

## Hybridization between *Juniperus grandis*, *J. occidentalis* and *J. osteosperma* in northwest Nevada I: Terpenes, Leviathan Mine, Nevada

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### ABSTRACT

The volatile leaf oils of *J. grandis*, *J. occidentalis*, *J. osteosperma* and putative hybrids from near Leviathan Mine, NV were analyzed. No evidence of hybridization involving *J. occidentalis* was found. There appears to be hybridization between *J. grandis* and *J. osteosperma*. Only one tree, morphologically typical of *J. grandis*, was found in the Leviathan mine population. One shrub appeared to be, morphologically, pure *J. osteosperma*. PCO, using 49 terpenes, with character matches weighted by F (from ANOVA between parental species) produced no evidence that *J. occidentalis* was involved in hybridization with *J. osteosperma* in this population. PCO analysis (with 42 terpenes), revealed hybrids between *J. grandis* and *J. osteosperma*, and possible backcrosses to *J. osteosperma*. Analyses of 32 of the largest terpene components revealed 6 intermediates, 8 dominant/ recessives; 18 terpenes were transgressive, beyond the range of *J. grandis* or *J. osteosperma*. These transgressive components were truncated to values in the range of the putative parental species and a new PCO indicated the plants to be more intermediate. The terpene analysis seems in agreement with the haplotype data of Terry (2010).

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**KEY WORDS:** *J. osteosperma*, *J. grandis*, *J. occidentalis*, hybridization, Cupressaceae, terpenes, Leviathan mine, Nevada.

Hybridization among species of *Juniperus* in north-western Nevada was first reported by Vasek (1966) and confirmed by Terry et al. (2000) and Terry (2010). Terry et al. (2000) found cpDNA (trnL-trnF, trnS-trnG) haplotypes of *J. occidentalis* in Nevada populations of *J. osteosperma*, with lower frequencies occurring in Utah, Colorado, and Wyoming. Subsequently, Terry (2010) analyzed trnL-trnF and trnS-trnG (cpDNA) haplotypes and reported similar results (Fig. 1). Notice, all 15 trees of *J. occidentalis* in Oregon have the same haplotype and that this haplotype is also present in northwest Nevada. The Leviathan mine population was one of the most diverse populations and contained 5 haplotypes (Fig. 1).

Recently, Adams (2012a) analyzed geographic variation in the leaf essential oils of *J. osteosperma* (Torr.) Little and reported differences among the populations. However, the putative hybrid populations of northwest Nevada were not included in that study.

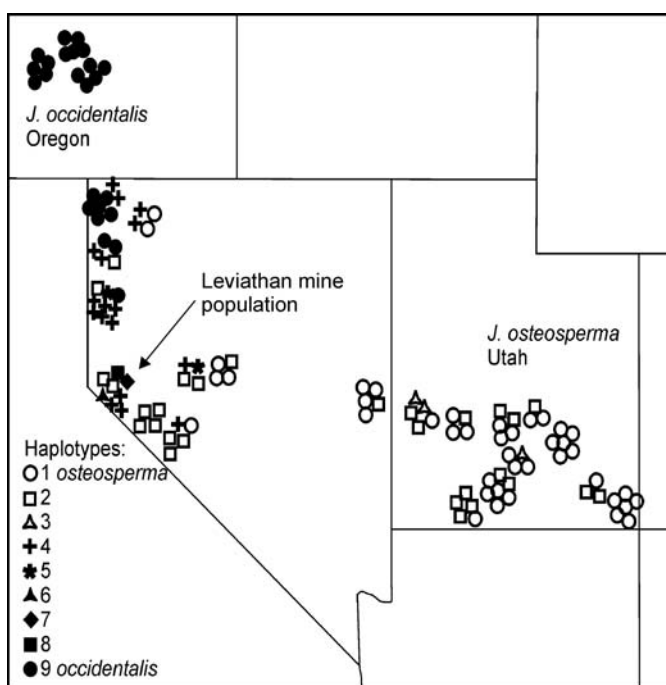


Figure 1. Distribution of haplotypes (trnL-trnF and trnS-trnG) in *J. occidentalis* and *J. osteosperma* (based on Terry, 2010).

These three western junipers occupy generally allopatric ranges (Fig. 2), with *J. grandis* favoring granitic outcrops in the high Sierra, *J. occidentalis* growing on lava beds at lower elevations in northern California and Oregon, and *J. osteosperma*, preferring the intermediate elevations in the Basin and Range region of Nevada, Utah and adjacent states; a fourth species, *J. californica*, grows in the Mojave desert foothills of southern California, thence northward in the central valley foothills (Adams 2011). Adams (2012b) found that *Juniperus grandis* and *J. occidentalis* appear to hybridize in the Beckwourth, CA area (Fig. 2) but, otherwise no evidence of gene flow between these species was found.

The Leviathan mine population, sampled by Terry (2010, popn. 16) appears to be an area of sympatry between *J. grandis* and *J. osteosperma* and subject to ancestral as well as possible current hybridization. Analysis of plants from the Leviathan mine population is the focus of this paper.

### MATERIALS AND METHODS

Plant material: *J. grandis*, Adams 11963-11967, Jct. US 50 & CA 89, 38° 51.086' N, 120° 01.244' W, 1937 m, Meyers, El Dorado Co.; CA; Adams 11968-11972, 16 km w of Sonora Jct., on CA. 108, 38° 18.289' N, 111° 35.598' W, 2585 m, Tuolumne Co.; CA, *J. osteosperma*, Adams 1689-1699, 1701-1705, on US 6, Thistle, 40° 00' 6.9" N, 111° 29' 4.6" W, 1650 m, Utah Co., UT, Adams 12067-12071, 4 km n of Sedona, AZ, at Grasshopper Point, on Alt US 89, 34.888° N, 111.733° W, 1380m, Coconino Co., AZ, 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 US 375, 37° 26.404' N, 115° 22.703' W, 1675 m, Lincoln Co. NV; Adams 11125-11127, McKinney Tanks Summit on US 6, 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 I 80, 40° 45.598' N, 115° 55.942' W, 1535 m, Elko Co., NV; Adams 11144-11146, 8 km e of Wells, on I 80, 41° 06.533' N, 114° 51.441' W, 1876 m, Elko Co., NV; Adams 11960-11962, 56 km n of Reno, NV; on US 395, 39° 54.458' N, 120° 00.322' W, 1383 m, Lassen Co., CA; Adams 11973-11977, 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, Mahogany Flats Campground, Panamint Mtns., 36° 13.783' N, 117° 04.102' W, 2477 m, Inyo Co., CA, Adams 12323-12327, Basin, San Bernardino Mtns., 34° 16.910' N, 116° 45.306' W, 1820 m, San Bernardino Co., CA, Adams 12210-12214, ca. 1 km e of CA 18, ca. 16 km s of jct CA 18 & CA 247, n slope San Bernardino Mtns., 34° 21.213' N, 116° 50.607' W, 1393 m, San Bernardino Co., CA, Adams 12215-12219, on I15, at Bailey Rd., 35° 27.938' N, 115° 31.709' W, 1431 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 OR 20, shrubs, 0.5 - 1m tall, 43° 53.922' N, 120° 59.187' W, 1274 m, Deschutes Co., OR; Adams 11952-11954, 14 km e of Jct. OR66 & I 5, on OR66, 42° 08.044' N, 122° 34.130' W, 701 m, Jackson Co., OR; Adams 11957-11959, on CA 299, 10 km e of McArthur, CA, 41° 05.313' N, 121° 18.921' W, 1091 m, Lassen Co., CA; Adams 11995-11998 (Kauffmann A1-A3,

