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Geographic Variation in the Leaf Oils and DNA Fingerprints (RAPDs) of *Juniperus thurifera* L. from Morocco and Europe

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Abstract

Samples of *Juniperus thurifera* L. were collected from the Atlas Mts., Morocco, northern and southern Spain, the Pyrenees, France, Fench Alps and Corse Isaland, France. The leaf oils were analyzed and found to be polymorphic for several major compounds (sabinene, limonene, linalool, piperitone, linalyl acetate and sesquiterpenes). In general, the Moroccan trees were higher in sabinene, γ-terpinene, *cis*-sabinene hydrate and terpinen-4-ol, but lower in limonene, δ-2-carene, and piperitone than trees from Europe. Analysis based on Random Amplified Polymorphic DNAs (RAPDs) for the aforementioned population plus *J. foetidissima* (as an outgroup), revealed that the Moroccan *J. thurifera* populations were most similar to plants from southern Spain, then to populations from France. Although the trees generally clustered by populations, there appear to be some differentiation in the RAPDs between the European *J. thurifera* populations and the Moroccan populations. Combining previous studies on seeds per cone, proanthrocyanidins, and the current report on the leaf essential oils and RAPDs, there is some support for the continued recognition of *J. thurifera* var. *africana* Maire [syn.: *J. Africana* (Maire); *J. thurifera* ssp. *africana* (Maire) Gauquelin, Hassani et Lebreton] in Algeria and Morocco.

Key Word Index

Juniperus thurifera, *Cupressaceae*, composition of essential oil, RAPD, DNA fingerprinting, systematics.

Introduction

The genus *Juniperus* consists of approximately 67 species and 26 varieties, using the more widely accepted variety category instead of the subspecies category (1). All the taxa grow on the Laurasian land mass, except *J. procera* Hochst. ex Endl., which grows along the rift mountains in East Africa into the southern hemisphere (2) 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).

The genus is divided (3) into three sections: *Caryocedrus* (one species, *J. drrupacea* Labill.); *Juniperus* (= *Oxycedrus*) with 12-13 species, and *Sabina*, the remaining, with approximately 50 species. A previous study using RAPDs (3) indicated that section of *Sabina* could be further divided

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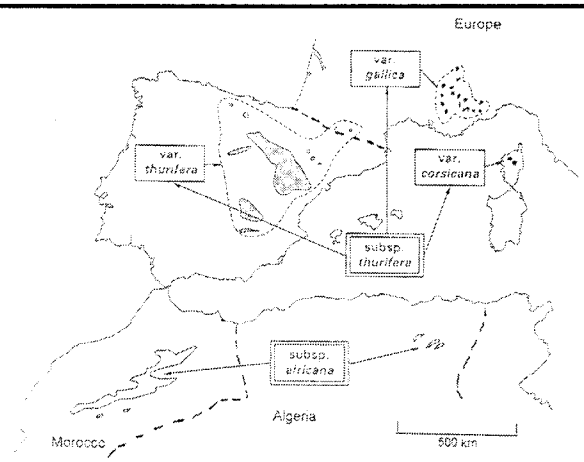

Figure 1. Distribution of *J. thurifera*, *J. thurifera* ssp. *africana* and chemivars *corsicana*, *gallica* and *thurifera*; adapted from (5)

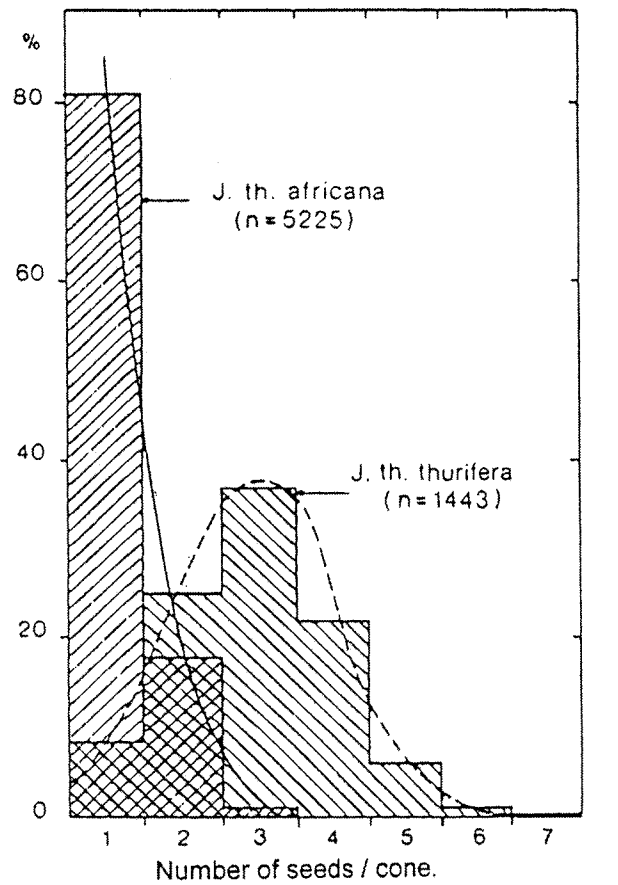

Figure 2. Histogram of the number of seeds per cone; *J. thurifera* samples, averaging about three seeds per cone, were taken from Europe; *J. th. africana* samples, averaging 1.21 seeds per cone, were collected in Morocco—adapted from (5)

