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Analysis of Leaf Waxes as a Taxonomic Guide to *Rhododendron* Subsection *Taliensia*

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Analysis of the hydrocarbon component of leaf waxes for a large number of specimens of *Rhododendron* subsection *Taliensia* Sleumer has shown that the distribution of *n*-alkanes, C_nH_{2n+2}, can be a useful taxonomic feature. There is a clear distinction between taxa for which the maximum of the distribution is at $C_{27}H_{56}$ and those with a maximum at $C_{31}H_{64}$, but more subtle differences between the distributions are also evident. The extent of variation between samples from the same clone taken at different times has been estimated, and the additional variation between different specimens of the same taxon has been shown to be smaller than this. Evidence is presented to show that hybrids between C_{27} and C_{31} taxa may have a distinct alkane distribution pattern. This can be used to identify parents of some natural hybrids. The data for some taxa indicate that the present taxonomic classifications based on morphology alone are not entirely satisfactory, and provide information which may help to elucidate problems that arise within populations consisting of closely allied taxa.

Key words: *Rhododendron*, subsection *Taliensia*, Ericaceae, leaf wax analysis, gas chromatography, alkanes.

INTRODUCTION

The genus *Rhododendron* is a member of the family Ericaceae and contains around 1000 species. Molecular studies (Kron and Judd, 1990; Chamberlain and Hyam, 1998) indicate that this genus is monophyletic, although the true relationship with the genus *Menziesia* remains to be resolved. These studies have confirmed the integrity of most of the eight sections recognized in modern classifications (Argent *et al*., 1997) and clearly point to the distinctness of section *Ponticum*. A molecular study using ITS sequences (Hyam, 1997) has confirmed that section *Ponticum* is indeed monophyletic, but did not confirm any meaningful subdivision that could be applied to the 250 species included within it.

Within section *Ponticum*, 23 subsections, of which subsection *Taliensia* is one, are recognized. The most important of the morphological characters used to delimit these subsections concern the form of the complex branched hairs (when present) that make up the indumentum. These are found especially on the undersurfaces of the leaves, though they may occur on all parts of the plants. Hybridization occurs widely between species from different subsections within this section. The boundaries between the subsections are therefore sometimes difficult to define, reflecting the reticulate nature of their origins.

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Plants have an enormous variety of chemical constituents, and some compounds may be characteristic of a particular genus or even of a single species. In principle, therefore, one could devise a purely chemical system of taxonomy, in which the chemical components of a chosen part or parts of a plant are separated and identified, with appropriate keys yielding the required identification. Such a procedure, while technically possible, would at present be prohibitively expensive. However, as part of a wider study of the constituents of *Rhododendron* leaves, it was shown that the distribution of hydrocarbons in the leaf waxes was sufficiently variable to provide useful taxonomic information (Evans *et al*., 1980). The waxes, which are found on the surfaces of the leaves, can be easily isolated and then separated and identified by gas chromatography, which is a relatively cheap and rapid technique.

This early work on *Rhododendron* leaf waxes showed that the hydrocarbon components consisted of mixtures of compounds, with the large majority being straight-chain saturated alkanes of formula C_nH_{2n+2}, where *n* is an odd number between 21 and 35 (Evans *et al*., 1980). Within this range, each specimen contained several compounds, mainly between C_{23} and C_{33}. There were also small amounts of straight-chain saturated hydrocarbons with an even number of carbon atoms, and even smaller amounts of several other series, which included branched-chain alkanes and possibly (since confirmed) also alkenes, C_nH_{2n}. Most subsections of the genus *Rhododendron* contained species which all had maxima in their distributions at C_{29}, C_{31} or C_{33}, but in

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subsection *Taliensia* [we use the classification of Cullen (1980) and Chamberlain (1982) based on Sleumer (1949), with the most recent listing of names taken from Argent *et al.* (1997)], some species had maxima at C_{31}, others at C_{27}, while for a few species, individual specimens had different maxima in their wax distributions. *Rhododendron canadense* was the only species not in subsection *Taliensia* that gave a C_{27} maximum. As there are major taxonomic difficulties with subsection *Taliensia*, with extensive populations of apparent hybrids, and variations within species so great that the boundaries with other species are extremely difficult to define, it appeared that wax analysis could provide a valuable taxonomic guide.

We have therefore analysed the waxes of a large number of specimens of plants in *Rhododendron* subsection *Taliensia*, in living collections, in herbaria, and collected in the wild, with the following objectives:

1. assessment of the validity of the technique, by checking the consistency of data for specimens collected from one plant on different occasions, comparing data for young leaves, mature fresh leaves and herbarium material, and checking the consistency of data for several plants of the same taxonomic unit;
2. establishing a reliable set of typical data for as many taxa within the subsection as possible;
3. constructing a partial key to the subsection using only wax data;
4. identifying taxa which require further study; and
5. assessing the possibility of deducing the parents of specimens from hybrid populations.

All this is necessary in the context of the difficulties associated with subsection *Taliensia*. Amongst the 50 species comprising this subsection (Argent *et al.*, 1997), there is a particularly confused complex of taxa, including *Rhododendron phaeochrysum*, *R. agannniphum*, *R. roxieanum* and *R. proteoides*. These species are sympatric, in an area that spans the boundaries of NE Burma and the adjacent Chinese provinces of Yunnan and Xizang (Tibet). Field observations have suggested that all four species form complex hybrid swarms with one another. In N Sichuan and Gansu there is another complex involving *R. phaeochrysum* and *R. przewalskii* which may also result from past or present hybridization. The extreme forms within these complexes may be referred unequivocally to the component species, using their morphology. However, a significant proportion of the individual plants are morphologically intermediate between the 'species'. Several of these intermediates have been formally named. These named entities have been defined by subtle differences in the leaf indumentum and in the size and shape of the leaves—characters that are difficult to interpret.

By contrast, leaf waxes can be quantified and defined by their chemical structure. They therefore offer the potential for an analysis of the complex populations that is more scientifically rigorous than that which is possible using ill-defined morphological characters. Evans *et al.* (1980) have demonstrated the potential use of leaf wax phytochemistry in *Rhododendron* in elucidating taxonomic problems within the genus. The present study refines and extends the use of one family of these compounds—the hydrocarbon fraction. We are not aware of any other published accounts of the use of leaf waxes to elucidate problems associated with hybridization in plant taxonomy.

The differences in the leaf wax profiles do confirm the distinctness of some of the species that are recognized on morphological grounds within subsection *Taliensia*. However, some species that are morphologically so distinct that their identity is beyond doubt are not distinguished by their leaf waxes. Therefore, leaf-wax profiles alone cannot be used to establish a reliable phylogeny for the species of subsection *Taliensia*. However, analysis of leaf waxes is informative where hybrid populations occur involving parents for which morphological differences are directly correlated with significant differences in the wax profiles. It may then provide a quantitative assessment of the relationship between individuals that exhibit an intermediate morphology and may be used to define the morphological limits of the parent taxa. In this paper, we present the results of this study of the leaf waxes of plants in subsection *Taliensia*.

MATERIALS AND METHODS

Extraction

Samples of *Rhododendron* leaves were removed from stalks, and their weight, condition and the number of leaves were recorded. The surface area of the sample of leaves (typically 100–200 cm^2, but samples as small as 10 cm^2 were used in the later stages of this work) was determined by photocopying, weighing the exposed area and taking the ratio to the known weight of 1 cm^2 of paper.

Leaves were placed in a 500 ml conical flask and covered with AnalaR chloroform (approx. 25–100 ml, depending on the area of leaf used). To this was added 1 ml of a standard chloroform solution containing 1 mg each of n-$C_{20}H_{42}$ and n-$C_{36}H_{74}$ per ml, to provide markers in the gas chromatography (GC) traces. After approx. 1 min the solution was filtered through filter paper into a round-bottomed flask, and then evaporated to dryness at room temperature using a rotary evaporator. The residue was redissolved in a small amount (approx. 2 ml) of chloroform and passed through an alumina (neutral Brockmann 1, 60 mesh) column, 6 cm long by 5 mm wide, to remove the polar fraction. The solution of the non-polar fraction was allowed to evaporate to dryness, and the extracted material was weighed.

