MULTIVARIATE DETECTION OF HYBRIDIZATION USING CONIFER TERPENES I: ANALYSIS OF TERPENE INHERITANCE PATTERNS IN *CRYPTOMERIA JAPONICA* F_1 HYBRIDS

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ABSTRACT

The leaf volatile oils of two cultivars of *Cryptomeria japonica*, cv. Haava and cv. Kumotooshi were analyzed, along with their 22 hybrids. The compositions of leaf oils of cv. Haava and cv. Kumotooshi and several hybrids are reported. The oil of Haava contains appreciable amounts of cis-thujopsene, widdrol and cedrol (not found in Kumotooshi oil) that appear to be inherited as a group in the hybrids in a Mendelian fashion, with a second (dominant/ recessive) gene involved. PCO (Principal Coordinates analysis) using character weights of Fs (Fs from ANOVA between the parents) was found to be the most effective method to separate the parents and their hybrids. PCA (Principal Components Analysis) and PCO using equally weighted characters were found to be ineffective in detecting hybrids. The hybrids clustered in two groups: those with and those without the cis-thujopsene/ widdrol/ cedrol suite. Several hybrids' oils were very similar to the Haava parents' oil. *Phytologia 94(2):253-275 (August 1, 2012).*

KEY WORDS: *Cryptomeria japonica,* cv. Haava, cv. Kumotooshi, hybrids, essential oil, terpenes, inheritance, genetics.

There are few studies on methods for the detection of hybridization using conifer terpenes from known crosses. Adams (1982) used leaf terpenoids to compare Wells' hybrid distance diagrams, PCA, PCO, and canonical variate analysis, but he had to use putative natural hybrids in *Juniperus*. He found that PCO, using character weighting of F-1 (F ratios from ANOVA between the putative parents), was the most effective method tested.

Hanover (1966) analyzed the genetics of monoterpenes from the oleoresin in clones, F_1 hybrids and S_1 progeny of *Pinus monticola*. He found the inheritance of each terpene (except camphene) to be additive, with some heterotic or epistatic effects. Re-analysis of the Hanover (1966) data for parents and F_1 progeny (Fig. 1) shows that α-pinene is intermediate in 6/17 and transgressive in 11/17 F_1 individuals. β-pinene had 7/17 intermediate and 10/17 transgressive (Fig. 1) values. δ-3-carene appears to be mostly intermediate (14/17) with only 3/17 being transgressive (Fig. 1), as was the case for limonene (11/17 intermediate, 6/17 transgressive).

Hanover (1971) expanded his study on *P. monticola* and concluded that :

1. Monoterpenes were under strong, predictable genetic control involving one to several loci.
2. One compound, β-pinene, consistently occurred in larger concentrations in the progeny, which may be due to age effects.
3. A strong positive correlation was found between concentrations of δ-3-carene and terpinolene and negative correlations between α-pinene/ β-pinene and myrcene/ δ-3-carene. Otherwise, the compounds appeared to be independently inherited.
4. Negative correlations were found between monoterpene concentrations and progeny height growth rate.

To determine the number of genes controlling terpenes, Irving and Adams (1973) crossed *Hedeoma drummondii* x *H. reverchonii* and analyzed the parents, F_1, and F_2 progeny. They reported the terpenes were controlled by a minimum of 1 to 7 genes. Tucker and Kitto (2011) and Tucker (2012) have recently published an informative review of the genetics of *Mentha* and discusses transgressive variation.

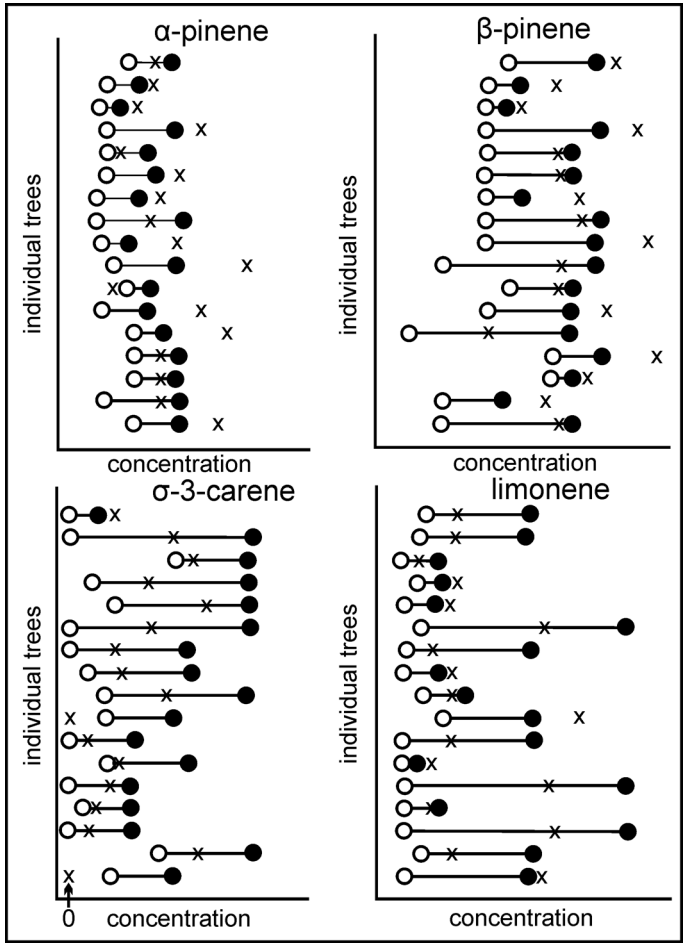

Figure 1. Graphs for four terpenes for 17 F_1 trees. Open and closed circles are the concentrations for parents 1 and 2 in the cross. x is the concentration in the F_1 individual tree (data from Hanover, 1966).

