

VARIATION IN nrDNA AND cpDNA OF *JUNIPERUS CALIFORNICA*, *J. GRANDIS*, *J. OCCIDENTALIS* AND *J. OSTEOSPERMA* (CUPRESSACEAE)

Robert P. Adams

Biology Department, Baylor University, Box 97388, Waco, TX
76798, USA, email Robert_Adams@baylor.edu

and

Michael E. Kauffmann

4312 Lentell Road, Eureka, CA, 95501

ABSTRACT

Single Nucleotide Polymorphisms (SNPs) of nrDNA and cpDNA (petN-psbM, trnD-T, trnL-F, trnS-G) of *Juniperus grandis* were examined from throughout its range. All the sequences showed *J. californica* to be quite distinct. 27 SNPs from nrDNA displayed mosaic variation among *J. grandis-occidentalis-osteosperma* individuals. Four SNPs from petN-psbM showed the Yolla Bolly plants with no differences from *J. occidentalis* (Sisters, OR), supporting the terpene data that the Yolla Bolly Mtns. putative *J. grandis* population is a form of *J. occidentalis*. No systematic differences were found in petN-psbM between *J. grandis* and *J. osteosperma*. 2 SNPs from trnD-trnT separated *J. occidentalis* from *J. osteosperma*-*J. grandis*. Analysis of trnL-F gave no SNPs of systematic use. *Juniperus grandis* (Meyers, CA) was separated from *J. occidentalis* by 2 SNPs from trnS-trnG and from *J. grandis* (Big Bear)-*J. osteosperma* by 2 SNPs. These data concur with the terpene data in showing the divergence of *J. grandis* from the central High Sierra from the disjunct San Bernardino Mtns. (Big Bear), *J. grandis* population. None of the five regions sequenced could consistently separate *J. grandis* (Big Bear) from *J. osteosperma*. *Phytologia* 92(2): 266-276 (August 2, 2010).

KEY WORDS: *Juniperus grandis* (= *J. occidentalis* var. *australis*), *J. californica*, *J. occidentalis*, *J. osteosperma*, Cupressaceae, nrDNA, petN-psbM, trnD-trnT, trnL-trnF, trnS-trnG, SNPs, geographic variation.

The western junipers consist of 4 species: *Juniperus grandis* R. P. Adams (= *J. occidentalis* var. *australis* (Vasek) A. & N. Holmgren), *J. californica* Carr., *J. occidentalis* Hook. and *J. osteosperma* (Torr.) Little (Adams, 2008). Adams and Kauffmann (2010, this issue), using leaf terpenoid data, found that the oil of the disjunct Yolla Bolly Mtns. (nw CA) population, often included in *J. grandis*, was slightly more similar to *J. occidentalis* than any population of *J. grandis* (Fig. 1). In

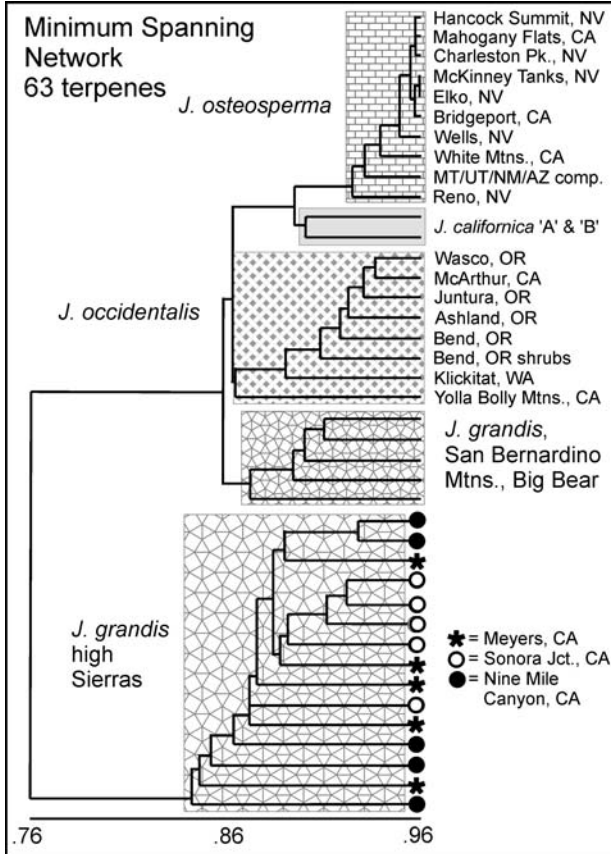


Figure 1. Minimum spanning network based on 63 terpenoids (from Adams and Kauffmann, 2010).

addition, they found that the oil of *J. grandis* from the High Sierras was quite different from *J. grandis* from the disjunct San Bernardino Mts. (Fig. 1). In fact, the oils of *J. grandis* from the San Bernardino Mts. were most similar to *J. occidentalis* (Fig. 4).

