (R.P.A.) has examined the leaf margins of these taxa and they appear smooth (or entire) at 40× magnification; nor has he found any serrate leaf

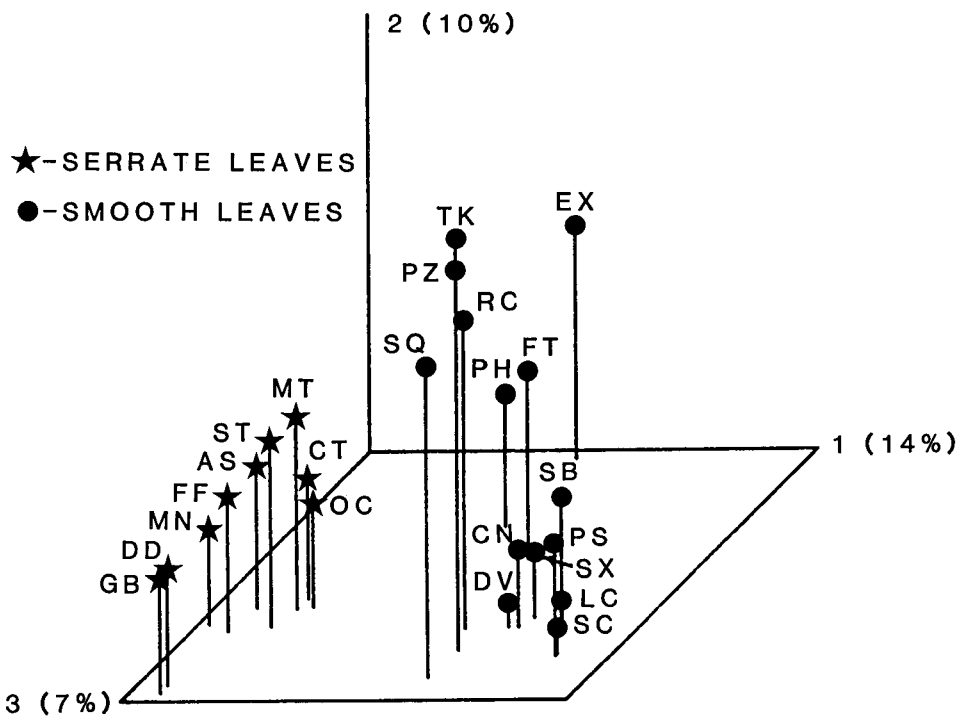

Fig. 5. PCO for taxa in *Juniperus* sect. *Sabina* using 317 RAPDs. See Table 1 for acronyms. Note the separation of the serrate and smooth leaf margined junipers.

margined junipers in the eastern hemisphere. So it was with considerable interest that these new DNA data were brought to bear on this question.

RAPDs were run on 23 juniper species, 9 of which were scored as serrate and 14 as smooth leaf margined. Examination of the eigenroots from PCO of this data set (317 RAPDs) showed that there were likely many trends, but the first three show the major trends. Graphically, one can see (Fig. 5) that all the taxa scored as serrate (stars) were separated by the first coordinate from the smooth leaf margined taxa (circles). *Juniperus phoenicea* and *J. pseudosabina*, listed as serrate by Gaussen (1968), do not ordinate with the serrate junipers (all from the continental North America). The hypothesis that the serrate junipers originated in central Mexico is supported by these data. All are xeric or xeric derived species presently distributed in the western United States, Mexico and on the volcanic peaks on the Mexico-Guatemala border. The greatest diversity of these serrate junipers is around the Chihuahuan Desert in Mexico. Their origin likely dates to the Madro-Tertiary geoflora (Axelrod, 1958).

It can be very misleading to examine three-dimensional figures when the number of eigenroots extracted is large. Because much of the variance is distributed on many other axes, the viewer can see apparently closely related taxa on some axes that are fully separated on subsequent axes. Therefore, it is desirable to divide the data set into subsets for subsequent PCO analyses. For example, if one analyses only the serrate (or smooth) leaf junipers, coordinate 1 will be removed. The reduction of the problem from 22 dimensional (23-1) space to 8 dimensional space (9-1) aids greatly. A second strategy is to superimpose a minimum spanning network onto the 3-D ordination so that actual similarities can be seen.

