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CHEMICAL VARIATION WITHIN THE GENUS *ZIERIA**

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Key Word Index—*Zieria*; Rutaceae; essential oils; chemical variation.

Abstract—Comparison of the essential oil components of about 200 samples representing most of the known *Zieria* taxa showed considerable chemical variation. The existence of interspecific, intraspecific and interpopulational chemotypes was established.

INTRODUCTION

The genus *Zieria* Sm. (tribe Boronieae) consists of prostrate shrubs to small trees with small white or pink flowers and mostly trifoliolate leaves. All but one species, *Z. chevalieri* from New Caledonia, are endemic to eastern Australia. The taxonomic revision [2–4] and concurrent identification of essential oil constituents of all available taxa within the genus [1] have presented a rare opportunity to record chemotaxonomic trends within an entire genus. Although previous investigators of *Zieria* essential oils were primarily interested in the structural elucidation of components [1], Penfold pointed to the "probable occurrence of physiological forms" due to the predominance of either safrole, methyl eugenol or elemicin in the essential oils of *Z. smithii* [5, 6]. We now attempt to correlate the essential oil composition of all *Zieria* subspecies with their taxonomic divisions.

The *Zieria* nomenclature used in this paper is defined by Powell and Armstrong [2] and is summarized in Table 1.

RESULTS AND DISCUSSION

The division of *Zieria* taxa into eight chemical groups based on essential oil constituents [1] has provided appropriate divisions for discussion of chemical variation between taxa.

* Part 6 in the series "Phytochemistry of the Genus *Zieria*". For Part 5, see ref. [1].

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Table 1. *Zieria* nomenclature and accession numbers used in this study

Taxa sampled	Taxa as defined in ref. [2]	Accession Nos
1. *Z. arborescens* sens. strict.	*Z. arborescens* subsp. 'a'	403, 406, 516, 517, 518, 951, 1138, 1306, 1359, 1378, 1388, 1436, 1437, 1442, 1478, 1479
2. *Z. arborescens* 'b' form (ridged stem form)	*Z. arborescens* subsp. 'b'	398
3. *Z. arborescens* 'c' form (broad leafed form)	*Z. arborescens* subsp. 'c'	118, 862, 1374, 1449a, 1455b, 1458a, 1458b, 1459a, 1464, 1465, 1471, 1473, 1474, 1476, 1477
4. *Z. arborescens* 'd' form (glabrous form)	*Z. arborescens* subsp. 'd'	1211
5. *Z. arborescens* 'e' form (hairy stem form)	*Z. arborescens* subsp. 'e'	455, 465, 1375, 1469, 1470
6. *Z. arborescens* 'f' form (narrow leafed form)	*Z. arborescens* subsp. 'f'	1314, 1316, 1317, 1435
7. *Z. aspalathoides* sens. strict.	*Z. aspalathoides* subsp 'a'	519, 942, 1154, 1156, 1171, 1187, 1305, 1356, 1357, 1424, 1432, 73–091, 78–075
8. *Z. aspalathoides* 'b' form (broad leafed form)	*Z. aspalathoides* subsp. 'b'	1303, 1447, 1463
9. *Z. aspalathoides* var. nov. 1	*Z.* sp. nov. 'B'	729
10. *Z. aspalathoides* var. *obovatum*	*Z.* sp. nov. 'A'	856, 1168
11. *Z. chevalieri*	*Z. chevalieri*	1296
12. *Z. collina*	*Z. collina*	
13. *Z. compacta*	*Z. fraseri* subsp. 'b'	207, 829, 943, 943b, 1244, 1485, 75–018

Table 1. (*Continued*)