Juniperus grandis has an interesting distribution (Fig. 2) with populations in the high Sierras and the San Bernardino Mtns. (Fig. 2),

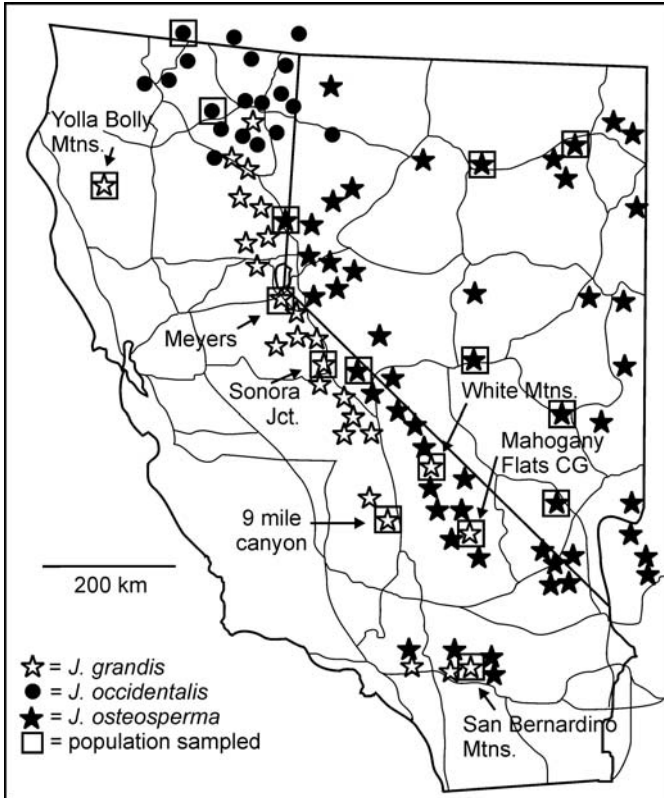


Figure 2. Distribution of *J. grandis* showing populations sampled. Partial distributions of *J. occidentalis* and *J. osteosperma* are also mapped in this region.

and, according to Vasek (1966), with putative outlying populations in the Yolla Bolly Mtns., White Mtns., and Panamint Range (see Mahogany Flats CG, Fig. 2). However, the oils from putative *J. grandis* from the White Mtns. and Panamint Range (Mahogany Flats) appeared to be typical *J. osteosperma* (Fig. 1).

The purpose of this study was to examine DNA sequences from the same trees of *J. grandis* sampled for leaf oils (Adams and Kauffmann, 2010). nrDNA has been widely used in *Juniperus* studies along with petN-psbM (Adams, 2009; Adams et al., 2009; Adams et al., 2010a, b, c, d; e Terry et al., 2000). Recently, Mao et al. (2010) have reported on the utility of several cpDNA regions for systematics of *Juniperus*. Three of their most promising cp regions were trnD^{GUC}-trnT^{GGU}, trnL^{UAA}-trnF^{GAA} and trnS^{GCU}-trnG^{UCC}. These 3 cp regions were also utilized in this study.