PCO of the 9 serrate junipers analysed (254 RAPDs) yielded eigenroots that appeared to asymptote after four roots (% variance: 19; 16; 15; 13). A plot of the first three principal coordinates (Fig. 6) does not reveal much clustering of these taxa. Due to project constraints, only about half of the serrate taxa were analysed. The nine taxa analysed were chosen to cover the diversity of the serrate group in North America and the diversity is well shown in Fig. 6. It is noteworthy that *Juniperus monticola* f. *monticola* and *J. standleyi*, both high elevation species occurring on extinct volcanos and with very similar morphology, were most similar in their RAPDs.

Perhaps of most interest is the close similarity between *Juniperus deppeana* var. *deppeana* and *J. gamboana*. Both of these taxa have trunk bark exfoliating in rectangular plates ("alligator bark") that is unique in the genus. In analyses of the morphology and leaf volatile oils (Zanoni & Adams, 1976) these taxa did not cluster together. From the RAPDs, it appears that the 'alligator bark' may well be of greater phylogenetic significance than previously thought. *J. gamboana* has smaller, fleshy female cones with 1(-2) seeds, whereas *J. deppeana* has larger, fibrous pulp cones with usually 2-3 seeds (often more). In *Juniperus*, a considerable emphasis has been placed on the female cones (pulp type, number of seeds, size, etc.). However, a new variety of *J. flaccida* with only a single seed per female cone has recently been discovered (Pérez de la Rosa, 1985). This variety (*J. flaccida* var. *martinezii*) is morphologically and chemically very similar to other *J. flaccida* varieties with several (6-13) seeds per female cone (Adams & al., 1990). It appears that the female cone characters in *Juniperus* may not be as important as traditionally accepted.

PCO of 296 RAPDs for 14 smooth leaf margined junipers of *Juniperus* sect. *Sabina* gave eigenroots that asymptoted after six roots (% variance: 17; 12; 10; 9; 9; 7). Fig. 7 shows the ordination on the first three axes. Several groupings are evident. The smooth leaf margined "sabinoid" junipers from North America cluster and link together (*J. saxicola*, Cuba; *J. lucayana*, Cuba; *J. scopulorum*, western United States). One should note that *J. saxicola* has only decurrent (juvenile type) leaves, as also found in *J. squamata* (Table 1). However, previous work on the volatile leaf oil indicated that *J. saxicola* was in fact most similar to *J. lucayana* (Adams, 1989; Adams & al., 1987). The RAPDs data confirm the terpenoid data. *J. saxicola* is apparently merely genetically fixed in the juvenile leaf stage (neotony) and this is not a major accumulation of genetic differentiation.

The most similar taxa (Fig. 7) are *Juniperus sabina* and *J. pseudosabina*. As the names suggest, these species are difficult to separate morphologically. Additional research is in progress to determine their taxonomic status. Two other taxa appear to be very similar, *J. turkestanica* and *J. prezewalskii*, and they are associated with two other central Asian junipers, *J. recurva* var. *coxii* and *J. squamata*. Some authors have treated *J. squamata* as a variety of *J. recurva*, but the RAPDs gives support to maintaining it as a distinct species. A fairly close similarity is seen between *J. chinensis* and the little known species *J. davurica*. These two eastern Asian species have both juvenile (decurrent) and adult (scale) type leaves on mature plants. Addi-

