Phytochemistry, Vol 27, No 11, pp 3499-3507, 1988
Printed in Great Britain

0031-9422/88 $3.00+0.00

INTERSPECIFIC VARIATION IN EPICUTICULAR WAXES OF *CHIONOCHLOA*

MARION G. SAVILL, ROY BICKESTAFFE and HENRY E CONNOR*

Department of Biochemistry, Lincoln College, Canterbury, New Zeland, *Centre for Resource Management, University of Canterbury, Christchurch, New Zealand

(*Received in revised form* 16 *February* 1988)

Key Word Index—*Chionochloa*, Gramineae, epicuticular wax, esters, alkanes, aldehydes; alcohols; acids, chemotaxonomy

Abstract—Interspecific variation in 19 species of *Chionochloa* was investigated by examining the major carbon chain lengths of fatty acids, alcohols, aldehydes, wax esters and alkanes of the epicuticular waxes. The chain lengths ranged from C_{24} to C_{32} in the acids, alcohol and aldehydes C_{21} to C_{33} in the alkanes and C_{34} to C_{52} in the wax esters. The degree of similarity between samples in terms of chain length distribution in the various lipid classes was calculated. In general, each sample has its own characteristic sequence of groups in the sequence order acid, alkane, alcohol, aldehyde and ester Using this sequence, it is possible to identify a species of *Chionochloa* if its original locality is known. Complete analysis of the major chain lengths of all five lipid fractions demonstrated the chemotaxonomic inter-relationship between the 19 species of *Chionochloa*. One Australian species, *C. frigida*, was chemically similar to some of the New Zealand species.

INTRODUCTION

Chionochloa as treated by Zotov is an austral genus of 23 long-lived perennial species, 21 of which occur only in New Zealand [1] Ecologically, they are among the most important grasses in New Zealand occurring from sea level to 2000 m in widely diverse sites. Chemotaxonomic studies in the genus have revealed interspecific and intraspecific variations in cellulose and hemicellulose [2-4], inorganic elements [2, 5, 6] and in the kinds and distribution of triterpene methyl ethers [7, 8] The carbon chain lengths found in the lipid components of epicuticular waxes of four species, *C flavescens*, *C. pallens*, *C. rigida* and *C rubra*, have also demonstrated that there are interspecific and intraspecific differences between them [9, 10]

Although now considerably illuminated by these extensive biochemical investigations, taxonomic interpretation of the genus is still limited because some of the biochemical markers have not occurred in all species, a constraint which cannot apply to the fatty acyl lipid constituents of epicuticular wax In this paper we examine interspecific variation in the distribution of the major carbon chain lengths of wax esters, alkanes, aldehydes, acids and alcohols of epicuticular wax of 18 New Zealand, together with one Australian species, to determine whether lipid composition could be useful as a means of chemotaxonomic analysis

RESULTS AND DISCUSSION

The major lipid components of the epicuticular waxes identified by TLC were alkanes, esters, aldehydes, alcohols and fatty acids in all 19 species examined GLC and mass spectrometry of each lipid fraction showed that the esters ranged from C_{34} to C_{52} (Table 1) alkanes from C_{21} to C_{33} (Table 2) aldehydes from C_{24} to C_{32} (Table 3) alcohols from C_{24} to C_{32} (Table 4) and fatty acids from C_{18} to C_{32} (Table 5) as would be expected from the earlier report of Cowlishaw *et al.* (1983) [9]. Small amounts of two unidentified series were found in some of the alcohol and acid fractions and one unidentified series in the aldehyde fraction [9].

The distribution of chain lengths in each lipid class of each sample was examined in a Biomedical Computer Program (BMDP2M), which arranges in a common order of similarity all those samples with a particular chain length or a sequence of chain lengths. Different levels of similarity are separated from each other by a quantity termed the amalgamation distance The closer the similarity the shorter the distance. In this way, the program allocated each lipid fraction of every sample into one of ten possible groups (Table 6). In general, each sample has its own characteristic sequence of groups, in the sequence order fatty acid, alcohol, aldehyde, ester and alkane. Some sequences are identical or nearly so, reflecting that the samples were members of the same or closely related species.

To assess the inter-relationship among the different species of *Chionochloa*, and of samples within the same species, the known chain lengths of all five lipid fractions of the epicuticular wax were examined, in one analysis, by the BMDP2M 'Clustering of Cases' Biomedical Computer Program. Each of the five lipid fractions was given equal weighting The groups of samples obtained from the programme with similar characteristics are shown in dendrogram form in Fig. 1. In the dendrogram the groups of samples are listed on the *x* axis and the amalgamation order and distance that separates the groups along the *y* axis.