MATERIALS AND METHODS

Plant material J. californica, 'A', Adams 10154-10156, Victorville, CA, Adams 85-8697, 13 km n of Amboy/Kelso exit on I40, on road to Kelso at Granite Pass, 34° 48.41N, 115° 36.54'W, 1280 m; *J. californica*, 'B', Adams 8698-99, 27 km se of SE of Yucca, AZ on Alamo Road, 34° 44.91N, 113° 58.19'W, 920 m; *J. grandis*, Adams 11963-11967, Jct. US 50 & CA 89, 38° 51.086N, 120° 01.244'W, 1937 m, Meyers, CA, Adams 11968-11972, 16 km w of Sonora Jct., on CA Hwy. 108, 38° 18.289'N, 111° 35.598'W, 2585 m, Tuolumne Co., CA, Adams 11984-11988, Nine Mile Canyon Rd., 20 km w of Jct. with US 395, 35° 54.003'N, 118° 02.078'W, 2059 m, Tulare Co., CA, Adams 11989-11993, 5km n Big Bear City on CA 18, 34° 17.533'N, 116° 49.153'W, 2053 m, San Bernardino Co., CA; *J. occidentalis*, Adams 11940-11942, 12 km e of Jct. WA 14 & US 97 on WA 14, 45° 44.392'N, 120° 41.207'W, 170 m, Klickitat Co., WA, Adams 11943-11945, 2 km s of jct. US 97 & US 197 on US 97, 38 km ne of Madras, OR, 44° 53.676'N, 120° 56.131'W, 951 m, Wasco Co., OR, Adams 11946-11948, 3 km sw of Bend, OR, on OR 372, 44° 02.390'N, 121° 20.054'W, 1132 m, Deschutes Co., OR, Adams 11949-11951, 32 km e of Bend, OR on OR20, shrubs, 0.5 - 1 m tall, 43° 53.922'N, 120° 59.187'W, 1274 m, Deschutes Co., OR, Adams 11952-11954, 14 km e of Jct. OR66 & I5, on OR66, 42° 08.044'N, 122°

34.130'W, 701 m, Jackson Co., OR, *Adams 11957-11959*, on CA299, 10 km e of McArthur, CA, 41° 05.313'N, 121° 18.921'W, 1091 m, Lassen Co., CA, *Adams 11995-11998* (*Kaufmann A1-A3, B1*), Yolla Bolly-Middle Eel Wilderness, 40° 06' 34"N, 122° 57' 59W, 1815- 2000 m, Trinity Co., CA;

J. osteosperma, *Adams 10272-10276*, on NV157, Charleston Mtns. 36° 16.246'N, 115° 32.604'W, 1795 m, Clark Co., NV, *Adams 11122-11124*, Hancock Summit, mile 38 on US375, 37° 26.404'N, 115° 22.703'W, 1675 m, Lincoln Co. NV, *Adams 11125-11127*, McKinney Tanks Summit on US6, 38° 07.005'N, 116° 54.103'W, 1933 m, Nye Co., NV. *Adams 11134-36*, 8 km s of Bridgeport, on US395, 38° 12.639'N, 119° 13.846'W, 2004 m, Mono Co., CA; *Adams 11141-11143*, 13 km w of Elko, on I80, 40° 45.598'N, 115° 55.942'W, 1535 m, Elko Co., NV, *Adams 11144-11146*, 8 km e of Wells, on I80, 41° 06.533'N, 114° 51.441'W, 1876 m, Elko Co., NV, *Adams 11960-11962*, 56 km n of Reno, NV, 39° 54.458'N, 120° 00.322'W, 1383 m, Lassen Co., CA, *Adams 11973-1977*, 10 km n of CA 168 on White Mtn. Rd., 37° 20.143'N, 118° 11.346'W, 2607 m, Inyo Co., CA, *Adams 11978-11982*, 36° 13.783'N, 117° 04.102'W, 2477 m, Inyo Co., CA. Voucher specimens are deposited at the Herbarium, Baylor University (BAYLU).

One gram (fresh weight) of the foliage was placed in 20 g of activated silica gel and transported to the lab, thence stored at -20° C until the DNA was extracted. DNA was extracted from juniper leaves by use of a Qiagen mini-plant kit as per manufacturer's instructions.

PCR amplification ITS (nrDNA), petN-psbM amplifications were performed in 30 µl reactions using 6 ng of genomic DNA, 1.5 units Epi-Centre Fail-Safe Taq polymerase, 15 µl 2x buffer E (petN, trnD-T, trnL-F, trnS-G) or K (nrDNA) (final concentration: 50 mM KCl, 50 mM Tris-HCl (pH 8.3), 200 µM each dNTP, plus Epi-Centre proprietary enhancers with 1.5 - 3.5 mM MgCl₂ according to the buffer used) 1.8 µM each primer. See Adams, Bartel and Price (2009) for the ITS and petN-psbM primers utilized.