into junipers with serrate and those with entire (smooth) leaf margins. The serrate leaf margined junipers are confined to the western hemisphere, although Gaussen (4) scored several eastern hemisphere species as having leaves with serrate margins. Gaussen (4) followed the classical treatment in dividing the genus into three sub-genera (sections)—*Caryocedrus*, *Oxycedrus* (= *Juniperus* by nomenclatural convention) and *Sabina*—and her further divided *Sabina* into two series: denticulees (denticulate) and entires (entire) leafed species. Gaussen (4) even subdivided these series into sections. The section *phoenicioides* was put in the class with serrate leaf margins (4). However, the leaves of all the taxa listed in Gaussen's treatment (4, p. 81) have entire leaf margins at 40 X (RPA). Furthermore, DNA evidence shows that Gaussen's *phoenicioides* is not related to the serrate junipers of the western hemisphere (3).

The *Juniperus* of section *Sabina*, of the eastern hemisphere can be further divided into two groups based on the number of seeds per female cone (often called berry) and female cone shape. The single seed/cone (single seeded) *Juniperus* of the eastern hemisphere have cones that are ovoid with a noticeable pointed tip, whereas the multi-seeded *Juniperus* are generally round and often have an irregular surface. Based on DNA fingerprinting data (RAPDs), Adams (1) found that *J. thurifera* (from Spain) is loosely associated with *J. foetidissima* Willd. and in turn with *J. polycarpos* K. Koch and *J. excelsa* M.-Bieb.

Juniperus thurifera from North Africa has been the subject of several nomenclatural changes having been treated as a variety [*J. thurifera* var. *africana* Maire, Bull. Soc. Hist. Nat. Afrique N. 17:125(1926)], then as a distinct species [*J. africana* (Maire) Villar, Types Sols Afrique N. 1:91 (1947)].

More recently, a study (5) using proanthrocyanidins and the number of seeds per cone, resulted in the naming of a new subspecies [*J. thurifera* ssp. *africana* (Maire) Gauquelin, Hassani et Lebreton] and three chemivars (*hispanica*, Spain; *gallica*, France; and *Corsicana*, Corse—Figure 1). They found (5) that the number of seeds per cone was significantly smaller (1.21 seeds/cone) in Morocco than in Europe where the number of seeds/cone ranged from 2.9-3.1 (Figure 2). Barbero et al. (6) reported additional morphological measurements that seemed to support the recognition of the subspecies. Barbero et al. (6) discussed the chemivars as varieties, but they did not appear to actually name the chemivars as varieties in a nomenclatural sense. In a recent treatment of the conifers, Farjon (7) recognized *J. thurifera* and treated *J. africana* and *J. thurifera* var. *africana* as synonyms. There have been several reports on the oils of *J. thurifera*. Teresa et al. (8) found the berry oil of *J. thurifera* to be dominated by limonene (88.5%). San Feliciano et al. (9) reported a number of sesquiterpenes and diterpenes from the leaf oil of *J. thurifera*. Akimov et al. (10) found the leaf oil of *J. thurifera* to have small amounts of monoterpenes (< 2.6% for α-pinene, the largest monoterpene hydrocarbon), but 86.7% for the combined oxygenated mono- and sesquiterpene fraction (individual compounds not reported). Adams (1) reported that the leaf oil of *J. thurifera* from central Spain contained a large amount of limonene (51.5%) and moderate amounts of linalool, piperitone, linalyl acetate and α-terpinyl acetate.

In this paper, both the leaf oils and DNA fingerprints will be examined for populations of *J. thurifera* from Morocco, Spain

Table I. Comparisons of the % total oil for leaf essential oils for *Juniperus thurifera* from different populations