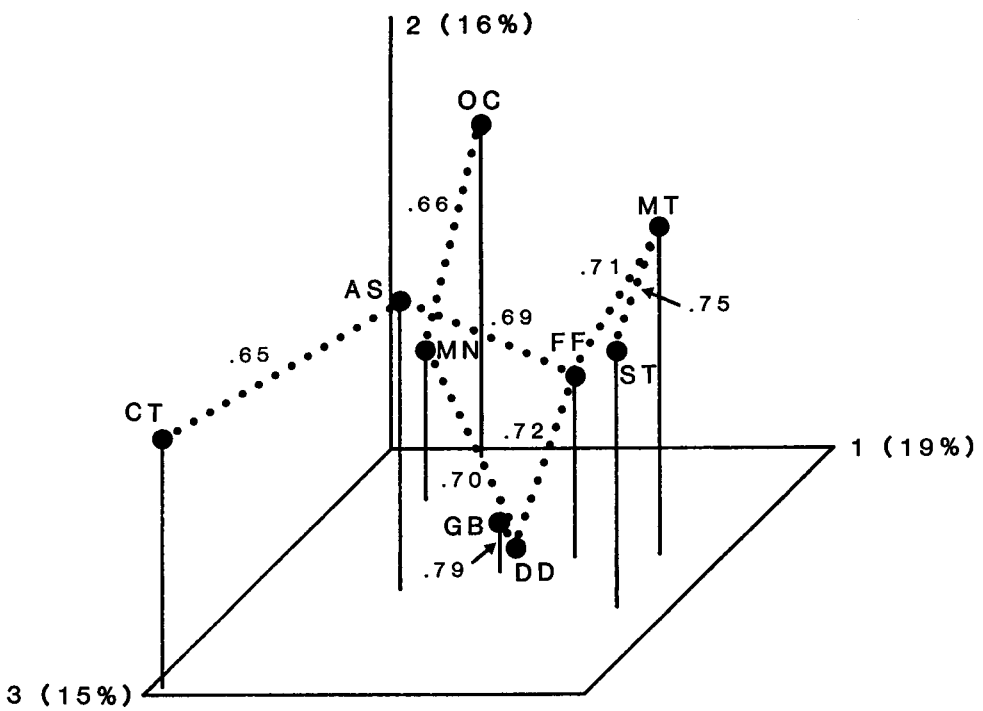

Fig. 6. PCO for taxa in *Juniperus* sect. *Sabina*, with serrate leaf margins (all North American), using 254 RAPDs, with a minimum spanning network (dotted lines) superimposed. The numbers next to the minimum spanning network (dotted lines) are the similarity values. See text for discussion and Table 1 for acronyms.

tional materials are needed from *J. davurica* for study. Both *J. excelsa* and *J. phoenicea* appeared to be rather removed from the other "sabinoid" junipers (Fig. 7, and also in Fig. 5).

A closely related group of one-seeded junipers from northern Mexico and the southwestern United States *(Juniperus erythrocarpa, J. monosperma* var. *monosperma, J. monosperma* var. *gracilis,* and *J. pinchotii)* continue to present taxonomic problems. *J. erythrocarpa* and *J. pinchotii* have recently been shown to hybridize in west Texas (Adams & Kistler, 1991) and *J. monosperma* var. *gracilis* appears to intergrade with *J. erythrocarpa* in Coahuila, Mexico. Furthermore, *J. monosperma* var. *gracilis* is more similar in its morphology and terpenes to *J. erythrocarpa* than to *J. monosperma* var. *monosperma* (Adams & al., 1981). In addition, *J. erythrocarpa* appears to have two chemical groups, one in west Texas and Coahuila, Mexico and another in New Mexico and Arizona (Adams & al., 1981).

PCO of 136 RAPDs from *Juniperus erythrocarpa, J. monosperma* var. *monosperma, J. monosperma* var. *gracilis,* and *J. pinchotii* yielded four eigenroots of fairly similar size (% variance: 36; 25; 22; 18). Fig. 8 shows the most similar taxa as *J. erythrocarpa* (Texas) and *J. monosperma* var. *gracilis* (northeastern Mexico, 0.7 similarity). *J. monosperma* var. *monosperma, J. pinchotii* and *J. erythrocarpa* (New Mexico) each appear rather distinct. The fourth principal coordinate (18 % variance)