The primers for trnD-trnT, trnL-trnF and trnS-trnG regions:
trnD for ACC AAT TGA ACT ACA ATC CC cf. Grivet et al., 2001
trnT rev CTA CCA CTG AGT TAA AAG GG

trnL for CGA AAT CGG TAG ACG CTA CG cf. Taberlet et al. 1991
trnF rev ATT TGA ACT GGT GAC ACG AG

trnS for GCC GCT TTA GTC CAC TCA GC cf. Shaw et al., 2005
trnG rev GAA CGA ATC ACA CTT TTA CCA C

The PCR reaction was subjected to purification by agarose gel electrophoresis (1.5% agarose, 70 v, 55 min.). In each case, the band was excised and purified using a Qiagen QIAquick gel extraction kit. The gel purified DNA band with the appropriate primer was sent to McLab Inc. (San Francisco) for sequencing. Sequences for both strands were edited and a consensus sequence was produced using Chromas, version 2.31 (Technelysium Pty Ltd.). Alignments and NJ trees were made using MAFFT (<http://align.bmr.kyushu-u.ac.jp/mafft/>). Minimum spanning networks were constructed from SNPs data using PCODNA software (Adams et al., 2009).

RESULTS AND DISCUSSION

Sequencing nrDNA for 30 individuals revealed 37 mutational events with 10 single mutations (occurred only once among the 30 individuals) and 27 mutations that occurred more than once, implying systematic information might be present. A minimum spanning network based on these 27 SNPs is shown in Figure 3. *Juniperus californica* is quite separated by at least 20 SNPs from the other taxa. However, none of the other three species are separated. There are neither specific nor geographic differences among *J. grandis*, *J. occidentalis* nor *J. osteosperma*. The pattern is completely mosaic. Clearly, nrDNA data is quite conserved among these species. This is similar to the situation in *Cupressus* from the western hemisphere (now *Hesperocyparis*, see Adams, Bartel and Price, 2009), where Little (2006) reported no variation in nrDNA among 11 *Cupressus* species!

Sequencing petN-psbM resulted in 4 SNPs from 853 - 855 bp of data. The petN-psbM SNPs show *J. californica* to be distinct, with 1 SNP separating *J. occidentalis* (Sisters, OR and Yolla Bolly, CA) from *J. grandis* (12 individuals from 4 populations) and *J. osteosperma* (3 trees, Salt Lake City, UT) (Figure 4, left).

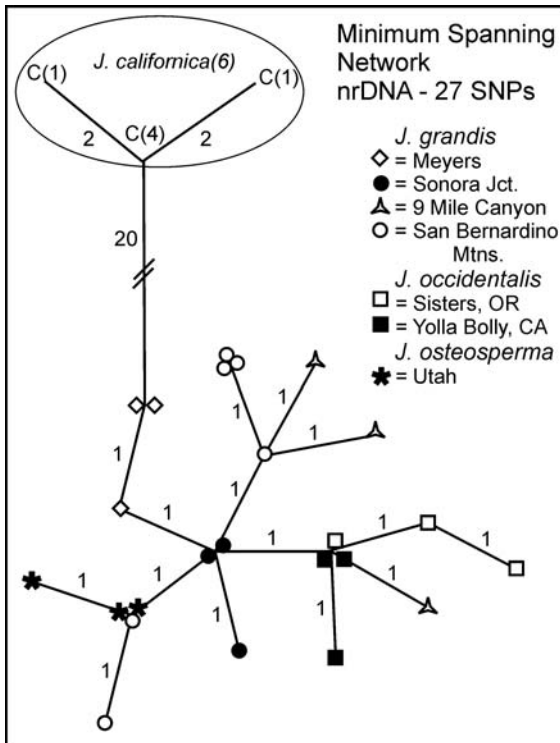


Figure 3. Minimum spanning network based on 27 SNPs from nrDNA sequencing (1250 - 1273 bp).

Sequencing the trnD-trnT region gave 6 systematically useful SNPs from 685 - 686 bp of data. Again, *J. californica* is well separated by 4 SNPs (Figure 4, right). No systematic variation was found among *J. osteosperma* (2), *J. grandis* (Meyers, 2; Big Bear 2) nor among the *J. occidentalis* (Sisters, OR, 2). No SNPs were found that separated *J. osteosperma* from *J. grandis*, or the Meyers and Big Bear *J. grandis* populations.