Squillace (1971) examined inheritance of monoterpenes in oleoresin of *Pinus elliotii* and conclude that β-pinene and myrcene were controlled by two alleles at a single locus, with high amounts being

dominant over low. Interestingly, this same pattern is evident in Fig. 1. Notice, that of the 30 transgressive individuals, 27 are in larger concentrations than the parents (Fig. 1).

Both quantitative variation and simple dominance has been reported in the inheritance of terpenes of Douglas fir (von Rudloff, 1984; von Rudloff and Rehfeldt, 1980) and Scots Pine (Pohjola, et al., 1989).

In the Cupressaceae, there have been very few studies on the inheritance of terpenes. One significant study in *Cupressus* (now *Hesperocyparis*) is that of Cool et al. (1975). They examined the leaf oils of *H. sargentii*, *H. macnabiana* and their putative natural hybrids. They analyzed oils from 36 trees in a single population and concluded that 12 were *H. sargentii*, 13 were *H. macnabiana* and 10 were intermediate in their oils. PCO analysis, using their terpene data with equal weights (Cool et al., 1975, Table 2), shows (Fig. 2) that

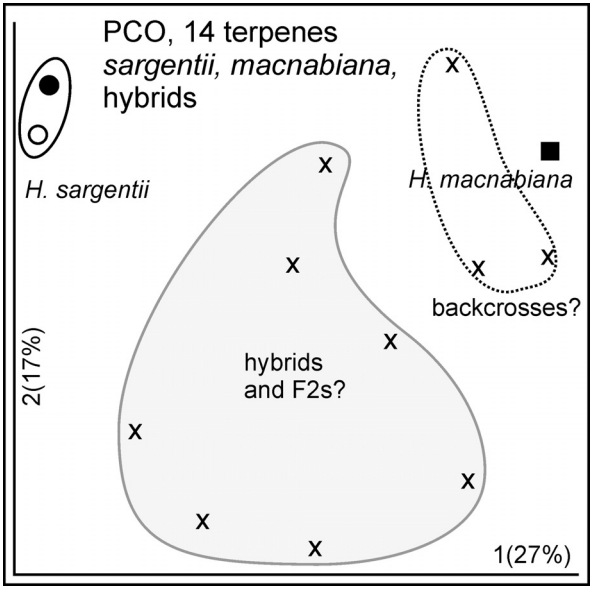

Figure 2. PCO of *H. sargentii, H. macnabiana* and putative hybrids (x). Data from Cool et al. (1975, Table 2).

the first and second eigenroots accounted for 27 and 17% of the variance among their samples. These low amounts of variance are likely due to the fact that only two samples of *H. sargentii* and one of *H. macnabiana* were present in the data set. Ordination (Fig. 2) reveals that the putative hybrids are quite dispersed between the parental species. From this ordination, it would appear that the putative hybrids (x) likely contain some F_{2s} and backcrossed individuals. However, if transgressive inheritance is involved, that could also explain the wide variation in the putative hybrids.

Seven of thirteen (7/13) terpenes (from Cool et al. 1975, Table 2) are transgressive. Only 4 of the 13 terpenes are mostly intermediate (camphene, sabinene, myrcene and β-phellandrene). The proportion of transgressive terpenes (7/13) is similar to that found in *P. monticola* (11/17, Fig. 1 above; Hanover, 1966). It appears that mixing biosynthetic genes can lead to over and under-expression of some terpenes.

Cryptomeria japonica D. Don (Sugi) is a monotypic genus (Farjon, 2005; Tsumura, 2011), endemic to Japan. Farjon (2005) argues that *C. fortunei* Hooibr. is conspecific, and a study (Kusumi et al. 2000) based on DNA sequencing, found no support for the recognition of *C. fortunei* separate from *C. japonica*. *Cryptomeria japonica* appears to have been introduced into China many years ago (Farjon, 2005) and is now widely cultivated in Japan, Taiwan, Korea, China and the Azores Islands (Tsumura, 2011). It is a very important commercial forest tree in Japan and the object of many detailed studies (see review, Tsumura, 2011) at the Forestry and Forest Products Research Institute and other institutes in Japan. The composition of the wood oil has been reported in the careful work of Nagahama and colleagues (Nagahama, 1964; Nagahama and Tazaki, 1993; Nagahama et al. 1996, 1998). Nagahama and Tazaki (1993) reported on the oil from wood of 14 sources and found an interesting polymorphism in appreciable amounts of cis-thujopsene/ cedrenes/ cedrol/ eudesmols/ elemol in 8 samples, and either absence or trace amounts in 6 samples.

The leaf oil of *Cryptomeria japonica* has been less examined. Shieh et al. (1981) reported taxon to have considerable amounts of elemol, cedrol, α-eudesmol, β-eudesmol, β-eudesmol that were

confirmed combined GCMS, IR and NMR, but the other components were only identified by GCMS. The development of new libraries utilizing both GCMS and retention time data (Adams 2007) make identification much more certain than in 1981. Nagahama et al. (1993) analyzed sesquiterpenes in leaf oils from 5 cultivars and found all contained appreciable amount of elemol, germacrene D-4-ol and hedycaryol with smaller amounts the eudesmols and α-cadinol. The leaf oil of two cultivars (Garin and Tosaaka) contained cedrol. Nagahama et al. (2001) compared the sesquiterpenes and diterpenes from leaves of 6 elite clones of *Cryptomeria japonica* and found that all were very high in *ent*-kaurene (kaur-16-ene), with moderate amounts of germacrene D-4-ol. Two clones had cedrol and α-thujopsenol. Most of the clones had elemol, eudesmols, and considerable hedycaryol.