Taxa sampled	Taxa as defined in ref. [2]	Accession nos.
14. *Z. cytisoides* sens. strict.	*Z. cytisoides* subsp. 'a'	94, 112, 522, 1030, 1111, 1113, 1155, 1301, 1431, 1431b1, 1431b2, 1431c1, 1431c2, 1431c3, 1448b, 79–074, 79–075
15. *Z. cytisoides* 'b' form (coastal form)	*Z. cytisoides* subsp. 'b'	91, 248, 307, 399, 436, 927,
16. *Z. furfuracea* sens. strict.	*Z. furfuracea* subsp. 'a'	212, 371, 1454, 1462c, 1462d
17. *Z. furfuracea* 'b' form (warty leafed form)	*Z. furfuracea* subsp. 'b'	
18. *Z. granulata* var. *granulata*	*Z. granulata*	402, 1373b, 1468, 1481
19. *Z. granulata* var. *adenodonta*	*Z.* sp. nov. C subsp. 'b'	311, 896, 1457b, 1460
20. *Z. involucrata*	*Z. involucrata*	486, 1346
21. *Z. laevigata* sens. strict.	*Z. laevigata* subsp. 'a'	390, 525, 1428, 1430, 1440, 1444, 1445, 1484
22. *Z. laevigata* var. *fraseri*	*Z. fraseri* subsp. 'a'	1043, 1184, 1185, 1186, 1188, 1197, 1201, 1202, 1204, 1387, 1422
23. *Z. laxiflora*	*Z. laevigata* subsp. 'b'	350, 457, 794, 795, 796, 797, 1183, 1404, 1405, 1419a, 1419b, 1455
24. *Z. minutiflora* sens. strict.	*Z. minutiflora* subsp. 'a'	416, 1110
25. *Z. minutiflora* 'b' form (hairy fruited form)	*Z. minutiflora* subsp. 'b'	526, 1166, 1169, 1172, 1322, 1410, 1410b, 1482
26. *Z. murphyi*	*Z. murphyi*	1027, 1288, 1400, 1439, 1443
27. *Z. obcordata*	*Z. obcordata*	1302, 1354
28. *Z. pilosa*	*Z. pilosa*	528, 906, 1109, 1401a, 1401b, 1427
29. *Z. robusta*	*Z. robusta*	167, 483, 1221, 1222, 1290, 1344, 1358
30. *Z. smithii* sens. strict.	*Z. smithii* subsp. 'a'	127, 325, 372, 400, 1081, 1084, 1085,1094, 1102, 1128, 1189, 1203, 1227, 1385, 1389, 1402, 1403, 1416, 1420, 1425, 1429, 1446, 1449b 1459b, 1459c, 1462b, 1472, 1475, 1480, 1486, 80–049
31. *Z. smithii* 'b' form (tomentose form)	*Z. smithii* subsp. 'b'	750, 751, 752, 753, 1164, 1173, 1179, 1411
32. *Z. smithii* 'c' form (glabrous form)	*Z. smithii* subsp. 'c'	367, 375, 1021, 1022, 1453, 1457a, 80–045, 80–046
33. *Z. smithii* 'd' form (robust mountain form)	*Z.* sp. nov. 'E' subsp. 'a'	1199, 1205, 1332, 1462
34. *Z. smithii* 'e' form (glabrous mountain form)	*Z.* sp. nov. 'E' subsp. 'b'	1406, 1407, 1408, 1409
35. *Z. smithii* 'f' form (prostrate mountain form)	*Z.* sp. nov. 'E' subsp. 'c'	1176
36. *Z.* sp. aff. *arborescens*	*Z.* sp. nov. 'F'	391, 523, 1101, 1280, 1390, 1399
37. *Z.* sp. aff. *aspalathoides*	*Z.* sp. nov. 'H'	1421, 1483
38. *Z.* sp. aff. *involucrata*	*Z.* sp. nov. 'I'	199, 420, 908
39. *Z.* sp. aff. *laevigata*	*Z.* sp. nov. 'D'	1304, 1355
40. *Z.* sp. aff. *smithii*	*Z.* sp. nov. 'J'	1278, 1376, 1450, 1451, 1452, 1456
41. *Z.* sp. nov. 1	*Z.* sp. nov. 'K'	1226
42. *Z.* sp. nov. 11	*Z.* sp. nov. 'G'	387, 401
43. *Z. veronicea*	*Z. veronicea*	1433, 1434

Group 1

Within this aldehyde-rich group, *Z. aspalathoides* var. nov. 1 and *Z. veronicea* have chemical affinities as they are the only *Zieria* taxa containing the 'lemon scent' citronellal. They do not, however, possess close morphological similarities. *Z. aspalathoides* var. nov. 1 is closely related to *Z. aspalathoides*. *Z. veronicea* is morphologically a very distinct species but is probably also closest in affinity to *Z. aspalathoides*.

