



Pergamon

Available online at [www.sciencedirect.com](http://www.sciencedirect.com)

SCIENCE @ DIRECT®

biochemical  
systematics  
and ecology

Biochemical Systematics and Ecology 31 (2003) 1271–1278

[www.elsevier.com/locate/biochemsyseco](http://www.elsevier.com/locate/biochemsyseco)

# Analysis of *Juniperus communis* and its varieties based on DNA fingerprinting

Robert P. Adams<sup>a,\*</sup>, Ram N. Pandey<sup>b</sup>

<sup>a</sup> Biology Department, Baylor University, P.O. Box 97388, Waco, TX 76798, USA

<sup>b</sup> Department of Biology, Eastern New Mexico University, Portales, NM 88130, USA

Received 29 April 2002; accepted 6 January 2003

---

## Abstract

Plants of *Juniperus communis* L. var. *communis*, *J. c.* var. *depressa* Pursh, *J. c.* var. *hemispherica* J. & C. Presl, *J. communis* var. *megistocarpa* Fern. & St. John, *J. c.* var. *nipponica* (Maxim.) Wils., *J. c.* var. *oblonga* hort. ex Loudon and *J. c.* var. *saxatilis* Pall. were sampled and DNA fingerprinting (RAPDs, Random Amplified Polymorphic DNAs) was performed. Based on 191 RAPD bands, there was little evidence to support the recognition of *J. communis* var. *hemispherica*, *J. c.* var. *oblonga* and *J. c.* var. *nipponica*. *Juniperus communis* var. *communis* (Sweden) was found to be most similar to *J. c.* var. *hemispherica* from Sicily, and also very similar to *J. c.* var. *saxatilis*. The recognition of *J. c.* var. *saxatilis*, sensu stricto, and var. *hemispherica* (from Sicily) was not supported by the RAPD data in this study. All of the *J. c.* var. *depressa* populations sampled from the Western Hemisphere formed a distinct group. *Juniperus communis* var. *megistocarpa*, endemic to maritime eastern Canada, was the most distinct variety of *J. communis*. *Juniperus communis* var. “*saxatilis*” populations from the Kamchatka peninsula and *J. c.* var. “*hemispherica*” from the Sierra Nevada, Spain, were very distinct from other *J. c.* var. *communis*–*saxatilis* populations.

© 2003 Elsevier Science Ltd. All rights reserved.

**Keywords:** *Juniperus*; Cupressaceae; RAPD; DNA fingerprinting; *J. communis*; Varieties

---

---

\* Corresponding author.

E-mail address: [robert\\_adams@baylor.edu](mailto:robert_adams@baylor.edu) (R.P. Adams).

## 1. Introduction

The genus *Juniperus* consists of approximately 68 species and 36 varieties (using the more widely accepted variety category instead of the subspecies category) (Adams, 1999, 2000a, b, c, d, 2001; Adams et al., 2002a, b, 2003). All the taxa grow on the Laurasian land mass, except *J. procera* Hochst. ex Endl., which grows along the rift mountains in East Africa and thence into the Southern Hemisphere (Adams et al., 1993) and some of the Mediterranean *Juniperus* species such as *J. oxycedrus* L., *J. phoenicea* L., and *J. thurifera* L. that grow in the mountains of the northernmost part of Africa (Morocco, Algeria).

*Juniperus communis* is the only *Juniperus* species that occurs in both hemispheres (Fig. 1). Farjon (1998) recognized var. *communis* L. (northern Europe), var. *depressa* Pursh (North America), var. *megistocarpa* (eastern Canada); and var. *saxatilis* Pall. (Europe, Siberia, Central Asia, Far East, Greenland, Iceland, and far western North America). Farjon did not recognize var. *hemispherica* (J. & C. Presl.) Nyman (Sicily, Mediterranean) or var. *oblonga* (M.-Bieb.) Parl. (Caucasus Mts.). In addition, Adams et al. (2002a) have recently reported that in Japan, var. *saxatilis* and var. *nipponica* (Maxim.) E.H. Wilson form a loose group with vars. *communis* and *saxatilis* from