More recently, Cheng et al. (2005) reported that the leaf oil of *Cryptomeria japonica* was dominated by *ent*-kaur-16-ene (40.6%), valencene (19.9%), eudesma-3,7(11)-diene (8.4%) and α-eudesmol (5.9%). However, the oil components were identified by the use of Wiley/NBS database of mass spectra, which, in the experience of the senior author, is not reliable for the unequivocal identification of terpenoids.

The purposes of the present paper are to report on a complete analysis of the volatile leaf essential oil of two cultivars of *Cryptomeria japonica* and their F_1 hybrids, and to compare various multivariate methods in the recognition of hybrids using terpenoid data.

MATERIALS AND METHODS

Leaves (2 branchlets, 15-20 cm long) were collected from *Cryptomeria japonica* cv. Haava (*Adams 13142*) and cv. Kumotooshi (*Adams 13143*) and 22 F_1 hybrids (#48, Adams 13144; #70, Adams 13145; #83, Adams 13146; #56, Adams 13147; #77, Adams 13123; #23, Adams 13149; #37, Adams 13150; #65, Adams 13151; #62, Adams 13152; #81, Adams 13153; #4, Adams 13224; #10, *Adams 13225*; #27, *Adams 13226*; #36, *Adams 13227*; #60, *Adams 13228*; #73, *Adams 13229*; #74, *Adams 13275*; #76, *Adams 13231*; #78, *Adams 13232*; #78, *Adams 13233*; #80, *Adams 13234*; #89, *Adams 13235,* growing at the Forestry and Forest Products Research Institute,

Tsukuba, Ibaraki, Japan. Voucher specimens are deposited in the Herbarium, Baylor University (BAYLU).

Air dried (30ºC, 24h) leaves (200 g) were steam distilled for 2 h using a circulatory Clevenger-type apparatus and trapped in a layer of diethyl ether (Adams, 1991). Nagahama et al. (1993) noted that steam distillation (actually hydrodistillation, where the leaves are placed into the boiling flask and may be subjected to reactions with the plant acids) resulted in the loss of elemol, germacrene D-4-ol and hedycaryol compared to hexane extraction. It should be noted that the modified Clevenger-type apparatus, having a diethyl ether solvent trap (see Fig. 4, Adams, 1991) utilizes a chamber to suspend the plant material, so only steam (not plant acids) is in contact with the materials. However, Nagahama et al. (1993) further demonstrated that germacrene D-4-ol and hedycaryol declined by 16% and 40%, respectively, when subjected to neutral steam. Because each sample in this study was distilled by neutral steam, the amount of degradation of germacrene D-4-ol and hedycaryol is proportional in all samples. The oil samples were concentrated with nitrogen and the samples stored at -20ºC until analyzed. The extracted leaves were oven dried (100ºC, 48 h) for determination of oil yields.

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 the HP Chemstation software with a J & W DB-5, 0.26 mm x 30 m, 0.25 micron coating thickness, fused silica capillary column run under the same conditions as the GCMS analysis (above).

Terpenoids (as percentage of total oil) were compared between the parents (3 replicate analyses) by ANOVA and SNK (Student-Newman-Keuls) analyses as described by Steele and Torrie (1960). Gower or Manhattan metric similarities (Gower, 1971; Adams, 1975) were computed among all individuals using character weighting of F-1

(F from ANOVA), and equal weights (wts = 1.0). Principle Component Analysis (PCA) and Principal Coordinate Ordination (PCO) were performed by factoring the associational matrix using the formulation of Gower (1966) and Veldman (1967).

RESULTS AND DISCUSSION

The compositions of the leaf essential oils of *C. japonica* cv. Haava and cv. Kumotooshi are given in Table 1. The oil of cv. Haava is dominated karu-16-ene (47.7%) with moderate amounts of sabinene and cis-thujopsene as well as α-cuprenene, widdrol and cedrol. The oil of cv. Kumotooshi is also dominated by karu-16-ene (28.4%), but contains moderate amounts of α-pinene, sabinene, limonene, β-phellandrene, bornyl acetate, elemol, and α-, β- and γ-eudesmols. The oils are highly significantly different in 21 components (Table 1) and significantly different in 5 components.

The oils in the hybrids were found to comprise two groups: 16 that showed complementation (cf. H x K 23, Table 1) and 6 that did not have the cis-thujopsene/ widdrol/ cedrol suite from parent cv. Haava (cf. H x K 74, Table 1).

Of the 17 major compounds, 7 were intermediate in concentration in the hybrids and 10 were transgressive. The distributions of values of components having intermediate inheritance (bornyl acetate, elemol, α-, β- and γ-eudesmols, bulnesol and kaur-16-ene) are shown in Figure 3. The concentration of bornyl acetate is skewed towards cv. Haava (Fig. 3). However, the other 6 compounds are generally scattered between parents, suggestive of multi-genic inheritance. Kaur-16-ene is generally intermediate, but three samples were slightly beyond the parents (Fig. 3).

The distributions of 8 of the 10 major compounds with transgressive inheritance are shown in Figure 4. Camphene and α-pinene are mildly transgressive in that most samples fall on or between the parents. Sabinene is the most extreme transgressive component, with parents' values of 8.5 and 11.6%, but hybrids ranged from 7.4 to 17.9%. cis-thujopsene, widdrol and cedrol all show similar patterns

