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The concordance of terpenoid, ISSR and RAPD markers, and ITS sequence data sets among genotypes: an example from *Juniperus*

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Abstract

Twelve individual genotypes selected from *Juniperus* populations, varieties and species were analyzed using ITS sequences, RAPDs, ISSRs, and leaf volatile terpenoids. These four data sets, all analyzed in the same manner, illustrated that these data sets can be used at different organizational levels: specific, inter-specific and intraspecific. Similarity matrices for the ITS, RAPD, and ISSR data sets were highly correlated (r ranged from 0.83 to 0.95). In contrast, the terpenoid matrix had a low correlation with the other data matrices (r ranged from -0.04 to 0.38). Because these 12 genotypes were taken from several species, the terpenoids, having been shown to most useful at the infra-specific level, were not concordant at this taxonomic level. The high correlation between DNA markers and ITS sequence data implies that these data are measuring concordant patterns among these genotypes.

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1. Introduction

Recently, Issakainen (1999) wrote “We easily forget that different parts of a single organism’s genome may have a different evolutionary history.” Scientists involved in biosystematic research are opportunistic. At each technological advance, we have seen biosystematists attempt to harness the new technology for use in the analysis of evolutionary problems. These data collection technologies include: the microscope (micro characters and chromosomes); micromolecular equipment (UV, IR, NMR, GC, GCMS, HPLC for the analyses of flavors and fragrances, free amino acids, lipids, pigments, waxes, etc.); macromolecular equipment (electrophoresis and DNA sequencers for the analyses of isozyme, DNA fingerprinting and sequences). Billie Turner (pers. comm.) used to tell his class “Characters are where you find them”. By that he meant that if you are studying populational differentiation, you must find a set of characters that is useful for addressing that problem. If you are examining differences among species, you need to find a set of characters that will be useful. This seemed to work well with morphological characters, but seems more difficult to apply to various kinds of DNA data now available.

Each bit of data gives us only a very small window into the very large genome of an individual. Current estimates suggest that plant genomes contain between 20,000 and 30,000 genes (Somerville and Somerville, 1999). Traditionally, biosystematists have relied on morphology as their primary data. Of course, a plant’s morphology arises through interactions between genes and the environment. It is not clear how many of genes contribute to the gross morphology of a plant, although ca. 5% of genes of known function in *Arabidopsis* contribute to cell wall development (Somerville and Somerville, 1999). An even smaller subset of genes is likely responsible for the visible differences in morphology used to classify species. In a classical study of two species of goldenrod (*Solidago*), Charles and Goodwin (1953) found 35 as the minimum number of genes for six key taxonomic characters (5.8 genes/trait). Their estimates are not very different from the modern estimates of quantitative trait loci (QTL), where 3.73 and 4.0 QTL/trait were reported for *Helianthus* (Kim and Rieseberg, 1999) and *Mimulus* (Bradshaw et al., 1998; Kearsley and Farquhar, 1998).

The number of independent genes is further complicated by pleiotropy (a single gene that has multiple effects) and epistasis (gene interactions). For example, many of the QTL identified in sunflower (Kim and Rieseberg, 1999) and *Mimulus* (Bradshaw et al., 1998) mapped to the same genomic location, possibly indicative of pleiotropy. If this interpretation is correct, then due to pleiotrophy, the number of genes contributing to species differences may actually be overestimated.

Micromolecules are generally under simple gene control. Irving and Adams (1973) used a biometric approach to estimate the minimum number of genes controlling monoterpenes in *Hedeoma*. They found that 20 monoterpenoids were controlled by a minimum of 39 genes (1.95 genes/trait).

With respect to secondary compounds such as terpenoids, a single gene may affect an entire class of compounds. For example, Vogel et al. (1996) show that geranylgeranyl diphosphate (GGDP) may be cyclized by enzymes leading to (–) (abietate-