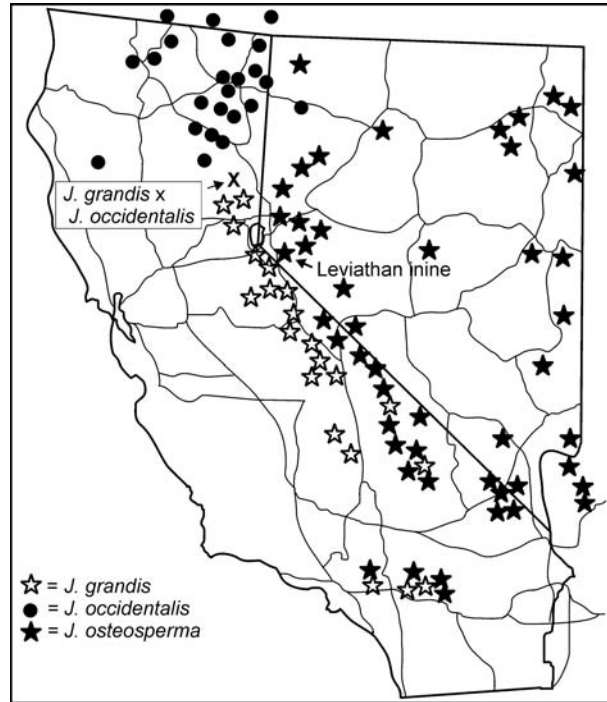


Figure 2. Distributions of *J. grandis*, *J. occidentalis* (in part) and *J. osteosperma* (in part) with Leviathan mine population noted.

B1), Yolla Bolly-Middle Eel Wilderness, 40° 06' 34" N, 122° 57' 59" W, 1815- 2000 m, Trinity Co., CA, Adams 12342-12346, 19 km WSE of Susanville, CA, on CA 36, 40° 22.178' N, 120° 50.211' W, 1570 m, Lassen Co., CA, Adams 12347-12351, on US 395, 5 km n of Madeline, 41° 05.867' N, 120° 28.456' W, 1695 m, Lassen Co., CA. **Leviathan mine population:** Adams 12368-12382, on Leviathan Mine Rd. (= Randall Terry popn.#16), 4 mi sw of US395, 38° 46.412' N; 119° 36.268' W, 6047 ft. Voucher specimens are deposited in the herbarium, Baylor University (BAYLU).

*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 taxon 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 Adams, 2007 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. Terpenoids (as per cent total oil) were coded and compared among the species by the Gower metric (1971). Principal coordinate analysis was performed by factoring the associational matrix using the formulation of Gower (1966) and Veldman (1967). Principal components analysis (PCA) follows the formulation of Veldman (1967).

## RESULTS AND DISCUSSION

Only one of the junipers in the Leviathan mine population appeared to be typical *J. grandis* (1, Table 1) and one plant appeared very similar to *J. osteosperma* (7, Table 1). The other 13 plants sampled were somewhat intermediate in morphology, but generally appeared more like *J. osteosperma*.

The oils of *J. osteosperma* are dominated by camphor (23.7%), bornyl acetate (16.6%) and sabinene (10.%, Table 2), with moderate amounts of  $\alpha$ -pinene, borneol and terpinen-4-ol. Whereas, typical oils of *J. grandis* and *J. occidentalis* (Table 2) have little camphor (0, 2.5%) or borneol (0, 2.2%). The oil of *J. occidentalis* has large amounts of sabinene, p-cymene, citronellol and bornyl acetate (Table 2), whereas *J. grandis* oil is dominated by  $\delta$ -3-carene,  $\alpha$ -pinene and  $\beta$ -phellandrene (Table 2).

The oil of tree 1, field identified as *J. grandis*, is very similar to *J. grandis* (Meyers, CA, Table 2). Hybrids 9 and 11 have some intermediated components, and generally complementary components. The oils of trees 10 and 15 are similar to *J. osteosperma*, but differ in several components. They could be backcrosses or just unusual oils of *J. osteosperma*.

As a first approximation, PCO was calculated using oils from the 15 Leviathan mine plants, *J. grandis* (10, Meyers, CA, Sonora Jct., CA), *J. occidentalis* (Mc Arthur, CA, 10) and *J. osteosperma* (8 population averages, see Materials). This PCO used character weighting (in similarities) of Fs (from ANOVA between the putative parents). The ordination clearly shows that none of the Leviathan mine plants resembles *J. occidentalis* in their terpenes (Fig. 3).

Eliminating the *J. occidentalis* plants, and running a second ANOVA between *J. grandis* and *J. osteosperma*, followed by PCO analysis, focused on differences in the oils between putative parental species, *J. grandis* and *J. osteosperma*. The first two principal components removed 62 and 6% of the

variance among samples. Ordination (Fig. 4), shows the *J. grandis* individual from Leviathan mine (1, Fig. 4) to be closely allied with typical *J. grandis*. Plants 9

Table 1. Morphological observations on plants of the Leviathan mine population.