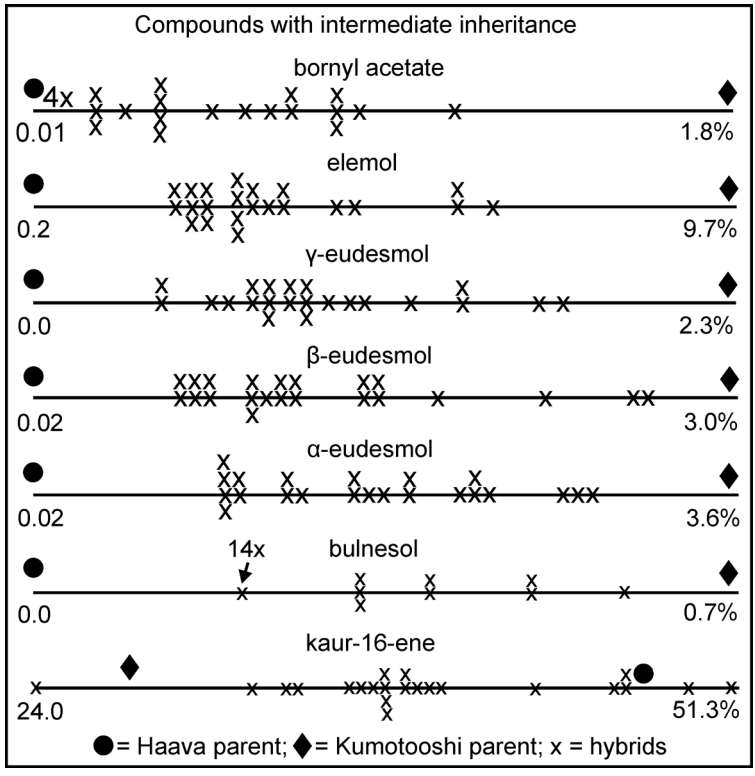

Figure 3. Distribution of compounds with intermediate inheritance.

with 6 of the same individuals having 0.0 amounts and 16 having amounts intermediate to greater than the Haava parent (Fig. 4). The ratio of 6:16 is very near 1:3 for a single locus, dominant gene (cis-thujopsene, widdrol, cedrol synthesized) vs. recessive gene (compounds absent). Due to the structural differences, it is very unlikely that cis-thujopsene, widdrol, and cedrol are produced by a single gene. It is more likely that the pathway is switched on by a gene leading to the synthesis of cis-thujopsene, widdrol and cedrol (along with other compounds associated with cedrol in the Cupressaceae: α- and β-cedrene, widdra-2,4(14)-diene, α-, γ-, and δ-cuprenene,

thujopsan-2-α-ol, and allo-cedrol). Notice that all of these compounds are present in Haava and absent in Kumotooshi parents (Table 1). In a related genus, *Juniperus*, these compounds are nearly always found only in the heartwood and not in the leaves (Adams, 2011), whether such compounds are found in the wood of Haava is not known, but Nagahama et al. (2001) reported α- and β-cedrene, thujopsene (cis?), cuparene and cedrol in the wood oil of cv. Tosaaka, whereas these

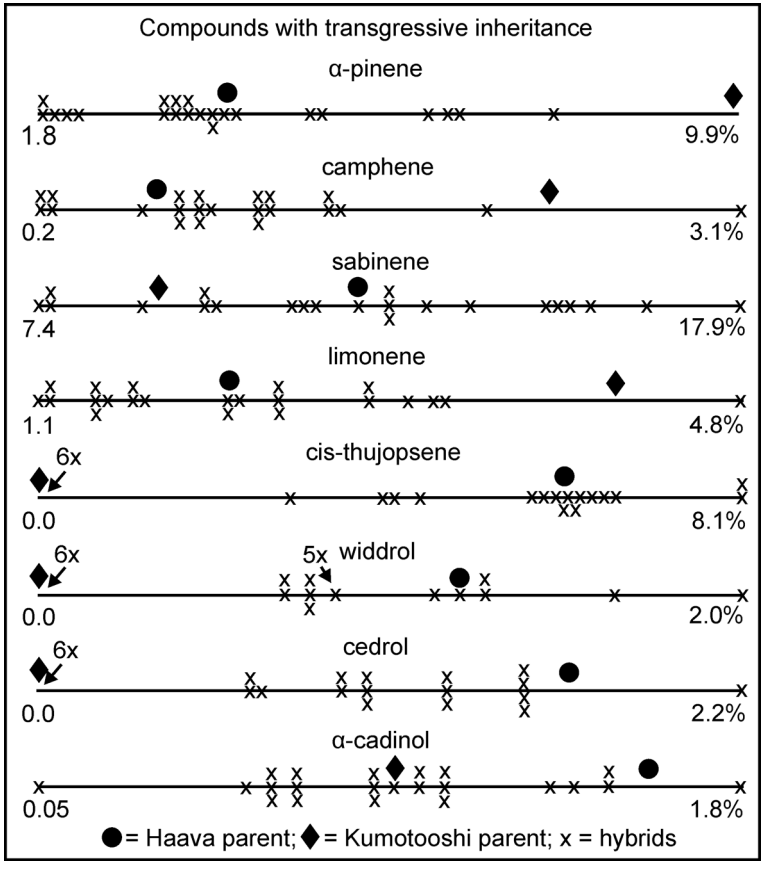

Figure 4. Distributions of compounds with transgressive inheritance.

compounds were absent in 5 other cultivars examined (they did not analyze the wood oils of cv. Haava or cv. Kumotooshi). Nagahama and Tazaki (1993) found cedrol, etc. to be present in the wood oil of cv obisugi, but absent in 6 other Sugi accessions.