7(8),13(14)-diene, abietic acid) or to (+) (kaurene, kaurenoic acid) compounds. They identified kaurene synthase B as the key enzyme for the synthesis of the (+) set of compounds. The loss (or mutational inactivation) of kaurene synthase B, will result in the loss of any derived compounds in this entire pathway. This can easily account for chemotypes as reported in *Juniperus* (Adams, 2000b,d). The composition of the leaf oils of *Juniperus* from the western hemisphere are completely different from the heartwood oils (Adams, 1991; Adams, 2000b,d). However, the leaf oils from some trees of *J. excelsa* and *J. polycarpus* in the eastern hemisphere contain large amounts (40%) of cedrol, the major component of the wood oils (Adams, 2000c) as well as thujopsene, α - and β -cedrenes (other wood oil components). These trees display no morphological differences nor differences in their RAPDs (Adams, 2000c). It appears that the pathway for the synthesis of the cedrol/thujopsene/cedrene compounds is occasionally turned on in the foliage. It is interesting to note that the converse case has not been observed (ie., leaf terpenoids expressed in the cedarwood oil), but we have analyzed far fewer samples of wood than leaves at this time. Recently, Steele et al. (1998) reported that two genes, δ -selinene synthase and γ -humulene synthase, (found in wound response tissue in *Abies grandis*) produced three major terpenoids and 34 and 52 sesquiterpenes, respectively in wounded tissue! Thus, if one were computing a similarity measure, a single gene difference could be counted as 37 (3+34) or 55 (3+52) differences in this example.

So whether using morphology or terpenoids (or other secondary compounds), we do not know precisely how big our viewing window is, but it is clear that we are observing only a very small sample of information from the 25,000–30,000 genes from a genotype. The human genome project (Venter et al., 2001) reported that expressed DNA (exons) comprise only 1.1 to 1.4% of the human genome. For *Arabidopsis*, one of the smallest genomes in plants, 25,498 genes were reported (The Arabidopsis Genome Project, 2000) with the exons being 33,249,250 bp out of 115,409,949 bp sequenced. In that case, exons account for 28.8% of the total DNA, in this very small genome. The average gene was reported as 2013 bp long.

With the enormous size of the genome, it is actually surprising that we do find characters are useful for analyzing geographical, intraspecific and specific variation in biosystematics. But through trial and error, biosystematists have selected characters to use at each of these levels of variation. We are now presented with the opportunity to use even more new tools and characters. In this paper, we investigate the applicability of terpenoids, ISSRs (intersimple Sequence Repeats), RAPDs (Random Amplified Polymorphic DNAs) and ITS sequence data at various levels of use.

It is admitted that comparison of such diverse data sets presents some difficult problems. We have assembled a set of 12 genotypes (individuals) of *Juniperus*. It is important to note that this study was not constructed to answer taxonomic questions, but rather to determine the kinds of information that one may gather from these 12 genotypes and to compare the concordance of the data sets.

The 12 individuals were selected from six taxa: one species (*J. drupacea* Labill.) in the monotypic section *Caryocedrus* and four closely related taxa in section *Juniperus*, that Adams (2000a), using RAPDs, terpenoids and morphological data, recognized at the specific rank: *J. oxycedrus* L., Spain; *J. badia* H. Gay (= *J. o.* subsp.

badia (H. Gay) Debeaux), *J. navicularis* Grand. (= *J. o.* subsp. *transtagana* Franco) and *J. macrocarpa* Sibth. & Sm. (= *J. o.* subsp. *macrocarpa* (Sibth. & Sm.) Neilr.). Farjon (1998) treated these four taxa as a single species, *J. oxycedrus* L.

We anticipated a data set with two very distinct genotypes (sect. *Caryocedrus* and *Juniperus*), and four closely related genotypes within sect. *Juniperus*. An additional collection of *J. oxycedrus* from northern Greece was added to represent populational variation within that species.

We should mention that obtaining reproducible RAPD patterns does present a problem in many labs. In fact, Penner et al. (1993) have investigated reproducibility in RAPDs using the same target DNA and primers in different laboratories. They found most RAPD markers to be reproducible with differences between PCR machines accounting for most of the differences.