		Morocco				Spain			Pyrenees		Fr Alps	Crsca.
Compound	**RI**	**M1**	**M2**	**TM**	**OM**	**S1**	**S2**	**CS**	**P1**	**P2**	**MF**	**CR**
tricyclene	926	t	0.8	t	t	-	-	-	t	-	-	t
α-thujene	931	2.5	2.3	0.7	1.3	t	-	0.1	0.1	0.5	0.3	t
α-pinene	939	4.6	4.2	17.1	4.0	0.5	6.3	2.0	3.2	2.5	4.9	6.8
α-fenchene	953	t	t	0.1	t	-	-	-	t	t	t	t
camphene	953	t	0.7	0.1	t	-	t	t	t	t	t	t
sabinene	976	45.8	40.2	12.2	37.4	1.5	0.3	1.5	0.4	9.7	4.9	0.3
β-pinene	980	0.4	0.2	1.2	0.3	t	0.5	0.1	0.3	0.2	t	0.5
myrcene	991	3.9	3.8	1.8	1.8	1.5	4.0	2.5	4.4	2.8	2.3	2.6
δ-2-carene	1001	-	-	-	-	2.0	0.9	2.0	4.4	1.3	2.9	2.5
α-phellandrene	1005	0.1	t	-	t	t	-	t	t	t	t	t
δ-3-carene	1011	-	1.7	3.1	2.0	-	-	0.5	-	-	0.8	t
α-terpinene	1018	1.8	2.0	0.5	1.6	0.1	t	0.1	0.1	0.5	0.2	t
p-cymene	1026	0.4	1.9	0.4	2.6	t	t	t	0.1	t	t	t
limonene	1031	0.8	3.1	1.3	5.2	28.8	61.8	52.8	75.1	53.0	52.1	60.6
β-phellandrene	1031	0.8	1.0	t	t	t	t	t	-	t	t	t
(Z)-β-ocimene	1040	-	-	-	-	t	-	t	-	t	t	-
(E)-β-ocimene	1050	0.3	0.1	-	0.2	0.1	0.2	0.2	0.1	0.8	0.4	0.1
γ-terpinene	1062	3.0	3.3	0.9	2.5	0.2	t	0.2	0.1	0.9	0.4	0.1
cis-sabinene hydrate	1068	2.4	2.2	0.4	2.3	0.3	-	0.1	t	0.4	0.3	t
trans-linalool oxide (furanoid)	1074	-	-	-	-	0.7	-	-	-	-	t	-
terpinolene	1088	1.4	1.3	0.7	1.1	0.8	0.9	0.8	0.9	1.3	0.8	1.1
trans-sabinene hydrate	1097	-	-	t	1.2	t	t	-	t	1.3	t	-
linalool	1098	1.6	1.8	1.2	3.7	13.4	0.8	5.7	t	1.6	2.6	1.1
cis-thujone (=α-thujone)	1102	0.1	-	2.6	t	-	-	-	-	-	-	-
isopentyl isovalerate	1103	-	-	-	-	-	-	-	-	-	0.1	t
trans-thujone (=β-thujone)	1114	0.1	0.1	2.2	0.1	-	-	t	-	-	-	-
trans-p-mentha-2,8-dien-1-ol	1118	-	-	-	-	-	-	-	-	-	0.1	-
cis-p-menth-2-en-1-ol	1121	0.4	0.3	-	0.6	0.6	-	0.2	t	0.2	0.3	0.3
cis-p-mentha-2,8-dien-1-ol	1134	-	-	-	-	-	-	-	-	-	t	-
trans-limonene oxide	1139	-	-	-	-	-	-	-	-	0.1	t	-
trans-p-menth-2-en-1-ol	1140	0.2	0.1	-	0.3	0.4	-	0.1	-	0.1	0.1	0.2
camphor	1143	-	1.0	-	-	-	-	t	-	-	-	-
camphene hydrate	1148	-	0.2	-	-	-	-	-	-	-	-	-
citronellal	1153	-	-	-	-	-	-	-	-	-	t	t
sabina ketone	1156	-	t	-	-	-	-	-	-	-	-	-
borneol	1165	-	0.7	-	-	-	t	-	t	t	-	-
terpinen-4-ol	1177	5.1	4.6	2.6	6.3	1.0	0.1	0.5	0.1	1.8	0.8	0.2
m-cymen-8-ol	1180	-	t	-	-	-	-	-	-	-	-	-
p-cymen-8-ol	1183	t	0.1	t	0.2	t	t	t	-	t	t	t
α-terpineol	1189	0.2	0.1	0.1	0.3	1.4	0.2	0.6	0.1	0.3	0.3	0.2
α-p-methyl phenylethanol	1205	-	0.1	-	-	-	-	-	-	-	t	-
trans-carveol	1217	-	-	-	-	-	-	-	-	-	t	-
endo-fenchyl acetate (= α-)	1220	-	-	-	-	-	-	-	-	t	t	-
citronellol	1228	-	-	-	-	0.1	-	0.4	-	0.2	0.6	0.1
piperitone	1252	-	t	-	0.1	8.3	0.1	3.5	1.3	1.1	4.0	3.8
trans-sabinene hydrate acetate	1252	t	t	-	0.2	-	-	-	-	-	-	-
linalyl acetate	1257	0.8	2.2	2.6	7.7	13.6	3.3	13.7	0.3	3.5	13.2	2.4
methyl citronellate	1261	-	-	-	-	-	-	-	-	-	0.3	-
bornyl acetate	1285	t	10.8	0.3	0.6	-	0.1	0.3	0.3	0.4	0.5	0.3
trans-linalool oxide acetate (pyranoid)	1286	-	-	-	0.1	0.7	-	-	-	t	0.4	-
decadienal isomer	1312	1.9	0.7	0.7	0.8	2.2	t	1.0	-	0.9	0.9	0.5
α-terpinyl acetate	1350	0.2	0.4	0.6	0.5	4.2	1.5	3.6	2.0	1.1	3.8	1.9
neryl acetate	1365	-	-	-	-	0.2	t	-	-	-	t	-
(E)-caryophyllene (= β-)	1418	0.1	t	t	0.2	t	0.1	-	t	t	t	0.2
cis-muurola-3,5-diene	1446	-	-	-	-	0.1	-	-	0.1	-	-	-
α-humulene	1454	-	-	-	-	-	-	-	0.1	-	t	t
cis-muurola-4(14),5-diene	1461	0.2	-	-	-	-	0.1	-	0.4	-	-	t
γ-muurolene	1477	0.2	t	-	t	-	0.1	-	-	t	-	t
germacrene D	1480	0.3	t	-	0.1	-	0.2	t	t	t	t	t
trans-murrola-4(14),5-diene	1491	t	-	t	t	-	-	-	-	-	t	t
α-muurolene	1499	0.5	t	t	t	-	0.6	-	t	0.1	t	0.2