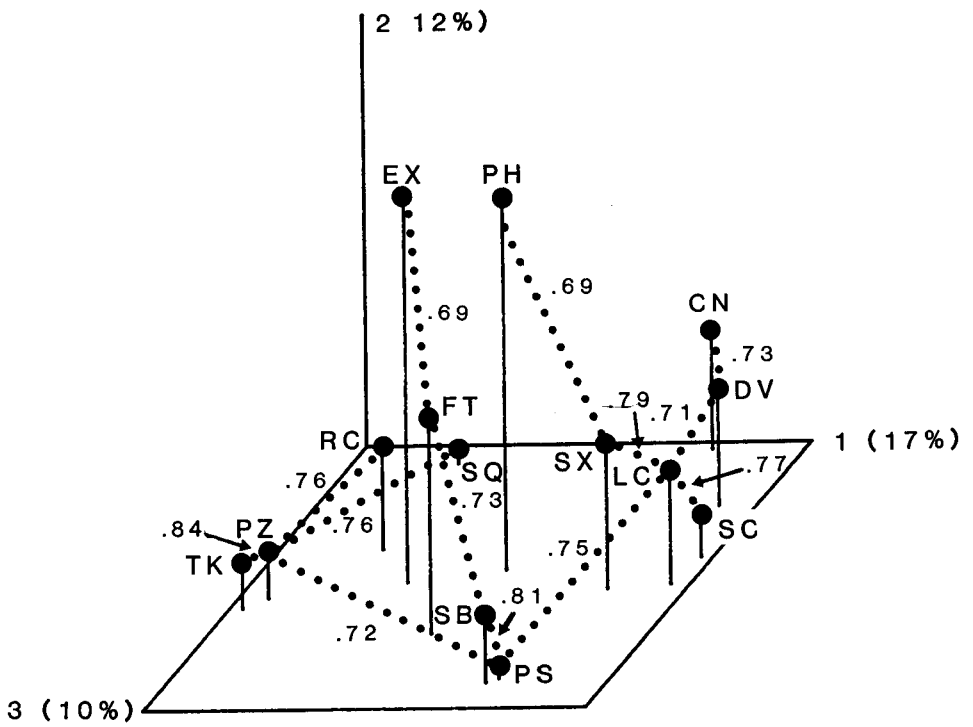

Fig. 7. PCO for taxa in *Juniperus* sect. *Sabina,* with smooth leaf margins using 296 RAPDs, with a minimum spanning network (dotted lines) superimposed. The numbers next to the minimum spanning network (dotted lines) are the similarity values. All the taxa are from the eastern hemisphere, except SX, LC and SC, which are from North America. See text for discussion and Table 1 for acronyms.

