

GEOGRAPHIC VARIATION IN THE LEAF ESSENTIAL OILS OF *JUNIPERUS CALIFORNICA*

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ABSTRACT

The volatile leaf oils of *Juniperus californica* were analyzed from throughout the species range in the United States. Three groups of *J. californica* were found: the Central Valley, the deserts of southern California, and a northwestern Arizona group. The oils of the Central Valley populations were very uniform and very low in α -pinene, with a moderate amount of sabinene, and high in camphor. Their oils contain 8 diterpenes not found in other *J. californica* populations. The oils from the southern California desert populations ranged from Vasek and Scora's 'Cal A' oil [high in α -pinene (30.3%), sabinene (19.3%) and low in camphor (5.8%)] to 'Cal B' oil [moderate amounts of α -pinene and sabinene and a high concentration of camphor (21.9%)]. However, the chemical races of Vasek and Scora (1967) were found as a mosaic that did not fit any geographic pattern in southern California. The differentiation of the Central Valley populations appears to be due to a post-Pleistocene migration from germplasm in the southern California deserts. *Phytologia* 93(2): 245-259 (August 1, 2011).

KEY WORDS: *Juniperus californica*, leaf essential oils composition, geographic variation.

Vasek and Scora (1967) presented preliminary analysis of the leaf essential oils of *Juniperus californica* and suggested that there were two chemical races (Cal A and B). Adams et al. (1983) re-analyzed the leaf oils of the same populations that Vasek and Scora (1967) studied and found that Cal A oil was high in sabinene, β -pinene, camphor and terpinen-4-ol, whereas these compounds were low in Cal B. In contrast, α -pinene was found to be high in Cal B oil and low in Cal A. Adams (2000, 2011) further characterized the oils of these chemical types.

To date, no comprehensive geographic study of the leaf essential oil of *J. californica* has been published. The purpose of the present paper is report on geographic variation in the leaf essential oil of *J. californica* and to attempt to clarify the purported chemical races, A and B.