The non-polar components were then dissolved once more in chloroform, to give a concentration of 10 mg ml^{-1}. Aliquots of 0·5 μl were injected into the gas-liquid chromatograph.

Gas chromatography analyses

GC analyses were carried out in five separate batches. The different procedures merely reflect facilities available at different times, and will give directly comparable data.

(1) In Glasgow University Botany Department, 1974–1978, using packed columns (2·7 m, 3 % OV-17 coated

on Gas Chrom Q), temperature programmed from 180–290°C at 3° or 4° min^{-1}, then held at 290°C for 20 min, with nitrogen carrier gas.

(2) In Glasgow University Botany Department, 1980, using a support-coated, open-tubular (SCOT) column with OV-17 as the stationary phase (from S.G.E. Pty Ltd.), operated from 240–290°C at 1° min^{-1} after holding at 240°C for an initial period of 5 min, with helium carrier gas.

(3) In Glasgow University Chemistry Department, 1991, using a 25 m × 0·3 mm ID fused silica capillary coated with CP sil-5 CB, operated by injection at 80°C then held for 2 min before raising to 220°C at 30° min^{-1}, holding for 1 min and then raising to 280°C at 2° min^{-1}. The injection split ratio was 50 : 1 and helium carrier gas was used.

(4) In Edinburgh University Chemistry Department, 1993, using a column similar to that in method 3 but with splitless injection and hydrogen carrier gas. The initial temperature was 100°C followed by an increase to 300°C at 2° min^{-1}. In a few cases, analyses were duplicated in Glasgow using method 3. These are indicated by a footnote in Table 1.

(5) As in method 3, but with hydrogen as the carrier gas and the temperature programmed as in method 4; all analyses since 1996.

Relative proportions of each of the compounds measured were determined by measuring peak heights in methods 1 and 3, whilst an integrator was coupled in parallel across the input terminals of the GC recorder in the second series of analyses and an integrator was incorporated as a component of the recorder in the fourth and fifth series.

RESULTS AND DISCUSSION

Table 1 gives analytical results for all species samples analysed, in alphabetical order, together with an indication of the origin of each sample and the method of GC analysis. Many of the samples were obtained from the living collections at the Royal Botanic Garden Edinburgh (RBGE), and accession numbers are given both for these and for other samples from living collections, including those of the Rhododendron Species Foundation (RSF), Federal Way, Washington, USA. A specific plant from the RBGE is indicated by a letter following the accession number. Original collectors' numbers are also given, where these are available. These include some wild-collected specimens of our own, numbered in the SDR series.

The relative abundances of the *n*-alkanes of odd carbon number from $C_{25}H_{52}$ to $C_{33}H_{68}$ are displayed with the most abundant hydrocarbon scaled to 100. Amounts of $C_{21}H_{44}$, $C_{23}H_{48}$ and $C_{35}H_{72}$ were also measured, but these were invariably small, $<10\,\%$ relative to the most abundant alkane; this was true even for $C_{35}H_{72}$ in waxes in which the distribution peaked at $C_{33}H_{68}$. The mass of leaf wax was also measured, relative to both total leaf mass and surface area. The first of these is a highly variable parameter, as the leaf mass changes so much during growth and during drying. The mass of leaf wax per unit area of leaf should be much more consistent, for samples ranging from fresh young leaves to herbarium specimens. However, the extraction efficiency was lower for the small samples of leaves used in the later stages of this project, so even this second parameter was too unreliable to be of use. The only general observation worth making is that the amount of wax extracted was greater for those species which had $C_{27}H_{56}$ as the dominant constituent than for those dominated by other hydrocarbons.

Statistical significance of the alkane distributions in waxes

To assess the consistency of wax analytical data, and thus its usefulness, one must consider a number of factors.

(1) How reproducible are the results for several analyses of the same extract of waxes?

(2) Is there significant variation between results for young and mature leaves from the same plant, and between data for fresh and herbarium samples?

(3) How much variation is there between results for leaves collected from different parts of the same plant or on different occasions?

(4) How much variation is there between different plants of the same taxonomic unit?

We did not set out to perform rigorous statistical studies of all of these factors, as such work would be prohibitively expensive and time-consuming. However, there is sufficient information in the data which we have obtained to make reasonable estimates of the natural and experimental variations on which the applicability of our results depend.

Wax extracts for a few specimens (all with C_{31} maxima) have been analysed twice. For each of these we have, as usual, normalized the C_{31} intensities to 100, and have then compared the intensities for C_{29} for each pair of analyses and of C_{33} for each pair. The mean modulus of the differences between these intensities was 3 percentage units, and the standard deviation for any one normalized percentage intensity is thus approximately 2 %. This is substantially smaller than the other variations, and is therefore of no significance.

Both mature and young leaves were collected in the summer from ten plants of seven different species, and wax analyses were performed. As some had C_{27} maxima and others C_{31}, we calculated the ratios of the intensities for mature and young leaves, for the alkanes with C_{max-2} and with C_{max+2}. For C_{max-2} the ratio mature : young was $1{\cdot}1 \pm 0{\cdot}3$, while the ratio for C_{max+2} was $1{\cdot}0 \pm 0{\cdot}5$. (The quoted uncertainty is the estimated standard deviation.) There is therefore no significant systematic difference between results for young and mature leaves, although there are sizeable differences in one or two cases. A similar comparison was made for mature leaves studied when fresh, and after storage in the herbarium for about 4 years. For C_{max-2} the ratio fresh : herbarium was $1{\cdot}02 \pm 0{\cdot}12$ and for C_{max+2} the ratio was $0{\cdot}95 \pm 0{\cdot}28$. Again the difference is not significant.

Specimens were collected from nine individual plants on two or more occasions. For the alkanes with C_{max-2} and C_{max+2} we compared the percentage intensities for the