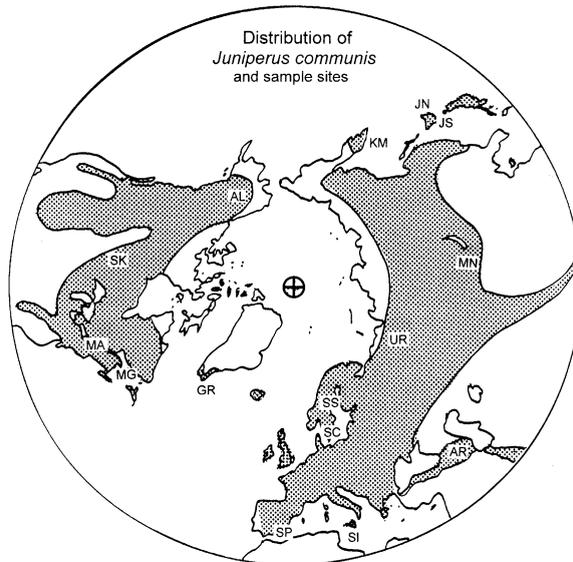


Fig. 1. Distribution of *J. communis* with sample sites noted. AL = Alaska, *J. c.* var. *depressa*, USA; SK = Saskatchewan, Canada, *J. c.* var. *depressa*; MA = Massachusetts, USA; MG = Magdalen Island, Canada, *J. c.* var. *megistocarpa*; GR = Greenland, *J. c.* var. *saxatilis*; SS = Umea, Sweden, *J. c.* var. *saxatilis*; SC = Stockholm, Sweden, *J. c.* var. *communis*; SP = Sierra Nevada, Spain, *J. c.* var. *hemispherica*; SI = Sicily, *J. c.* var. *hemispherica*; AR = Armenia, *J. c.* var. *oblonga*; UR = Ural Mts., Russia, *J. c.* var. *saxatilis*; MN = Altair Mts., Mongolia, *J. c.* var. *saxatilis*; KM = Kamchatka Peninsula, Russia, *J. c.* var. *saxatilis*; JS = Hokkaido, Japan, *J. c.* var. *saxatilis*; JN = Hokkaido, Japan, *J. c.* var. *nipponica*.

Europe. A study of Arctic populations of *J. communis* (Adams et al., 2003) revealed that these Arctic populations clustered by continent with the populations in Greenland and Iceland showing the highest affinities to populations from Europe, not North American populations.

Ashworth et al. (1999, 2001) used DNA fingerprinting to examine *J. communis* plants identified as *J. communis* var. *depressa*, *J. c.* var. *jackii* Rehder, *J. c.* var. *montana* Aiton (= *J. c.* var. *saxatilis* Pall., see Farjon, 1998) collected from California, Oregon, Nevada and Utah in the southwest and west coast of the US. They did not get a clear pattern separating these taxa, and concluded that their samples represent a single taxon (variety). *Juniperus communis* var. *jackii* is quite distinctive in forming longer, more sparsely branched lateral branches and is found only on serpentine soil. However, when *J. c.* var. *jackii* plants were transplanted to normal soil, the new growth reverted back to typical *J. c.* var. *montana* (*saxatilis*) growth (Adams, 1993). This led to the conclusion that the habit of *J. c.* var. *jackii* is merely environmentally induced.

In this study, we have attempted to compare all the known varieties (or subspecies) of *J. communis* to try and discern whether these varieties are distinct in their RAPDs.