Yu and Pauls (1992) explored various PCR programming details to optimize the reactions for RAPDs production. Levi et al. (1993) evaluated a range of concentrations of Triton X-100, gelatin, dNTPs, primer, template DNA, TAQ, MgCl₂, as well as various times for annealing, elongation and denaturation. More recently, Bielawski et al. (1995) examined changes in protocols to obtain reproducible RAPD markers in vertebrate DNA (striped bass, plus a few other vertebrate species). However, there are ways to minimize this variation (Adams et al., 1998) and these methods have been applied to the ISSR and RAPD data gathering procedures in this study. In this study we select two genotypes (individuals) from each of the six taxa in order to (1) eliminate spurious and non-informative bands in the RAPDs and ISSRs data sets and (2) detect and remove intra-taxon polymorphisms from the data sets. The purpose of this study was not to make taxonomic decisions, but rather to compare genotype sampling using a variety of methods, therefore the use of only two individuals (genotypes) from each taxon should not necessarily be taken to be representative of any particular taxon discussed in this paper. But it is important for the reader to note that exactly the same genotypes (individuals) were used for each data set, so sampling errors are not a factor in these comparisons.

2. Materials and methods

The following collections were used in this study: *J. drupacea* Labill., Adams 5651, 5652, 8795, 8796, Greece; *J. navicularis* Grand. (= *J. oxycedrus* subsp. *transtagana* Franco), Adams 8239–8243, Lisbon, Portugal; *J. oxycedrus*, Adams 7080–7082, El Penon, Spain, and Adams 5988, 5999, 8787, 8788, Lemo, n. Greece; *J. badia* H. Gay (= *J. oxycedrus* subsp. *badia* (H. Gay) Debeaux), Adams 7795–7800; *J. macrocarpa* (= *J. o.* subsp. *macrocarpa*), Adams 7205–7207, Tarifa, Spain. Voucher specimens are deposited at BISH.

Details on the extraction and analysis of the terpenoids for these taxa have been previously reported (Adams, 1997, 2000a; Adams et al., 1999).

For DNA work, one gram (fresh weight) of the foliage was placed in 20 g of activated silica gel and transported to the lab, where it was stored at –20°C until the DNA was extracted. DNA was extracted using the Qiagen DNeasy mini kit.

The RAPD analyses follow that of Adams and Demeke (1993). Ten-mer primers were purchased from the University of British Columbia and Genset: (5'-3') 116: TAC GAT GAC G; 134: AAC ACA CGA G; 153: GAG TCA CGA G; 204: TTC GGG CCG T; 212: GCT GCG TGA C; 218: CTC AGC CCA G; 239: CTG AAG CGG A; 244: CAG CCA ACC G; 250: CGA CAG TCC C; 265: CAG CTG TTC A; 327: CTA GAG GTC C; 338 CTG TGG CGG T; 346: TAG GCG AAC G; 347: TTG CTT GGC G; 375: CCG GAC ACG A. ISSR primers (Univ. British Columbia) 807: AGA GAG AGA GAG AGA GT; 808: AGA GAG AGA GAG AGA GC; 811: GAG AGA GAG AGA GAG AC; 812: GAG AGA GAG AGA GAG AA; 818: CAC ACA CAC ACA CAC AG; 823: TCT CTC TCT CTC TCT CC; 824: TCT CTC TCT CTC TCT CG; 825: ACA CAC ACA CAC ACA CT; 835: AGA GAG AGA GAG AGA GYC; 836: AGA GAG AGA GAG AGA GYA; 846: CAC ACA CAC ACA CAC ART; 847: CAC ACA CAC ACA CAC ARC; 857: ACA CAC ACA CAC ACA CYG; 858: TGT GTG TGT GTG TGT GRT; 895: AGA GTT GGT AGC TCT TGA TC. [note: R, Y=mixed base positions, R=(A, G), Y=(C, T)].

ITS amplifications were performed in 50 µl reactions using 0.6 ng of genomic DNA, 1.5 units Promega Taq polymerase, 5 µl Promega 10× buffer (final concentration: 50 mM KCl, 10 mM Tris-HCl (pH 9), 2.0 mM MgCl₂, 0.01% gelatin and 0.1% Triton X-100), 2 mM MgCl₂, 0.4 mM each dNTP, 0.23 µM each primer (ITSA, ITSB), and 8% (by vol.) DMSO. ITSA=GGA AGG AGA AGT CGT AAC AAG G; ITSB=CTT TTC CTC CGC TTA TTG ATA TG. The sequences for the ITSA and ITSB primers were based on Blattner (1999). The following PCR conditions were used: MJ Research Programmable Thermal Cycler, 45 cycles, 94°C (1 min.), 50°C (1 min.), 72 °C (1 min.), with a final step of 72 °C (5 min.). The PCR reaction was subjected to purification by agarose gel electrophoresis (1.5% agarose, 1 h) and the ITS band (approx. 1100 bp) was excised and purified by use of a Qiagen QIAquick gel extraction kit.