| Tree #                          | habit                               | bark color   | bark exfoliation  | leaf glands                       |
|---------------------------------|-------------------------------------|--------------|-------------------|-----------------------------------|
| 1                               | 7m tree, 3 stems, <i>J. grandis</i> | cinnamon     | shaggy strips     | visible, w white exudate          |
| 2                               | 5 m shrub, 5m x 5m                  | gray         | shaggy strips     | visible, ruptured                 |
| 3                               | 7m tree, 1 stem                     | brown        | interlaced strips | visible, few ruptured             |
| 4                               | 6m tree, branched at 4m             | gray         | strips            | visible, w white exudate          |
| 5                               | 4m tree, 3 stems, twisted           | gray, orange | shaggy strips     | vis. only on whip lvs., few rupt. |
| 6                               | 6m tree, 5 stems                    | gray-brown   | thin strips       | vis., with white exudate          |
| 7                               | 4m shrub, <i>J. osteosperma</i>     | gray-brown   | strips            | not vis., v. few ruptured         |
| 8                               | 3m shrub                            | gray         | shaggy strips     | vis., w white exudate             |
| 9                               | 4m shrub-tree, 10 stems             | brown        | shaggy strips     | vis, w white exudate              |
| 10                              | 3m tree, 1 stem, <i>osteo</i> BC?   | gray         | shaggy strips     | vis, very few ruptured            |
| 11                              | 3m shrub                            | gray         | shaggy strips     | vis., w white exudate             |
| 12                              | 3m shrub                            | gray-brown   | shaggy strips     | vis., few w clear exudate         |
| 13                              | 3m shrub                            | gray         | shaggy strips     | vis., few w white exudate         |
| 14                              | 5m tree, 1 stem                     | gray         | shaggy strips     | vis., not ruptured                |
| 15                              | 1.5m shrub x 3 m, <i>osteo</i> BC?  | gray         | shaggy strips     | few vis., v. few ruptured         |
| <i>J. grandis</i> (typical)     |                                     |              |                   |                                   |
|                                 | trees, 1-3 stems                    | cinnamon     | shaggy strips     | vis., few w clear/ white exud.    |
| <i>J. osteosperma</i> (typical) |                                     |              |                   |                                   |
|                                 | shrubs, trees (1- few stems)        | gray-brown   | thin strips       | not vis., not ruptured            |

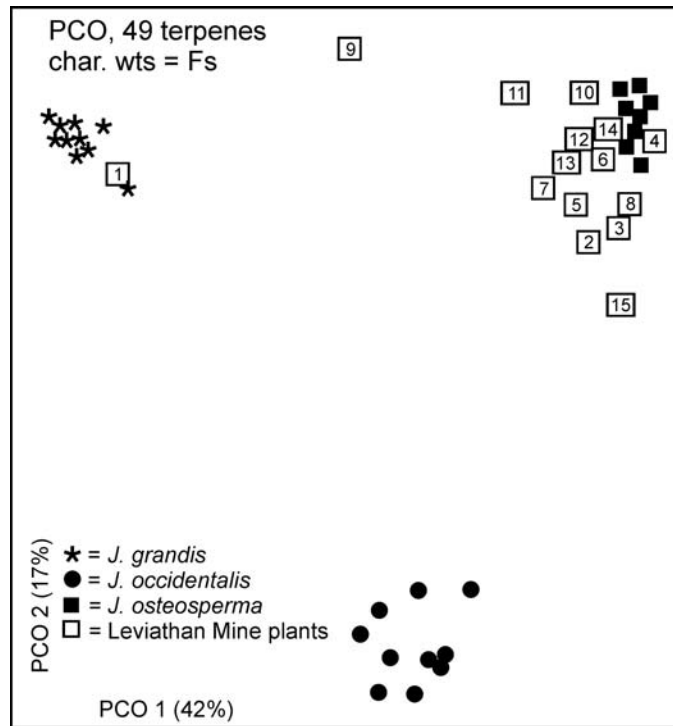


Figure 3. PCO using 49 terpenes with character weights = Fs from ANOVA between *J. grandis*, *J. occidentalis*, and *J. osteosperma*.

and 11 are intermediate and presumably hybrids. Individuals 10 and 13 are closely allied with *J. osteosperma* (Fig. 4). Nine other Leviathan mine plants are clustered between individual 11 and *J. osteosperma*.

Analysis of variation among the putative hybrids revealed that, of 32 major terpenes, 6 were intermediate (between *J. grandis* and *J. osteosperma*), 8 appeared as dominant/ recessive traits having values like one of the two species and 18 terpenes were transgressive (i.e., larger or smaller than either *J. grandis* or *J. osteosperma*). Adams and Tsumura (2012), in a study of artificial hybrids within *Cryptomeria japonica*, reported that of the 17 major terpenes, 7 were intermediate and 10 were transgressive in the F<sub>1</sub> hybrids. Three compounds, cedrol, widdrol and cis-thujopsene, appeared to be genetically linked and inherited as a dominant/ recessive traits with some modifying genes. This group of linked, dominant/recessive compounds interfered with the ordination of hybrids between parents, such that hybrids with large amounts of cedrol, widdrol and cis-thujopsene were very difficult to separate from the Haava parent. A second study (Adams and Stoehr, 2013) of artificial hybrids of Douglas fir (*Pseudotsuga menziesii* var. *menziesii* and var. *glauca*) found that of 19 terpenes in the F<sub>1</sub> hybrids, 3 were intermediate, 4 dominant/ recessive and 12 transgressive. When the 12 transgressive terpenes were truncated to values between the parents, PCO ordination was improved, with the hybrids depicted as more intermediate between the parents (Adams and Stoehr, 2013).

To investigate the effects of truncation of transgressive terpenes, the 18 transgressive terpenes were truncated to values between those of *J. grandis* and *J. osteosperma*. Extraction of eigenroots showed an increase in variance in coordinate 1 (75%) and a slight decrease in coordinate 2 (5%). Ordination shows (Fig. 5) that the overall pattern is somewhat affected. The most noticeable change is in the placement of several putative hybrids as intermediate (2, 5, 7, 12, 15, Fig. 5) and ordination of several plants towards *J. osteosperma* (3, 4, 8, 10, 13, 14, Fig. 5). The ordination in fig. 5 suggests that individuals 9

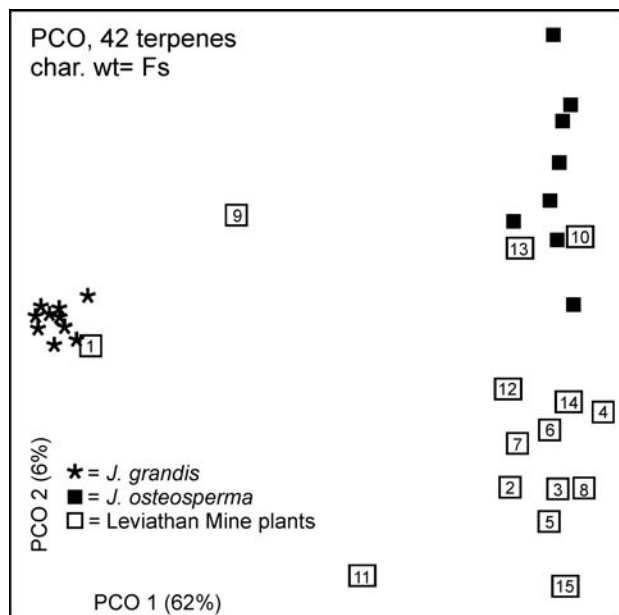


Figure 4. PCO, 42 terpenes, F weighted.