Table I. Continued

Compound	RI	Morocco				Spain			Pyrenees		Fr. Alps	Crsca.
		M1	M2	TM	OM	S1	S2	CS	P1	P2	MF	CR
γ-cadinene	1513	1.1	t	t	0.4	t	0.4	0.1	t	0.2	t	0.4
δ-cadinene	1524	2.3	0.1	0.6	0.5	0.1	2.3	0.4	0.2	0.7	0.1	1.1
α-cadinene	1538	0.2	t	t	t	-	0.2	-	-	t	t	t
elemol	1549	2.9	0.5	14.7	2.0	3.2	1.3	0.3	0.3	2.8	0.4	1.2
germacrene B	1556	0.5	t	0.1	0.4	0.3	0.4	0.2	t	0.2	0.1	0.8
germacrene D-4-ol	1574	3.7	0.2	1.3	0.4	0.5	1.5	0.4	t	0.7	0.2	0.4
cedrol	1596	-	0.1	4.4	1.9	-	-	0.2	-	0.1	t	0.3
β-oplopenone	1606	0.5	0.3	t	0.3	0.1	0.6	0.5	0.2	0.5	t	t
1-epi-cubenol	1627	0.3	-	-	-	0.3	0.4	t	t	0.3	t	0.2
γ-eudesmol	1630	0.3	-	3.0	0.5	0.8	0.2	0.1	t	0.5	t	0.5
epi-α-cadinol	1640	0.8	-	1.1	0.2	t	2.4	0.1	0.3	0.5	t	0.6
epi-α-muurolol	1640	0.8	-	1.1	0.3	t	t	0.1	-	0.5	-	0.6
α-muurolol	1645	0.3	-	t	t	-	0.4	t	-	0.1	t	t
β-eudesmol	1649	0.5	t	5.1	1.0	1.4	0.5	0.4	0.3	1.0	0.3	1.1
α-eudesmol	1652	2.4	0.2	6.1	0.6	1.7	t	0.5	0.4	1.0	0.1	1.2
α-cadinol	1653	2.4	0.2	t	0.5	-	4.0	0.4	0.5	1.0	0.6	1.1
bulnesol	1666	-	t	1.0	0.1	t	-	-	-	0.1	t	t
sesquiterpene alcohol, FW 222	1688	-	t	t	t	0.4	1.4	-	-	0.3	0.1	1.1
8-α-acetoxyelemol	1789	0.3	0.3	0.4	1.1	0.9	-	t	0.4	t	t	t
manoyl oxide	1989	-	-	-	-	0.1	t	1.1	t	t	1.2	0.1
abietatriene	2054	-	-	-	-	-	t	-	t	t	-	-
abietadiene	2080	-	-	-	-	-	0.1	t	0.5	0.5	t	0.1
abieta-8(14),13(15)-diene	2147	-	-	-	-	-	-	-	t	t	-	-
4-epi-abietal	2288	-	t	-	-	-	-	-	-	-	t	0.2
trans-totarol	2314	-	-	-	-	-	-	-	-	-	-	0.1
trans-ferruginol	2332	-	-	-	-	-	-	-	-	-	-	0.2

MI = 9221, Atlas Mts., Morocco; M2 = 9223, Atlas Mts., Morocco; TM = average from samples from Tizi-n-Tichka, Morocco; OM = average from samples from Oukaimeden, Morocco; S1 = 7085, Ruidera, Spain; S2 = 9051, Ruidera, Spain; CS = average of samples from Consuegra, Spain; P1 = 9151, Pyrenees, France; P2 = 9152, Pyrenees, France; MF = average from samples from Montdauphin, France; CR = average from samples from Corse Island, France; components that tend to separate the Moroccan population from the European populations are bolded; RI = Kovat's Index on DB-5 (= SE54) column; t = compositional values < 0.1%; unidentified components < 0.5% are not reported

and France to aid in understanding the variation in this species.