MATERIALS AND METHODS

Plant specimens (populations shown in Figure 1): *Juniperus*

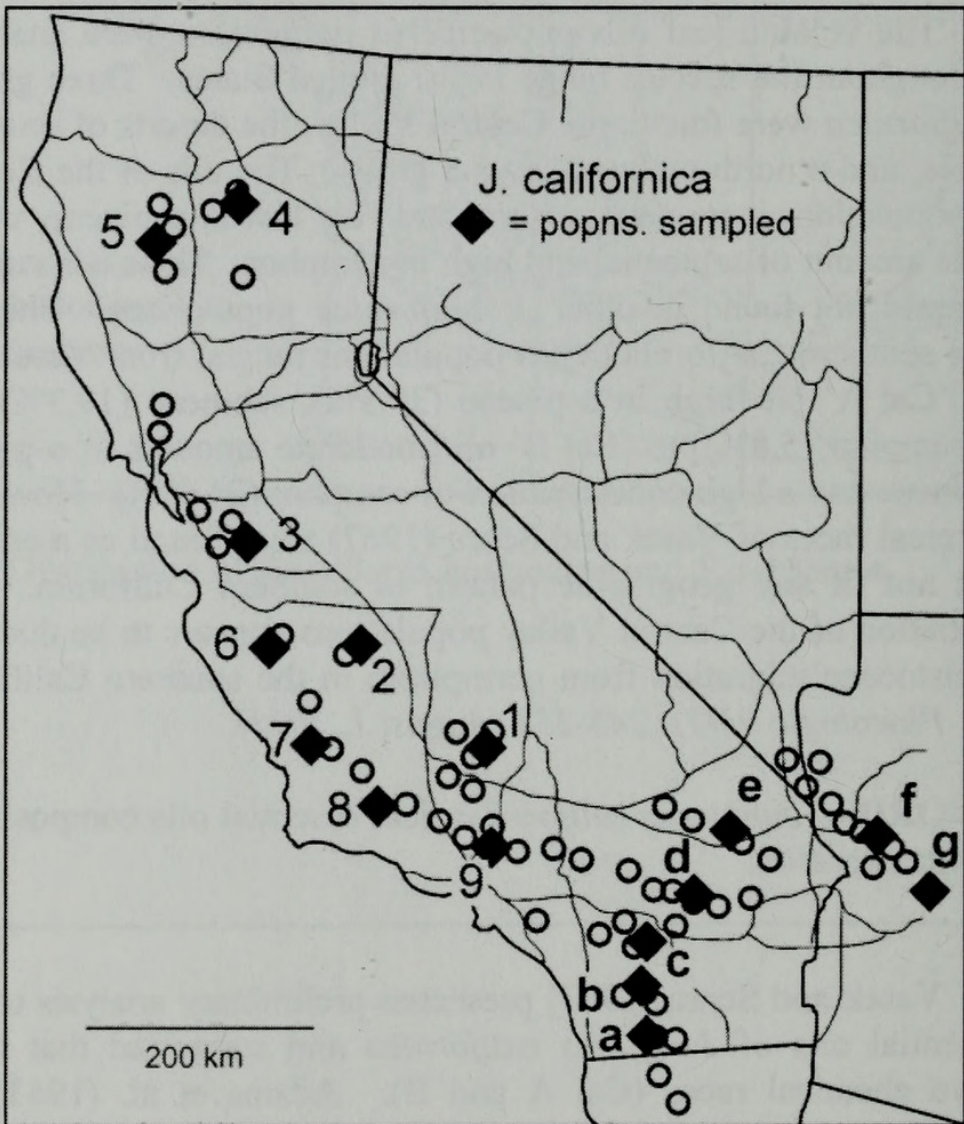


Figure 1. Distribution of *J. californica* (based on Vasek, 1966) and populations sampled (black quadrangles).

californica: Popn. 1, *Adams 12145-49*, Bodfish, CA; Popn. 1, *Adams 12145-49*, 4.8 mi. s of Bodfish, CA on CA483, Lat. 35° 33.252' N; Long. 118° 30.385' W, 1023 m; Popn. 2, *Adams 12150-54*, 8 mi. SW of Coalinga, CA on CA198, ca. 20 mi. w of hwy I5, Lat. 36° 05.762' N; Long. 120° 27.245' W, 315 m; Popn. 3, *Adams 12155-12159*, on Del Puerto Canyon Rd., 12 mi w of hwy. I5. Lat. 37° 26.186' N; Long. 122° 19.494' W, 256 m, Del Puerto Canyon, CA; Popn. 4, *Adams 12160-12164*, 8 mi ne of Red Bluff, CA on CA 36, Lat. 40° 17.066' N; Long. 122° 07.006' W, 272 m; Popn. 5, *Adams 12165-12169*, 4 mi sw of Lakeport, CA, Lat. 38° 59.709' N; Long. 122° 55.802' W, Elev. 424 m; Popn. 6, *Adams 12170-12174*, 3.5 mi. e on CA146 at west entrance to Pinnacles Natl. Park, CA, Lat. 36° 28.417' N; Long. 121° 13.513' W, 605 m, Popn. 7, *Adams 12175-12179*, 19 mi. w. of US101, 17 mi e of Santa Margarita, CA, Lat. 35° 28.137' N; Long. 120° 22.753' W, Elev. 450 m, Popn. 8, *Adams 12180-12184*, on CA33, 12 mi s of jct of CA33 and CA166, ~25 mi sw of Maricopa, CA, Lat. 34° 46.010' N; Long. 119° 25.241' W. Elev. 981 m; Popn. 9, *Adams 12185-12189*, on CA N2, ~2mi w of Palmdale, CA, Lat. 34° 35.007' N; Long. 118° 10.489' W. Elev. 844 m; Popn. a, *Adams 12190-12194*, on hwy I8, mile 76. 11 mi. sw of Ocotillo, CA, Lat. 32° 38.175' N; Long. 116° 07.103' W, Elev. 989 m; Popn. b, *Adams 12195-12199*, on CA S2, 12-15 mi s of Scissors Crossing, CA, Lat. 33° 01.053' N; Long. 116° 25.789' W. Elev. 801 m; Popn. c, *Adams 12200-12204*, on CA 74, Pinyon Flats campground. ~10 mi sw of Palm Desert. Lat. 33° 34.981' N; Long. 116° 27.383' W. Elev. 1228m; Popn. d, *Adams 12205-12209*, on CA 62, 1.5 mi s of Yucca Valley City center, Lat. 34° 06.724' N; Long. 116° 28.361' W. Elev. 1044 m; Popn. e, *Adams 5067-5071*, 8.0 mi. N of I40 on Rd to Kelso, CA at Microwave Station, 34° 48' 40.24" N, Long. 115° 36' 32.62" W, Elev. 1300 m; Popn. f, *Adams 5072-5076*, 17 mi se of Yucca, AZ, on road to Alamo Lake, AZ, 34° 42' 53" n, 113° 54' 49" w, 950 m; Popn. g, *Adams 12117-12121*, 5 mi. NW of Jct. of AZ97 and US93 on w side of US93, 2 mi. se of Mohave/ Yavapai Co. line, Lat. 34.46695°N; Long. 113.31133°W, Elev. 987 m. All specimens are deposited in the BAYLU herbarium.

A summary of the populations sampled is given in Table 1.

Table 1. Summary of populations sampled.