To examine correlations among the components, PCA was performed on 29 terpenoids (> 0.4% conc.) utilizing components from three replicates of each parent and 22 hybrids. The first component (PC) accounted for 38% of the variance among the samples and the second PC removed 18%. A plot of the components on the first 2 PCs (Fig. 5) shows several inheritance patterns. Notice the tight clusters

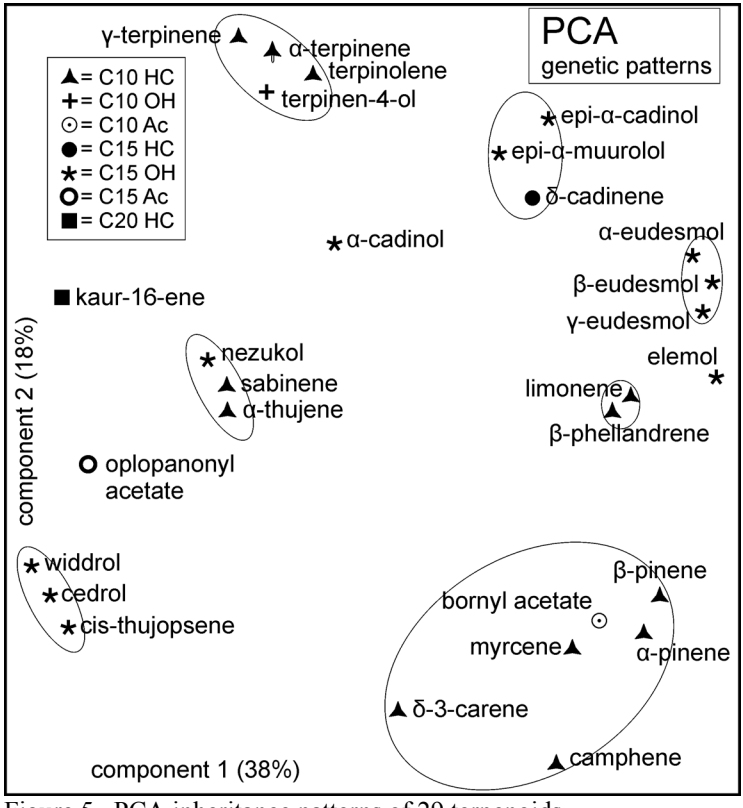

Figure 5. PCA inheritance patterns of 29 terpenoids.

clusters of cis-thujopsene/ cedrol/ widdrol; nezukol/ sabinene/α-thujene; α- and γ-terpinene/ terpinolene/ terpinen-4-ol; epi-α-cadinol/ epi-α-muurolol/ δ-cadinene; α-, β- and γ-eudesmols; and limonene/ β-phellandrene. The monoterpenes (α- and β-pinene, myrcene, δ-3-carene and camphene) form a loose group with bornyl acetate.

Histograms of cis-thujopsene, sabinene and kaur-16-ene are shown in Figures 6, 7, and 8. The histogram of cis-thujopsene (Fig. 6) shows a 3:1 inheritance. However, a 3:1 Mendelian inheritance would presume that each parent was heterozygous. Yet, the absence of cis-thujopsene/ widdrol/ cedrol) in parent Kumotooshi (Fig. 4) suggests it is homozygous recessive. There appears to be a modifier gene involved.

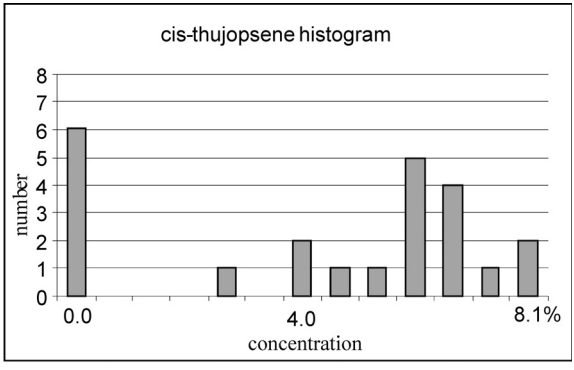

Figure 6. Histogram of cis-thujopsene (widdrol and cedrol had similar histograms).

The histogram of sabinene shows a second mode of variation in that the distribution is nearly continuous (Fig. 7). This suggests multi-genic control.

The histogram for kaur-16-ene displays a third pattern of variation that suggests two or more genes controlling the expression of kaur-16-ene.

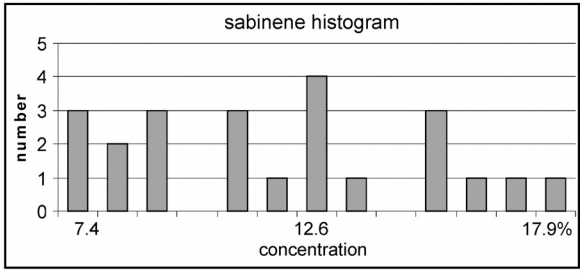

Figure 7. Histogram for sabinene.

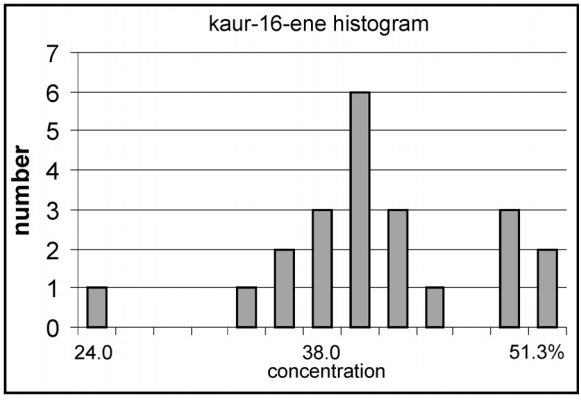

Figure 8. Histogram for kaur-16-ene.

The detection of hybrids in nature using terpenoids can present a challenge. Ordination of the parents and their artificial hybrids using PCA (Fig. 9) gives an incomplete separation. This was also found by Adams (1982) in putative natural hybridization of *Juniperus* species. Notice (Fig. 9) that the hybrids (cf. 80, 89) are depicted very near parent H (Haava). However, the group of 6 individuals, that do not contain the dominant compounds (cis-thujopsene, cedrol, widdrol), are intermediate between parents H and K (Fig. 9). In general, Adams (1982) found that correlation was not as useful as similarities in classifying hybrids.