Preliminary sequencing of the trnL-trnF region (701-702 bp) for *J. grandis* (2), *J. occidentalis* (2) and *J. osteosperma* (2) yielded only one

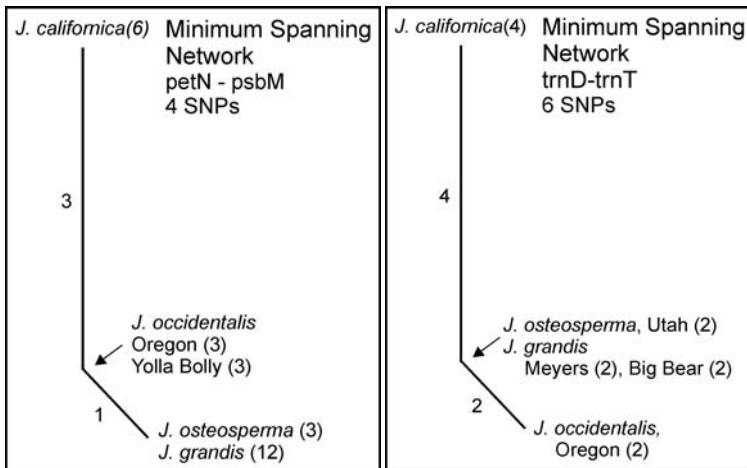


Figure 4. Minimum spanning networks. Left: petN-psbM. Right: trnD-T.

single point insertion in one individual, so sequencing of additional individuals was not continued.

Sequencing trnS-trnG resulted in 815 - 818 bp of data with 9 SNPs. Analysis of these SNPs (Fig. 5) shows *J. californica* well resolved (5 SNPs) with *J. occidentalis*, *J. grandis* (Meyers, CA) and *J. grandis* (Big Bear) - *J. osteosperma* separated by 2 SNPs each. Interestingly, *J. grandis* (Big Bear) - *J. osteosperma* is separated by 4 SNPs from *J. occidentalis* (Fig. 5).

Overall, only trnS-trnG separated *J. grandis* into two groups (Meyers and Big Bear) as found in the terpene data (Adams and Kaufmann, 2010). The Big Bear *J. grandis* appeared to be more like *J. occidentalis* in its terpenes and more like *J. osteosperma* in its SNPs.

Vasek (1966) reported finding *J. grandis* (*J. occidentalis* var. *australis*) individuals with affinities to *J. osteosperma* in the San Bernardino Mtns. as well as what he considered to be typical *J. grandis* and *J. osteosperma*. In the High Sierras, *J. grandis* grows on very xeric exposed granite domes, but its habitat in Big Bear (San Bernardino Mtns.) is on alluvial deposits. It may be found on exposed granite at higher sites

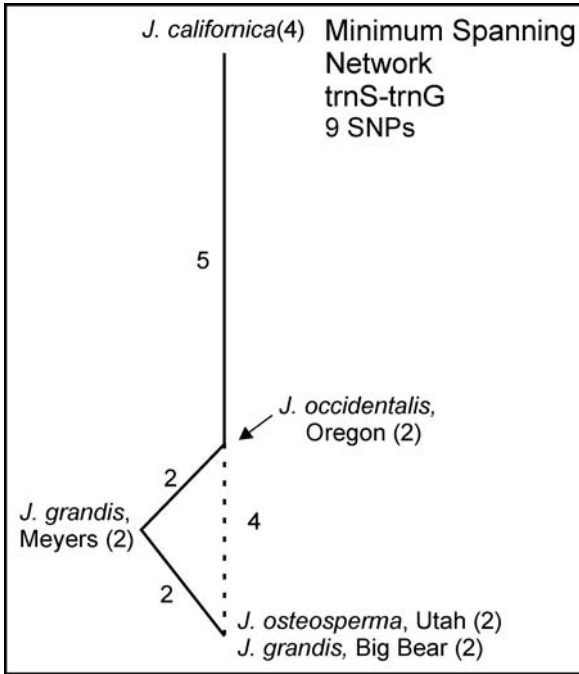


Figure 5. Minimum spanning network based on trnS-trnG SNPs.

and additional collections are being made from the San Bernardino Mtns. to further investigate the interactions between *J. grandis* and *J. osteosperma*. At present, the Big Bear population of *J. grandis* does not appear to be typical of *J. grandis* from the High Sierras in its oil or DNA. Additional DNA markers are being screened for use in resolving this taxonomic problem.