Dideoxy terminating PCR reactions were in 10 µl: 2 µl ABI Big Dye Sequencing Kit (Perkin-Elmer Corp.), 2 µl Half Term Dye Terminator (Genepak), 3 µl (45 ng) template DNA, 1 µl primer (ITSA or ITSB,=0.29 µM). PCR program: 96 °C(30 sec.), 50 °C(15 sec.), 60 °C(4 min.), 25 cycles. Sequencing was performed on an ABI 377 automated sequencer. Sequences for both strands were edited and a consensus sequence was produced using Sequencher version 3.1 (GeneCodes Corp., 1998). The alignment was done manually in Sequencher.

For RAPDs and ISSRs, PCR was performed in a volume of 15 µl containing 1.5 µl Promega 10X buffer(see above), 0.2 mM of each dNTPs, 0.36 µM primers, 0.3 ng genomic DNA, 15 ng BSA and 0.6 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. DNA amplification was performed in an MJ Programmable Thermal Cycler (MJ Research, Inc.). RAPDs were run as: 94 °C (1.5 min) for initial strand separation, then 40 cycles of 38 °C (2 min), 72 °C (2 min), 91 °C (1 min). Two additional steps were used: 38 °C (2 min) and 72 °C (5 min) for final extension. ISSRs were run as: 94 °C (1.5 min) for initial strand separation, then 40 cycles of 50 °C (2 min), 72 °C (2 min), 91 °C (1 min) with two additional steps: 50 °C (2 min) and 72 °C (5 min) for final extension.

RAPD, and ISSR bands that were unique to a single sample or did not show fidelity within the two replicated samples of each taxon were eliminated and not used in the computation of similarities (Table 1). It should be noted that these bands contain very useful information for the study of genetic variance and individual variation, but are merely ‘noise’ in the present taxonomic study. RAPD, and ISSR bands, and terpenoids were scored as present or absent. The ITS sequence data were also treated as simple matches. Indels were treated as matching if present, matching if absent and a mismatch (present vs absent) in a comparison. Thus, all of the data sets were analyzed in the same manner to facilitate comparisons. For the terpenoids, each compound was scored as present or absent in each genotype. Minimum spanning networks follow Adams (1975). PCA ordination follows the formulation of Gower (1966).

3. Results and discussion

ITS Sequences are deposited in GenBank (*J. drupacea*, Adams 8795–AY046515, Adams 8796–AY046516; *J. navicularis*, Adams 8329–AY046517, Adams 8239–AY046518; *J. oxycedrus*, Greece, Adams 8787–AY046519, Adams 8788–AY046520; *J. macrocarpa*, Adams 7206–AY046521, Adams 7205–AY 046522; *J. oxycedrus*, Spain, Adams AY046523, Adams 7080–AY046524; *J. badia*, Adams AY046525, Adams 7800 AY046526).

Each of the data sets had unique characteristics (Table 2). In general, the ITS sequences were least variable and the ISSRs the most variable (Table 2). Note that only 4 ISSRs (of 346 bands) were present in all 12 of the samples, whereas 1073 bp (of 1120 bp) of ITS sequences were present in all 12 samples. The ratio of single events to constant events was lowest in the ITS sequences (0.007%) and highest in the ISSRs (37.3%, Table 2). In general, it appeared that the ISSRs would be more useful for analysis of individual differences, the ITS sequence data for interspecific and intergeneric differences and the RAPD data for work at intermediate taxonomic levels. However, it should be noted that RAPD data is greatly influenced by the selection of primers used. Demeke et al. (1992) found primers that distinguished between cultivar lines of cabbage and cauliflower and Adams et al. (1998) found

Table 1

RAPD and ISSR primers used showing (numbers in parenthesis) the total bands present/primer and # bands shared by both individuals from a given taxon

RAPDs:					
116 (20,7)	134(36,16)	153(22,13)	204(23,15)	212(32,19)	218(23,17)
239(24,14)	244(30,19)	250(21,11)	265(14,7)	327(30,11)	338(21,14)
346(20,8)	347(25,14)	375(13,12)			
ISSRs:					
807(18,11)	808(23,14)	811(27,11)	812(23,10)	818(17,10)	823(24,17)
824(22,11)	825(17,14)	835(20,12)	836(19,9)	846(27,13)	847(22,15)
857(35,21)	858(18,10)	895(34,18)			