Materials and Methods

Specimens used in this study: *J. thurifera*, Adams 7083-7085, 9050, 9051, Ruidera, Spain; Adams 9451-53, Consuegra, Spain; Adams 9221-9223, Tizi-n' Ait-Imi, Atlas Mts., Morocco (ex. W. Badri), Adams 9415-9417, Oukaimeden, Atlas Mts., Morocco, Adams 9420, 9421, Tizi-m-Tichka/Kabah Telouet, Atlas Mts., Morocco; Adams 9151-9153, Pyrenees, France (ex. T. Gauquelin); Adams 9036-9038, Montdauphin, France; Adams 9492-9494, Valee du Golo, Corse Island, France (ex. Camille Peyre) and *J. foetidissima*, Adams 8789-8790, Lemos, Greece. Voucher specimens for all collections were deposited at Baylor University.

Fresh leaves (200 g fresh weight) were steam distilled for 2 h using a circulatory Clevenger-type apparatus (11). 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 (48 h, 100°C) for determination of oil yields. After initial GC/MS analyses, composite oil samples were made for each of the taxa in this study. These composite (average) oil samples were then subjected to GC/MS for compound identification and quantification by TIC.

The oils were analyzed on an HP MSD 5973, directly coupled to an HP 58900 gas chromatograph, using a J&W DB-5, 0.26 mm x 30 m, 0.25 μm coating thickness, fused silica capillary column (see 12 for operating details). Identifications were made by library searches of our volatile oil library (12), using the HP library search routines, coupled with retention time data of reference compounds.

One g (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 using the Qiagen Dneasy plant mini kits. The RAPDs analyses follow that of Adams and Demeke (3). Ten-mer primers were purchased from the University of British Colombia (5'-3') 153: GAG TCA CGA G; 184: CAA ACG GAC C; 212: GCT GCG TGA C; 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: ATA CGG CGT C; 338: CTG TGG CGG T; 346: TAG GCG AAC G; 347: TTG CTT GGC G; 375: CCG GAC ACG A; 389: CGC CCG CAG T; 391: GCG AAC CTC G; 399: TTG CTG GGC G; 413: GAG GCG GCG A; 431: CTG CGG GTC A; 478: CGA GCT GGT C.

PCR was performed in a volume of 15 μL containing 50 mM Tris-HCl (pH 9), 2.0 mM $MgCl_2$, 0.01% gelatin and 0.1% Triton X-100, 0.2 mM of each DNTPs, 0.36 μM primers, 0.25

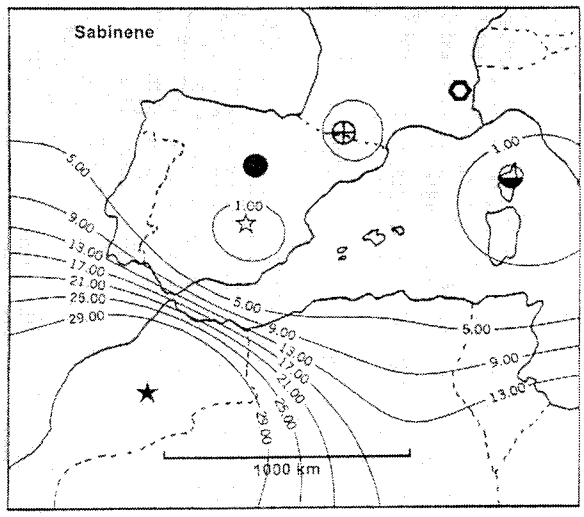

Figure 3. Contour map of the percent sabinene concentration in the leaf oils (notice the high level in average Morocco population and the lower levels in the European populations); shaded star = Morocco; open star = Ruidera, Spain; shaded circle = Consuegra, Spain; cross circle = Pyrenees, France; open hexagon = French Alps; half-shaded circle = Corsica, France

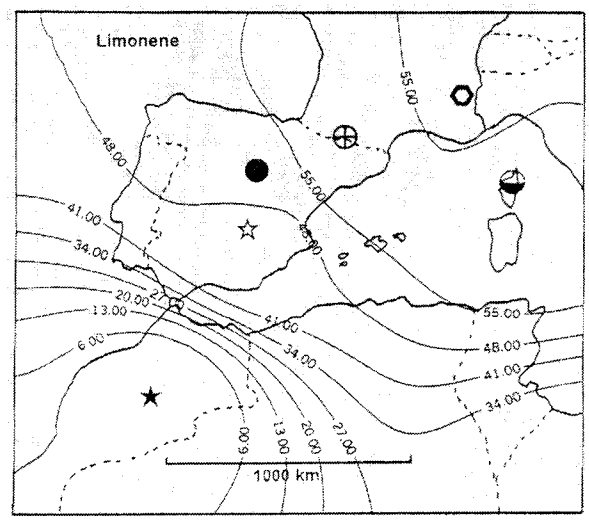

Figure 4. Contour map of the percent limonene concentration in the leaf oils; there were high levels in the European populations and a low level in the Morocco populations (average)—symbols defined in Figure 3