TABLE 1. *Wax analysis for* Rhododendron *species*

Taxon	Collector's number	Source[a]	*n*-alkane chain length relative abundances[b]					Age[c]/GC method
			C_{25}	C_{27}	C_{29}	C_{31}	C_{33}	
R. adenogynum		19698332	+	8	73	100	18	M/1
Diels		19698332	8	4	53	100	30	M/2
		19698332B	3	5	55	100	28	M/4
		19764107	7	16	56	100	29	M/3
	SDR 936	wild	13	14	35	100	24	M/5
	SDR 932	wild	6	6	41	100	40	M/5
		wild	4	7	45	100	52	M/5
R. aganniphum var. aganniphum	Forrest 16472	19614570	10	20	67	100	33	M/3
Balf.f. & Kingdon-Ward	Forrest 16472	19614570A	58	70	98	100	30	M/4
	Forrest 16472	19614570A	33	55	100	94	32	M/4
	Forrest 16472	19614570A	26	75	98	100	+	M/5
	Forrest 16472	19614570A	42	26	55	100	31	Y/5
	Forrest 19574	RSF 74/055	4	5	11	100	63	M/5
	Forrest 19574	RSF 74/055	23	25	90	100	32	M/5
	dongshongense	Glendoick	12	20	41	100	27	M/5
R. aganniphum var. flavorufum		19698582	17	25	83	100	24	M/3
(Balf.f. & Forrest) D.F.Chamb.		19698582	16	29	100	99	23	M/3
	Forrest 14368	19698581C		52	78	100	30	M/4
	Forrest 14368	RSF 70/407	21	37	75	100	27	M/5
	Forrest 14368	RSF 95/084	19	27	89	100	45	M/5
R. alutaceum var. alutaceum	Rock 11100	19614571	16	17	58	100	46	M/3
Balf.f. & W.W.Sm.	Forrest 19574	RSF 76/156	4	7	44	100	31	M/5
R. alutaceum var. iodes	Forrest 19567	19614564A	23	100	22	14	4	M/4
(Balf.f. & Forrest) D.F.Chamb.	Forrest 19567	19614574	15	100	26	16	2	M/2
		19698820A	43	100	19	15	3	M/4
		19698820A	29	100	19	13	10	M/5
		19698820A	32	100	40	46	13	Y/5
R. alutaceum var. russotinctum		RBGE	16	100	+	20	4	M/1
(Balf.f. & Forrest) D.F.Chamb.		19698820	30	100	18	18	5	M/3
		RBGE	27	100	21	28	8	M/3
		19698820D	32	97	55	100	46	M/4
		19698820D	26	86	55	100	56	M/5
		19698820D	40	100	56	91	38	Y/5
	Rock 33	Glendoick	16	100	34	20	21	M/5
		Glendoick	40	100	61	28	5	M/5
		Glendoick	9	13	33	100	46	M/5
		Glendoick	45	100	37	38	4	M/5
		Glendoick	22	100	44	27	14	M/5
		Glendoick	13	13	26	100	61	M/5
R. balfourianum	Forrest 16811	19191004	7	10	30	100	39	M/3
Diels	Forrest 16811	19191004	10	15	44	100	59	M/3
	Forrest 16811	19191004E		6	33	100	38	M/4
	Forrest 16316	19698392	8	6	31	100	30	M/2
	Forrest 29256	19698394	3	4	19	100	66	M/3
R. balfourianum var. *aganniphoides*		19698393A	4	6	31	100	43	M/4
Diels								
R. beesianum		19698409	3	7	20	100	68	M/3
Diels		19698409		+	14	100	43	M/4
	Forrest 10195	19698411		11	34	100	79	M/3
	Forrest 30526	19724039	12	32	40	100	72	M/3
	Forrest 30526	19724039A	+	50	53	100	56	M/4
	Forrest 30526	19724039A	55	24	65	100	57	M/5
	Forrest 30526	19724039A	42	48	70	100	66	Y/4
	Forrest 30526	19724039A	70	66	53	100	20	Y/5
	SDR 752	wild	44	20	54	100	48	M/5
	SDR 774	wild	17	24	52	100	61	M/5
	SDR 774	wild	17	25	45	100	74	Y/5

Table continued on next page

TABLE 1. *Continued*

Taxon	Collector's number	Source[a]	*n*-alkane chain length relative abundances[b] C_{25}	C_{27}	C_{29}	C_{31}	C_{33}	Age[c]/ GC method
R. bhutanense	CHM 3091	Glendoick	15	12	32	100	77	M/5
Long & Bowes Lyon	CHM 3091A	Glendoick	16	19	30	100	63	M/5
	BB 889	RSF 91/015	13	1	37	100	63	M/5
R. bureavii	Forrest 15609	19180017	7	11	30	100	60	M/2
Franch.	Forrest 15609	19181009	8	10	40	100	52	M/1
	Forrest 15609	19181009D	18	29	44	100	49	M/4
	Forrest 15609	19181009E	10	6	39	100	54	M/4
	Forrest 25439	19331022	13	14	57	100	57	M/3
	Forrest 25439	19331022C	9	11	50	100	64	M/4
	Forrest 25439	19331022B	4	7	40	100	68	M/4
	Forrest 25439	19331022B	32	16	42	100	55	M/5
	Forrest 25439	19331022B	6	24	49	100	60	Y/5
		19698425	+	10	22	100	63	M/4
		19698425A	9	11	51	100	45	M/4
		19698425A	70	58	72	100	20	M/5
		19698425A	30	25	55	100	46	Y/5
R. bureavioides	CEE 344	19913300	33	21	39	100	39	H/4
Balf.f.	CEE 344	19913300	23	17	25	100	28	H/4
	SB 8305	Warren Berg	4	9	17	100	98	M/5
	Cox 5072/a	Glendoick	9	10	25	100	66	M/5
	Cox 5072/b	Glendoick	11	16	31	100	20	M/5
	Cox 5072/c	Glendoick	11	20	31	100	69	M/5
	Cox 5076B/a	Glendoick	4	9	5	100	69	M/5
	Cox 5076B/b	Glendoick	2	4	23	100	40	M/5
	Cox 5076B/c	Glendoick	4	8	19	100	86	M/5
	M Sinclair	wild	0	0	65	100	60	M/5
	M Sinclair	wild	18	32	35	100	96	M/5
	SB	RSF 94/249	4	7	22	100	44	M/5
R. clementinae	Rock 25401	19330314		5	38	100	36	M/2
Forrest		19698477	4	7	39	100	45	M/3
	Rock 25401	RSF 73/337	5	3	26	100	52	M/5
	Forrest 25705	RSF 75/045	16	7	30	100	69	M/5
		Glendoick	4	4	10	100	70	M/5
R. elegantulum		19698331	12	6	28	100	37	M/2
Tagg & Forrest		19698331	9	9	45	100	37	M/3
		19698331A	13	10	35	100	32	M/4
		19698331A	46	27	38	100	48	M/5
		19698331A	11	17	34	100	45	Y/5
		RSF 81/129	20	23	28	100	19	M/5
R. faberi	K Rushforth/a	Glendoick	19	22	23	100	89	M/5
Hemsl.	K Rushforth/b	Glendoick	19	19	4	49	100	M/5
	Cox/a	Glendoick	33	37	28	100	94	M/5
	Cox/b	Glendoick	18	1	39	91	100	M/5
R. lacteum		19490491	11	14	28	100	92	M/3
Franch.		19490491	5	11	37	100	93	M/3
		19490491	+	3	30	100	66	M/4
		19490491	+	19	42	100	69	M/4[d]
		19490491	+	26	36	100	64	M/4
	Forrest 6778	19764034	10	28	38	100	79	M/3
	SDR 710	wild	8	15	41	100	78	M/5
	KGB 806	Gothenburg	11	13	14	56	100	M/5
	KGB 427	Gothenburg	7	9	8	100	38	M/5
R. lanatoides D.F.Chamb.		Glendoick	10	22	100	99	35	M/5
R. mimetes		19698727	7	13	23	100	66	M/3
Tagg & Forrest		19698727D	10	+	72	100	84	M/4
R. mimetes var. simulans	Forrest 20428	19825082	5	8	20	100	63	M/3
Tagg & Forrest	Forrest 20428	RSF 76/168	23	26	24	100	76	M/5
	Forrest 20428	19825082B	6	5	23	100	63	M/4
R. nigroglandulosum		Glendoick	9	15	62	100	72	M/5
Nitzelius		Glendoick	1	2	15	100	60	M/5