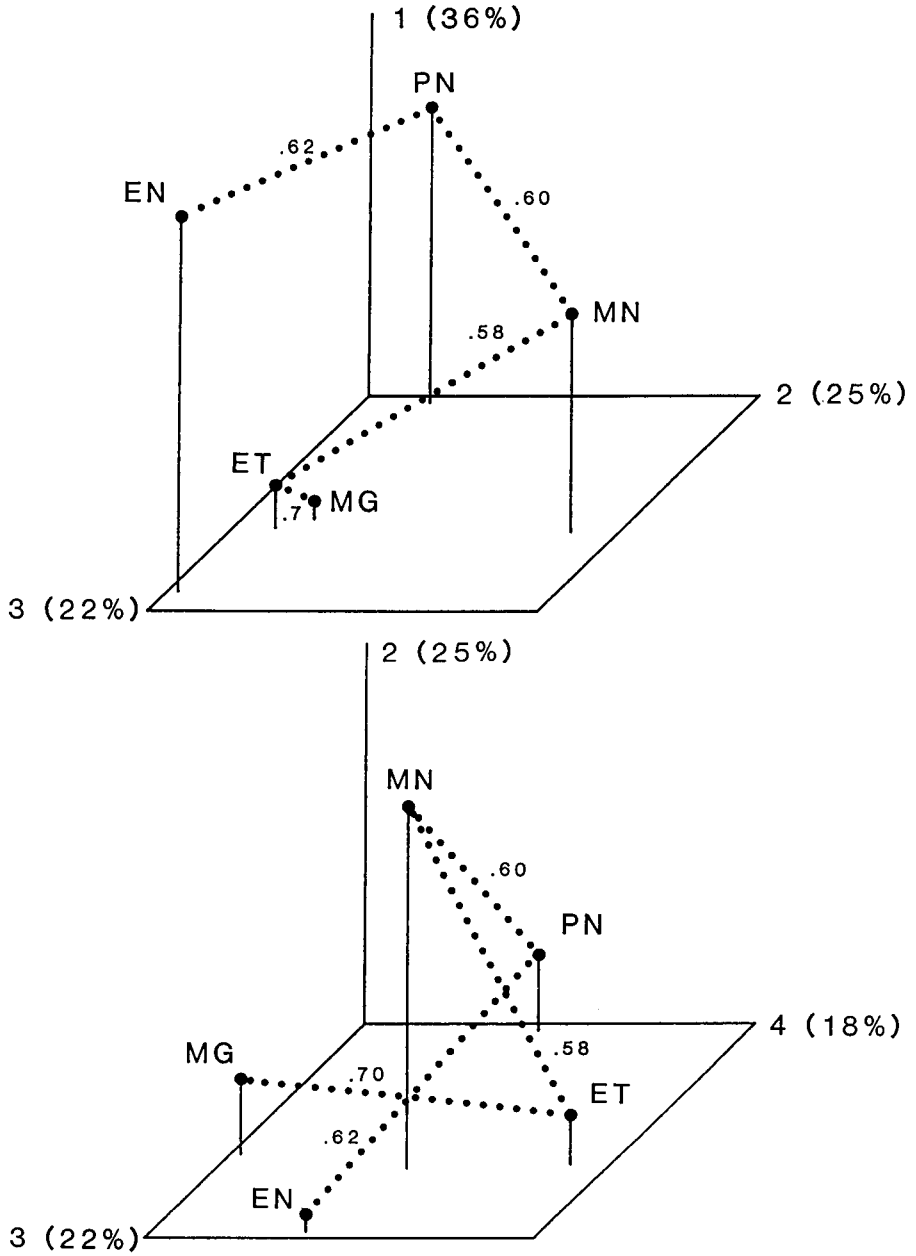

Fig. 8 (above). Plot of axes 1-3 for PCO for three closely related monospermous junipers, using 136 RAPDs, with a minimum spanning network (dotted lines) superimposed. The numbers next to the minimum spanning network (dotted lines) are the similarity values. EN = *Juniperus erythrocarpa*, New Mexico; ET = *J. erythrocarpa*, Texas; MN = *J. monosperma* var. *monosperma*; MG = *J. monosperma* var. *gracilis*; PN = *J. pinchotii*. Note the separation between the two *J. erythrocarpa* sources (EN, ET).

Fig. 9 (below). Plot of axes 2-4 for PCO for three closely related monospermous junipers, using 136 RAPDs, with a minimum spanning network (dotted lines) superimposed. Note the separation of *Juniperus monosperma* var. *gracilis* from all taxa.

contains significant biological information. Note (Fig. 9) that axis four separates *J. erythrocarpa* (Texas) from *J. monosperma* var. *gracilis*. This emphasizes the point that one often needs to examine the smaller eigenroots for biological information, especially when the eigenroots account for considerable variance as in this case. A study using populational data analyses is in progress to attempt resolve this taxonomic problem (Adams, in prep.)

In order to examine the utility of RAPDs at the lower taxonomic levels, we analysed four varieties of *Juniperus communis*, four varieties of *J. deppeana* and three varieties of *J. flaccida*. PCO of 227 RAPDs for four *J. communis* varieties yielded three eigenroots (% variance: 39, 33, 28). The four varieties are well separated (Fig. 10) but no subgrouping is apparent. PCO of *J. flaccida* and *J. deppeana* varieties resulted in three major eigenroots (% variance: 36, 19, 15). This first axis separates *J. deppeana* from *J. flaccida* (Fig. 11). The second axis separates *J. deppeana* var. *robusta* and var. *zacatacensis* from var. *deppeana* and *J.* var. *patoniana*. The third axis separates the *J. flaccida* varieties. A close similarity between *J. deppeana* var. *robusta* and var. *zacatacensis* has also been reported for the terpenoids (Zanoni & Adams, 1976). On the basis of morphology and terpenoids, Adams & al. (1990) recognized that *Juniperus martinezii*, was in fact part of the *J. flaccida* group (*J. flaccida* var. *martinezii*) in spite of the fact that it has only a single seed per