The chemical data then support the morphological evidence in indicating that *Z. aspalathoides* var. nov. 1 warrants elevation to species rank [2] to parallel the *Z. veronicea* separation (Table 1).

Group 2

Z. laevigata var. *fraseri*, *Z. compacta* and *Z. cytisoides* sens. strict. (groups 5.2 and 6) consistently contain benzaldehyde at varying levels (Table 2). This supports the proposal [2] to group *Z. laevigata* var. *fraseri* with *Z. compacta* as subspecies of *Z. fraseri* because of morphological similarities. On the other hand, the morphological distinctness of *Z. cytisoides* overrules chemical implications that *Z. cytisoides* is associated with this species.

Table 2. Essential oil compositions of *Zieria* species with benzaldehyde-rich chemovars

	Z. compacta				*Z. cytisoides* sens. strict.													*Z. laevigata* var. *fraseri*			
Collection No. / RR_t component	207	829	943 a	943 b	94	522	1113	1301	1431 a	1431 b1	1431 b2	1431 c1	1431 c2	1431 c3	1448 b	79/ 074	79/ 075	1043	1201	1387	1422
17 α-Pinene					34.6	36.9	47.5	19.6	22.8	7.2	0.1	7.0	8.2	2.1	14.4	4.8	21.9	19.4			23.4
26 Limonene	6.0	0.8	22.0		3.6	3.3	5.0	2.5							24.8				7.0		18.2
27 β-Phellandrene					15.2	13.1	20.2	8.5		6.3	0.2	7.3	7.3	2.9	14.7						
59 Chrysanthenone	14.0				5.8	0.2		1.8													
60 Benzaldehyde	48.2	27.1	10.8	91.6	7.2	10.9	7.2	1.1	5.9	8.2	31.9	15.0	14.5	58.9	4.9	2.5	9.8	11.8	84.4		
61 *trans*-Chrysanthenyl acetate										59.7	57.4	50.5	45.9	24.3							
67 *cis*-Chrysanthenyl acetate									51.0						7.3	50.4	54.1				
71 β-Caryophyllene	2.6	20.7	6.1													15.7					
79 Humulene	0.6	6.7	1.9																		
85 Naphthalene	5.7	13.0	13.7		15.0	4.7	4.0	15.7							3.5			10.3	2.0	9.3	11.3
86 $C_{15}H_{24}$	2.2	5.4						10.0							4.5			8.0			12.6
87 Carenone			12.4																		
100 Safrole	2.8	2.5	1.5	0.7														2.4		15.4	6.8
115 Methyl eugenol					5.0			12.3										15.9		33.8	
130 M^+ 216	1.0	3.8	5.1			1.8		6.0							2.4			4.9	14.1		