Table continued on next page

TABLE 1. *Continued*

Taxon	Collector's number	Source[a]	*n*-alkane chain length relative abundances[b]					Age[c]/ GC method
			C_{25}	C_{27}	C_{29}	C_{31}	C_{33}	
R. phaeochrysum var. agglutinatum (Balf.f. & Forrest) D.F.Chamb.		19845025	12	13	24	100	49	M/2
		19845025	7	7	24	100	62	M/3
		19845025	30	100	36	25	5	M/3
	SDR 907	wild	12	100	44	42	11	M/5
	SDR 908	wild	8	9	30	100	56	M/5
	Rock 11335	19790979	42	90	70	100	3	M/3
	Rock 11335	19790979	33	100	88	98	17	M/5
		19845025	3	5	25	100	57	M/5
	Cox 5058A	Glendoick	12	9	25	100	41	M/5
	Cox 5124	Glendoick	4	6	27	100	50	M/5
		Glendoick	12	12	20	100	70	M/5
	R Lancaster	Glendoick	12	100	30	20	4	M/5
		Glendoick	15	31	36	100	66	M/5
R. phaeochrysum var. levistratum (Balf.f. & Forrest) D.F.Chamb.	Smith 13982	19644530	36	100	22	11	2	M/3
		19698537	32	100	39	25	5	M/3
		19698537A	40	100	25	19	+	M/4
		19698537A	58	100	28	17	16	M/5
		19698537A	32	100	21	13	4	Y/5
	Forrest 20442	19698538	10	100	35	27	5	M/2
	Forrest 20442	19698538	14	100	35	27	4	M/2
	Forrest 20442	19698538	15	100	30	21	5	M/1
	Forrest 20442	19698538	19	100	32	19	4	M/3
		19698874	15	100	32	31	8	M/3
	SDR 863	wild	55	100	16	11	1	M/5
	SDR 787	wild	45	100	33	32	13	M/5
	Forrest 29327	RSF 71/509	17	100	38	33	9	M/5
	Cox 5057	Glendoick	60	100	47	32	33	M/5
	Cox 5132	Glendoick	29	100	51	25	3	M/5
	Smith 13982		26	100	22	10	+	M/2
	Smith 13973	19644529	21	100	18	7	+	M/2
	SDR 865	wild	60	100	30	22	13	M/5
		19380299	32	100	46	18	5	M/1
R. phaeochrysum var. phaeochrysum Balf.f. & Forrest	Forrest 10547	RBGE	12	18	36	100	43	M/2
	Forrest 10547	19698780	16	47	32	100	58	M/1
	Forrest 10547	19698780	7	12	40	100	52	M/3
	Forrest 10547	19698780A	12	16	49	100	45	M/4
	Forrest 10547	19698780A	7	13	44	100	36	M/4
	Forrest 16811	19190018	4	8	27	100	41	M/2
	SDR 753	wild	34	25	59	100	33	M/5
	SDR 775	wild	6	12	54	100	31	M/5
	SDR 754	wild	11	10	39	100	47	M/5
	Smith 13977	RSF 79/146	19	11	32	100	36	M/5
	Forrest 14368	W Berg	21	37	75	100		M/5
	Smith 13973	19644529	3	3	18	100	43	M/2
		19698781			18	100	58	M/4
		19698781			18	100	58	M/4[d]
		Glendoick	41	37	71	100	61	M/5
		Glendoick	17	27	39	100	73	M/5
		Glendoick	23	44	36	100	55	M/5
	SB 8305	W Berg	2	12	19	100	86	M/5
R. prattii Franch.		Glendoick	6	12	19	83	100	M/5
		Glendoick	28	22	27	68	100	M/5
		Glendoick	40	35	62	94	100	M/5
		Glendoick	18	39	33	100	39	M/5
	Wilson 1547	19698801	0	9	25	90	100	M/1
	Wilson 1547	19698801	3	5	13	80	100	M/2
	Wilson 1547	19698801	11	14	28	100	92	M/3
	SB 9014	RSF 94/148	10	16	24	100	53	M/5
R. principis Bureau & Franch.	L & S 2794	19370106	0	+	64	100	59	M/1
	L & S 2794	19370106	0	6	50	100	36	M/2
	L & S 2738	19832552	10	10	80	100	42	M/3
	L & S 2738	19832552A	31	35	86	100	37	M/5
	L & S 2738	19832552A	13	5	59	100	43	Y/5

Table continued on next page

TABLE 1. *Continued*

Taxon	Collector's number	Source[a]	*n*-alkane chain length relative abundances[b] C_{25}	C_{27}	C_{29}	C_{31}	C_{33}	Age[c]/ GC method
R. principis Bureau & Franch.		19698905	0	15	78	100	44	M/4
		19698905	5	11	73	100	27	M/4[d]
		19698905A	8	9	84	100	20	M/4
	LS 15797/a	Glendoick	13	12	55	100	35	M/5
	LS 15797/b	Glendoick	17	12	48	100	38	M/5
	LS 15797/c	Glendoick	9	24	48	100	58	M/5
	LS 15797/d	Glendoick	13	9	47	100	41	M/5
	LS 15797/e	Glendoick	26	28	61	100	+	M/5
		R. Adams	15	17	59	100	40	M/5
R. pronum Tagg & Forrest	Forrest 30880	19731826	10	12	37	100	37	M/3
		Glendoick	13	18	36	100	44	M/5
		RSF 78/080	6	12	21	100	50	M/5
R. proteoides Balf.f. & W.W.Sm.	ROC 151	19491025	5	6	32	100	41	M/3
		Glendoick	13	34	52	100	17	M/5
		Glendoick	20	5	26	100	36	M/5
	KGB 695	Gothenberg	17	40	54	100	37	M/5
R. przewalskii Maxim.	B 8857	RSF 94/008	12	33	43	100	52	M/5
	SB 8303	RSF 94/015	8	46	52	100	43	M/5
	SB 8303	RSF 97/045	7	10	29	100	58	M/5
		RSF 82/103	20	4	47	100	56	M/5
	SB 8302	RSF 94/023	34	92	81	100	30	M/5
		Glendoick	12	19	39	100	65	M/5
R. przewalskii ssp. *dabanshanense* (W.P.Fang & S.X.Wang) W.P.Fang & S.X.Wang	CCH 3946	Glendoick	10	10	19	100	50	M/5
	CCH 3946	Glendoick	2	8	36	100	77	M/5
		Glendoick	5	4	15	100	86	M/5
		Glendoick	4	4	14	100	56	M/5
		Glendoick	12	22	51	100	56	M/5
R. roxieanum var. *roxieanum* Forrest		RSF 74/116	5	100	23	51	10	M/5
R. roxieanum var. *cucullatum* (Hand.-Mazz.) D.F.Chamb.	Rock 10920	19241048	64	100	20	27	5	M/3
		Glendoick[e]	13	34	22	100	43	M/5
		Glendoick	8	26	25	100	34	M/5
R. roxieanum var. *oreonastes* Balf.f. & Forrest	Rock 11312	19241042	30	100	75	30	3	M/1
	Rock 11312	19241042	21	100	50	21	4	M/2
	Rock 11285	19653450	21	100	43	24	5	M/2
	Rock 11285	19653450	35	100	83	42	6	M/3
	Rock 11285	19653450	25	56	100	52	8	Y/3
	Rock 25422	19734059	59	100	46	18	2	M/2
	Rock 25422	19734059A	55	100	43	15	11	M/5
	Rock 25422	19734059A	45	100	61	26	6	Y/5
	SDR 785	wild	76	100	91	69	22	M/5
R. rufum Batalin	Hummell 31	19500299	10	20	25	100	56	M/2
	Hummell 31	19501047	8	11	27	100	67	M/3
	CHM 2591	Glendoick	3	6	10	100	81	M/5
	CHM 2591	Glendoick	7	7	27	100	74	Y/5
	CHM 2531	Glendoick	33	42	52	100	95	M/5
R. sphaeroblastum Balf.f. & Forrest	Forrest 17110	19191007	11	13	22	100	62	M/1
		19698855	5	7	31	100	52	M/3
		19698855B	3	3	28	100	66	M/4
	Forrest 17110	RSF 76/185	7	7	22	100	61	M/5
R. taliense Franch.	Forrest 6772	19568652	37	100	46	53	16	M/2
	Forrest 6772	19568652	35	100	44	58	16	M/3
	Forrest 6772	19568652B	53	100	35	34	8	M/5
		19698873	40	100	50	40	5	M/1
		19698873	38	100	45	40	10	M/2
		19698873	42	100	39	35	9	M/3
		19754131A	47	100	42	40	11	M/4
		19754131A	54	100	44	39	12	M/5
		19754131A	37	100	64	69	17	M/5
		19754131A	59	100	29	19	4	Y/4
		19754131A	54	100	78	62	12	Y/5