Popn.	Location	Lat/ Long	Elev.
Central Valley			
1	Bodfish	35° 33.252' N 118° 30.385' W	1023 m
2	Coalinga	36° 05.762' N 120° 27.245' W	315 m
3	Del Puerto Canyon	37° 26.186' N 122° 19.494' W	256 m
4	Red Bluff	40° 17.066' N 122° 07.006' W	272 m
5	Lakeport	38° 59.709' N 122° 55.802' W	424 m
6	Pinnacles Natl. Park	36° 28.417' N 121° 13.513' W	605 m
7	Santa Margarita	35° 28.137' N 120° 22.753' W	450 m
8	sw of Maricopa	34° 46.010' N 119° 25.241' W	981 m
9	Palmdale	34° 35.007' N 118° 10.489' W	844 m
southern California desert			
a	sw of Ocotillo	32° 38.175' N 116° 07.103' W	989 m
b	Scissors Crossing	33° 01.053' N 116° 25.789' W	801 m
c	Pinyon Flats CG	33° 34.981' N 116° 27.383' W	1228 m
d'Cal B'	Yucca Valley City	34° 06.724' N 116° 28.361' W	1044 m
e'Cal A'	s of Kelso	34° 48.671' N 115° 36.544' W	1300 m
northwestern Arizona			
f	se of Yucca, AZ	34° 42.883' N, 113° 54.817' W	950 m
g	2 mi. se of Mohave/ Yavapai Co. line, AZ	34° 28.017' N 113° 18.680' W	987 m

Isolation of Oils - Fresh leaves (200 g) were steam distilled for 2 h using a circulatory Clevenger-type apparatus (Adams, 1991). The oil samples were concentrated (ether trap removed) with nitrogen and the samples stored at -20°C until analyzed. The extracted leaves were oven dried (100°C, 48 h) for determination of oil yields.

Chemical Analyses - Oils from 10-15 trees of each of the taxa were analyzed and average values reported. The oils were analyzed on a HP5971 MSD mass spectrometer, scan time 1 sec., directly coupled to a HP 5890 gas chromatograph, using a J & W DB-5, 0.26 mm x 30 m, 0.25 micron coating thickness, fused silica capillary column (see 5 for operating details). Identifications were made by library searches of our volatile oil library (Adams, 2007), using the HP Chemstation library search routines, coupled with retention time data of authentic

reference compounds. Quantitation was by FID on an HP 5890 gas chromatograph using a J & W DB-5, 0.26 mm x 30 m, 0.25 micron coating thickness, fused silica capillary column using the HP Chemstation software.

Data Analysis - Terpenoids (as percent total oil) were coded and compared among the species by the Gower (1971) metric. Principal coordinate analysis was performed by factoring the associational matrix using the formulation of Gower (1966) and Veldman (1967). Associational measures were computed using absolute compound value differences (Manhattan metric), divided by the maximum observed value for that compound over all taxa (= Gower metric, Gower, 1971; Adams, 1975). Principal coordinate analysis was performed by factoring the associational matrix based on the formulation of Gower (1966) and Veldman (1967).

RESULTS AND DISCUSSION

The compositions of leaf oils from various populations are listed in Table 2. All the components (>0.5% total oil) are now identified, except two diterpenes. The Central Valley populations (Bod, RBf, Lkp) are each very low in α -pinene, have a moderate amount of sabinene, and are high in camphor (Table 2). But the most characteristic components are the diterpenes: rosa-5,15-diene (ent-), pimaradiene, unknown diterpene, KI 1973, manoyl oxide, geranyl linalool (E,E), abietatriene, sandaracopimarinal, sandaracopimarinal, trans-totarol and trans-ferruginol (Table 2). Most of these are only present in the Central Valley populations. All the Central Valley populations except Palmdale (on the very southernmost end) were very uniform among tree oils. In contrast, the oils of all the southern California desert and Arizona populations were very variable. The oil of the Kelso population (Kel) was called 'Cal A' by Vasek and Scora (1967) was high in α -pinene (30.3%), sabinene (19.3%) and low in camphor (5.8%) characteristic of 'Cal A'. The Yucca Valley population (Cal B) was extremely diverse in its oils. It does have moderate amounts of α -pinene and sabinene and a high concentration of camphor (21.9%), but with one individual (YV-3, Adams 12207) that is practically devoid of mono-terpene hydrocarbons (Table 2). The latter oil composition is the most unusual I have ever encountered in

Juniperus; it appears that mono-terpene synthase(s) have been inactivated in this individual. The good population would be worthy of additional study, especially as regards to examine terpene synthases and their expression. The oils of the two Arizona populations were very similar, so only the southeastern-most population (AZ, Table 2) is shown in detail. The Arizona oil is very high in α -pinene (45.4%), very low in sabinene (0.4%) with moderate amounts of camphor (14.7%).

To examine variation among populations in their total oil components, PCO was performed using 40 terpenes. This resulted in eigenroots that accounted for 36, 13 and 8% of the variance among populations. Ordination (Fig. 2) reveals two major groups: Central Valley, and NW Arizona - s. California desert populations. The latter group can be further subdivided into the NW Arizona and s. California desert populations (Fig. 2).

