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CHEMOTAXONOMY (PHENOLIC SPOT PATTERN) OF THE SUBGENUS *COPROSMA* (RUBIACEAE) FROM NEW ZEALAND

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Abstract

The study is based on the phenolic spot patterns of 10 species of the surgenus *Coprosma* from New Zealand. *C. baueri* Endl. (Norfolk Is.) is compared where appropriate, as it has been a source of considerable taxonomic confusion. The spot pattern reflects the variation and major trends as shown by conventional morphological patterns.

Introduction

The pioneer chemotaxonomic contribution in the genus is by Taylor. (1960, 64) who successfully utilized polyphenols as taxonomic markers for two *Coprosma* species and their hybirds. May (1968, 1970) working mainly on New Zealand species used flavonoid information for the genus *Coprosma* on the basis of species specific spots contributed to an understanding of species differentiation. Clark (1973) utilized the same method of study as May for *C. robusta* Raoul, *C. macrocarpa* Cheeseman and their hybrids. Wilson (1979, 1984) found flavonoid study a useful approach for solving hybridization and related problems in *C. robusta, C. repens* Richard and *C. crassifolia* Colenso.

These investigations indicate that flavonoid chemistry can play a comparatively important part in the better understanding of the genus and its species definitions. In the present study, a comparative survey of the leaf flavonoids of 10 species belonging to the subgenus *Coprosma* was carried out to point out flavonoid variation within populations and single taxa and to relate flavonoid diversity to the systematics of the subgenus.

Material and Methods

Thin layer chromatography/electrophoresis technique was used. Mostly dried mature leaf material, with samples ranging from 0.05 g to 0.1 g were extracted in Isoamyl alcohol. $FeCl_3$ 2% w/v (Smith, 1960), p-nitroaniline, NH_3 (Aq.) (Bate-Smith, 1962) and diazotized benzidine (May, 1968) were used as spray reagents. The chemicals were BDH Analar grade.

The spots are comparable because of their relative position on the chromatograms. Generally more than one specimen from more than one locality was studied for each

species, except in the case of off-shore island endemics. Intensity of the spots was based on the frequency of one attribute in the specimens, on its fluorescent properties and size of the attribute. Individual specimen differences were ignored. Spot concentration is graded in detail, elsewhere it is represented by presence (+) absence (–) or weakness (±) of the attribute.

Results

A total of 52 major UV-fluorescent spots resulting from combined chromatography/electrophoresis were observed in 10 species of the subgenus *Coprosma.*

Flavonoid pattern and the subgenus Coprosma:

The major regular spots 1, 2, 3, 4, 5, 6, 7, 14, 38 and 46 appear to be constant for the subgenus (Table 1 & 2). Generally these major spots are selected on the basis of stronger intensity both in terms of their frequency, their presence in a greater number of species, and fluorescent properties of the spots. In addition there are less commonly occurring spots , which are sporadic in their distribution e.g., 8, 9, 10, 11, 12, 13, 32, 33, 36, 37 are represented in more than two species, and are in addition to the major, section-specific and species-specific spots. Though spot 11 is present in a considerable number of species, it is weaker in intensity and is treated as a minor spot.

Conspicuous quantitative differences in some of the spots within populations and different species were observed. In section *Petiolatae* there is a reduction in fluorescent intensity of spots 1, 2, 3 and 4. A similar reduction in *C. robusta* occurs in spots 1, 2, 3 and 4. These quantitative differences are very frequent and represent variation within the populations, but, in spite of this variation, consistency in the major tendency makes it possible to delimit the taxa at various levels.

Flavonoid pattern and sectional differentiation:

At the sectional level chemical distinction is based on the presence or absence of a considerable number of spots. Section *Coprosma* can be differentiated on the basis of spots 41, 42, 43, *Petiolatae* on spots 24, 25, 26, whereas section *Australes* generally lacks all of these spot.

Sectional affinities might be deduced on the basis of less commonly occurring spots. A considerable number of such spots are present in *Australes,* e.g. common spot 32 and comparatively less common are 10, 11 and 33. Section *Coprosma* collectively has more chemical affinities with *Australes* (spots 8, 9, 10, 11, 33 and less common 12, 13 and 33) but it is less closely related to *Petiolatae* with which it shares only 22 and

Table 1. Phenolic spot-pattern within the subgenus *Coprosma*. The appearance of Rf values and occurrence of spots found in chromatograms of mature leaves. Species are arranged alphabetically within their respective section (Allan, 1961).