Table continued on next page

TABLE 1. *Continued*

Taxon	Collector's number	Source[a]	*n*-alkane chain length relative abundances[b] C_{25}	C_{27}	C_{29}	C_{31}	C_{33}	Age[c]/GC method
R. taliense		19754131A	34	100	68	58	13	Y/5
Franch.	SDR 708	wild	72	100	80	67	24	M/5
	SBEC 0350/a	Glendoick	50	100	43	48	34	M/5
	SBEC 0350/b	Glendoick	32	100	50	85	35	M/5
	SBEC 0350/c	Glendoick	31	100	35	49	20	M/5
	SBEC 0350/d	Glendoick	38	100	53	75	27	M/5
	SBEC 0350/e	Glendoick	30	100	41	55	27	M/5
	SBEC 0350/f	Glendoick	38	100	41	62	19	M/5
R. traillianum aff.		19698884	31	100	39	37	12	M/2
R. traillianum	Forrest 14774	19764105	20	100	20	20	5	M/1
Forrest & W.W.Sm.	Forrest 14774	19764105	27	100	51	68	15	M/2
R. traillianum var. *dictyotum*	Rock 18438	19851755	20	100	51	65	5	M/3
(Balf.f. ex Tagg) D.F.Chamb		Glendoick	6	23	57	100	56	M/5
R. traillianum var. *traillianum*	Rock 18444	19614564	25	100	24	14	3	M/3
Forrest & W.W.Sm.	Rock 18444	19614564	30	100	18	11	+	M/4
	Rock 18444	19614564	24	100	23	12	3	M/4
	Rock 18444	19614564A	27	100	18	10	2	M/5
	Rock 18444	19614564A	23	100	34	19	8	Y/5
R. wasonii var. *wasonii*	CCH 3926/a	Glendoick	0	20	39	100	50	M/5
Hemsl. & E.H.Wilson	CCH 3926/b	Glendoick	0	20	39	100	50	M/5
R. wasonii		19698920		4	25	100	78	M/1
Hemsl. & E.H.Wilson		19698920	2	4	18	100	55	M/2
		19698920	2	3	27	100	84	M/3
R. wasonii aff. McLaren AD106		Glendoick	5	10	16	100	76	M/5
		Glendoick	4	4	35	100	75	Y/5
R. wasonii var. *wenchuanense*		19835004	3	3	21	100	68	M/3
Rhododactylum Group		19835004A			33	100	55	M/4
		Glendoick	3	6	10	100	17	M/5
R. wasonii var. *wenchuanense*	Cox 4056		23	18	17	100	89	M/5
L.C.Hu								
R. wightii		19170032	5	10	45	100	35	M/3
Hook.f.		19698924	6	14	53	100	26	M/3
		19698924B	8	14	61	100	48	M/4
		Glendoick	23	74	39	100	26	M/5
		RSF 98/246	27	32	35	100	71	M/5
		Glendoick	6	12	27	100	74	M/5
R. wiltonii		19698928	14	20	25	100	66	M/1
Hemsl. & E.H.Wilson		19698928		13	22	100	96	M/2
		19698928	21	16	21	100	72	M/3
		Glendoick	6	9	27	100	86	M/5
		Glendoick	5	10	21	100	94	M/5
	SB 9215	RSF 95/158	7	9	24	100	96	M/5
	SB 9215	RSF 95/115	7	10	20	90	100	M/5
	SB 9215	RSF 95/199	23	30	48	100	35	M/5

[a] Reference codes are as follows:
19xxxxxx RBGE accession number.
SDRxxx Collected wild from Yunnan, May 1997.
RSF xx/xxx Rhododendron species foundation numbers.
[b] +, trace.
[c] H, herbarium; M, mature (one year old); Y, young (current season's growth).
[d] Analysis performed in Glasgow in 1993 according to method 3.
[e] May be *R. proteoides*.

samples collected on different occasions, and used the differences between these pairs of values to determine the estimated standard deviation in the measurement of any one such percentage intensity. The value obtained was just over 10 % (i.e. 10 percentage points in the intensity, not 10 % of the intensity). The contribution to this from the analysis of the wax solution is just 2 %, which leaves the standard deviation for the variation in the wax content of the leaves themselves at just under 10 %.

The variation across different samples from the same taxon was investigated by selecting six taxa which raise no questions of identification, and for which specimens from several different plants were available. For each of these six we calculated the mean and standard deviation for the percentage intensities for the alkanes with C_{max-2} and C_{max+2}. The average value of these 12 standard deviations was 11·3 %. This figure includes a contribution of over 10 % from the variation due to sampling at different times or from different parts of the plant, as discussed previously, so the variation between different specimens of the same taxon is much smaller. Combination of errors in the standard manner indicates that a standard deviation of 4–5 % can be attributed to this variation within the taxon.

A taxon with a distribution centred at exactly C_{27} and with full width at half height (FWHH) of 3·5 carbon units (see below) should therefore have intensities of 40 ± 11 for C_{25} and C_{29}. The uncertainty range is small enough to allow taxonomic use of wax measurements, but care should be taken in interpretation, as with any other botanical measurement, particularly when working with a single specimen.

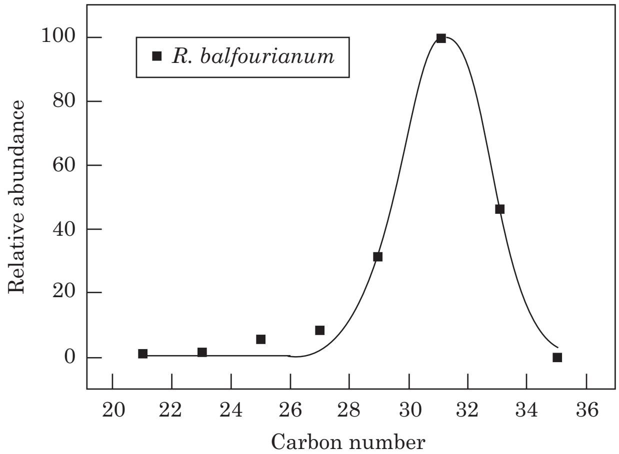

FIG. 1. Distributions of *n*-alkanes C_nH_{2n+2} in leaf waxes of five specimens of *Rhododendron balfourianum*. The composite data have been fitted by a Gaussian distribution, centred at 31·1 carbon atoms and with full width at half height (FWHH) 3·3 carbon atoms.

A key to subsection Taliensia *based on waxes*

It is immediately apparent from Table 1 that almost all specimens studied in this work have a clear maximum in the distribution of odd-numbered straight-chain alkanes at either $C_{27}H_{56}$ or $C_{31}H_{64}$. It is also evident that for the large majority of taxonomic units all specimens have the same maximum. Thus there is a sound basis for taxonomic classification on the basis of wax analysis. The critical question is whether other, less strikingly defined, features of the waxes can also be used to prepare a reliable key, or a least partial key, to the taxa within *Rhododendron* sub-section *Taliensia*.

Several attempts were made to construct such a key, using the relative amounts of the alkanes immediately above and below the maximum in the distribution, C_{max+2} and C_{max-2}. These efforts confirmed that there is indeed a lot of useful information within the distributions, but that it was impossible to define a set of criteria which would not split taxonomic units between two or more categories. However, the process drew our attention to a feature of the distributions, which allows the preparation of a key based on a single question, and yielding an ordered series of categories, with all analyses of specimens of any one good taxonomic unit falling either entirely into one category or into two adjacent categories.