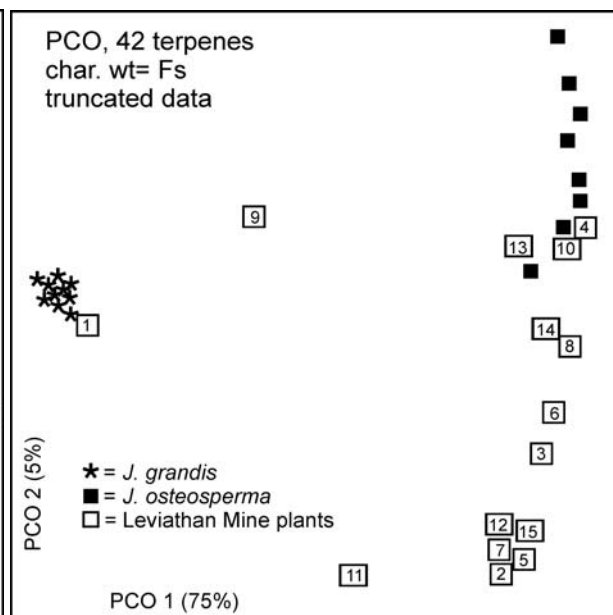


Figure 5. PCO, 42 terpenes, F weighted, data truncated between *J. grandis* and *J. osteosperma* values.

11 are hybrids with plants 2,5,7,12,15 (plus 3 and 6?) being backcrosses to *J. osteosperma*, and the remaining 5 plants are *J. osteosperma*.

Additional analyses of the variation among the Leviathan plants' terpenes was made by plotting the values along with those of *J. grandis* and *J. osteosperma*. Analyses of the 6 intermediate and 8 dominant/recessive terpenes, shows that even among those scored as intermediate ( $\alpha$ -fenchene, verbenene,  $\beta$ -pinene,  $\alpha$ -cadinol, terpinen-4-ol and borneol), many Leviathan plants had zero or trace amounts, and these low values were typical of *J. osteosperma* (Fig. 6). Only terpinen-4-ol and borneol appeared to have intermediate values (Fig. 6).

For eight dominant/ recessive compounds (3-carene, 2-carene, neo-isopulegyl acetate, KI 1092, KI 1230, trans-p-menth-2-en-1-ol, neo-isopulegol and piperitone), the Leviathan plants contained zero or trace amounts (Fig. 6). For each of the 8 compounds, the zero or trace amount is typical of *J. osteosperma*. So it is easy to see why most Leviathan plants are ordinated near *J. osteosperma* (Figs. 4, 5). Of course, it may be that most of the Leviathan plants are not hybrids, but *J. osteosperma* as suggested in Figs. 4, 5.

Analyses of the 18 transgressive terpenes found they were in 7 groups: (bornyl acetate, sabina ketone,  $\gamma$ -thujene, cis- and trans-sabinene hydrate, camphene hydrate), (camphor, p-mentha-1,4-dien-7-ol), (sabinene,  $\gamma$ -terpinene), ( $\alpha$ -terpinene), (myrcene, KI 1154, KI 1389), (terpinolene,  $\alpha$ -phellandrene), and ( $\alpha$ -pinene,  $\beta$ -phellandrene). Several of the terpenes show extreme transgressive variation (Fig. 7, bornyl acetate, camphor,  $\alpha$ -terpinene). Several transgressive terpenes might also be considered as dominant/ recessive traits (myrcene, terpinolene,  $\alpha$ -pinene, Fig. 7).

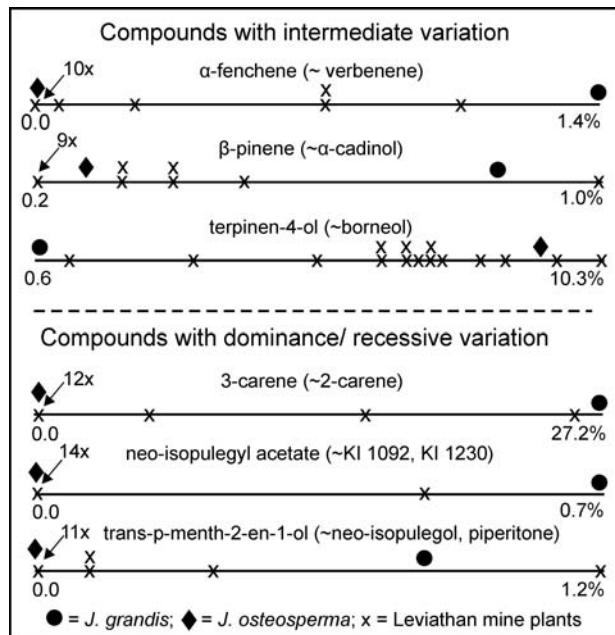


Figure 6. Terpenes with intermediate or dominant/ recessive variation.

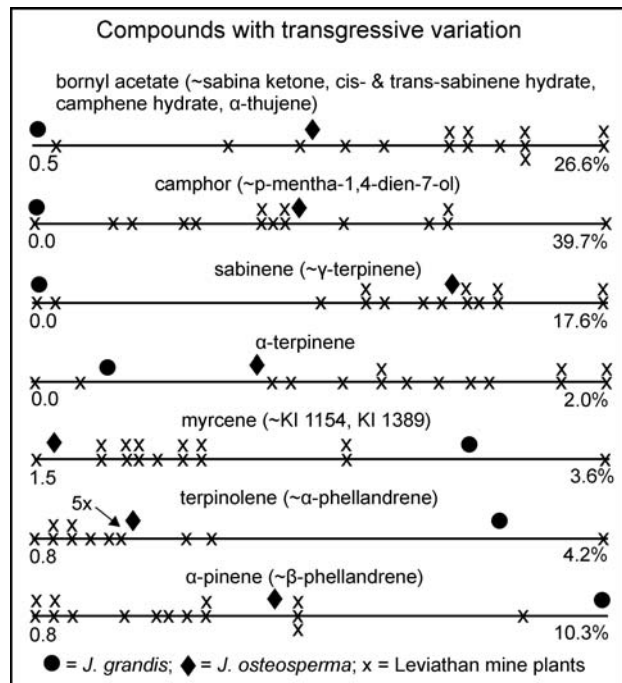


Figure 7. Terpenes with transgressive variation.

Recently, Adams and Stoehr (2013) investigated patterns of variation among Douglas fir hybrids, and reported that the parents and hybrids showed compounds that are zero or near zero in one parent were often zero in the hybrids. This pattern was unbalanced and many more terpenes had this pattern in the

inland parent than in the coastal parent. Thus, the similarities were biased towards the inland parent. Removing some of the redundant terpenes, led to a more intermediate ordination of the hybrids (Adams and Stoehr, 2013).