Group 3

Methyl eugenol-rich oils are almost entirely restricted to *Z. smithii* sens. strict. The only exceptions were occasional samples of *Z. laevigata* var. *fraseri* and *Z. cytisoides* 'b' form. *Z. arborescens* sens. strict. and 'c' form, *Z. smithii* sens. strict., 'e' and 'f' forms and *Z.* aff. *smithii* all have safrole chemotypes with minor components indicating further subdivisions. The safrole chemotype of *Z. arborescens* sens. strict. has a complex oil with major component safrole at low levels (13–15%). *Z. smithii* 'e' form has significant quantities of chrysanthenone as a secondary component whereas 'f' form contains eugenol (28%) in association with safrole. *Z.* sp. aff. *smithii* consistently gives an oil containing predominantly safrole and is thus almost identical chemically to the safrole chemotypes of *Z. smithii* sens. strict. and *Z. arborescens* 'c' form which contain associated quantities of α-pinene. Several members of this taxonomic group (*Z. arborescens* 'c' and 'e' forms and *Z. smithii* sens. strict., 'c' and 'd' forms) also have elemicin chemotypes. In *Z. arborescens* 'e' form, which is only known from the one location, safrole or α-pinene can replace elemicin as the major component as all three compounds are major constituents. Again it is members of this group along with *Z. chevalieri* that possess methoxystyrene chemotypes. *Z. arborescens* 'c' form is rich in either 2,3,4,6-tetramethoxystyrene or 2,6-dimethoxy-3,4-methylenedioxystyrene. The former styrene is the predominant component of *Z. smithii* 'b' form and the latter the predominant component of *Z. smithii* 'd' form. The styrene component of *Z. chevalieri* is the distinctively different trimethoxystyrene which is consistent with its unique geographical location (New Caledonia) but also indicating some chemical affinities with the *Z. arborescens–smithii* group to which it is only distantly related. *Z. chevalieri* is morphologically more closely related to the *Z. fraseri* and *Z. laevigata* groups.

The predominance of aromatic methyl ethers in this group indicates a positive correlation between essential oil constituents and classical morphology within the closely related *Z. arborescens–smithii*–sp. aff. *smithii* group. Moreover, *Z. smithii* sens. strict. provides the best example of clearcut infrasubspecific variation as all 26 samples fall cleanly into one of three chemical varieties: chemovar A with 40–90% methyl eugenol, chemovar B with 65–85% safrole and chemovar C with 35–85% elemicin. This is consistent with the earlier variation observed by Penfold [5, 6] but has no obvious geographical correlation (Fig. 1). Some geographical correlations are apparent, however, with the *Z. arborescens*–aff. *arborescens–smithii* group, which shows a decline in terpenoid (e.g. zierone) content and a corresponding increase in phenolic ethers as collections move north over the 25° latitude of their distribution range (Fig. 2). Investigation of a *Z. smithii* 'e'

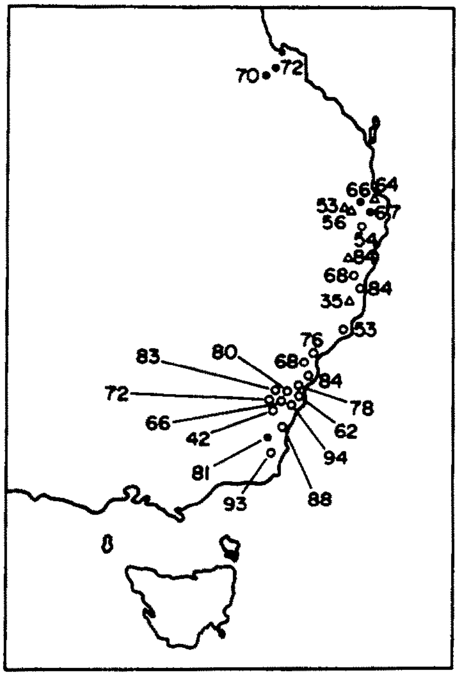

Fig. 1. Geographical variation in the essential oil composition of *Zieria smithii* sens. strict. showing the percentage contributions of methyl eugenol (○), safrole (●) and elemicin (△).