For all 'good' taxonomic units (i.e. excluding those which appear in fact to be a mixture of two or more taxa, or which include a number of hybrids) the distribution of odd-carbon alkanes can be fitted to a reasonable approximation by a normal (Gaussian) distribution with a width (FWHH) of about 3·5 carbon units. Figure 1 shows as an example the fitted normal distribution for all analyses of specimens of *Rhododendron balfourianum*. The internal consistency between the analyses is good. If such a distribution is centred exactly at an odd number, say C_{27}, then the relative abundances of the adjacent alkanes, with C_{25} and C_{29}, would be approx. 40 %. Centring the distribution at an even number (while still of course considering only the distribution of the odd hydrocarbons), say at C_{28}, would give the maximum of 100 % at both C_{27} and C_{29}, while C_{25} and C_{31} would have intensities of approx. 10 %. The centre of the distribution does not have to lie at an integer; it can take any value. For example, if the centre was at 27·5, the distribution around the maximum would be C_{25} 25, C_{27} 100, C_{29} 63 and C_{31} 5 %. In practice, the intensities well away from the centre are rather higher than predicted by a Gaussian distribution, but use of this type of fitted function allows rapid and quite precise analysis of the most important part of the data.

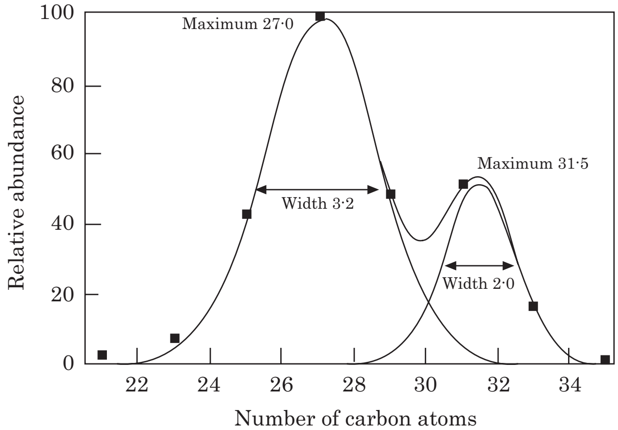

FIG. 2. Distributions of *n*-alkanes C_nH_{2n+2} in leaf waxes of 13 specimens of *Rhododendron taliense*. The composite data have been fitted by the sum of two Gaussian distributions, one centred at 27·1 carbon atoms with FWHH 3·7 carbon atoms, and the second with relative intensity of 34 %, centred at 31·5 and with FWHH 3·1.

For a few taxonomic units, mainly those with a C_{27} maximum, such as *R. taliense*, and some specimens of *R. traillianum* and *R. alutaceum*, a double normal distribution was required, with a minor component centred near C_{31} (Fig. 2). As large amounts of wax were found for C_{27} taxa, it may be that the amount of C_{31} is more or less constant, but that the extra wax in the plants is of a second kind, centred near C_{27}.

On this basis the key shown in Fig. 3 was constructed. The criteria have been selected so that each category corresponds

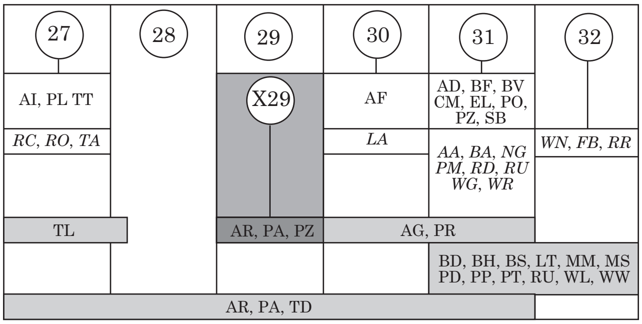

FIG. 3. Key to *Rhododendron* subsection *Taliensia* according to the maxima of leaf wax distributions. Codes in italics represent taxa for which less than five specimens were studied. Areas lightly shaded show taxa covering more than one category. The heavily shaded area represents the special category, X29 (see text). The two-letter codes represent taxa as follows: *R. adenogynum*, **AD**; *R. aganniphum* var. *aganniphum*, **AG**; *R. aganniphum* var. *flavorufum*, **AF**; *R. alutaceum* var. *alutaceum*, **AA**; *R. alutaceum* var. *iodes*, **AI**; *R. alutaceum* var. *russotinctum*, **AR**; *R. balfourianum*, **BF**; *R. balfourianum* var. *aganniphoides*, **BA**; *R. beesianum*, **BS**; *R. bhutanense*, **BH**; *R. bureavii*, **BV**; *R. bureavioides*, **BD**; *R. clementinae*, **CM**; *R. coeloneuron*, **CO**; *R. elegantulum*, **EL**; *R. faberi*, **FB**; *R. lacteum*, **LT**; *R. mimetes*, **MM**; *R. mimetes* var. *simulans*, **MS**; *R. nigroglandulosum*, **NG**; *R. phaeochrysum* var. *agglutinatum*, **PA**; *R. phaeochrysum* var. *levistratum*, **PL**; *R. phaeochrysum* var. *phaeochrysum*, **PP**; *R. prattii*, **PT**; *R. principis*, **PR**; *R. pronum*, **PM**; *R. proteoides*, **PO**, *R. przewalskii*, **PZ**; *R. przewalskii* ssp. *dabanshanense*, **PD**; *R. roxieanum* var. *cucullatum*, **RC**; *R. roxieanum* var. *oreonastes*, **RO**; *R. roxieanum* var. *roxieanum*, **RR**; *R. rufum*, **RU**; *R. sphaeroblastum*, **SB**; *R. taliense*, **TL**; *R. traillianum* aff., **TA**; *R. traillianum* var. *dictyotum*, **TD**; *R. traillianum* var. *traillianum*, **TT**; *R. wasonii* McLaren AD106, **WM**; *R. wasonii* var. *wasonii*, **WW**; *R. wasonii* var. *wenchuanense*, **WN**; *R. wasonii* var. *wenchuanense* rhododactylum group, **WR**; *R. wightii*, **WG**; *R. wiltonii*, **WL**.

to distributions with maxima in a range $N \pm 0{\cdot}5$, where N is an integer. Given data for several specimens, it should be possible to define the mean maximum for each taxonomic unit to one decimal place. Thus, for example, we can place *R. taliense* at 27·0, *R. adenogynum* at 30·8, *R. balfourianum* at 31·2 (Fig. 1) and *R. bureavii* at 31·4. Mean maxima for taxa for which five or more specimens have been analysed are listed in Table 2.

It should be noted that in some cases we only had access to material from a single plant or a single clone, so there may be variability which we have not taken into account. Nevertheless, we believe that we have shown that wax analysis provides a valuable taxonomic parameter, and that the assignment of taxa to categories in the key is a sound basis for the study of problematic species.

The numbers of taxa in the categories of the key are far from uniform. In particular, there are none at C_{29}, and few in the immediately adjacent categories. The occasional distribution centred near C_{29} invariably has a FWHH much greater than 3·5 carbon units; this is discussed below. The primary separation into C_{27} and C_{31} specimens is thus unequivocal.

Wax characteristics of hybrids

Although alkane distributions for the very large majority of specimens fall distinctly into categories in which the maximum is near C_{27} or near C_{31}, there are a few which either have double maxima, at C_{27} and C_{31}, or have unusually broad distributions, with large amounts of C_{27}, C_{29} and C_{31}. We have devised the key to place all of these specimens in a separate category, which we label X29. This sort of distribution is much more common in populations of hybrids between C_{27} and C_{31} species (see below), and on that basis alone it is reasonable to suppose that its occurrence indicates such a hybrid. In Table 1 these distributions occur only for specimens of taxa which are widely believed to include, or to consist entirely of, hybrids. We therefore propose the hypothesis that a wax distribution in category X29 is indicative, but not a necessary indicator, of a hybrid between a C_{27} and a C_{31} taxon.