Symbols: ++++, +++, ++, +, ± and – refer to the presence and relative strength of the particular constituent.

1.	+++	++++	++++	++	++++	++++	+++	++++	++++	++++
2.	++	++++	++++	++	+++	++++	+++	+	+++	++
3.	++	+	++++	±	+++	+++	+	+	++	–
4.	+	++++	++++	±	++++	+++	+++	±	+	+
5.	+++	++++	++++	+++	++++	++++	++++	++	+++	++
6.	±	++++	++++	+++	++++	++++	+++	+	++++	++++
7.	+++	++	+++	++	++++	++	++	±	++	+++
8.	–	±	+	±	++	±	+	–	–	–
9.	–	–	+	±	±	±	±	–	–	–
10.	–	–	+++	+	++++	±	±	–	–	++
11.	–	+	+++	±	±	++	±	+	±	+++
12.	–	–	++	++	–	+++	±	–	–	–
13.	–	+	++	++	–	–	+++	–	–	–
14.	±	+	+	+	+	+	+	+	+	+
15.	–	+	+	+	±	±	±	+	+	–
16.	–	–	–	–	–	–	–	–	–	+
17.	–	–	+	–	–	–	–	–	–	+
18.	–	–	+	–	+	–	+	–	–	+
19.	–	–	±	–	–	++	±	–	–	–
20.	–	–	–	–	–	–	±	–	–	–
21.	–	–	–	–	–	–	±	–	–	–
22.	–	–	–	–	–	–	+++	–	–	–
23.	–	+	–	–	–	–	–	–	–	–
24.	–	–	–	–	–	–	–	+	+++	++++
25.	–	–	–	–	–	–	–	±	–	++++
26.	–	–	–	–	–	–	–	–	±	++++
27.	–	–	–	–	–	++++	–	–	–	–
28.	–	–	+	–	–	++	±	–	–	–
29.	–	–	–	–	–	++	–	–	–	–
30.	–	++++	–	–	–	–	–	–	–	–
31.	–	–	–	–	–	+++	–	–	–	–
32.	+	+++	+	±	++	–	+++	–	–	–
33.	+	±	+	±	–	+++	+++	–	–	–
34.	–	–	–	–	–	+	–	–	–	–
35.	–	–	–	–	–	+	–	–	–	–
36.	–	±	+	+	–	–	–	–	–	++
37.	–	±	+	+	–	–	–	+	+	+
38.	++	+	+	+	+	+	+	+	+	+
39.	–	+	–	–	–	–	±	–	–	–
40.	–	–	–	–	–	–	+	–	–	–
41.	–	–	–	–	–	+	+	–	–	–
42.	–	–	–	–	–	+++	+++	–	–	–
43.	–	–	–	–	–	++++	++++	–	–	–
44.	–	+++	–	–	–	–	–	–	–	–
45.	–	–	+	–	+	–	–	–	–	–
46.	±	+	+	+	+	+	±	+	+	+
47.	–	–	–	–	–	+	+	–	–	–
48.	–	–	–	–	–	±	±	–	–	–
49.	–	–	+	±	+++	–	–	–	–	–
50.	–	–	–	+	–	–	–	–	–	–
51.	–	–	–	–	–	+	–	–	–	–
52.	–	–	–	–	+	–	–	–	–	–

Spots *C. acutifolia* *C. grandifolia* *C. macrocarpa* *C. robusta* *C. tenuifolia* *C. dodonaefolia* *C. lucida* *C. chathamica* *C. petiolata* *C. repens*

the less commonly occurring spot 10. One spot 11 is common to all sections. *Petiolatae* is further linked to *Australes* by having spot 37, though this is not strongly present in *Australes.*

It would appear that sectional segregation is even stronger when minor spots are combined with section specific spots. The sections are linked phytochemically into the subgenus by a few common spots which tend to connect *Coprosma* more closely with *Australes* than with *Petiolatae.*

Section: Australes:

Six clear spots are responsible for species definition in this section. *Coprosma robusta* is specific in having spot 50, *C. grandifolia* is distinguished on the basis of spots 23, 30 and 44, although 44 and 23 while specific, are not constantly occurring spots within the species. *Coprosma tenuifolia* Cheeseman is clearly differentiated on the basis of spot 52 and from *C. grandifolia* Hook f. and *C. acutifolia* Hook f., by spot 49, which is also present in *C. robusta* and *C. macrocarpa. Coprosma macrocarpa* might be identified on the basis of less frequently occurring spots.*C. acutifolia* has abscure distinction (weak spot representation) and can not consistently be differentiated by this method alone.