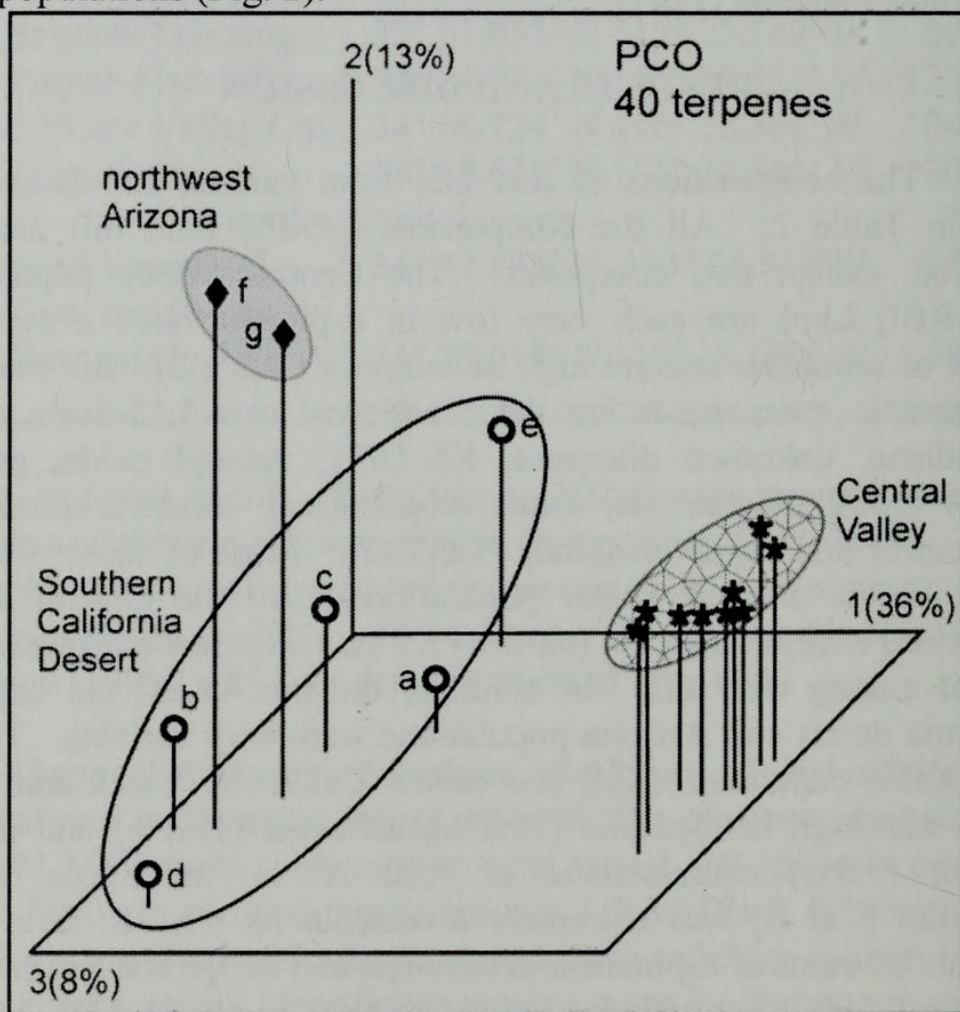


Figure 2. PCO of 16 *J. californica* populations based on 40 terpenes.

The differentiation of the Central Valley populations is clearly seen by contour mapping the clustering (Fig. 3). Notice a small difference between the more inland (1, 2, 3) and coastal range populations (6, 7, 8). The Yucca Valley population (d) is somewhat differentiated from the other southern California desert populations (a, b, c, e, Fig. 3). The NW Arizona populations form a low level group.