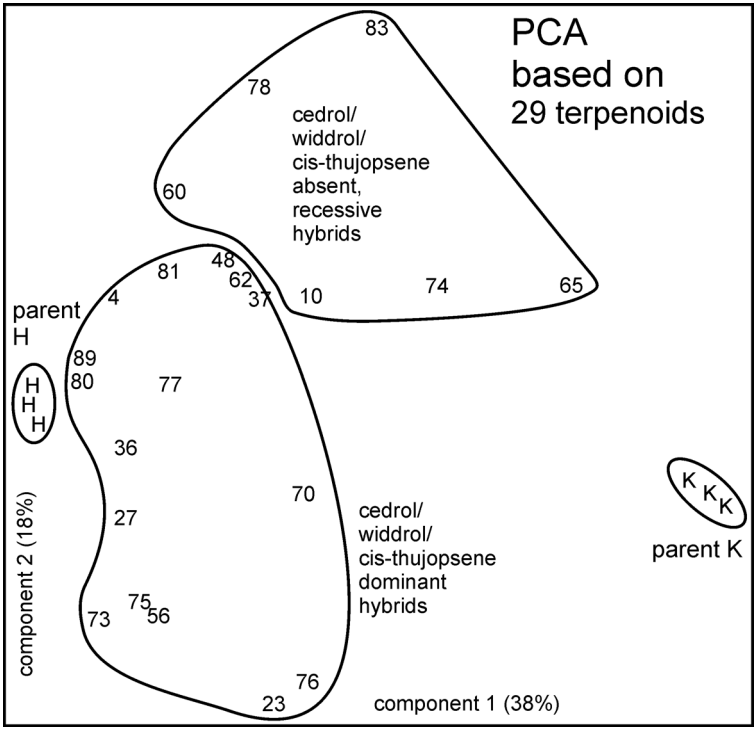

Figure 9. PCA of parents and hybrids using 29 terpenoids.

PCO using equally weighted terpenoids (Fig. 10) in the calculation of similarity measures gave some improvement over PCA (cf. Figs. 9, 10). The cis-thujopsene/ widdrol/ cedrol-absent hybrids are more intermediate between the parents. This PCO accounted for 27% and 13% of the variance among samples. However, plants 80, 4, 89, and 36 are very near the H (Haava) parent and their separation little improved from the PCA analysis (cf. Figs. 9, 10).

Adams (1982) investigated the use of statistically derived character weighting and found that weighting the similarity by F ratios (F from ANOVA between the parents) improved the detection of

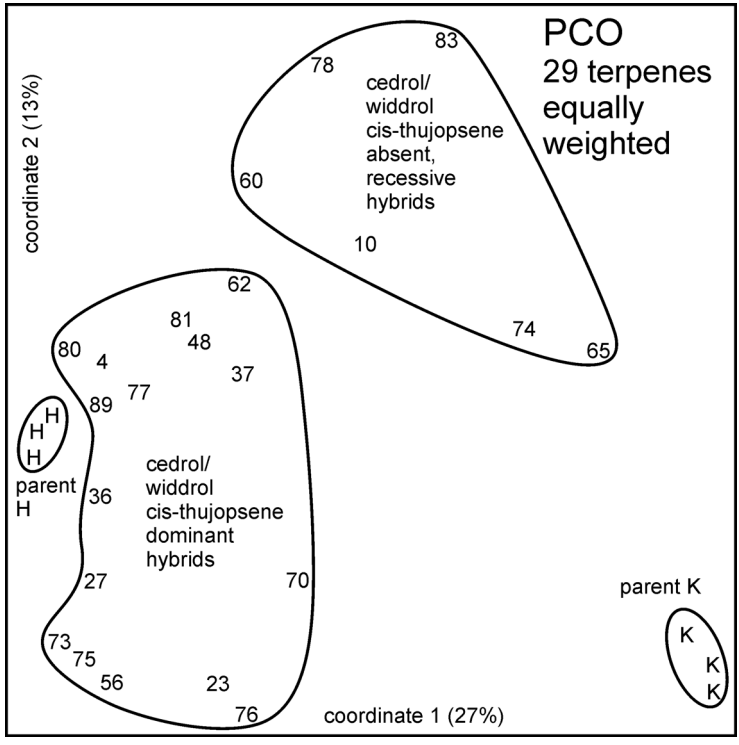

Figure 10. PCO based on 29 terpenoids, equally weighted.

hybridization. PCO using F ratio weighted characters (Fig. 11) tightened the cluster of the cis-thujopsene/ widdrol/ cedrol-absent group and placed them in a very intermediate position between the parents. F weighed PCO accounted for 43% and 11% of the variance among samples. Thus, the use of F ratio weighting increased the separation between parents H and K from 27% to 43% making the ordination of the hybrids much more distinct. The group cis-thujopsene/ widdrol/ cedrol group is still quite near parent H (Fig. 11).