Analysis of the 24 terpenes with the largest Fs (from ANOVA between *J. grandis* and *J. osteosperma*) revealed that 7 are intermediate (Table 3) and their character weighting (as % total weight) ranged from 0.71% to 4.53%. Eight of the 24 appeared as dominant/ recessives with 3 compounds were more like *J. grandis* in Leviathan plants and 5 compounds were more like *J. osteosperma* in Leviathan plants (Table 3). Character weights ranged from 0.72% to 5.59%. Nine of the 24 terpenes were transgressive; 6 compounds were more like *J. osteosperma* in Leviathan plants (Table 3) and character weights ranged from 2.48% to 15.42%. To balance the number of characters that are like *J. grandis*

Table 3. Patterns of variation for the 24 terpenoids with the highest F ratios in ANOVA. Variation among *J. grandis* and *J. osteosperma* and Leviathan plants. x denotes the terpene occurrence pattern in *J. grandis*, Leviathan plants and/ or *J. osteosperma*. char wt = F, scaled as % total weight. char wt 1 is the original weighting based on 42 characters (Fs, scaled to % total), char wt 2 is the char weight based on 16 selected characters to balance modes between the parents (Fs, scaled to % total).

| cpd   | <i>J. grandis</i> | Leviathan | <i>J. osteosperma</i> | char wt 1 | char wt 2 |
|---|-------------------|-----------|-----------------------|-----------|-----------|
| <u>intermediate (7)</u>   |                   |           |                       |           |           |
| $\alpha$ -terpinene   | x                 | x         | x                     | 2.45      | 3.97      |
| borneol   | x                 | x         | x                     | 1.15      | 1.86      |
| terpinen-4-ol   | x                 | x         | x                     | 4.16      | 6.73      |
| p-mentha-1,4-dien-7-ol  | x                 | x         | x                     | 4.53      | 7.33      |
| germacren-D-4-ol  | x                 | x         | x                     | 0.71      | 1.14      |
| epi- $\alpha$ -cadinol  | x                 | x         | x                     | 0.77      | 1.25      |
| $\alpha$ -cadinol   | x                 | x         | x                     | 1.08      | 1.74      |
| <u>dominant/ recessive (8), 3 cpds more like <i>J. grandis</i> in Leviathan plants, 5 cpds more like <i>J. osteosperma</i> in Leviathan plants.</u> |                   |           |                       |           |           |
| $\alpha$ -fenchene  | x                 | x         |                       | 2.26      | 3.66      |
| trans-carveol   | x                 | x         |                       | 1.44      | 2.34      |
| carvone   | x                 | x         |                       | 1.97      | 3.19      |
| camphene  |                   | x         | x                     | 5.59      | 9.05      |
| 3-carene  |                   | x         | x                     | 1.17      | 0         |
| KI 1154   |                   | x         | x                     | 0.72      | 0         |
| KI 1230   |                   | x         | x                     | 0.93      | 0         |
| KI 1389   |                   | x         | x                     | 0.72      | 0         |
| <u>transgressive (9), 6 cpds more like <i>J. osteosperma</i> in Leviathan plants.</u>   |                   |           |                       |           |           |
| sabinene  |                   | x         | x                     | 2.48      | 0         |
| $\gamma$ -terpinene   |                   | x         | x                     | 6.28      | 0         |
| cis-sabinene hydrate  |                   | x         | x                     | 8.19      | 0         |
| camphor   |                   | x         | x                     | 3.34      | 5.42      |
| camphene hydrate  |                   | x         | x                     | 12.17     | 0         |
| bornyl acetate  |                   | x         | x                     | 3.27      | 5.29      |
| $\alpha$ -thujene   | x                 | x         | x                     | 5.63      | 9.11      |
| trans-sabinene hydrate  | x                 | x         | x                     | 15.42     | 24.96     |
| sabina ketone   | x                 | x         | x                     | 7.98      | 12.92     |

and those like *J. osteosperma*, 4 terpenes were selected from the dominant/ recessive group and 5 were selected from the transgressive group along with the 7 intermediate terpenes, to make a set of 16 terpenes for PCO analysis. Note that char wt 2 values of zero (0) were not included in this group of 16 'selected' terpenes.

PCO based on 16 'selected' terpenes, with hybrids' values truncated and F weighted, produced an ordination (Fig. 8) that has only very small differences from PCO using 42 terpenes, truncated, and F weighted (Fig. 7). So, although this technique of balancing terpene characters between parents had a positive effect in Douglas fir (Adams and Stoehr, 2013), it does not seem to have an effect on the present data set. It might be noted that trans-sabinene hydrate has a very large percentage of the total weight (24.96%) in this analysis. The F value for trans-sabinene hydrate was changed to that of sabina ketone, so it had a much lower weight (i.e., equal to that of sabina ketone in the similarity), but only very, very minor differences were seen in the ordination.

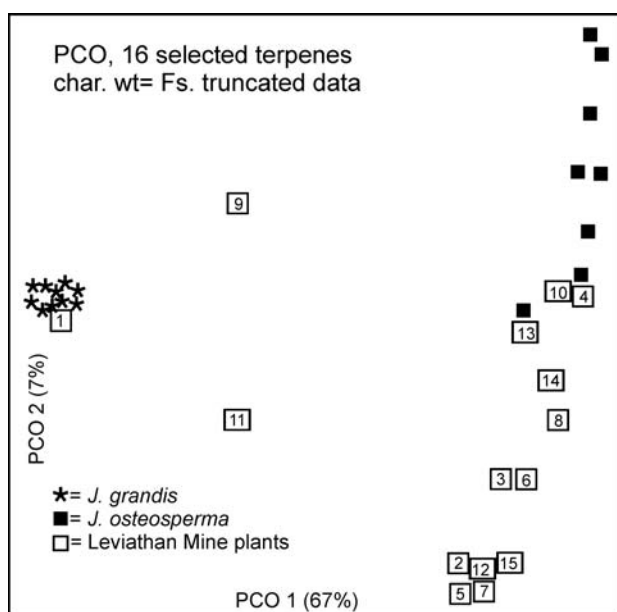


Figure 7. PCO, 16 selected terpenes, wt. = Fs, and truncated terpene values for the Leviathan plants.

giving excessive weight to some characters. The present study, using putative hybrids, mirrors the previous studies (Adams and Tsumura, 2012; Adams and Stoehr, 2013) that encountered problems with transgressive variation, linked terpenes and dominant/ recessive suites of terpenes in artificial hybrids. These problems make it difficult to accurately identify backcrossed plants.