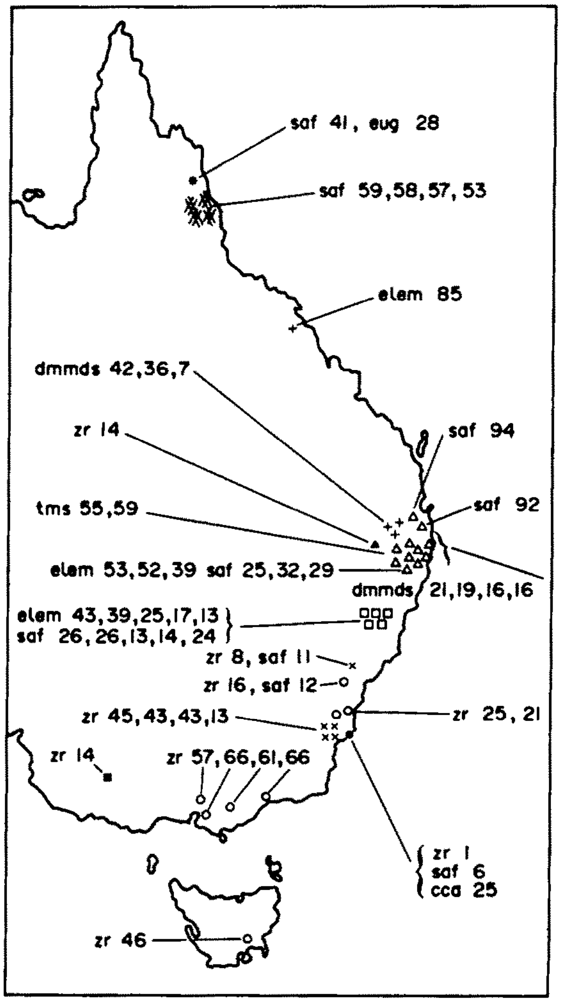

Fig. 2. Geographical variation in the essential oil composition of the *Zieria arborescens–smithii* group showing zierone (zr), safrole (saf), dimethoxymethylene dioxystyrene (dmmds), tetramethoxystyrene (tms), elemicin (elem) and eugenol (eug) percentages for *Z. arborescens* sens. strict. (○), 'b' form (●), 'c' form (△), 'd' form (▲), 'e' form (□), 'f' form (■), *Z.* sp. aff. *arborescens* (×) and *Z. smithii* 'd' form (+), 'e' form (⋕) and 'f' form (•).

form population on Thornton Peak, North Queensland showed remarkable constancy of essential oil composition (Table 3) in contrast with the varying benzaldehyde composition of a *Z. cytisoides* sens. strict. population in the Wolgan Valley area of NSW (see Table 2, 1431 c1–1431 c3).

The chemistry of this complex group supports the close morphological relationship between *Z. arborescens* and *Z. smithii* and more distant associations to *Z. cytisoides*, *Z. laevigata* and *Z. chevalieri*.

Group 4

Several morphologically distinct species have car-3-en-2-one as their major component along with smaller quantities of the structurally related terpinolene, car-3-ene and eucarvone monoterpenes. Three samples of *Z. granulata* var. *adenodonta* collected from the two known distribution centres of this uncommon species were all rich in this ketone. Similarly, *Z.* sp. nov. 11 samples collected from its only known location were rich in car-3-en-2-one. *Z. aspalathoides* sens. strict., which is much more common and geographically widespread, was generally (but not exclusively) rich in car-3-en-2-one. One fresh and two aged samples from the more northern end of the distribution contained very little of this ketone. Similarly, three *Z. furfuracea* sens. strict. samples from two locations were rich in car-3-en-2-one. The one low car-3-en-2-one exception (0.1 % oil yield) was, however, collected from an identical environment about 1 km from one of the typical carenone oil bearing shrubs (1.5 % oil yield). On the other hand, of the eight *Z. laevigata* sens. strict. samples investigated, only one was rich in carenone, the others containing predominantly hydrocarbons (group 6), along with *Z. laevigata* var. *fraseri*, which is also represented in group 6. Morphologically two of the members of this (group 4) chemical group, *Z. granulata* var. *adenodonta* and *Z.* sp. nov. 11, are closely related to *Z. granulata* and *Z.* sp. nov. 1 from the chrysanthenone group (group 5.1) and are in turn closely related to *Z. furfuracea* sens. strict. (also in group 4), which in turn shows affinities with *Z. smithii* sens. strict. (group 3). Moreover, *Z.* sp. aff. *involucrata*, presumably a hybrid (*Z.* sp. aff. *arborescens* × *Z. cytisoides* sens. strict.) is represented in both this car-3-en-2-one group (group 4) and the chrysanthenone group (group 5.1). The low level of the major component and the corresponding complexity of the *Z.* aff. *involucrata* oils were not typical of the rest of the group. *Z. granulata* var. *adenodonta* is closely related to the very rare *Z. collina* which has only recently been recollected and is at present awaiting chemical investigation.