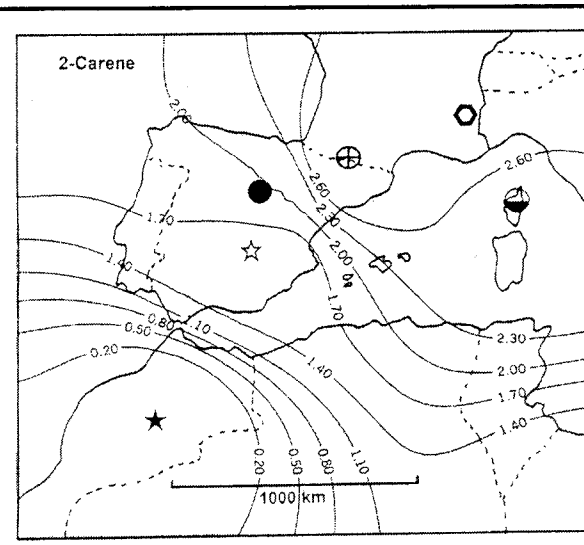

Figure 5. Contour map of the percent δ-2-carene concentration in the leaf oils; the European populations have moderate amounts, but δ-2-carene is missing in all samples from Morocco—symbols defined in Figure 3

ng genomic DNA, and 0.5 unit of Taq DNA polumerase (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 cycfles 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 were scored in four classes: very bright (= 6), medium bright (= 5), faint (= 4) and absent (= 0). See Adams and Demeke (3) 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 (13-15). Principal coordinate analysis (PCO) of the similarity matrix follows Gower (16).

Results and Discussion

The leaf oils were found to be polymorphic in the Tizi-n' Ait-Imi, Morocco, Ruidera, Spain and Pyrenees population samples, so the highest and lowest types are reported in Table I. There was only one unidentified compound (great then 0.5% concentration): RI 1688 (on DB-5) FW 204 (m/e) 41(78), 55(62), 67(41), 84(100), 93(26), 109(17), 119(33), 121(26), 137(13), 161(20), 204(2).

In the Tizi-n' Ait-Imi, Morocco samples (M1, M2, Table I), these represented the extremes of the samples analyzed. δ-3-Carene was missing in M1, but about 1.7-3.1% in the other Moroccan samples (Table I); it was often missing in the European plants. The major differences between M1 and M2 was that M2 had smaller amounts of sesquiterpenes such as γ- and δ-cadinene, elemol, germacrene B, germacrene D-4-ol, α- and β-eudesmol, and α-cadinol (Table I). In these respects, the oil of M2 was more similar to the oils of some of the European samples. The population at Tizi-n-Tichka (TM) was only about 100 km from the population at Tizi-n' Ait-Imi (M1, M2), but the oil was quite different in several major compounds (α-pinene, sabinene, γ-terpinene, *cis*-sabinene hydrate, terpinen-4-ol, elemol and cedrol (Table I). Cedrol, normally restricted to heartwood oils, was found in several populations (Table I).

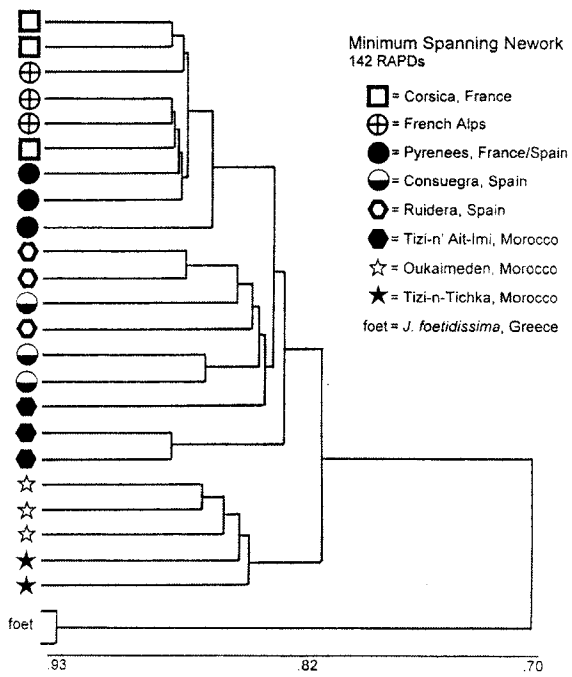

Figure 6. Minimum spanning network based on 142 RAPD bands of the *J. thurifera* with *J. foetidissima* added as an outgroup (notice that most individuals cluster by population, but that one of the Moroccan plants [M1 in Table I] clusters with the plants from Spain)