Table 2

Comparison of data sets used in this study. The # single events are the number of characters that occurred in only one individual of a taxon or randomly between taxa. s/c=# single events/# constant

	# characters	# constant	# single events	s/c	# chars/# used ^c
ITS sequences	1120	1073	8	0.007%	3.48%
RAPDs	347	18	157	8.7	56.77
ISSRs	346	4	149	37.3	56.94
Terpenes	128	22 ^a	49 ^b	2.2	100.0

^a for the terpenes, # constant=# cpds present in all taxa.

^b terpene data were based on averages of several plants, so single events are based on analysis of the population average concentration of each component.

^c # char=total number of characters scored. # used=number of characters used in the computation of similarity measures.

primers that generated markers associated with habitat preferences within a single juniper population. Conversely, Adams and Demeke (1993) found markers that separated subgenera of *Juniperus*. The terpene data of *Juniperus*, being both quantitative and qualitative has been very useful at the populational level (Adams, 1975, 1977, 1983, 1986). And, in fact, the ancestral/modern populations of *J. ashei* (Adams, 1977) were found to also be distinct in their RAPDs (Adams et al., 1998). These results are consistent with findings from other studies that have employed two or more of these data sets (e.g., McGregor et al., 2000; Esselman et al., 1999; Tredway et al., 1999).

Minimum spanning network analyses for the twelve genotypes are shown in Figs. 1–4 (Note: no phylogenetic inferences should be made from these networks, phylogenetic work for the entire genus will be published elsewhere, Schwarzbach et al., in prep.). Networks generated from ITS sequences and RAPD similarity matrices were very similar. The most surprising result is the clear separation of *J. oxycedrus*—Greece from *J. oxycedrus*—Spain in both networks. Morphological examination of the specimens revealed that the leaves from Greece were broader than those from Spain. Although we included these two sets of *J. oxycedrus* samples to investigate intraspecific variation, it appears that the Grecian taxon is quite distinct from *J. oxycedrus* from Spain. Additional field research is in progress to define this Grecian taxon (Adams, in prep.). It might be noted that phylogenetic analysis (PAUP) using ITS data (with *Cupressus* as an outgroup) generated a diagram that was essentially identical to the minimum spanning network in Fig. 1.

The ISSR data (Fig. 3) also show the divergence of *J. oxycedrus*—Greece from *J. oxycedrus*—Spain, as well as the same general clustering of taxa as seen in the ITS and RAPD networks. The only major discrepancy is that *J. oxycedrus*—Spain is not allied with *J. badia* in the ISSR network. The terpenoids network (Fig. 4), by contrast, is noticeably different in that *J. badia* is very distinct. In fact, the *J. badia* essential oil has several unidentified components that are unique to *J. badia* (see Adams et al., 1999; Adams, 2000a for discussion). Possibly, it represents a ‘cryptic’ species, as few morphological differences have been found that distinguish it from