#### ACKNOWLEDGEMENTS

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#### CONCLUSION

It appears that the Leviathan mine population samples contain one typical *J. grandis*, 2 hybrids, 5-10 backcrossed (to *J. osteosperma*) individuals and 3 plants whose oils are fairly typical of *J. osteosperma*. The terpene data support the haplotype data of Terry (2010). It is interesting that Terry (2010) and figure 1 (above) show 5 haplotypes in the Leviathan mine population, of which only 2 of the 5 haplotypes appear in *J. osteosperma* populations (none of the 5 haplotypes appears in *J. occidentalis* populations). It seems likely that haplotypes 6, 7, and 8 are from *J. grandis* germplasm.

Finally, it should be noted that the detection of hybridization using terpenoid data and multivariate methods is subject to considerable difficulty due to the dominant/recessive and transgressive traits and genetic linkage groups



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Table 2. Leaf essential oil compositions for *J. osteosperma* (McKinney Tanks, NV) plus putative *J. osteosperma* backcrosses: #10, 15, putative hybrids, #9, 11, and putative *J. grandis*: #1, along with *J. grandis* (Meyers, CA) and *J. occidentalis* (Mc Arthur, CA). Compounds in boldface indicate hybridity.

| KI          | compound                               | osteo       | #10         | #15         | #9          | #11         | #1          | grand       | occid       |
|-------------|--|-------------|-------------|-------------|-------------|-------------|-------------|-------------|-------------|
| 921         | tricyclene                             | 0.8         | 0.4         | 0.7         | 0.4         | 0.6         | t           | -           | 1.1         |
| 924         | $\alpha$ -thujene                      | 0.5         | 0.5         | 0.7         | 0.1         | 0.5         | t           | -           | 1.0         |
| 932         | $\alpha$ -pinene                       | 4.4         | 0.9         | 3.9         | 5.0         | 1.0         | 9.3         | 14.0        | 5.0         |
| <b>945</b>  | <b><math>\alpha</math>-fenchene</b>    | -           | -           | <b>t</b>    | <b>0.8</b>  | -           | <b>1.1</b>  | <b>1.5</b>  | <b>t</b>    |
| <b>946</b>  | <b>camphene</b>                        | <b>1.1</b>  | <b>0.6</b>  | <b>1.1</b>  | <b>0.7</b>  | <b>0.8</b>  | -           | -           | <b>1.0</b>  |
| 953         | thuja-2,4-diene                        | t           | -           | -           | t           | -           | t           | t           | t           |
| <b>961</b>  | <b>verbenene</b>                       | -           | -           | <b>0.2</b>  | <b>1.3</b>  | -           | <b>0.4</b>  | <b>2.9</b>  | -           |
| <b>969</b>  | <b>sabinene</b>                        | <b>10.2</b> | <b>10.7</b> | <b>15.0</b> | <b>0.5</b>  | <b>13.6</b> | t           | -           | <b>12.0</b> |
| 974         | $\beta$ -pinene                        | 0.2         | 0.2         | 0.3         | 0.4         | 0.1         | 1.0         | 1.3         | 0.4         |
| 988         | myrcene                                | 1.7         | 1.5         | 2.7         | 2.2         | 1.8         | 3.6         | 3.1         | 1.3         |
| <b>1001</b> | <b><math>\delta</math>-2-carene</b>    | -           | -           | -           | <b>t</b>    | -           | <b>0.2</b>  | <b>1.1</b>  | <b>t</b>    |
| 1002        | $\alpha$ -phellandrene                 | 0.3         | 0.3         | 0.2         | 0.4         | 0.1         | 2.4         | 1.6         | 0.8         |
| <b>1008</b> | <b><math>\delta</math>-3-carene</b>    | -           | <b>0.2</b>  | <b>0.3</b>  | <b>15.8</b> | <b>t</b>    | <b>26.3</b> | <b>27.3</b> | <b>1.0</b>  |
| 1014        | $\alpha$ -terpinene                    | 1.3         | 1.3         | 1.9         | 0.3         | 1.4         | 0.5         | 0.4         | 1.7         |
| 1020        | p-cymene                               | 2.4         | 1.4         | 0.7         | 3.1         | 0.8         | 1.3         | 1.4         | 10.7        |
| 1024        | limonene                               | 2.1         | 1.9         | 3.4         | 2.2         | 2.4         | 1.4         | 1.2         | 0.9         |
| <b>1025</b> | <b><math>\beta</math>-phellandrene</b> | <b>3.2</b>  | <b>2.9</b>  | <b>2.2</b>  | <b>4.4</b>  | <b>1.5</b>  | <b>12.4</b> | <b>10.6</b> | <b>3.5</b>  |
| 1044        | (E)- $\beta$ -ocimene                  | t           | t           | 0.4         | 0.6         | 0.1         | t           | t           | 0.1         |
| <b>1054</b> | <b><math>\gamma</math>-terpinene</b>   | <b>2.1</b>  | <b>2.3</b>  | <b>3.1</b>  | <b>1.0</b>  | <b>2.4</b>  | <b>0.3</b>  | <b>0.3</b>  | <b>3.0</b>  |
| <b>1065</b> | <b>cis-sabinene hydrate</b>            | <b>0.8</b>  | <b>1.3</b>  | <b>1.4</b>  | <b>0.1</b>  | <b>1.6</b>  | <b>0.3</b>  | -           | <b>0.9</b>  |
| 1078        | camphenilone                           | t           | t           | t           | -           | t           | -           | -           | -           |