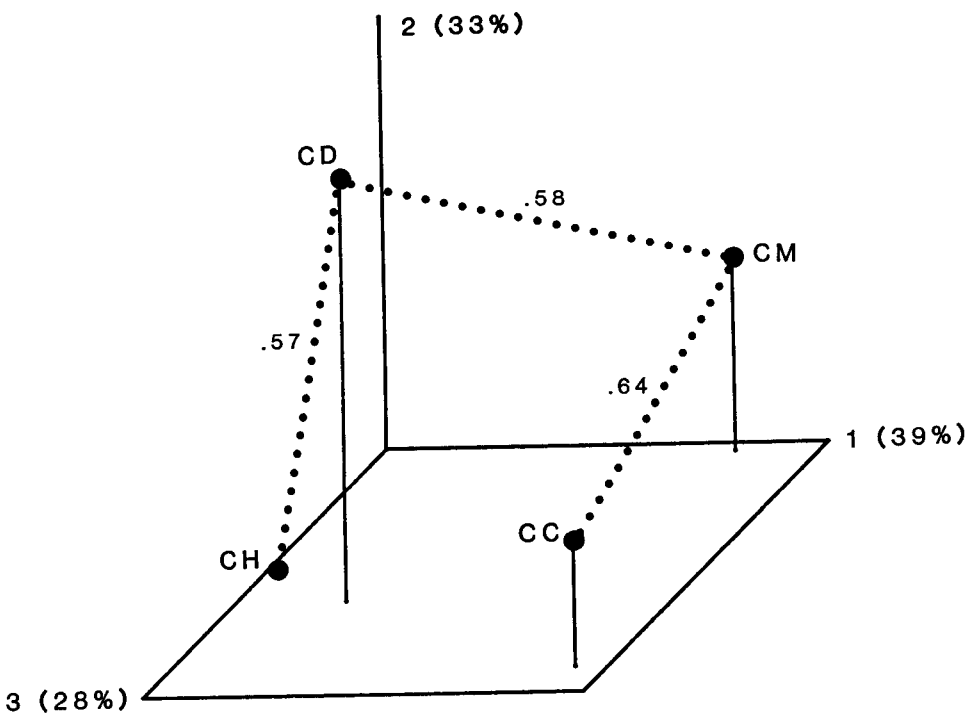

Fig. 10. PCO for *Juniperus communis* varieties using 227 RAPDs, with a minimum spanning network (dotted lines) superimposed. The numbers next to the minimum spanning network (dotted lines) are the similarity values. CC = *J. communis* var. *communis*; CD = *J. communis* var. *depressa*; CH = *J. communis* var. *hemispherica*; CM = *J. communis* var. *montana*. See text for discussion.

female cone. The RAPDs data bear out this close relationship and support the maintenance of *J. martinezii* as a variety of *J. flaccida*.

Conclusions

In this work we have shown that RAPDs can be utilized at taxonomic levels ranging from varietal to sectional in *Juniperus*. With the use of more powerful computer algorithms, polymorphic bands within species could be eliminated and RAPDs will be even more useful at these higher levels. Certainly at the species and populational level RAPDs offer an enormous potential because they are fast and inexpensive. Of course, there is a trade-off. The RAPD bands are homologous in that they have the same 10-mer nucleotides on each end and their length (number of bases) is the same (as far as can be ascertained with today's gel electrophoresis technology). Obviously, sequence data for each band would determine homology more precisely. However, consider the sequencing time and cost that would be needed in this study. Taxa analysed: 44. Total number of RAPD bands analysed: 1624. Time required to extract DNA, do PCR and score data: about 8 weeks. We hesitate to even estimate the cost and time to sequence 1624 oligo-nucleotides ranging from 220 to 2500 bp with current sequencing technology. Alternatively, one