Within *Australes, C. acutifolia* shows decrease in spots 2, 3, 4, 5, 6 and 46. The spots are fewer and some are obscure. *C. robusta* is very complex where spot differences occur between specimens and only prominent and related spots are considered for species definition. Plants from different localities show qualitative differences. It would appear that some localities show greater correlation than others e.g., plants from Swanson and Anawhata show strong similarities, and therefore, seem to be very closely related to each other.

In *C. macrocospa* common spots are 10, 11, 13 and 19 and less commonly occurring ones are 12, 17, 33, 45 and 49. Plants from the Three Kings show fewer spots, but on overall distribution they are related to mainland plants, which show greater morphological differentiation and provide comparatively complex quantitative and qualitative phytochemical information. *C. tenuifolia* seems to be fairly uniform, showing little variability.

C. macrocarpa and *C. robusta* seem to be closely related phytochemically. *C. acutifolia* has only spots 32 and 33 in common with the other species of the section and is thus phytochemically remote. *C. grandifolia* is fairly close to *C. macrocarpa* and *C. robusta.* There are a considerable number of unidentified spots in the section particularly in *C. robusta* and *C. macrocarpa.* Close affinities between *C. robusta* and *C. macrocarpa* have been shown (May, 1968, 1970; Clark 1973).

Table 2. Rf. and Colour reactions of the spots

Symbols b = bright; bl = blue; br = brown; d = deep; l = invisible; p = purple; r = red; s bl = sky blue v = violet; y = yellow; w = weak; Benz = Benzidine.

Spots	Rf of spots		Colour reactions			
	1-D	2-D	UV	NH_3 + UV	Benz	p-nitro aniline
1.	0.4105	0.2565	bb	bl	bl	w bl
2.	0.4690	0.9881	w bl	bl	w pale bl	bl
3.	0.5300	0.1529	pale bl	bl	pale bl	pale bl
4.	0.6481	0.0978	bl	bl	bl	pale bl
5.	0.2571	1.2885	p bl	bl	bl	bl
6.	0.6677	1.1799	s bl	bl	w bl	pale bl
7.	0.7350	1.076	s bl	bl	w bl	pale bl
8.	0.4644	1.497	w bl	w bl	w pale bl	pale bl
9.	0.6563	2.237	bl	w bl	w bl	pale bl
10.	0.7666	2.301	w bl	w bl	w bl	w bl
11.	0.7589	2.240	v	w bl	w p bl	pale bl
12.	0.3830	1.7834	p	w bl	w bl	pale br
13.	0.4487	1.3414	w bl	–	bl	bl
14.	1.048	1.2371	w bl	pale	w bl	pale bl
15.	0.9782	1.0785	bl	pale	w bl	bl
16.	0.1252	0.944	w bl	w bl	w bl	pale bl
17.	0.757	1.1159	bl	w bl	w bl	pale bl
18.	0.8657	0.6438	w bl	w bl	w bl	pale
19.	0.8506	0.8018	w bl	w bl	w bl	pale
20.	0.6722	1.22	w bl	w bl	w bl	pale bl
21.	0.6501	2.1565	w bl	pale bl	w bl	pale bl
22.	0.4465	1.7021	w bl	w bl	w bl	pale bl
23.	0.5260	0.9142	l	–	pale bl	br & bl
24.	0.3682	1.1159	bl	w bl	pale bl	pale bl
25.	0.5714	1.652	bl	w bl	pale bl	pale bl
26.	0.3620	0.7284	bl	w bl	pale bl	pale bl
27.	0.4328	1.054	bl	w bl	pale bl	pale bl
28.	0.2489	0.241	pale br	–	pale br	pale br
29.	0.1075	0.826	l	pale br	br	br bl
30.	0.3484	0.3783	l	–	d v	d v
31.	0.457	0.7648	bl	–	w bl	br bl
32.	0.377	0.607	w bl	–	w bl	pale bl
33.	0.4104	0.6469	w bl	–	w bl	pale bl
34.	0.549	0.1676	red	–	br red	br red
35.	0.549	0.271	w bl	pale bl	–	pale bl
36.	0.797	1.534	w bl	–	w bl	pale bl
37.	0.821	2.019	w bl	–	w bl	pale bl
38.	0.1017	0.0138	y	br y	–	y
39.	0.7884	1.884	pale bl	w bl	pale bl	pale bl
40.	0.7807	2.3629	pale bl	w bl	w bl	w bl
41.	0.8249	0.2385	s bl	w bl	bl	pale bl
42.	0.9466	0.2596	pale bl	w bl	bl	pale bl
43.	1.008	0.0685	p	bl	bl	pale bl
44.	0.654	0.52	l	bl	d v	br bl
45.	0.035	0.93	w bl	bl	w bl	pale bl
46.	0.31	1.96	bl	w bl	w bl	w pale bl
47.	0.40	1.96	w bl	w bl	w bl	pale bl
48.	0.313	1.789	w bl	w bl	w bl	pale bl
49.	0.71	1.77	bl	w bl	w bl	w bl
50.	0.340	0.055	bl	w bl	w bl	pale bl
51.	0.505	0.2673	w bl	–	w bl	w bl
52.	0.5454	1.88	bl	–	w bl	pale bl