TABLE 2. *Maxima in wax distributions*

Species	Maximum	Double peaked*
R. adenogynum	30·8	
R. aganniphum var. *flavorufum*	30·1	
R. alutaceum var. *iodes*	27·0	
R. alutaceum var. *russotinctum*	28·5	26·9/(30·9)†
R. balfourianum	31·2	
R. bureavii	31·4	
R. clementinae	31·0	
R. elegantulum	31·0	(25·6)/31·1
R. phaeochrysum var. *agglutinatum*	30·3	(27·2)/31·4
R. phaeochrysum var. *levistratum*	27·0	
R. pronum	30·3	(26·2)/31·1
R. przewalskii	30·8	(28·0)/31·5
R. taliense	27·7	27·0/(31·5)
R. traillianum var. *traillianum*	27·0	

* Only carried out in cases where a second peak was clearly significant; figures in parentheses are the positions of the minor peaks.
† A Lorentzian fit was used because a Gaussian fit was inadequate.

Problematic taxa

The data for a few species do not fall neatly into the logical categories that work so well for most taxa. Chemical analysis may prove to be of particular value in such cases as these species are subject to confusion when morphological features alone are considered. A full discussion of both chemistry and morphology will be published in a separate paper. Here we merely draw attention to the apparent anomalies in the data.

Rhododendron alutaceum has three varieties, *alutaceum*, *iodes* and *russotinctum*. The first of these is a good C_{31} taxon (i.e. all analyses are in category 31, but on the basis of rather few clearly-defined specimens), while var. *iodes* is unequivocally C_{27}. It has been stated (Cox and Cox, 1997) that most cultivated plants of *R. alutaceum* var. *alutaceum* are forms of *R. roxieanum*, but the latter species usually has C_{27} as the maximum in its wax distribution, so the chemistry suggests that there may be some other explanation for the appearance of these plants. Some specimens of *R. a.* var. *russotinctum* have maxima at C_{27}, others have maxima at C_{31}, but another one is quite different, with a double maximum at C_{27} and C_{31} in all analyses. This is the pattern which we believe to be characteristic of a hybrid between C_{31} and C_{27} taxa. The specimen concerned, RBGE 19698820D,

TABLE 3. *Wax analyses for a hybrid population of* Rhododendron roxieanum *and* R. beesianum

Plant	Age*	*n*-alkane chain length relative abundances							GLC method†
		C_{21}	C_{23}	C_{25}	C_{27}	C_{29}	C_{31}	C_{33}	
Plant 1	H	7	10	58	100	49	80	66	5
Plant 2	H	7	14	44	100	50	28	13	5
Plant 3	H	1	4	30	100	32	14	3	5
Plant 4	H	4	8	33	100	42	26	11	5
Plant 5	H	1	13	29	100	43	27	8	5
Plant 6	H	1	6	27	100	56	40	10	5
Plant 7	H	3	10	40	100	40	30	8	5
Plant 8	H	2	12	70	100	14	7	3	5
Plant 9	H	8	12	16	25	66	100	49	5

* H, Herbarium.
† See Materials and Methods.

is one of a group of plants of unknown origin given this accession number in 1969 on the basis of their presumed identity as *R. a.* var *russotinctum*. Reinspection has shown that some plants clearly belong to var. *iodes*, while others, fitting the description of typical var. *russotinctum*, are probably hybrids. If such hybrids involve C_{27} forms of *R. roxieanum* in their ancestry, they must be derived from *R. alutaceum* var. *alutaceum*, not from var. *iodes*, to provide the C_{31} component of their waxes. However, *R. roxieanum* also shows a confused mixture of wax distributions, particularly in var. *cucullatum*. The chemistry of the three *R. roxieanum* varieties suggests that they are not simply a cline. Although *R. roxieanum* is usually identifiable unequivocally, particularly var. *oreonastes* with its distinctive narrow leaves, two specimens, SDR785 and Rock 11285, show wax distributions which indicate that they are probably hybrids. Thus even the most morphologically distinct taxa may include unsuspected hybrids, whose masquerading may be unmasked by chemical analysis.

The dramatic distinction between the chemistry of var. *alutaceum* and that of var. *iodes* raises the question of whether taxa distinguished in such a way should merely be separated as varieties. We will address that issue in a later publication. A similar question arises with *R. phaeochrysum*. On the one hand all specimens of *R. p.* var. *levistratum* are characterized by a maximum at C_{27}, whereas all *R. p.* var. *phaeochrysum* have wax with a maximum of C_{31}. We have found no evidence in the wax analyses for hybrids between these two varieties. This leaves *R. p.* var. *agglutinatum*, which should be readily identifiable by its agglutinated indumentum, but has a complex mixture of wax types: C_{27} maximum, C_{31} maximum, and double C_{27}/C_{31} maximum with high C_{29}. The clone of this last type (Rock 11335) thus shows the characteristics of a hybrid between C_{27} and C_{31} taxa. The possibility that all specimens of *R. a.* var. *agglutinatum* are hybrids cannot be ruled out.

Rhododendron aganniphum raises different issues. Most, if not all, specimens of *R. a.* var. *flavorufum*, although gathered from three different gardens, turned out to be of the same clone. Analyses for this variety consistently gave levels of C_{29} not much lower than those of C_{31}, a rare pattern otherwise only found in *R. principis* in this subsection. We cannot tell whether this clone is abnormal, perhaps a hybrid, or typical of an unusual taxon. Specimens of *R. a.* var. *aganniphum* also show high abundances of C_{29} and C_{31} waxes, but also in most analyses large amounts of C_{27}; again this suggests that some or all plants are hybrids. The status of *R. aganniphum* is therefore not satisfactorily defined by the present work.

Wild populations of hybrids

Populations which clearly contain hybrids provide the opportunity to relate the leaf waxes to plant morphology. Occasionally one finds small, localized populations of plants which are quite distinct from their neighbours. Sometimes both parents are adjacent, sometimes just one, and in the latter cases it is not necessarily obvious what the second parent is. These small populations are of much greater value for our purposes than the huge hybrid populations found in many areas of Western China and the Himalayas. These large populations may be derived from more than two species, and it is often difficult to be sure what these species are, particularly as it is usually the most ill-defined taxa which are involved.

In the simplest case, the population may be represented by two clearly distinct species and well-defined morphological hybrids. An example of this is shown in Table 3, which presents the analytical results for a group of plants which included *R. roxieanum* var. *oreonastes* × *beesianum*, from Bai Ma Shan in North-West Yunnan, China. These two species are very obviously different both morphologically and chemically; one (*R. roxieanum*) is a C_{27} species while the other is a C_{31} species. Five of the nine plants (numbers 2–6) have the appearance of good *R. roxieanum* var. *oreonastes*, and the wax distributions are also characteristic of that taxon, with the maximum at C_{27}, and with a tendency to have rather more C_{29} than C_{25}. Plant number 9 is similarly confirmed as typical *R. beesianum*. Plants 7 and 8 are clearly shown by their morphology to be hybrids of *R. roxieanum* and *R. beesianum*. However, the waxes from plant 7 are consistent with those from pure *R. roxieanum*, while those from plant 8 show a surprisingly large amount of C_{25}, more than we have observed in other

TABLE 4. *Wax analyses for a hybrid population of* Rhododendron przewalskii *and* R. phaeochrysum *var.* levistratum

Plant	Age*	*n*-alkane chain length relative abundances							GLC method†
		C_{21}	C_{23}	C_{25}	C_{27}	C_{29}	C_{31}	C_{33}	
Plant 1	M		6	40	100	17	9	2	3
Plant 2	M		7	36	100	69	38	5	3
Plant 2	H		5	34	100	88	76	21	5
Plant 3	M		4	8	33	60	100	51	3
Plant 4	M		3	8	9	27	100	55	3
Plant 4	H		19	30	21	24	100	63	5
Plant 5	M	2	4	6	7	27	100	53	3
Plant 5	H		3	5	12	32	100	39	5
Plant 6	M	2	4	5	5	33	100	45	3
Plant 6	H		11	20	23	32	100	57	5
Plant 7	M		4	8	8	20	100	81	3

* H, Herbarium; M, mature (1 year old).
† See Materials and Methods.