| 1086        | terpinolene                            | 1.4         | 0.9         | 1.4         | 1.9         | 0.9         | 4.2         | 3.7         | 1.3         |
| 1090        | 6,7-epoxymycene                        | 0.1         | -           | t           | -           | t           | -           | -           | -           |
| <b>1092</b> | <b>96, 109,43,152, C10-OH</b>          | -           | -           | <b>t</b>    | <b>0.7</b>  | -           | <b>0.3</b>  | <b>0.9</b>  | -           |
| 1095        | linalool                               | -           | 0.3         | 0.4         | 0.6         | 0.1         | 0.5         | t           | 0.5         |
| <b>1098</b> | <b>trans-sabinene hydrate</b>          | <b>1.0</b>  | <b>1.4</b>  | <b>1.5</b>  | -           | <b>1.7</b>  | -           | -           | -           |
| 1102        | isopentyl-isovalerate                  | 0.2         | -           | t           | -           | t           | -           | -           | -           |
| <b>1112</b> | <b>3-me-3-buten-methyl butanoate</b>   | <b>0.4</b>  | t           | <b>0.6</b>  | -           | <b>0.3</b>  | -           | -           | -           |
| 1118        | cis-p-menth-2-en-1-ol                  | 0.6         | 1.1         | 0.6         | 0.4         | 0.5         | 1.4         | 0.8         | 0.7         |
| 1122        | $\alpha$ -campholenal                  | 0.3         | 0.3         | t           | t           | t           | t           | t           | -           |
| 1136        | trans-p-menth-2-en-1-ol                | -           | -           | 0.4         | -           | t           | 1.2         | 0.9         | 0.9         |
| <b>1141</b> | <b>camphor</b>                         | <b>23.7</b> | <b>29.5</b> | <b>5.5</b>  | <b>22.9</b> | <b>27.8</b> | -           | -           | <b>2.5</b>  |
| 1144        | neo-isopulegol                         | -           | -           | -           | -           | -           | 0.8         | 0.5         | -           |
| 1145        | camphene hydrate                       | 1.5         | 1.5         | 1.9         | 1.1         | 2.3         | 0.2         | t           | 0.2         |
| 1154        | p-menth-1,5-dien-8-ol iso.             | -           | -           | -           | -           | -           | 1.0         | 0.6         | -           |
| 1154        | sabina ketone                          | 0.8         | 1.5         | 0.4         | 1.7         | 0.4         | -           | -           | 0.4         |
| 1161        | p-menth-1,5-dien-8-ol iso.             | -           | -           | -           | -           | -           | t           | 0.3         | -           |
| <b>1165</b> | <b>borneol</b>                         | <b>6.0</b>  | <b>1.5</b>  | <b>2.0</b>  | <b>1.4</b>  | <b>0.6</b>  | -           | -           | <b>2.2</b>  |
| 1166        | coahuilensol                           | -           | -           | 1.6         | -           | -           | 0.4         | t           | 0.6         |
| <b>1174</b> | <b>terpinen-4-ol</b>                   | <b>8.3</b>  | <b>8.3</b>  | <b>7.8</b>  | <b>1.2</b>  | <b>7.3</b>  | <b>0.5</b>  | <b>0.4</b>  | <b>6.7</b>  |
| 1176        | m-cymen-9-ol                           | -           | -           | -           | 1.1         | -           | 0.4         | 0.4         | -           |
| 1179        | p-cymen-8-ol                           | 0.5         | 1.4         | 0.4         | 0.4         | 0.2         | 0.3         | 0.4         | 0.5         |
| 1186        | $\alpha$ -terpineol                    | 0.4         | 0.4         | 0.5         | 1.9         | 0.5         | 3.4         | 1.2         | 0.4         |
| 1195        | myrtenol                               | 0.2         | 0.2         | t           | t           | 0.3         | -           | -           | -           |
| 1195        | cis-piperitol                          | 0.3         | 0.2         | t           | -           | -           | 0.6         | 0.4         | 0.2         |
| 1204        | verbenone                              | 0.2         | -           | -           | 0.8         | 0.1         | -           | -           | -           |
| 1207        | trans-piperitol                        | 0.3         | 0.3         | 0.3         | -           | 0.2         | 1.1         | 0.9         | 0.3         |
| 1215        | trans-carveol                          | 0.6         | 0.4         | t           | -           | t           | -           | -           | -           |
| 1219        | coahuilensol, me-ether                 | 0.2         | 0.2         | 1.6         | 0.4         | 0.3         | 1.3         | 0.4         | 1.1         |
| 1223        | citronellol                            | 8.3         | t           | t           | 0.2         | -           | 0.2         | t           | 8.4         |
| <b>1230</b> | <b>trans-chrysanthenyl ac.</b>         | -           | -           | -           | <b>0.7</b>  | -           | <b>0.5</b>  | <b>3.9</b>  | -           |