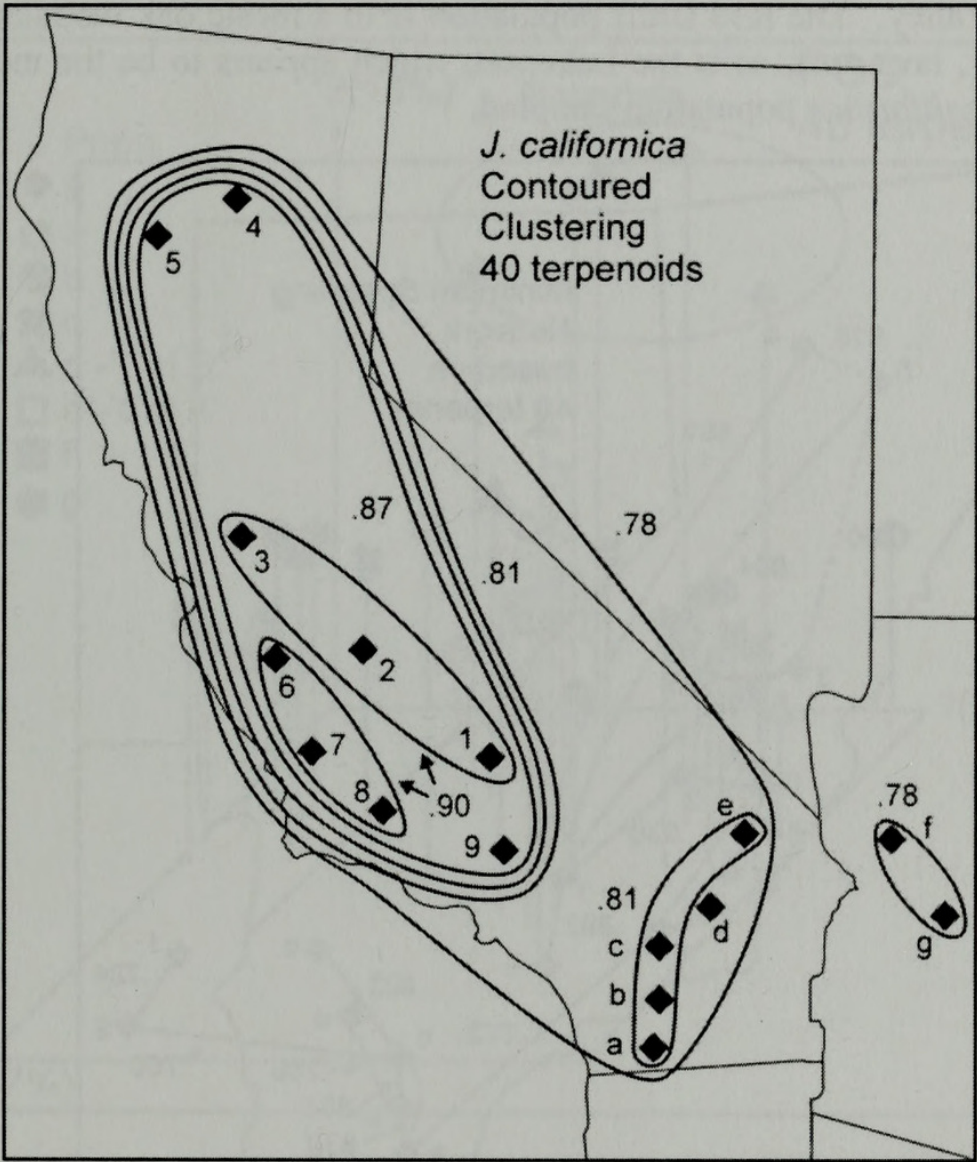


Figure 3. Contoured clustering of *J. californica* populations.

Additional insight is obtained by examining a minimum spanning network of the populations (Figure 4). An interesting aspect is that the northern populations (4, Red Bluff; 5, Lakeport) are more similar to southern Central Valley populations than to each other (note

dotted link = 0.816, Fig. 4). The Lakeport population (5, Fig. 4) has a very high secondary similarity (0.890) to the Del Puerto Canyon population (3, Fig. 4). The Central Valley group links with the Ocotillo population (a, Fig. 4) at a lower similarity (0.773). The NW Arizona populations link with Pinyon Flats CG at 0.760. One is impressed with the north-south linkages of populations in the Central Valley. In general, the sites appear more mesic as one goes northward in the Central Valley. The Red Bluff population is in a mesic oak woodland on grassy, lava rock, as is the Lakeport, which appears to be the most mesic *J. californica* population sampled.