Table 3. The infrapopulational variation in essential oil composition of a population of *Z. smithii* 'e' form from Thornton Peak

RR_t component	Collection No. 1406	1407	1408	1409
17 α-Pinene	8.3	4.9	3.3	4.2
29 γ-Terpinene	3.6	1.6	1.8	4.4
59 Chrysanthenone	10.9	19.4	27.0	21.9
86 $C_{15}H_{24}$	3.2	6.0	3.1	1.3
100 Safrole	57.7	57.0	52.5	59.1

Group 5

The closely related *Z. granulata* and *Z.* sp. nov. 1 together with *Z. aspalathoides* 'b' form, *Z. obcordata* and *Z. robusta* invariably contained chrysanthenone-rich oils. One of the two *Z.* sp. aff. *involucrata* samples gave a complex oil with chrysanthenone, although the major component (21 %) was present at a low level with respect to the rest of the group. The Tasmanian specimens of *Z. cytisoides* 'b' form contained significantly more chrysanthenone (55, 42, 49 %) than the New South Wales population (18, 15 %) where a geographical barrier, Bass Strait, separates the two (Fig. 3). The mainland samples contained more safrole or methyl eugenol.

The close morphological relationship between the dominant members of group 4 and subgroup 5.1 (*Z. granulata*, *Z. aspalathoides*, *Z. furfuracea*, *Z.* sp. nov. 11, *Z.* sp. aff. *involucrata*, *Z. obcordata*, *Z. robusta*, *Z.* sp. nov. 1) is consistent with the essential oil chemistry in that all species are rich in either car-3-en-2-one or chrysanthenone. This dichotomy provides excellent support for further separation within *Z. granulata* and *Z. aspalathoides* as suggested by Powell and Armstrong [2]. The elevation of *Z. granulata* var. *adenodonta* to species rank is consistent with the high car-3-en-2-one content (60 %) of this variety with respect to var. *granulata* with chrysanthenone (70 %) as the major component. Likewise, *Z. aspalathoides* 'b' form (61 % chrysanthenone) is justifiably separated on chemical grounds from *Z. aspalathoides* sens. strict. (61 % car-3-en-2-one) as a new subspecies (Table 1).

Although the chrysanthenone from subgroup 5.1 has greater chemical affinities with subgroup 5.2, the morphological affinities are closest to group 4.

The predominance of *cis*-chrysanthenyl acetate in *Z. arborescens* 'b' form essential oil indicates chemical affinities with *Z. smithii* 'c' form to which morphological affinities also exist. The morphologically distinct *Z. cytisoides* sens. strict., however, is also rich in chrysanthenyl acetate. The extraordinary feature of this form is the geographical separation of populations giving the opposite C7 acetate epimers. *Cis*-chrysanthenyl acetate is the predominant component of samples collected in the Riverina region of New South Wales whereas *trans*-chrysanthenyl acetate is the predominant component of material from the Wolgan Valley region of New South

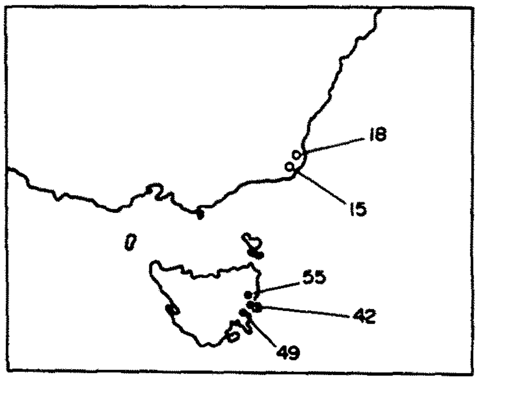

Fig. 3. Geographical variation in the essential oil composition of *Zieria cytisoides* 'b' form from Tasmania (●) and the mainland (○) showing the percentage contribution of chrysanthenone.