## 2. Materials and methods

Specimens used in this study: *J. communis* var. *communis*: Adams 7846, 7848, Stockholm, Sweden; *J. communis* var. *hemispherica*: Adams 9045, 9046, Mt. Etna, Sicily, Italy (type locality); Adams 7194, 7195, Sierra Nevada, Spain; *J. communis* var. *saxatilis*: Adams 9211, 9212 (ex. K. Hoegh), Qaqortoq, Greenland; Adams 8686, 8687 (ex. Jin Murata), Hokkaido, Japan; Adams 9213, 9214 (ex. G. Samuelson), Umea, Sweden; Adams 9178, 9179 (ex. J. W. Leverenz), 25 km NW of Labytnangi, near the Ural Mts., Russia; Adams 9181, 9182 (ex. J. W. Leverenz), Eso, Kamchatka Peninsula, Russia; Adams 7589, 7590, Altair Mts., Mongolia; *J. communis* var. *depressa*: Adams 7582, 7582, Denali National Park, Alaska, USA; Adams 7094, 7095, Neimembian Lake, Saskatchewan, Canada; Adams 8572, 8573, Boxborough, MA, USA; *J. c.* var. *megistocarpa*, Adams 8575, 8576, Magdalen Islands, Quebec, Canada (ME); *J. c.* var. *nipponica*, Adams 8579, 8690 (ex. Jin Murata), Hokkaido, Japan; *J. communis* var. *oblonga*: Adams 8764, 8765, Lake Sevan, Armenia. Voucher specimens are deposited at the Baylor University herbarium (BAYLU).

One gram (fresh weight) of the foliage was placed in 20 g of activated silica gel and transported to the laboratory, thence stored at  $-20^{\circ}\text{C}$ . DNA was extracted from the leaves by use of the Qiagen Dneasy Plant Mini Kit. The RAPD analyses follow that of Adams and Demeke (1993). Ten-mer primers were purchased from the University of British Columbia (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; 249: GCA TCT ACC G; 250: CGA CAG TCC C; 265: CAG CTG TTC A; 327: ATA CGG CGT C; 338: CTG TGG CGG T; 346: TAG GCG AAC G; 347: TTG CTT GGC G; 375: CCG GAC ACG A; 391: GCG AAC CTC G; 413: GAG GCG GCG A; and 431: CTG CGG GTC A.

PCR was performed in a volume of 15  $\mu$ l containing 50 mM Tris–HCl (pH 9), 2.0 mM MgCl<sub>2</sub>, 0.01% gelatin and 0.1% Triton X-100, 0.2 mM of each dNTP, 0.36  $\mu$ M of each primer, 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.). The thermal cycle was: 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.

Bands that occurred once or did not show fidelity within the two samples of each taxon were eliminated. 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. Bands were scored in four classes: very bright (=6); medium bright (=5), faint (=4) and absent (=0). See Adams and Demeke (1993) for details on electrophoresis and RAPD band scoring.

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). A minimum spanning network (=single linkage clustering) program was written (Adams, 1975). Principal coordinate analysis (PCO) of the similarity matrix follows Gower (1966) using a program written by the senior author.

### 3. Results and discussion

One hundred and ninety-one RAPD bands were found to vary among the populations and these were used to construct a minimum spanning network (Fig. 2). The largest cluster contains *J. c.* var. *communis*, var. *hemispherica*, var. *nipponica*, var. *oblonga* and var. *saxatilis*. This seems to imply that these varieties are not distinct. Surprisingly, *J. c.* var. *communis*, Sweden, was most similar to *J. c.* var. *hemispherica*, Sicily. The key character separating *J. c.* var. *communis* from other varieties is that it is an upright, small tree. It has been our field experience (RPA) that in many locations (Hungary, Switzerland, Sweden) it is easy to find individuals that are more shrubby than tree-like. This character appears to be controlled by only a few genes throughout the species. In fact, near Amherst, MA, USA, there are individuals of the normally shrubby *J. c.* var. *depressa* that exhibit tree-like growth, a further indication that the genes for apical dominance are not entirely well regulated in this species. This lack of distinctness of *J. c.* var. *communis* from *J. c.* var. *saxatilis* has previously been reported (Adams et al., 2002a, b).