The petN-psbM SNPs data gives support to the terpene data that the Yolla Bolly population is a variant of *J. occidentalis*. Additional research is needed to clarify its taxonomic status.

ACKNOWLEDGEMENTS

This research was supported in part with funds from Baylor University. Thanks to Tonya Yanke for lab assistance.

LITERATURE CITED

- Adams, R. P. 1975. Statistical character weighting and similarity stability. *Brittonia* 27: 305-316.
- Adams, R. P. 2008. The junipers of the world: The genus *Juniperus*. 2nd ed. Trafford Publ., Victoria, BC.
- Adams R. P.. 2009. Variation in *Juniperus durangensis* and related junipers (Cupressaceae): Analysis of nrDNA and petN SNPs. *Phytologia* 91:353-358.
- Adams, R. P. J. A. Bartel and R. A. Price. 2009. A new genus, *Hesperocyparis*, for the cypresses of the new world. *Phytologia* 91: 160-185.
- Adams, R. P. and M. M. E. Kauffman. 2010. Geographic variation in the leaf essential oils of *Juniperus grandis* (Cupressaceae) and comparison with *J. occidentalis* and *J. osteosperma*. *Phytologia* 92: 167-185.
- Adams, R. P., R. P. Chaudary, R. N. Pandey and L. Singh. 2009. *Juniperus recurva* var. *uncinata*, the hooked branchlet juniper, a new variety from Nepal. *Phytologia* 91: 361-382.
- Adams, R. P., B. Rumeu, S. S. Fontinha and M. Nogales. 2010a. Speciation of *Juniperus cedrus* and *J. maderensis* in the Canary Islands and Madeira based on terpenoids and nrDNA and cp petN sequences. *Phytologia* 92: 44-55.
- Adams, R. P. , B. R. Rumeu, M. Nogales, and S. S. Fontinha. 2010b. Geographic variation and systematics of *Juniperus phoenicea* L. Madeira and the Canary Islands: Analyses of SNPs from nrDNA and petN-psbM DNA. *Phytologia* 92: 59-67.
- Adams, R. P., G. Hunter, and T. A. Fairhall. 2010c. Discovery and SNPs analyses of populations of *Juniperus maritima* on Mt. Olympus, a Pleistocene refugium? *Phytologia* 92: 68-81.
- Adams, R. P., A. E. Schwarzbach and J. A. Morris. 2010d. *Juniperus zanonii*, a new species from Cerro Potosi, Mexico. *Phytologia* 92: 105-117.

- Adams, R. P., S. Terzioglu and T. Mataraci. 2010e. Taxonomy of *Juniperus oxycedrus* var. *spilinanus* in Turkey: Leaf terpenoids and SNPs from nrDNA and petN-psbM. *Phytologia* 92: 156-166.
- Grivet, D., B. Heinze, G. G. Vendramin and R. J. Petit. 2001. Genome walking with consensus primers: application to the large single copy region of chloroplast DNA. *Molec. Ecol. Notes* 1: 345-349.
- Mao, K, G. Hao, J-Q Liu, R. P. Adams and R. I. Milne. 2010. Diversification and biogeography of *Juniperus* (Cupressaceae): variable diversification rates and multiple intercontinental dispersals. *New Phytologist* (in press).
- Shaw, J., E. B. Lickey, J. T. Beck, S. B. Farmer, W. Liu, J. Miller, K. C. Siripun, C. T. Winder, E. E. Schilling and R. L. Small. 2005. The tortoise and the hare II: relative utility of 21 noncoding chloroplast DNA sequences for phylogenetic analysis. *Amer. J. Bot.* 92: 142-166.
- Taberet, P., L. Gielly, G. Pautou and J. Bouvel. 1991. Universal primers for the amplification of three non-coding regions of chloroplast DNA. *Plant Molec. Biol.* 17: 1105-1109.
- Terry, R. G., R. S. Novak and R. J. Tausch. 2000. Genetic variation in chloroplast and nuclear ribosomal DNA in Utah juniper (*Juniperus osteosperma*, Cupressaceae): Evidence for interspecific gene flow. *Amer. J. Bot.* 87: 250-258.
- Vasek, F. C. 1966. The distribution and taxonomy of three western junipers. *Brittonia* 18: 350-372.
- Vasek, F. C. and R. W. Scora. 1967. Analysis of the oils of western North American junipers by gas-liquid chromatography. *Amer. J. Bot.* 54: 781-789.