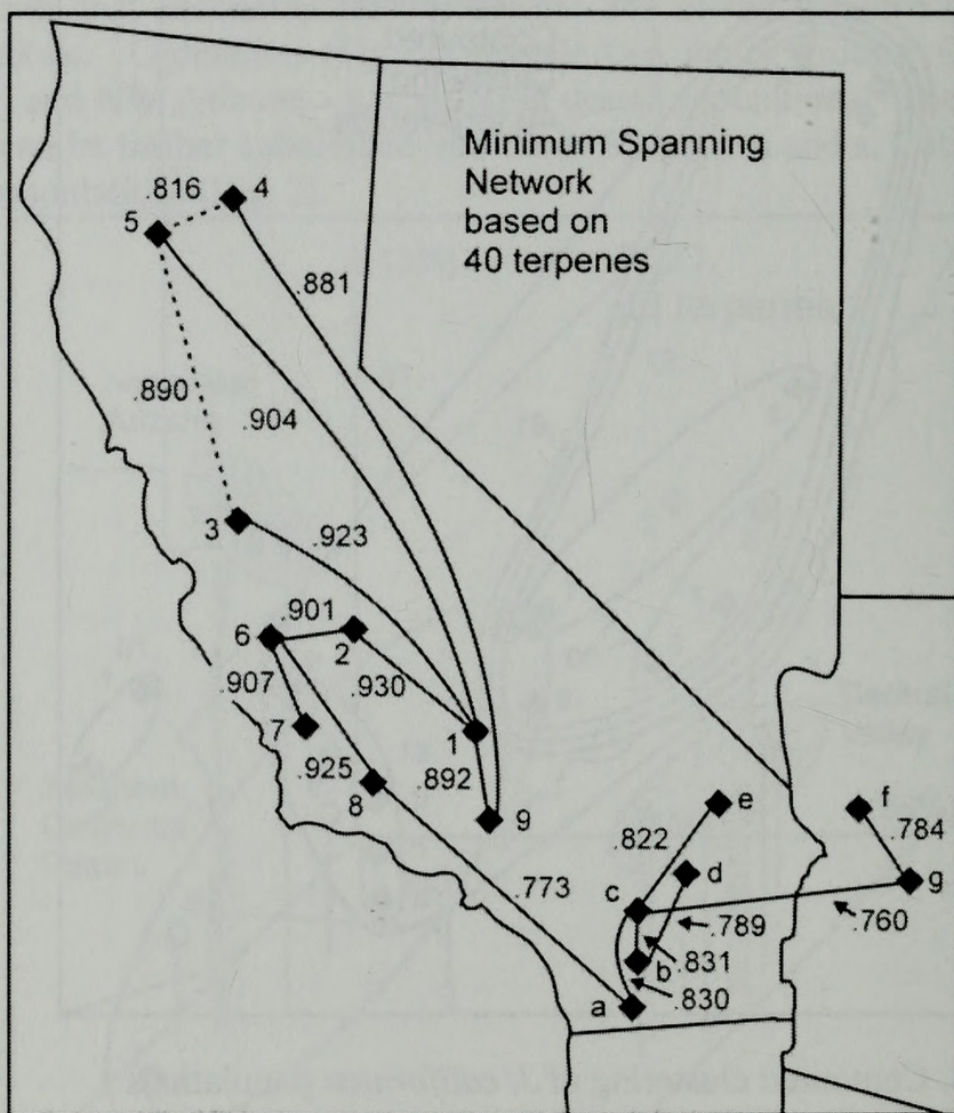


Figure 4. Minimum spanning network based on 40 terpenes. The dotted lines show secondary similarities.

Patterns of variation among individuals in the southern California and NW Arizona populations were examined by PCO using 40 terpenes. Figure 5 shows little clustering by population. The Palmdale population (9 of other figures) is part of the Central Valley group (Figs. 2-4), but very variable, so it was included. There is no evidence of intermixing of southern California desert plants with the Palmdale (9) population. However, given the diversity found among