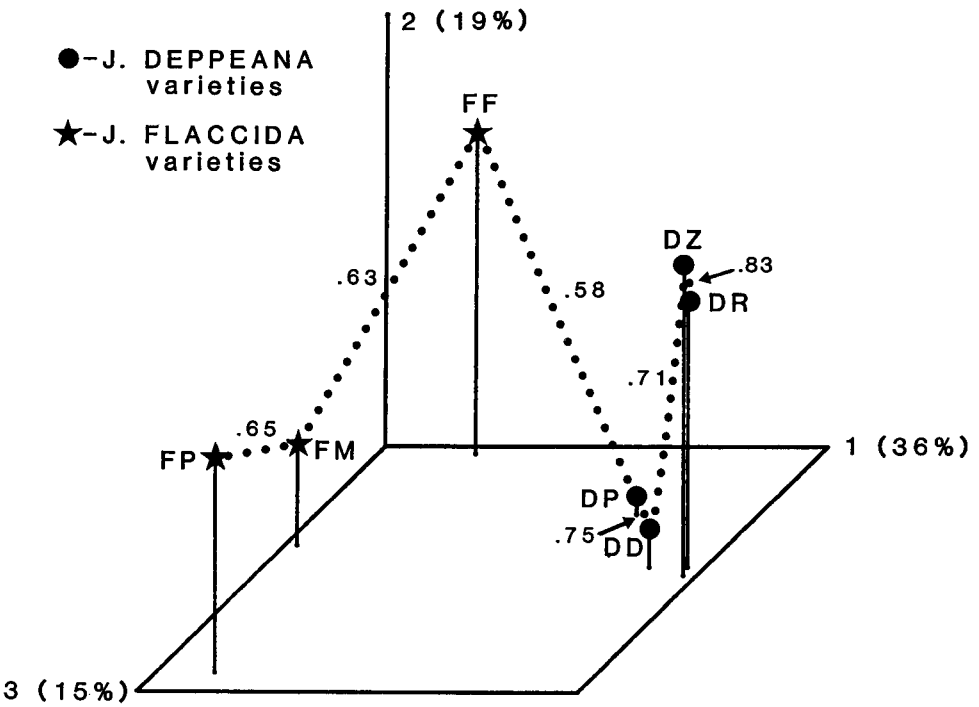

Fig. 11. PCO for *Juniperus deppeana* and *J. flaccida* varieties using 112 RAPDs, with a minimum spanning network (dotted lines) superimposed. The numbers next to the minimum spanning network (dotted lines) are the similarity values. DD = *J. deppeana* var. *deppeana*; DP = *J. deppeana* var. *patoniana*; DR = *J. deppeana* var. *robusta*; DZ = *J. deppeana* var. *zacatacensis*; FF = *J. flaccida* var. *flaccida*; FM = *J. flaccida* var. *martinezii*; FP = *J. flaccida* var. *poblana*. See text for discussion.

might excise a RAPD band, label it and then hybridize it with bands from other taxa. But this would not be rapid nor inexpensive! No doubt, non-homologous band comparisons will be scored as homologous but the methods utilized in this paper are robust to just such errors (Adams, 1975), because the sum of similarity is more than the parts. The user is cautioned about using parsimony based trees with RAPDs data because in those algorithms a few non-homologous characters can drastically affect the tree shape. Many of the systematic applications of RAPDs will be for studies of geographic variation (Demeke & Adams, 1993) and hybridization. The use of similarity measures and principal coordinates analysis is strongly encouraged for RAPDs, as errors are minimized due to the use of numerous, random characters. In addition, if errors of homology are random then they can be accounted for as noise in principal coordinates analysis.

A second factor to consider is reproducibility. Although we initially experienced some reproducibility problems with *Brassica*, *Juniperus*, and *Phytolacca*, the problems were eliminated by using small amounts of genomic DNA (1 ng or less per 25 µl PCR reaction). However, we have had difficulty in obtaining reproducible bands in wheat (Demeke, in progress). Ellsworth & al. (1993) showed RAPD banding patterns to vary based on primer concentration, target (genomic) DNA concentration, annealing temperatures, and MgCl concentrations. Therefore, we can not overemphasize the importance of critically investigating the effects of these parameters during the preliminary work and then adopt a strict methodology during the study.

RAPDs promise to be a significant new tool in systematics, particularly when used at the specific and populational levels.

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