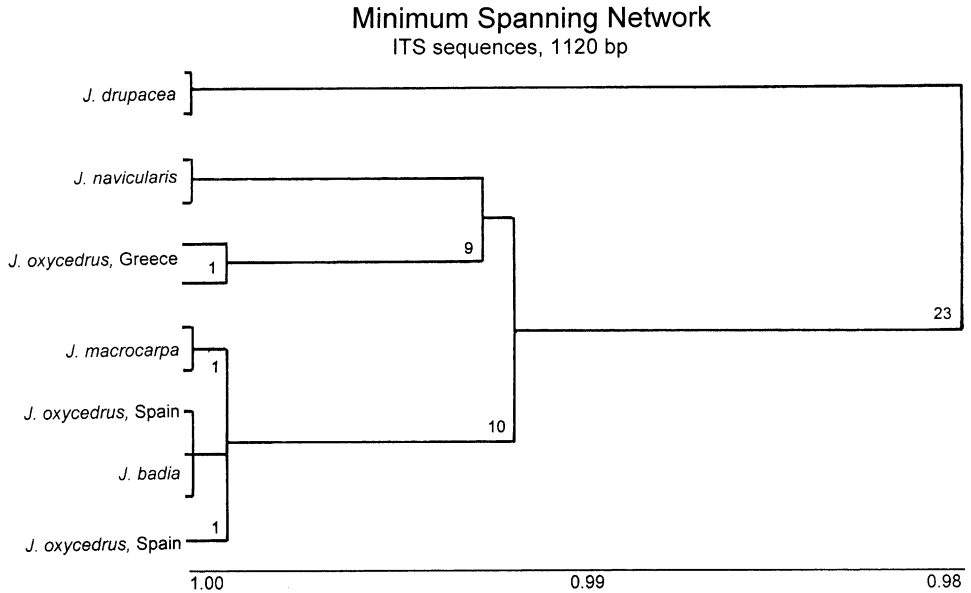


Fig. 1. Minimum spanning network based on ITS sequences, 1120 bp. The number at each branch point is the number of bp differences. Note the distinctness of the genotypes of *J. drupacea* (sect. *Caryocedrus*) from the other genotypes (sect. *Juniperus*) and the distinctness of genotypes of *J. navicularis* and *J. oxycedrus* from Greece.

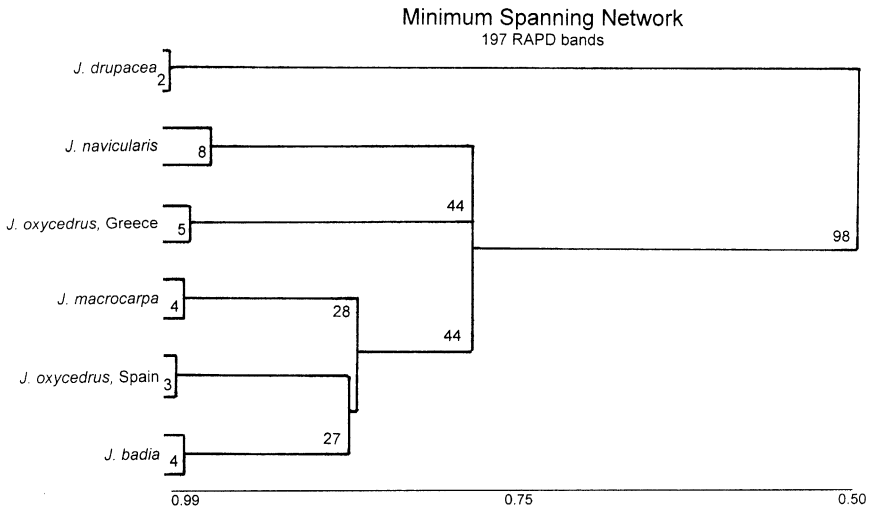


Fig. 2. Minimum spanning network based on 197 RAPD bands. The number at each branch point is the number of band differences. Note the distinctness of genotypes of *J. drupacea* (sect. *Caryocedrus*) from the other genotypes (sect. *Juniperus*) and the distinctness of genotypes of *J. navicularis* and genotypes of *J. oxycedrus* from Greece. In addition, one now sees the differences between genotypes taken from *J. badia*, *J. macrocarpa*, and *J. oxycedrus*, Spain.