TABLE 5. *Wax analyses for a hybrid population of* Rhododendron proteoides *and* R. aganniphum

Plant	Age*	*n*-alkane chain length relative abundances								GLC method†
		C_{21}	C_{23}	C_{25}	C_{27}	C_{29}	C_{31}	C_{33}	C_{35}	
Plant 1	H	12	42	73	90	100	79	32		5
Plant 2	H	7	34	35	22	60	100	34	11	5
Plant 3	H	5	8	54	75	100	93	42	17	5
Plant 4	H	5	5	3	6	45	100	55		5
Plant 5	H	3	3	8	7	51	100	35		5
Plant 6	H	4	4	8	9	53	100	41		5

* H, Herbarium.
† See Materials and Methods.

specimens of this species. Thus there is no chemical evidence for these two plants being hybrids. In contrast, plant 1 gives maxima for both C_{27} and C_{31}, with the latter at 80 % of the intensity of the former, but its appearance is close to that of *R. roxieanum*. Therefore, in this case, there is chemical evidence of hybridization with a C_{31} species, in the absence of any obvious morphological evidence. Where at least some specimens in a population show a wax profile that combines characteristics of two possible parent species, then it may be inferred that these specimens have a hybrid origin whether or not they are intermediate morphologically. However, conversely, absence in any individual of such a profile does not necessarily imply that that individual should be referred to as one of the parents.

Table 4 gives data for a small population of plants from the Zheduo Pass, above Kanding in Sichuan Province, China, which appear to include hybrids of *R. przewalskii* and a variety of *R. phaeochrysum*. Leaves from all of these plants were studied when they were fresh, and several were investigated from dried material after many years, with satisfyingly good agreement. Plant 1, which looks like pure *R. phaeochrysum*, is a C_{27} species, which is therefore most likely to be *R. phaeochrysum* var. *levistratum*. Plant 7 looks like *R. przewalskii*, and from the wax it probably is, although the C_{33} value of 81 is at the top end of the observed range for this species. Plant 2 looks like *R. phaeochrysum*, but it has what we have come to recognize as the hallmarks of a hybrid: high C_{29}, and moderate to high values for both C_{27} and C_{31}, particularly in the dried specimen. On the basis of the wax analyses for this plant and its neighbours, we can identify it as *R. przewalskii* × *R. phaeochrysum* var. *levistratum*. The chemical analysis in this case thus gives a strong indication of the second parent of the hybrids, distinguishing between *R. p.* var. *levistratum* and *R. p.* var. *phaeochrysum*. Plants 3–6 all have intermediate morphology, and are therefore presumably hybrids of the same parentage. However, plants 4–6 analyse as good *R. przewalskii* (C_{31} maximum), demonstrating again that not all hybrids show their mixed parentage in their waxes. The remaining plant, number 3, is less clear cut, but the high content of C_{29} and the moderate value for C_{27} indicate that it is also probably a hybrid of the same kind, in its chemistry tending more to the characteristics of its *R. przewalskii* parent, whereas plant 2 tends more to the *R. phaeochrysum* parent.

Table 5 presents data for a population of plants from Mei Li Shan, North-West Yunnan, China, which were believed to have *R. proteoides* and *R. aganniphum* in their parentage. Distributions of waxes for plants 4–6 are consistent with them being *R. proteoides*, while plant 2 has a similar distribution, but with rather more C_{29}, which might indicate a hybrid. Of these four plants, number 5 has the appearance

TABLE 6. *Wax analyses for a hybrid population of* Rhododendron proteoides *and* R. phaeochrysum

Plant	Age*	*n*-alkane length relative abundances								GLC method†
		C_{21}	C_{23}	C_{25}	C_{27}	C_{29}	C_{31}	C_{33}	C_{35}	
Plant 1	H	4	19	66	100	80	79	30		5
Plant 2	H	5	7	25	23	54	100	57		5
Plant 3	H	3	14	25	27	76	100	31		5
Plant 4	H	12	14	37	29	58	100	30		5
Plant 5	H	2	6	30	100	30	16	4		5

* H, Herbarium.
† See Materials and Methods.

of *R. proteoides*, while number 4 is also very close to the pure species. Plants 2 and 6 are clearly hybrids, despite their wax distributions, and are consistent with the description of *R. bathyphyllum* Balf.f. & Forrest, which is now believed to be *R. proteoides* × *aganniphum*. Plants 1 and 3 are clearly different, both having maxima for C_{29}, with large amounts of both C_{27} and C_{31}. Plant number 3 has the appearance of a hybrid, but plant 1 looks like pure *R. proteoides*. However, neither of the varieties of *R. aganniphum* is a C_{27} taxon, as all specimens we examined had either a C_{31} maximum (albeit often with a high C_{29} abundance) or were probably hybrids themselves. The data for plants 1 and 3 of this hybrid population have wax distributions which are quite similar to those of some cultivated plants labelled *R. a.* var. *aganniphum* (particularly of Forrest 16472), so it is difficult to make any unequivocal statements about their parentage.

Data for a second population, also from Mei Li Shan and which was also thought to have *R. proteoides* and *R. aganniphum* as parents, are shown in Table 6. The waxes for plants 2 and 4 clearly indicate *R. proteoides*, while those for plant 3 are similar, but with rather more C_{29}, which may indicate a hybrid. However, although the appearance of plant 4, as straight *R. proteoides*, conforms with its wax analysis, plant 3 also looks like pure *R. proteoides*, while plant 2 is probably a hybrid. Plant 5 looks like a hybrid, but its wax is that of a clear C_{27} species, which cannot therefore be *R. aganniphum*, on the basis of our knowledge of that species. The most likely candidate is *R. phaeochrysum* var. *levistratum*, which must therefore also be a likely parent of the hybrids in Table 5, even though this taxon was not observed in the immediate vicinity of the hybrid populations. Plant 1 in Table 6, with the pattern of distribution of waxes which we have come to recognize as characteristic of a $C_{27} \times C_{31}$ hybrid, with high concentrations of C_{27}, C_{29} and C_{31}, is presumably therefore *R. proteoides* × *R. phaeochrysum* var. *levistratum*, and its appearance is consistent with this assignment.

A final example illustrates the value of wax analyses for the confirmation of the identity of parents of a hybrid. Plants grown from *R. taliensia* seed (SBEC 0350) collected on the Cangshan mountains in Yunnan, China, included a few which had some characteristics of *R. lacteum*, which grows a little lower down the mountain. The two species have completely different alkane distributions, and a wax sample from a plant believed to be a hybrid had the pattern characteristic of *R. lacteum*. As the seed came from *R. taliense*, the identification of the hybrid as *R. taliense* × *lacteum* is unequivocal.

Overall, therefore, we may state the following conclusions.

(1) All well-defined taxa consist of specimens which consistently have maxima in the alkane components of their leaf waxes at either $C_{27}H_{56}$ or $C_{31}H_{64}$.
(2) The precise positions of the maxima in the alkane distribution can be a useful taxonomic tool.
(3) Populations of natural hybrids between C_{27} and C_{31} taxa include specimens with wax distributions which are additive combinations of those of their parents. An abundance of C_{29} in the wax usually, if not always, indicates the presence of a hybrid between C_{27} and C_{31} taxa.
(4) The utility of wax analysis as a means of identifying the parents of plants in hybrid populations has been demonstrated.
(5) A few taxa, notably *R. aganniphum*, *R. alutaceum* var. *russotinctum*, *R. phaeochrysum*, and to a lesser extent *R. roxieanum*, contain a confusing range of specimens with different wax characteristics. These taxa also present morphological problems, which we will address in a future paper.

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