The second large cluster (Fig. 2) is the *J. c.* var. *depressa* populations from North America. *Juniperus c.* var. *megistocarpa* appears very distinct in this analysis (Fig. 2), in contrast to a previous report (Adams et al., 2002a, b). This taxon, with its very large female cones (9–13 mm, Adams, 1993) is endemic to sand dunes on islands in Quebec, Nova Scotia and Newfoundland.

The Kamchatka population (Fig. 2) is quite distinct along with the Sierra Nevada,

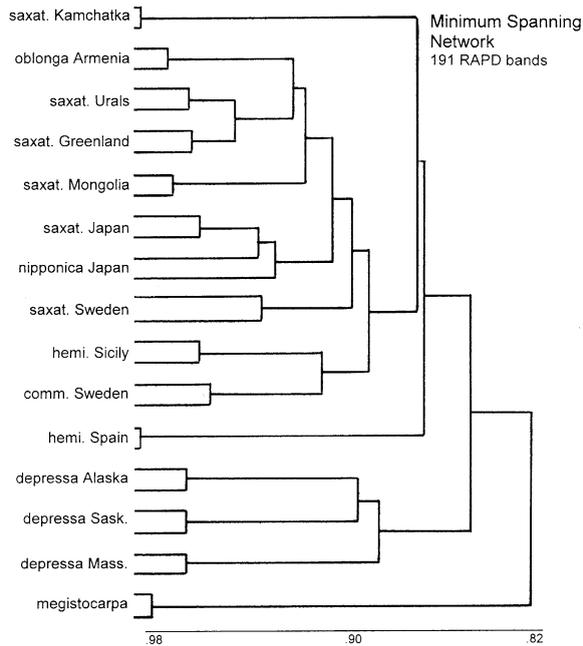


Fig. 2. Minimum spanning network based on 191 RAPD bands. See text for discussion. Scale on bottom is the similarity (0.0–1.0).

Spain, population. Both fit loosely with *J. c. var. communis*–*saxatilis* populations (Fig. 2). Examination of the specimens did not reveal any major morphological differences in the Kamchatka plants, but the Sierra Nevada plants' leaves appeared a little broader than those of *J. c. var. communis* from Sweden.

Several of the populations studied are recent (dating from the last glacial maximum, 12,000 a BP, Adams et al., 2003). These recent populations include all the var. *depressa* and var. *megistocarpa* populations sampled in North America, Greenland, Sweden, Urals, and Kamchatka. A geographically based minimum spanning network shows (Fig. 3) the complex patterns in Europe and Asia. Note that the recent population of *J. c. var. communis* in Sweden is most similar to the Sicily population. Sicily (and southern Italy) could have been refugia for *J. communis* during the last glacial maximum. The northern and central European populations of *J. communis* are recent (Adams et al., 2003). Armenia and Japan are other refugia. The Kamchatka population is most similar (0.87, Fig. 3) to *J. c. var. nipponica* from Japan. Adams et al. (2003) concluded that the Kamchatka population is recent (since 12,000 a BP) and likely founded by birds bringing seeds from Japan. This study supports their thesis. The relatively large divergence of the Kamchatka population from Japan (or any other populations) seems to favor founder's effect and genetic drift to account for these differences.

The link of Greenland and the Urals populations is a little misleading because additional populations linking these sites were not included in this study. It has

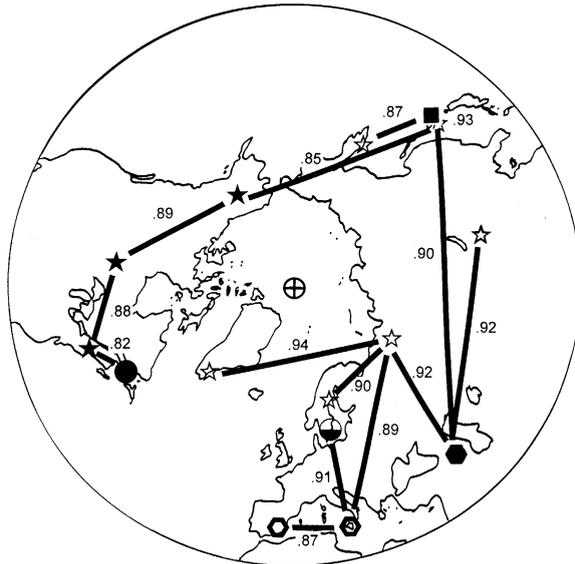


Fig. 3. Minimum spanning network superimposed on a geographic map. See text for discussion.

already been shown (Adams et al., 2003) that the Greenland population was derived from Iceland, thence Sweden.