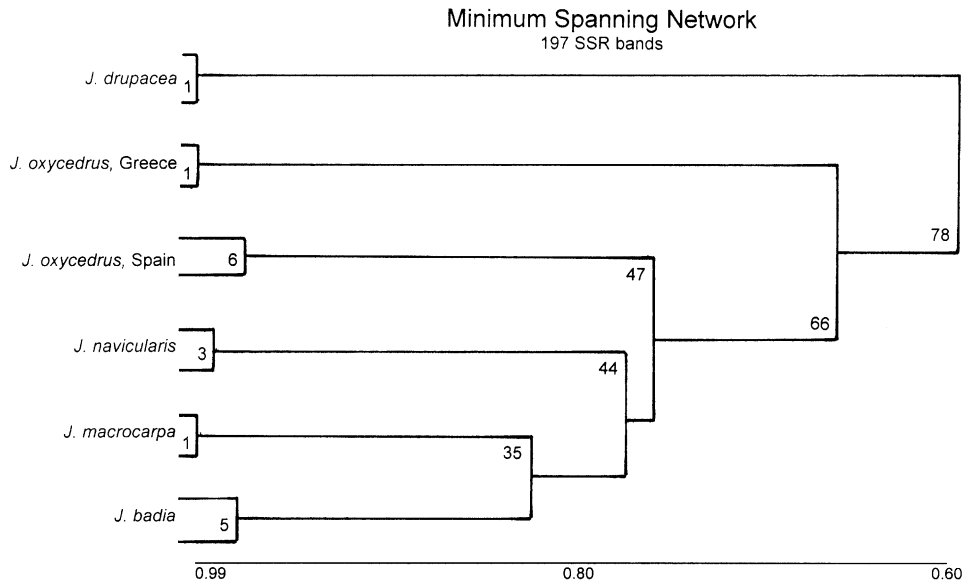


Fig. 3. Minimum spanning network based on 197 ISSR bands. The number at each branch point is the number of band differences. The genotypes of *Juniperus drupacea* (sect. *Caryocedrus*) is distinct but, genotypes of *J. oxycedrus*, Greece, are now shown as very distinct, as are genotypes of *J. badia*, *J. macrocarpa*, *J. navicularis* and *J. oxycedrus*, Spain.

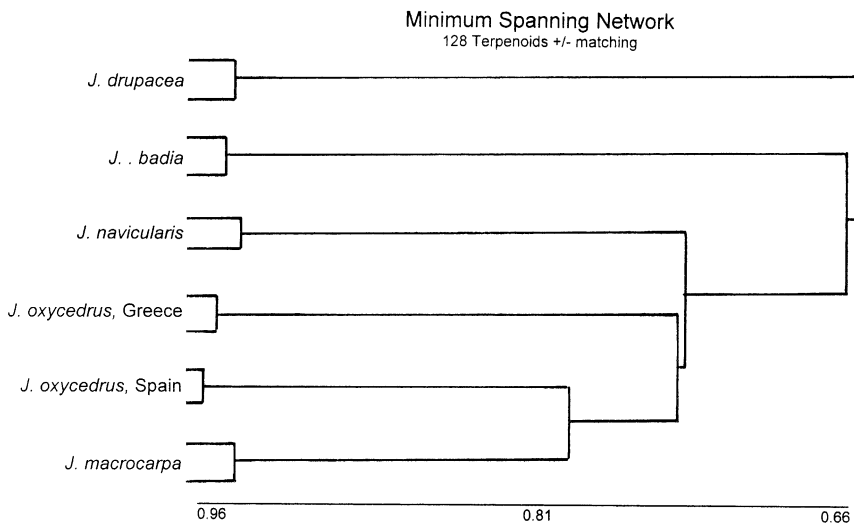


Fig. 4. Minimum spanning network based on 128 terpenoids. Each of the genotypes appear to be distinct with little clustering except by taxon.

J. oxycedrus of Spain (Adams, 2000a) or it may be a chemical race. For a complete listing of the terpenoids of the taxa in the present study, see Adams (1997, 2000a) and Adams et al. (1999).

To provide a quantitative estimate of similarity among the four data sets, pairwise correlations were calculated between the similarity matrices (Table 3). The highest correlation was between ITS sequences and RAPDs ($r=0.95$) followed by RAPD-ISSR ($r=0.84$) and ITS-ISSR ($r=0.83$). The terpenoid matrix was not highly correlated with any data set (Table 3).

An examination of five previous *Juniperus* studies (all at the specific level) involving the use of RAPDs and terpenoids revealed the following correlations between RAPDs and terpenoids: $r=0.44$ (Adams, 1999); $r=0.11$ (Adams, 2000a); $r=0.44$ (Adams, 2000b); $r=0.45$ (Adams, 2000c); $r=-0.32$ (Adams, 2000d). These values are similar to the RAPD-Terpenes correlation of $r=0.38$ (Table 3) in this study. It appears that the taxonomically useful limits of the terpenoids has been exceeded by the diversity of these data sets (e.g., at the specific levels).

Principal Component Analysis (PCA) of the correlations among the matrices generated by these four kinds of data sets is shown in Fig. 5.

Notice that ITS, RAPDs, and ISSRs data sets cluster together and the terpenoid data set clusters separately.

How do we account for the differences among these different data sets? It is possible that at least some of the differences result from sampling errors within individual data sets. That is, for some data sets, we may not have sampled enough characters to achieve a stable classification.