It may be that compounds that are clearly inherited as dominant/ recessive should be remove from the data. To test this idea,

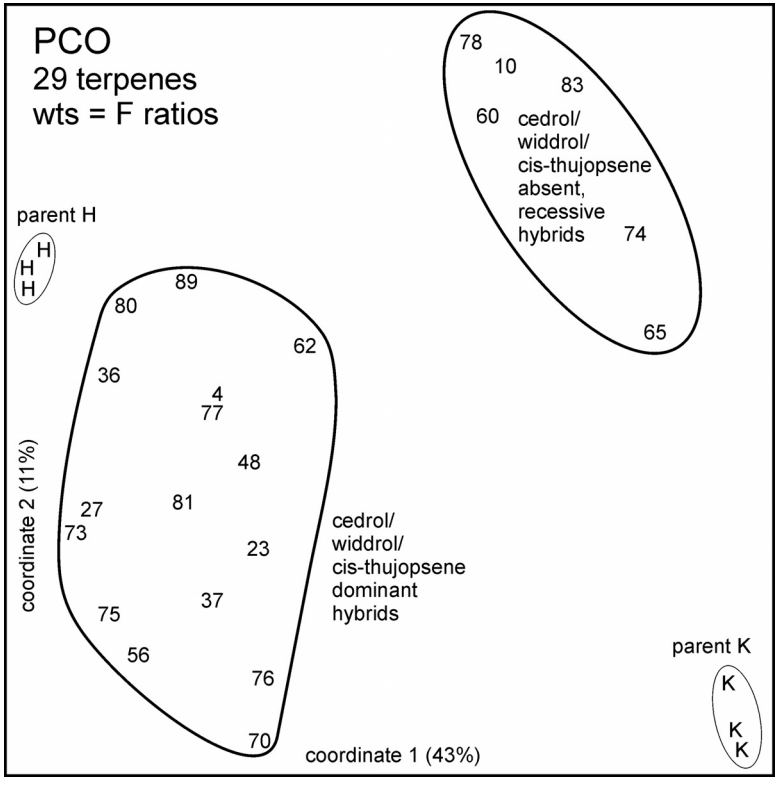

Figure 11. PCO based on 29 terpenes using F ratios for character weights.

cis-thujopsene, widdrol and cedrol were removed from the data set and a new PCO was run. Most of the hybrids that contained cis-thujopsene, widdrol and cedrol were moved into the intermediate group (Fig. 12). However, 6 hybrids (23, 27, 56, 73, 75, 76) remained near parent H (Fig. 12). It may be that some maternal inheritance is involved in these individuals. In general, the detection of hybrids is much better with the three dominant/ recessive cis-thujopsene/ widdrol/ cedrol components removed from the analyses. Although this was easily done with artificial hybrids, it may be more difficult to determine dominant/

recessive components in hybridization (including backcrossing) in natural populations.

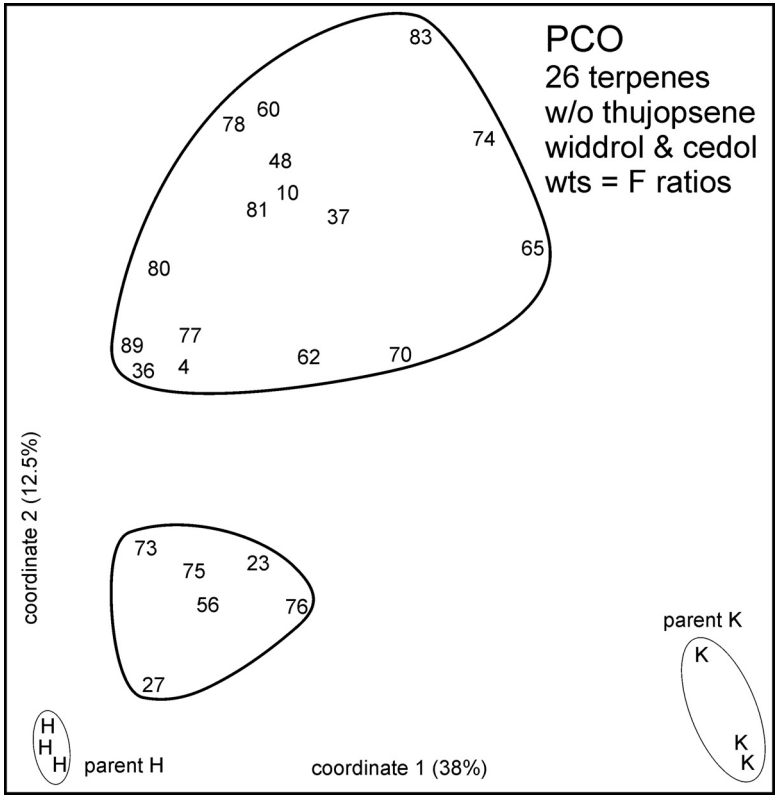

Figure 12. PCO with weighting by Fs, without cis-thujopsene, widdrol and cedrol.

In summary, the detection of hybrids by chemical means from artificial crosses of two cultivars of *C. japonica* was not easy, due to transgressive variation and Mendelian heritance of several compounds found only in one parent (cv. Haava). The use of F weighted similarities greatly improved the detection of hybrids and the removal

of dominant/ recessive components further added in the identification of hybrids. Additional artificial crosses of conifers will need to be examined to develop robust methods for the analysis of putative, natural hybridization situations.

ACKNOWLEDGEMENTS

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

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Table 1. Comparison of leaf essential oils of *Cryptomeria japonica*, cv. Haava, Kumotooshi, and selected hybrids: H x K 23, hybrid with compounds from each parent present, H x K 74, hybrid without cis-thujopsene/widdrol/cedrol suite of components (from Haava). Components that clearly differ between Haava and Kumotooshi are in bold face. F significance (F sig.): * = P 0.05, ** = P 0.01, ns = not significant, nt = not tested.