Another view of the overall relationships is seen from principal coordinates analysis (Fig. 4). In PCO, the eigenroots appeared to have reached asymptotes after five

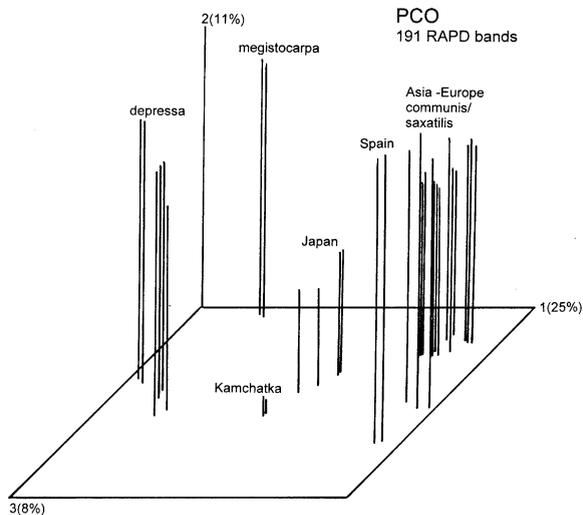


Fig. 4. Principal coordinate analysis (PCO) showing the major groups: *communis-saxatilis* in Asia-Europe, Spain, Japan, Kamchatka, var. *megistocarpa* and var. *depressa*.

eigenroots (24.85, 11.05, 8.30, 7.96 and 6.58). The major trend in this data set is the separation of var. *depressa*, var. *megistocarpa*, Spain, Kamchatka and all the other *communis saxatilis* from Asia and Europe (Fig. 4). The first three coordinates accounted for only 44% of the variation among the 30 OTUs. This suggests that the large amount of variation seen among individuals and sample sites is not part of clearly defined patterns. The Kamchatka plants are placed near the var. *saxatilis-nipponica* from Japan (Fig. 4) concordant with the linkage map (Fig. 3) that suggests that the Kamchatka plants may have come from Japan.

*Juniperus c.* var. *depressa* is separated from *J. c.* var. *saxatilis* (= *J. c.* var. *montana* Aiton) in that the former has a stomatal band about as wide as the green leaf margin, whereas in var. *saxatilis*, the stomatal band is twice or more as wide as the green margin (Adams, 1993). Plants in North America near the west coast have stomatal bands indicative of *J. c.* var. *saxatilis*. However, Ashworth et al. (1999, 2001) studied plants from var. *saxatilis* and var. *depressa* from the western US and found that these varieties were not separated by their RAPDs data. It appears that the *J. communis* plants of North America can be classified as either *J. c.* var. *depressa* or *J. c.* var. *megistocarpa*. Although specimens with broad stomatal bands have been found on the west coast, these do not appear to be *J. c.* var. *saxatilis* as found in Europe and Asia. The situation will need some additional study for final clarification.

*J. communis* is a very variable species. Small morphological differences and differences in habit were not found to be concordant with RAPDs DNA data in this study. Particularly noticeable is the lack of distinction between *J. c.* var. *communis* and *J. c.* var. *saxatilis*. In some populations, the growth habit is very variable. The recognition of *J. c.* var. *saxatilis*, sensu stricto, is not supported by the RAPD data of this study. Additional studies (in progress) using DNA sequence data may help resolve these taxonomic questions.

## Acknowledgements

Thanks to K. Hoegh, Greenland, J.W. Leverenz, Denmark, Jin Murata, Japan, and G. Samuelson, Sweden, for plant collections.