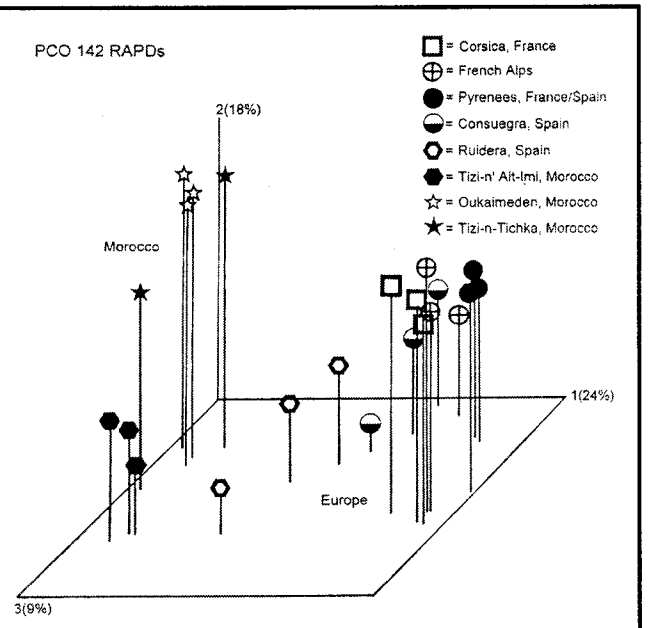

Figure 7. Principal coordinates ordination based on 142 RAPD bands; the Moroccan plants are well separated from the European plants; the plants from southern Spain (Ruidera) are somewhat intermediate

In spite of the variation among individuals, there was a clear pattern in the leaf oils. Notice (Table I) that sabinene, γ-terpinene, δ-3-carene, *cis*-sabinene hydrate and terpinen-4-ol were high in the Moroccan plants and low in the European plants. In a similar fashion, δ-2-carene, limonene and piperitone were high in the European plants, but low in the Moroccan plants. However, the rest of the 93 compounds listed in Table I did not show any major pattern.

Figure 3 shows the sabinene pattern. All the European populations were low in sabinene (0.3-9.7%), and the Moroccan composite sample was high (33.9%). A similar trend was seen with limonene (Figure 4) in that the European populations (28.8-61.8%) were quite different from the Moroccan composite (2.6%).

Figure 5 presents the δ-2-carene pattern showing 2.85-4.4% in the European populations and none in the Moroccan composite. It might be noted that the largest range of δ-2-carene was in the Pyrenees samples (1.3-4.4%, Table I). Each of these three compounds showed the same trend: divergence of the Moroccan plants, clinical variation from Morocco to Spain to France, and polymorphism.

The analysis using the DNA fingerprinting data (Random Amplified Polymorphic DNAs, RAPDs) resulted in 142 RAPD bands that showed fidelity within populations (bands that only occurred once or randomly among populations were not scored). A minimum spanning network (Figure 6) includes two *J. foetidissima* plants from Greece as an outgroup. The network shows that most plants from each population cluster together (Figure 6). The most distinct cluster was composed of the northernmost plants from France. Notice that one of the Moroccan plant clustered with the Ruidera, Spain individuals (Figure 6). The Oukaimeden and Tizi-n-Tichka, Morocco plants formed a distinct cluster (Figure 6), even though they were separated from the Tizi-n' Ait Imi population by only about 100 km.

To better visualize the relationships among these *J. thurifera* populations, the *J. foetidissima* plants were removed from the data set and a new similarity matrix was computed and factored. Principal coordinates analysis of this matrix resulted in eigenroots that accounted for 23.5, 10.4, 9.08, 6.4, 5.4 and 4.6% of the variance among the 23 OTUs. An ordination using the first three coordinates (Figure 7) shows separation between the Moroccan and European plants, with the plants from Spain being somewhat intermediate.

This is exactly the order that would be expected in the case of colonization from the Laurasian landmass to the African continent. It is also possible that the African populations were of more recent origin. Seeds could easily have been carried from the Iberian peninsula to Algeria and Morocco by birds. Birds (thrushes) are primary dispensers of *J. thurifera* seeds (17) and this would also be a route that birds might take. This is most certainly the case for the origin of *J. phoenicea* on the Canary Islands, which is more distant from Europe than is North Africa. In addition, it is likely that birds continue to carry seeds between Morocco and Spain, providing gene flow.

The previous study using seeds per cone and proanthrocyanidins (5,6) indicated that the Moroccan population was quite differentiated from the European populations and that study is supported by the essential oils and to some extent by the RAPDs data in this study. However,

although the major oil components show this pattern, most of the minor components do not show much differentiation between the Moroccan and European populations.