Unfortunately, the number of characters required varies depending on data type and OTU number (Thormann et al., 1994). In general, fewer characters are required to achieve stability if they all come from a single locus or if the number of OTUs in a data set is small. For example, 327 RAPD fragments were required to achieve a stable classification among 19 cruciferous accessions (i.e., a CV of less than 10%; Thormann et al., 1994), whereas only 93 RAPD bands were required to stably define six of the same OTUs in an earlier study (Demeke et al., 1992). However, it should be noted that part of the difference between these two studies (Thormann et al., 1994; Demeke et al., 1992) may have been in the Demeke et al. study, the primers were very carefully pre-screened (18 primers selected from over 200 primers screened) and this likely resulted in the use of far fewer primers and RAPD bands, but they were more stable and useful.

Table 3
Correlation among the similarity matrices of the four data sets

	ITS	RAPDs	ISSRs	Terpenes
ITS	–	0.95	0.83	0.030
RAPDs	0.95	–	0.84	0.38
ISSRs	0.83	0.84	–	–0.04
Terpenes	0.30	0.38	–0.04	–

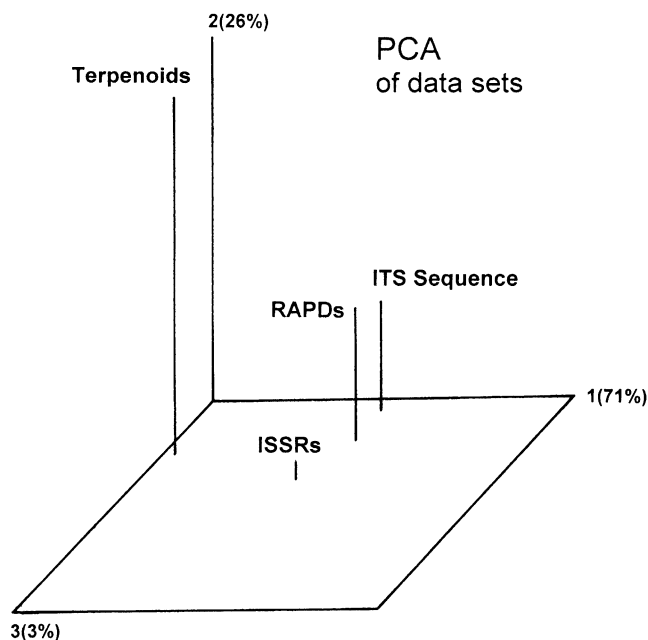


Fig. 5. PCA (Principal Components Analysis) of the similarity matrices based on ITS sequences, RAPDs, ISSRs, and terpenoids. The major grouping is between the terpenoids and the molecular data. The ITS and RAPDs data sets are most similar.

In the present study, the number of characters does not seem to be well correlated with the performance of a particular data set. The high correlation between the ITS and RAPD data sets suggests that they provide the best estimate of organismal relationships, yet they differed dramatically in character number (39 informative ITS sequence differences versus 197 RAPD bands).

It seems more likely that differences in the performance of these data sets results from a combination of intra-taxon polymorphism, a lack of homology among co-migrating fragments, and convergent evolution. For example, high levels of intra-taxon polymorphism in the ISSR data set may have reduced the amount of useful signal for detecting organismal relationships. It also seems likely that some fraction of the co-migrating RAPD and ISSR bands will not be homologous, particularly in the more distant comparisons (Rieseberg, 1996). Although this is unlikely to bias the relationships (Adams and Rieseberg, 1998), it may reduce the stability of the classification. A major factor in the terpenoid data sets may be epistatic gene effects and mutations eliminating a whole pathway synthesis branch (cf. the large number of unique, structurally related compounds in *J. badia* (Adams, 2000a) that accounts for much of its divergence in its similarity from *J. oxycedrus*).

As has been pointed out by numerous authors (e. g., Doyle, 1992; Rieseberg and Soltis, 1991), taxonomies based on single genes are highly suspect. Rather, concordance of different data sets seems to be the strongest evidence that we can use in

making taxonomic decisions. Because concordance is unlikely to be the result from chance alone, it provides a degree of assurance in organismal classification that may be difficult to obtain in any other manner.

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