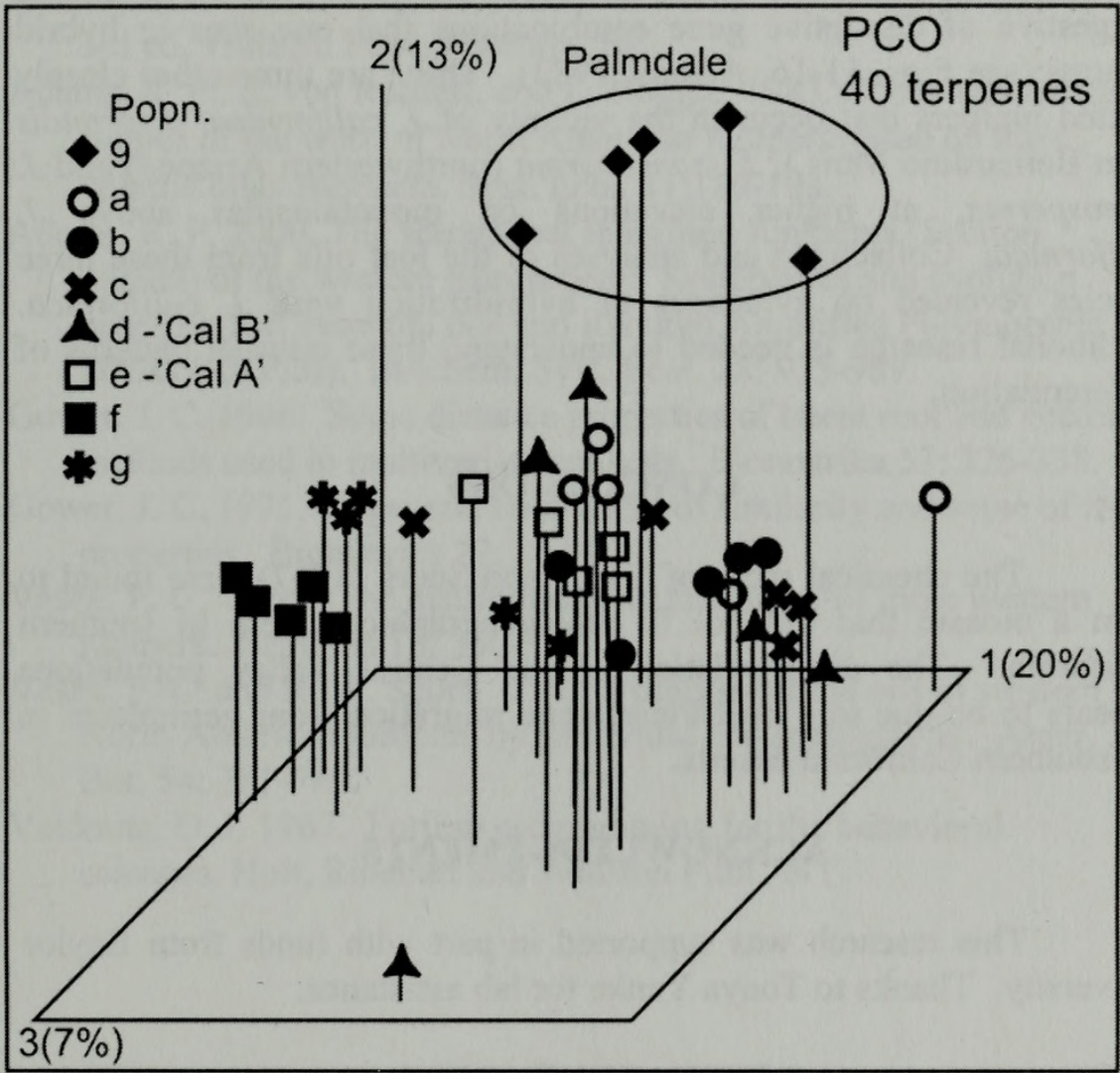


Figure 5. PCO of 40 *J. californica* individuals from the southern California desert based on 40 terpenes.

the southern California desert individuals, it might be difficult to clearly ascertain this. There is some clustering of the NW AZ individuals (particularly the Yucca, AZ plants, popn. f, Fig. 5). However, 'Cal A' (Kelso, e, Fig. 5) plants and 'Cal B' plants (Yucca Valley, d, Fig. 5) are

somewhat scattered to the center and left on PCO coordinate 1 (Fig. 5). Note the lone individual in the foreground (Fig. 5). This the unusual plant (*Adams 12207*) from Yucca Valley with essentially no monoterpenes.

It seems very unusual that the oils from plants in the northern portion of the range (Central Valley) of *J. californica* are so uniform and the oils from the southern California desert are so variable. This is suggestive of disruptive gene combinations that one sees in hybrid swarms (see Figs. 11-16, Adams, 1983). There are three other closely related junipers that occur in the vicinity of *J. californica*: *J. grandis* (San Bernardino Mtns.), *J. monosperma* (northwestern Arizona) and *J. osteosperma*, at higher elevations on mountainsides above *J. californica*. Collections and analyses of the leaf oils from these three species revealed no evidences of hybridization with *J. californica*. Additional research is needed to understand these unusual patterns of differentiation.

CONCLUSIONS

The chemical races of Vasek and Scora (1967) were found to from a mosaic that did not fit any geographic pattern in southern California. The differentiation of the Central Valley populations appears to be due to a post-Pleistocene migration from germplasm in the southern California deserts.

ACKNOWLEDGEMENTS

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Table 2. Comparison of leaf essential oils of *J. californica* from the Bodfish (Bod), Red Bluff (RBf), Lakeport (Lkp), Kelso (Kel), Yucca Valley (YV), Yucca Valley, - tree 3 (*Adams 12207*), and nw Arizona (AZ). Kelso and Yucca Valley are called 'Cal A' and 'Cal B' in Vasek and Scora (1967) and Adams et al. (1983). Compounds in bold face appear to separate the taxa. Compositional values less than 0.1% are denoted as traces (t). Unidentified components less than 0.5% are not reported. KI is the Kovat's Index using a linear approximation on DB-5 column. *= cpds used for PCO.

		'Cal A' 'Cal B'						
KI	Component	Bod	RBf	Lkp	Kel	YV	YV-3	AZ
921	tricyclene	0.2	0.1	0.1	0.1	0.1	-	t
924	α -thujene*	0.5	0.3	0.3	0.9	0.5	-	0.3
932	α-pinene*	1.7	0.6	0.5	30.3	12.2	0.2	45.4
945	α -fenchene*	-	-	-	-	-	-	t
946	camphene*	0.3	0.2	0.2	0.4	0.3	-	0.5
961	verbenene*	-	-	-	-	-	-	-
969	sabinene*	11.7	8.5	10.0	19.3	9.0	t	0.4
974	β -pinene*	0.2	0.1	0.1	0.8	0.4	-	0.4
988	myrcene*	1.3	1.0	1.2	2.1	1.1	1.1	1.5
1001	δ-2-carene*	-	t	0.8	0.6	-	-	0.3
1002	α -phellandrene*	0.2	0.2	0.2	0.2	0.2	t	t
1008	δ-3-carene*	-	-	-	-	1.3	-	t
1014	α-terpinene*	1.8	1.6	1.6	1.4	1.4	-	0.1
1020	p-cymene*	0.1	0.1	0.2	0.5	0.5	-	0.1
1024	limonene*	2.3	1.8	2.0	1.9	0.9	0.1	1.9