The present data correlates with previous studies on the seeds per cone, proanthrocyanidins. In a preliminary study of other *Juniperus* species (RPA, in prep.), the correlation between classifications based on essential oils, RAPDs, ISSRs, ant ITS sequence data revealed that the classifications based on RAPDs, ISSRs and ITS sequence data was highly correlated, but none of these were correlated with the essential oil classification. It is possible that the divergence in sabinene, etc. is controlled by only a few genes with major effects. Likewise, perhaps the number of seeds/cone is influenced by the more arid conditions in Morocco, where the fewest number of seeds per cone have been reported (5).

In summary, evolutionary divergence of the Moroccan populations in their seeds per cone, proanthrocyanidins, and leaf oils appear to support the recognition of *J. thurifera* var. *africana* Maire ([syn.: *J. africana* (Maire); *J. thurifera* ssp. *africana* (Maire) Gauquelin, Hassani et Lebreton] in North Africa until additional data can be gathered that is pertinent to this question. It is important to conserve these North African populations (18) because they may represent significant gene combinations.

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References

1. R.P. Adams, *Systematics of multi-seeded eastern hemisphere Juniperus based on leaf essential oils and RAPD DNA fingerprinting.* Biochem. Syst. Ecol., 27, 709-725 (1999).
2. R.P. Adams, T. Demeke and H.A. Abulfatih, *RAPD DNA fingerprints and terpenoids: clues to past migrations of Juniperus in Arabia and east Africa.* Theoret. Appl. Genetics, 87, 22-26 (1993).
3. R.P Adams and T. Demeke, *Systematic relationships in Juniperus based on random amplified polymorphic DNAs (RAPDs).* Taxon, 42, 553-572 (1993).
4. H. Gaussen, *Les Gymnosperms actuelles et fossils. X. Les Cupressacees.* Lab. Forest. Univ. Toulouse, p. 145 (1968).
5. T. Gauquelin, M.I. Hassani and P. Lebreton, *Le genevier thurifere, Juniperus thurifera L. (Cupressacees): analyse biometrique et biochimique; popositions systematiques.* Ecologia Mediterranea, 14, 31-42 (1988).
6. M. Barbero, P. Lebreton and R. Quezel, *Sur les affinities biosystematiques et phytoecologiques de Juniperus thurifera L. et du Juniperus excelsa Bieb.* Ecologia Mediterranea, 20, 21-37 (1994).
7. A. Farjon, *The taxonomy of the multiseed junipers (Juniperus sect Sabina) in southwest Asia and east Africa.* Edinb. J. Bot., 49, 251-283 (1992).
8. J. de P. Teresa, A.F. Barrero, A.S. Feliciano and M.C. Caballero, *Componentes de las arcestidas de Juniperus thurifera Linnaeus aceite esencial.* Rivista Ital. EPPOS., 62, 116-120 (1980).
9. A. San Feliciano, M. Medarde, J.L. Lopez, J.M. Miguel de Corral, P. Puebla and A.F. Barrero, *Terpenoids from leaves of Juniperus thurifera.* Phytochemistry, 27, 241-248 (1988).
10. A.P. Akimov, S.I. Kuznetsov, G.I. Nilov, N.N. Chirkina, A.P. Krylova and R.M. Litvinenko, *Essential oils of Junipers from ancient Mediterranean region. Composition, properties and prospectives of use.* Tr. Nkitsk. Botan. Sad., 69, 79-93 (1976).
11. R.P. Adams, *Cedar wood oil—analysis and properties. In: Modern Methods of Plant Analysis: Oils and Waxes.* Edits., H.F. Linskins, J.F. Jackson, p. 159-173, Springer Verlag, Berlin (1991).
12. R.P. Adams, *Identification of Essential Oil Components by Gas Chromatography/Quadrupole Mass Spectroscopy.* Allured Publ. Co., Carol Stream, IL (2001).
13. J.C. Gower, *A general coefficient of similarity and some of its properties.* Biometrics, 27, 857-874 (1971).
14. R.P. Adams, *Numerical-chemosystematic studies of infraspecific variation in Juniperus pinchotii Sudw.* Biochem. Syst. Ecol., 3, 71-74 (1975a).
15. R.P. Adams, *Statistical character weighting and similarity stability.* Brittonia, 27, 305-316 (1975b).
16. J.C. Gower, *Some distance properties of latent root and vector methods used in multivariate analysis.* Biometrika, 53, 326-338 (1966).
17. T. Santos, J.L. Telleria and E. Virgos, *Dispersal of Spanish juniper Juniperus thurifera by birds and mammals in a fragmented landscape.* Ecography, 22, 193-204 (1999).
18. T. Gauquelin, V. Bertaudiere, N. Montes, W. Badri and J.F. Asmode, *Endangered standards of thuriferous juniper in the western Mediterranean basin: Ecological status, conservation and management.* Biodiversity and Conservation, 8, 1479-1498 (1999).