KI	compound	Haava	Kumo	F sig.	HxK 23	HxK 74
921	tricyclene	t	0.3	nt	0.4	0.1
924	α-thujene	0.8	0.6	10.8*	1.1	0.5
932	**α-pinene**	**3.9**	**9.9**	**93.1****	**8.2**	**4.8**
946	**camphene**	**0.4**	**2.4**	**236****	**3.1**	**0.5**
969	**sabinene**	**11.6**	**8.5**	**18.0***	**17.9**	**8.3**
974	**β-pinene**	**0.3**	**0.8**	**102****	**0.5**	**0.3**
988	**myrcene**	**2.2**	**3.6**	**34.6****	**4.1**	**1.8**
1002	α-phellandrene	t	t	nt	-	t
1008	**δ-3-carene**	**0.3**	**0.9**	**120****	**1.4**	**0.1**
1014	α-terpinene	1.2	1.1	4.0 ns	1.1	0.9
1020	p-cymene	0.3	0.1	nt	0.3	0.1
1024	**limonene**	**2.1**	**4.2**	**66.2****	**1.5**	**3.0**
1025	**β-phellandrene**	**1.6**	**2.7**	**29.4****	**1.2**	**2.0**
1044	(E)-β-ocimene	t	t	nt	-	t
1054	γ-terpinene	2.1	1.7	7.0 ns	2.0	1.5
1065	cis-sabinene hydrate	0.4	0.3	nt	0.5	0.2
1086	terpinolene	0.8	0.9	2.1 ns	0.7	0.6
1098	trans-sabinene hydrate	0.4	0.4	nt	0.4	0.2
1110	1-coten-3-yl acetate	t	t	nt	-	-
1112	trans-thujone	t	t	nt	t	t
1118	cis-p-menth-2-en-1-ol	0.2	0.1	nt	0.2	0.1
1136	trans-p-menth-2-en-1-ol	0.1	t	nt	0.2	0.1
1141	camphor	-	t	nt	t	-
1145	camphene hydrate	-	t	nt	t	-
1165	borneol	-	-	nt	0.1	-
1174	terpinen-4-ol	2.9	2.3	8.3 *	3.2	2.0
1186	α-terpineol	0.1	0.1	nt	0.1	0.1
1254	linalyl acetate	t	t	nt	0.1	0.2
1287	**bornyl acetate**	**t**	**1.8**	**292****	**0.1**	**0.3**
1289	trans-sabinyl acetate	t	t	nt	-	t
1346	α-terpinyl acetate	t	0.2	nt	0.2	t
1389	β-elemene	-	0.1	nt	-	-

KI	compound	Haava	Kumo	F sig.	HxK 23	HxK 74
1407	longifolene	-	0.1	nt	-	-
1373	α-ylangene	t	-	nt	-	-
1396	α-chamipinene	t	-	nt	-	-
1410	α-cedrene	t	-	nt	t	-
1413	β-funebrene	t	-	nt	t	-
1419	β-cedrene	t	-	nt	0.2	-
1429	**cis-thujopsene**	**5.9**	**-**	**340****	**5.7**	-
1454	(E)-β-farnesene	t	-	nt	-	-
1478	γ-muurolene	0.3	0.2	nt	0.3	-
1481	widdra-2,4(14)-diene	0.3	-	nt	0.3	-
1480	germacrene D	t	0.1	nt	0.7	-
1485	β-selinene	-	t	nt	-	-
1493	trans-muurola-4,5-diene	-	0.2	nt	-	-
1500	**α-muurolene**	**-**	**0.4**	**298****	**0.3**	**0.1**
1505	**α-cuprenene**	**0.8**	**-**	**304****	**0.3**	-
1513	γ-cadinene	0.4	0.4	nt	0.4	0.5
1522	δ-cadinene	1.4	1.5	0.92ns	0.9	1.6
1532	γ-cuprenene	0.3	-	nt	0.4	t
1537	α-cadinene	t	0.1	nt	t	0.1
1542	δ-cuprenene	t	-	nt	t	-
1548	**elemol/hedycaryol**	**0.2**	**9.7**	**271****	**2.4**	**6.8**
1574	germacrene-D-4-ol	0.8	0.9	nt	1.2	1.3
1586	thujopsan-2-α-ol	0.1	-	nt	-	-
1589	allo-cedrol	t	-	nt	-	-
1599	**widdrol**	**1.2**	**-**	**432****	**0.6**	-
1600	**cedrol**	**1.6**	**-**	**675****	**0.7**	-
1607	β-oplopenone	0.1	-	nt	t	-
1630	**γ-eudesmol**	**-**	**2.3**	**339****	**0.7**	**1.8**
1638	epi-α-cadinol	0.6	0.7	16.2*	0.3	0.8
1638	epi-α-muurolol	0.5	0.7	3.5ns	0.3	0.8
1644	α-muurolol	t	t	nt	t	t
1649	**β-eudesmol**	**t**	**3.0**	**296****	**0.9**	**2.6**
1652	**α-eudesmol**	**t**	**3.6**	**268****	**1.0**	**3.0**
1652	**α-cadinol**	**1.6**	**0.9**	**42.4****	**0.5**	**1.1**
1670	**bulnesol**	**-**	**0.7**	**308****	**0.2**	**0.5**
1685	α-bisabolol	0.1	t	nt	0.1	-
1887	**oplopanonyl acetate**	**0.4**	**-**	**295****	t	-
1932	beyerene	-	t	nt	-	-
1932	C_{20}, 41,55,257,272	-	0.5	nt	-	0.1
1958	isopimara-8(14),15-diene	0.2	0.2	nt	0.1	0.3

KI	compound	Haava	Kumo	F sig.	HxK 23	HxK 74
1999	kaur-15-ene	0.2	0.3	nt	0.3	0.3
2009	epi-13-manoyl oxide	-	t	nt	t	-
2034	**kaur-16-ene**	**47.7**	**28.4**	**38.8****	**24.0**	**47.7**
2055	abietatriene	0.4	0.3	nt	0.3	-
2132	nezukol	0.8	0.6	12.0*	0.1	0.7
2331	trans-ferruginol	0.2	t	nt	0.2	0.2

KI = Kovat's Retention Index on DB-5(=SE54) column using alkanes.
Compositional values less than 0.1% are denoted as traces (t).
Unidentified components less than 0.5% are not reported.