KI	Component	Bod	RBF	Lkp	Kel	YV	YV-3	AZ
1025	β -phellandrene*	1.4	1.3	1.3	1.9	0.9	0.1	1.3
1044	(E)- β -ocimene	0.2	0.2	0.3	0.2	0.2	-	0.3
1054	γ-terpinene*	2.9	2.7	2.7	2.4	2.4	t	0.2
1065	cis-sabinene hydrate*	0.7	0.7	0.9	1.0	1.0	0.3	0.2
1067	cis-linalool oxide (furanoid)	-	t	t	t	t	-	t
1086	terpinolene*	1.0	0.9	1.0	1.1	0.9	t	0.4
1098	trans-sabinene hydrate*	0.7	0.9	0.9	1.4	1.3	-	0.3
1100	n-nonanal	-	0.3	t	-	-	-	-
1112	trans-thujone*	t	t	-	0.6	0.5	-	t
1118	cis-p-menth-2-en-1-ol*	0.7	0.5	0.8	-	-	0.6	0.5
1136	trans-p-menth-2-en-1-ol*	-	-	-	-	-	0.9	-
1141	camphor*	30.3	43.6	27.8	5.8	21.9	1.5	14.7
1145	camphene hydrate*	0.9	1.9	0.9	0.3	0.9	0.6	0.6
1148	citronellal*	0.5	0.2	0.5	2.3	0.6	-	1.0
1165	borneol*	t	0.1	0.2	0.1	0.4	1.4	0.8
1174	terpinen-4-ol*	7.8	7.2	8.2	5.5	8.0	9.2	0.9
1179	p-cymen-8-ol	t	-	-	-	-	t	-
1186	α -terpineol*	0.4	0.5	0.4	0.4	0.7	1.8	0.9
1195	cis-piperitol*	0.1	0.1	0.2	0.1	0.3	0.5	0.3
1207	trans-piperitol*	0.3	0.3	0.4	0.5	0.7	1.5	0.5

KI	Component	Bod	RBf	Lkp	Kel	YV	YV-3	AZ
1223	citronellol*	4.8	3.7	6.3	5.9	7.4	8.8	11.1
1239	carvone	-	-	-	-	0.1	0.2	-
1249	piperitone*	3.1	0.4	7.0	0.3	0.1	0.1	-
1257	methyl citronellate	-	0.2	0.3	-	t	t	-
1274	pregeijerene B	-	0.2	t	t	0.3	t	0.5
1287	bornyl acetate*	0.2	0.9	0.1	1.5	0.8	0.8	1.5
1298	carvacrol	-	-	-	-	-	0.4	-
1319	unknown	-	1.0	0.5	-	-	-	t
1396	duvalene acetate	-	-	-	-	-	0.2	-
1403	methyl eugenol*	t	-	-	0.3	0.8	0.2	0.1
1429	cis-thujopsene	-	t	t	t	t	-	t
1452	α -humulene	-	-	-	t	t	-	t
1505	β -bisabolene	-	0.1	t	t	t	0.1	t
1548	elemol*	2.7	2.4	2.0	2.1	8.5	29.6	3.2
1555	elemicin*	0.6	0.1	t	1.1	2.5	-	1.3
1561	(E)-nerolidol*	0.1	0.5	t	-	-	0.3	-
1630	γ-eudesmol*	0.3	0.3	0.3	0.4	1.0	4.1	0.5
1649	β-eudesmol*	0.5	0.3	0.4	0.6	2.1	8.8	1.5
1652	α-eudesmol*	0.4	0.3	0.4	0.6	1.8	6.3	1.1
1670	bulnesol*	0.1	0.1	0.1	0.1	0.3	1.4	0.1
1746	8- α -11-elemodiol*	0.4	0.5	0.4	0.3	1.3	6.3	0.5

KI	Component	Bod	RBf	Lkp	Kel	YV	YV-3	AZ
1792	8- α -acetoxylemol*	0.2	0.2	0.2	0.3	1.0	4.5	0.5
1931	rosa-5,15-diene(ent-)	0.1	t	t	-	-	-	-
1948	pimaradiene	0.1	t	t	-	-	-	-
1973	diterpene, 204, 41, 93 (272)	t	t	t	-	-	-	-
1988	manoyl oxide*	10.8	6.8	11.8	-	t	t	t
2026	geranyl linalool (E,E-)	t	t	t	-	-	-	-
2055	abietatriene*	0.3	t	t	-	-	-	-
2105	iso-abienol	4.4	3.6	4.1	-	-	-	-
2145	diterpene, 41, 69, 255, 298	-	-	-	-	-	1.9	-
2184	sandaracopimarinal*	0.7	0.5	1.2	-	-	-	-
2282	sandaracopimarinal*	0.2	0.2	0.3	-	-	-	-
2314	trans-totarol*	0.3	0.2	0.3	-	t	0.1	-
2331	trans-ferruginol	0.1	0.1	0.1	-	-	-	-



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