Section: Coprosma:

C. lucida Forster and *C. dodonaefolia* Oliver are correlated on the basis of spots 12, 28, 41, 42 and 43 (Table 1). There are considerable phytochemical differences between these two species. Spots 22 and 11 are consistent in their occurrence in *C. lucida.* In addition there are less commonly occurring species-specific spots, i.e. 20, 21 and 40. North Island plants are closely related phytochemically. There are a considerable number of unidentified spots (spot differences between specimens) in this species.

Species-specific spots in *C. dodonaefolia* appear to be 27, 29, 31 with 34 and 35 less commonly occurring. Quantitative differences between specimens are frequent. In section Coprosma each species has a considerable number of unidentified spots.

Section: Petiolatae:

The sectional differentiating spots are 24, 25 and 26. The distance between spot 1 and the group of 2, 3 and 4 is much greater than it is in the other two sections. Similarly the group 10, 11, 36 and 37 is well separated. Only *C. repens* in *Petiolatae* shows spots 16 and 17 and 10, which are of common occurrence in that species. There is a great deal of quantitative spot difference between the species which are not clearly identified on the basis of phenol phytochemistry. *C. chathamica* Cockyane lacks spot 26. *C. baueri* Endlicher (Norfolk Island) is closely related to *C. repens* phytochemically. *C. repens* is fairly constant although in some cases plants (e.g. Waiomu) are more variable in possessing a greater number of spots as compared to *C. petiolataa* Hook. f. or *C. chathamica.*

Discussion

Flavonoid spot patterns for each section and individual species when analysed show a substantial degree of species specific differentiation and thus may be used for segregation not only of species, but also of sections recognised on other grounds within the genus. There are a considerable number of spots which appear to be common throughout the subgenus, and the latter can easily be differentiated into three sections on the basis of quantitative spot differences. Consistency of the section-specific spots in the related species is the basis for grouping them in their respective sections. Likewise there is an ample amount of spot differentiation for species segregation.

In addition there are a number of spots representing individual specimen differences which indicate complexities within the species concerned. These however are ignored in obtaining comparisons at higher levels. Phytochemistry thus confirms that in most cases each species has a specific profile (Taylor, 1960; 64, May, 1968, 70; Clark, 1973; Wilson, 1979, 84) and also that there is considerable population-based variation within each species.

Morphologically variable species and/or those with a wide distribution range (e.g. *C. lucida, C. macrocarpa, C. robusta*) show a wide range of phytochemical complexity (with a large number of spots) which reflects their general variability, whereas morphologically stable species and those of restricted distribution generally have simpler phytochemical patterns. Island endemics show a further reduction in variation (e.g., *C. petiolata, C. acutifolia* and *C. macrocarpa* sub. sp. *macrocarpa*). Because of the range of individual specimen differences available, phytochemistry could be used for more elaborate surveys of intra-specific relationships. There was no significant deviation between results based on dried (herbarium) material and fresh material (including cultivated plants).

The genus *Coprosma* is old on the basis of the number of major and other spots as is also evident from the results of May (1968, 1970). Isolated endemic species with fewer spots are perhaps of secondary origin. With that exception the majority of species show considerable morphological and phytochemical variation which is more pronounced in species of wider distribution or where hybridization is common. Phytochemical evidence thus provides an independent set of criteria for inferring taxonomic relationships within the subgenus *Coprosma.*

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