## References

- Adams, R.P., 1975. Numerical–chemosystematic studies of infraspecific variation in *Juniperus pinchotii* Sudw. *Biochem. Syst. Ecol.* 3, 71–74.
- Adams, R.P., 1993. *Juniperus*. In: Morin, N.R. (Ed.), *Pteridophytes and Gymnosperms. Flora of North America*, vol. 2. Oxford University Press, New York, pp. 412–420.
- Adams, R.P., 1999. Systematics of multi-seeded eastern hemisphere *Juniperus* based on leaf essential oils and RAPD DNA fingerprinting. *Biochem. Syst. Ecol.* 27, 709–725.
- Adams, R.P., 2000a. Systematics of smooth leaf margin *Juniperus* of the western hemisphere based on leaf essential oils and RAPD DNA fingerprinting. *Biochem. Syst. Ecol.* 28, 149–162.
- Adams, R.P., 2000b. Systematics of *Juniperus* section *Juniperus* based on leaf essential oils and RAPD DNA fingerprinting. *Biochem. Syst. Ecol.* 28, 515–528.

- Adams, R.P., 2000c. Systematics of the one seeded *Juniperus* of the eastern hemisphere based on leaf essential oils and random amplified polymorphic DNAs (RAPDs). *Biochem. Syst. Ecol.* 28, 529–543.
- Adams, R.P., 2000d. The serrate leaf margined *Juniperus* (Section *Sabina*) of the western hemisphere: systematics and evolution based on leaf essential oils and Random Amplified Polymorphic DNAs (RAPDs). *Biochem. Syst. Ecol.* 28, 975–989.
- Adams, R.P., 2001. Geographic variation in leaf essential oils and RAPDs of *J. polycarpus* K. Koch in central Asia. *Biochem. Syst. Ecol.* 29, 609–619.
- Adams, R.P., Demeke, T., 1993. Systematic relationships in *Juniperus* based on random amplified polymorphic DNAs (RAPDs). *Taxon* 42, 553–572.
- Adams, R.P., Demeke, T., Abulfatih, H.A., 1993. RAPD DNA fingerprints and terpenoids: clues to past migrations of *Juniperus* in Arabia and East Africa. *Theor. Appl. Genet.* 87, 22–26.
- Adams, R.P., Hsieh, C., Murata, J., Pandey, R.N., 2002a. Systematics of *Juniperus* from eastern Asia based on Random Amplified Polymorphic DNAs (RAPDs). *Biochem. Syst. Ecol.* 30, 231–241.
- Adams, R.P., Pandey, R.N., Rezzi, S., Casanova, J., 2002b. Geographic variation in the Random Amplified Polymorphic DNAs (RAPDs) of *Juniperus phoenicea*, *J. p.* var. *canariensis*, *J. p.* subsp. *eu-mediterranea*, and *J. p.* var. *turbinata*. *Biochem. Syst. Ecol.* 30, 223–229.
- Adams, R.P., Pandey, R.N., Leverenz, J.W., Dignard, N., Hoegh, K., Thorfinnson, T., 2003. Pan-Arctic variation in *Juniperus communis*: history biogeography based on DNA fingerprinting. *Biochem. Syst. Ecol.* 31, 181–192.
- Ashworth, V.E.T.M., O'Brien, B.C., Friar, E.A., 1999. Fingerprinting *Juniperus communis* L. cultivars using RAPD markers. *Madrono* 46, 131–141.
- Ashworth, V.E.T.M., O'Brien, B.C., Friar, E.A., 2001. Survey of *Juniperus communis* L. varieties from the western United States using RAPD fingerprints. *Madrono* 48, 172–176.
- Farjon, A., 1998. *World Checklist and Bibliography of Conifers*. Royal Botanic Gardens, Kew, UK, pp. 298.
- Gower, J.C., 1966. Some distance properties of latent root and vector methods used in multivariate analysis. *Biometrika* 53, 326–338.
- Gower, J.C., 1971. A general coefficient of similarity and some of its properties. *Biometrics* 27, 857–874.