| KI          | compound                                | osteo       | #10         | #15         | #9          | #11         | #1         | grand      | occid      |
|-------------|---|-------------|-------------|-------------|-------------|-------------|------------|------------|------------|
| 1238        | cumin aldehyde                          | 0.3         | 0.3         | 0.1         | -           | -           | -          | -          | 0.2        |
| <b>1239</b> | <b>carvone</b>                          | <b>0.6</b>  | <b>0.4</b>  | <b>t</b>    | <b>0.2</b>  | <b>0.1</b>  | <b>0.2</b> | <b>t</b>   | -          |
| <b>1249</b> | <b>piperitone</b>                       | <b>t</b>    | -           | <b>t</b>    | <b>1.2</b>  | <b>t</b>    | <b>0.3</b> | <b>1.2</b> | <b>0.2</b> |
| 1255        | 4Z-decenol                              | -           | -           | -           | -           | -           | 0.6        | 0.4        | -          |
| <b>1257</b> | <b>methyl citronellate</b>              | -           | -           | <b>t</b>    | <b>0.9</b>  | <b>0.1</b>  | <b>0.2</b> | <b>0.2</b> | -          |
| 1274        | neo-isopulegyl acetate                  | -           | -           | t           | -           | -           | 0.5        | 0.3        | -          |
| 1283        | $\alpha$ -terpinen-7-al                 | 0.2         | -           | -           | -           | -           | -          | -          | -          |
| <b>1284</b> | <b>bornyl acetate</b>                   | <b>16.6</b> | <b>16.8</b> | <b>26.3</b> | <b>10.0</b> | <b>20.3</b> | <b>0.9</b> | <b>0.4</b> | <b>9.5</b> |
| 1285        | safrole                                 | -           | 0.2         | -           | -           | -           | 0.4        | 0.3        | -          |
| <b>1298</b> | <b>carvacrol</b>                        | <b>t</b>    | <b>t</b>    | <b>0.3</b>  | <b>0.2</b>  | <b>0.3</b>  | <b>t</b>   | <b>0.2</b> | <b>0.4</b> |
| 1319        | 149,69,91,164, phenolic                 | 0.4         | 0.6         | 0.7         | 0.5         | 1.9         | 2.5        | 0.4        | -          |
| <b>1318</b> | <b>methyl geranate</b>                  | -           | -           | <b>1.1</b>  | <b>0.5</b>  | -           | <b>0.4</b> | <b>0.4</b> | <b>1.0</b> |
| <b>1325</b> | <b>p-mentha-1,4-dien-7-ol</b>           | <b>0.5</b>  | <b>0.8</b>  | <b>0.3</b>  | -           | <b>0.2</b>  | -          | -          | <b>t</b>   |
| <b>1332</b> | <b>cis-piperitol acetate</b>            | -           | -           | -           | <b>t</b>    | <b>0.1</b>  | <b>0.3</b> | <b>0.4</b> | -          |
| 1343        | trans-piperitol acetate                 | -           | -           | -           | -           | -           | 0.1        | 0.3        | -          |
| 1387        | $\beta$ -bourbonene                     | -           | -           | -           | -           | -           | t          | 0.5        | 0.2        |
| <b>1388</b> | <b>79,43,91,180</b>                     | -           | -           | -           | <b>0.3</b>  | <b>t</b>    | <b>0.9</b> | <b>0.3</b> | -          |
| <b>1389</b> | <b>111,81,151,182</b>                   | -           | -           | -           | <b>1.2</b>  | <b>0.2</b>  | <b>3.3</b> | <b>1.0</b> | -          |
| 1403        | methyl eugenol                          | -           | -           | -           | -           | -           | 0.1        | t          | -          |
| 1429        | cis-thujopsene                          | 0.7         | -           | -           | -           | -           | -          | -          | 0.9        |
| 1448        | cis-muurolo-3,5-diene                   | -           | -           | -           | t           | -           | t          | t          | -          |
| 1451        | trans-muurolo-3,5-diene                 | -           | -           | -           | -           | -           | -          | -          | 0.1        |
| 1465        | cis-muurolo-4,5-diene                   | -           | -           | -           | -           | -           | -          | -          | 0.1        |
| 1468        | pinchotene acetate                      | 0.5         | t           | 0.8         | 0.2         | -           | 0.8        | -          | 0.6        |
| 1471        | 121,105,180,208,phenol                  | -           | -           | -           | -           | -           | -          | 0.3        | -          |
| 1475        | trans-cadina-1(6),4-diene               | -           | -           | -           | -           | -           | -          | -          | 0.3        |
| 1478        | $\gamma$ -muurolene                     | -           | -           | -           | -           | -           | t          | -          | 0.8        |
| 1484        | germacrene D                            | -           | -           | -           | -           | -           | 0.2        | 0.2        | 0.3        |
| 1493        | trans-murolo-4(14),5-diene              | -           | -           | -           | -           | -           | -          | -          | 0.4        |
| 1493        | epi-cubebol                             | -           | -           | -           | -           | -           | t          | -          | 0.4        |
| 1500        | $\alpha$ -muurolene                     | t           | -           | 0.2         | 1.0         | -           | 0.2        | 0.3        | 1.1        |
| 1513        | $\gamma$ -cadinene                      | t           | 0.2         | 0.3         | 0.3         | 0.2         | 0.6        | 1.3        | 3.7        |
| 1518        | epi-cubebol                             | -           | -           | -           | t           | t           | t          | 0.4        | 0.4        |
| 1522        | $\delta$ -cadinene                      | 0.2         | 0.3         | 0.6         | 0.4         | 0.3         | 0.7        | 1.1        | 4.1        |
| 1537        | $\alpha$ -cadinene                      | -           | -           | -           | -           | -           | t          | t          | 0.4        |
| 1544        | $\alpha$ -calacorene                    | -           | -           | -           | -           | -           | -          | -          | 0.3        |
| 1548        | elemol                                  | 0.9         | 0.6         | 0.1         | 0.5         | 0.7         | 0.2        | -          | -          |
| 1555        | elemicin                                | -           | -           | t           | -           | -           | 0.2        | 1.5        | -          |
| <b>1574</b> | <b>germacrene-D-4-ol</b>                | <b>t</b>    | <b>0.3</b>  | <b>0.6</b>  | <b>0.4</b>  | <b>0.3</b>  | <b>0.7</b> | <b>0.7</b> | <b>0.6</b> |
| 1582        | caryophyllene oxide                     | t           | t           | t           | t           | -           | -          | t          | -          |
| 1586        | gleenol                                 | -           | -           | -           | -           | -           | -          | -          | 0.3        |
| <b>1607</b> | <b><math>\beta</math>-oploponone</b>    | <b>t</b>    | <b>t</b>    | <b>t</b>    | <b>0.5</b>  | <b>t</b>    | <b>0.2</b> | <b>0.4</b> | <b>0.4</b> |
| 1608        | humulene epoxide II                     | t           | -           | t           | -           | t           | -          | -          | -          |
| 1618        | 1,10-di-epi-cubenol                     | -           | -           | -           | -           | -           | t          | t          | 0.2        |
| 1627        | 1-epi-cubenol                           | -           | -           | -           | -           | t           | t          | t          | 1.6        |
| 1630        | $\gamma$ -eudesmol                      | 0.2         | t           | -           | -           | -           | -          | -          | -          |
| <b>1638</b> | <b>epi-<math>\alpha</math>-cadinol</b>  | <b>t</b>    | <b>0.2</b>  | <b>0.3</b>  | <b>0.3</b>  | <b>0.2</b>  | <b>0.6</b> | <b>0.7</b> | <b>1.1</b> |
| <b>1638</b> | <b>epi-<math>\alpha</math>-muurolol</b> | <b>t</b>    | <b>0.2</b>  | <b>0.4</b>  | <b>0.4</b>  | <b>0.2</b>  | <b>0.6</b> | <b>0.7</b> | <b>1.2</b> |
| 1644        | $\alpha$ -muurolol                      | -           | t           | t           | t           | t           | t          | t          | 0.7        |
| 1649        | $\beta$ -eudesmol                       | 0.2         | t           | -           | t           | 0.1         | -          | 0.4        | -          |
| 1652        | $\alpha$ -eudesmol                      | 0.2         | 0.3         | -           | 0.5         | -           | -          | -          | -          |
| <b>1652</b> | <b><math>\alpha</math>-cadinol</b>      | <b>0.2</b>  | <b>0.3</b>  | <b>1.0</b>  | <b>0.6</b>  | <b>0.7</b>  | <b>1.2</b> | <b>1.6</b> | <b>1.8</b> |
| <b>1688</b> | <b>shyobunol</b>                        | -           | -           | <b>0.2</b>  | -           | <b>t</b>    | <b>0.2</b> | <b>0.2</b> | -          |
| 1739        | oplopanone                              | t           | t           | t           | 0.2         | t           | t          | t          | -          |
| 1987        | manoyl oxide                            | -           | -           | t           | t           | -           | t          | t          | 3.2        |

| KI   | compound               | osteo | #10 | #15 | #9 | #11 | #1 | grand | occid |
|------|------------------------|-------|-----|-----|----|-----|----|-------|-------|
| 2009 | epi-13-manoyl oxide    | -     | -   | -   | -  | -   | -  | -     | t     |
| 2056 | manool                 | -     | -   | t   | -  | -   | t  | t     | -     |
| 2055 | abietatriene           | -     | -   | t   | t  | -   | t  | t     | -     |
| 2298 | 4-epi-abietal          | -     | -   | t   | t  | -   | t  | t     | -     |
| 2312 | abieta-7,13-dien-3-one | 0.1   | -   | -   | -  | t   | -  | -     | -     |

KI = linear Kovats Index on DB-5 column. \*Tentatively identified. Compositional values less than 0.1% are denoted as traces (t). Unidentified components less than 0.5% are not reported.