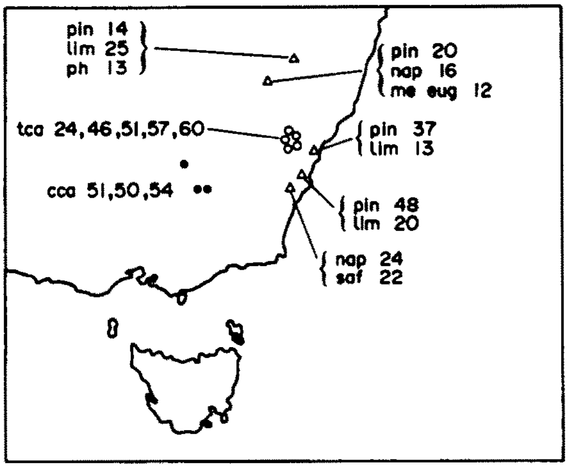

Fig. 4. Geographical variation in the essential oil composition of *Zieria cytisoides* sens. strict. showing the percentages of the principal constituents of the *trans*-chrysanthenyl acetate (tca) chemovar (○), the *cis*-chrysanthenyl acetate (cca) chemovar (●) and the hydrocarbon [α-pinene (pin), limonene (lim), β-phellandrene (ph), naphthalene (nap), methyl eugenol (me eug) and safrole (saf)] chemovar (△).

Wales (Fig. 4). Other hydrocarbon chemotypes were scattered across the central coast and northern regions of New South Wales. *Z. cytisoides* is closely related to *Z.* sp. aff. *arborescens* and hence to *Z. arborescens.*

Group 6

The limonene-rich oils in this group, typified by *Z. pilosa*, indicate chemical affinities to *Z.* sp. aff. *aspalathoides* and individual samples of *Z. aspalathoides* sens. strict., *Z. minutiflora* sens. strict. and 'b' form and *Z. cytisoides* sens. strict. The α-pinene-rich oils in this group, typified by *Z. cytisoides* sens. strict., indicate chemical affinities to *Z. laevigata* var. *fraseri.* Morphological similarities here exist between *Z.* sp. aff. *aspalathoides*, *Z. aspalathoides* sens. strict. and *Z. minutiflora.* The sesquiterpene-rich oils of this group are almost exclusive to *Z. laevigata* sens. strict. and *Z. laxiflora* and show chemical affinities as well as minor but distinctive chemical differences. Loose chemical affinities exist between *Z. murphyi* and the low zierone chemotypes of *Z.* sp. aff. *arborescens.*

Group 7

The presence of zierone in significant quantities is restricted to the *Z. arborescens* group and thus correlates morphological similarities with chemical composition for *Z. arborescens* sens. strict., 'd' and 'f' forms and *Z.* aff. *arborescens*, all of which are closely related. As this group distributes itself northward, the zierone content falls and the aromatic methyl ether content increases (Fig. 2).

Group 8

Apart from the consistent *Z. murphyi* Mt. Tomah chemotype and *Z. involucrata* where only one specimen was examined, this group contains only individual collections of chemically atypical specimens from the more variable taxa. The *Z. murphyi* Mt. Tomah chemotype shares a number of morphological similarities with *Z. involucrata*, which is in turn closely related to *Z. cytisoides* sens. strict.

CONCLUSIONS

Comparison of the essential oil composition of all available *Zieria* taxa showed both chemical similarities and chemical variation which establish the existence of interspecific, intraspecific and interpopulational chemotypes. Although variation within the genus is complex, sufficient criteria exist for the prediction of oil composition for the more constant taxa. The clearcut 'physiological forms' for *Z. smithii* proposed by Penfold [5, 6] were observed from *Z. smithii* sens. strict. However, most other *Zieria* taxa showed a gradation in chemical variation unamenable to further chemical subdivision.

EXPERIMENTAL

Details of plant material, distillation procedures and identification of constituents are as outlined in ref. [1]. Sampling generally reflected the abundance of the species: common species are well represented whereas rare species are represented by only one or two samples. The accession numbers of all samples examined are given in Table 1. Specimens are lodged with the National Herbarium of NSW and voucher details are available from the authors.

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