Table 1 Homologue composition (wt %) of major esters in epicuticular waxes of species of *Chionochloa*

		Carbon chain length								
Species	No	36	38	40	42	44	46	48	50	52
C acicularis	1	2	13	11	19	10	9	13	11	7
C antarctica	1	t	3	30	39	14	6	4	3	1
C. australis	1	1	1	13	24	12	6	8	38	2
	2	—	3	2	10	13	9	6	13	43
C beddiei	1	—	1	1	12	21	21	6	19	20
C bromoides	1	1	1	5	16	30	32	2	2	12
C cheesemanii	1	—	8	5	22	26	11	12	7	10
C conspicua	1	7	30	12	16	7	5	4	4	9
	2	10	8	22	11	7	8	7	8	18
	3	9	20	16	15	7	5	5	6	11
C. crassiuscula	1	—	t	2	7	11	14	12	36	18
C. defracta	1	t	2	16	16	9	7	20	20	11
C elata	1	1	4	3	11	13	10	14	22	22
C flavescens	1	t	2	21	19	17	22	9	6	2
	2	t	2	12	18	21	27	12	4	4
	3	t	2	19	13	21	26	8	7	4
	4	t	2	17	10	24	24	8	11	4
	5	t	2	22	21	11	12	9	10	12
	6	t	8	25	34	16	12	3	1	1
	8	t	3	23	33	5	1	2	8	26
	9	t	t	7	60	10	1	t	t	15
C. flavicans	1	2	7	33	11	10	13	10	13	2
C frigida	1	1	6	5	9	4	14	13	12	16
C macra	1	2	3	7	17	9	4	6	18	34
	2	4	3	9	31	16	7	6	8	10
	3	10	12	10	23	8	3	4	13	12
C pallens	1	2	3	6	30	16	5	10	16	11
	2	1	4	4	28	26	6	12	13	7
	3	4	5	11	8	11	10	5	13	32
	4	4	9	11	14	15	15	27	t	1
	5	20	55	5	7	5	2	4	2	1
C rigida	1	t	2	19	23	18	21	9	4	3
	2	t	3	17	29	12	16	7	4	12
	3	t	2	18	23	14	15	9	8	10
	4	t	2	14	20	15	19	10	12	10
	5	t	4	11	18	14	9	9	26	9
C. rubra	1	1	3	26	17	9	11	13	10	5
	2	t	4	25	29	11	11	10	6	1
	3	t	5	34	25	10	7	8	6	5
	4	t	3	10	21	15	15	9	8	20
	5	—	2	5	13	13	30	12	10	5
	6	2	11	65	7	7	3	2	3	
C. spiralis	1	t	3	5	13	19	16	18	13	5
C. teretifolia	1	t	1	7	14	6	2	14	55	—

t, Trace <0 5%, —, not detected

The position or grouping of a sample, relative to other samples, is determined by the variation in the carbon components of each sample. Consequently, the groups along the *x* axis, are arranged in order of similarity with the greatest difference being between the extremes P1a and Q7 The amalgamation distance between the successive groups, P1 to P5 and Q1 to Q7, is 0.5 or less, illustrating that the differences between the groups are similar The larger amalgamation distance 1.2 separates P5 and Q1

The large P1 group is subdivisible into three. The samples in P1a, P1b, P1c, P2 and P4 tend to be characterized by particular chain lengths in certain lipid fractions In general, P1a is characterized by C_{30} acids and C_{29} alkanes, P1b and P1c by C_{32} acids and C_{31} alkanes, and P1c by an increase in the chain length of the major esters. In P2 to Q7, one of the following differences mostly accounts for the position allocated to the samples (i) the absence of the same chain length component in the fatty acid, alcohol and aldehyde fractions or the dominance of a particular decarboxylation product in the alkane fraction (ii) The absence of high amounts of the major C_{29} or C_{31} alkanes [9, 10]. (iii) The absence of large quantities of C_{40} to C_{52} esters

Table 2 Homologue compositioion (wt %) of alkanes in epicuticular waxes of species of *Chionochloa*

		Carbon chain length						
Species	No	23	25	26	27	29	31	33
C acicularis	1	1	54		3	3	11	23
C antarctica	1	t	1		3	30	62	t
C australis	1	2	10		12	19	25	6
	2	3	8		10	18	32	8
C beddiei	1	t	28		28	12	12	8
C. bromides	1	—	2		16	64	12	t
C cheesemanii	1	—	1		2	4	21	65
C conspicua	1	t	*t*		1	6	63	25
	2	1	2		2	68	18	6
	3	t	*t*		t	5	65	25
C crassiuscula	1	t	1		4	12	53	24
C defracta	1	2	1		2	15	58	19
C elata	1	—	*t*		4	21	45	18
C flavescens	1	—	1		—	69	18	8
	2	t	t		5	67	13	12
	3	1	2		6	68	10	10
	4	t	1		4	57	27	6
	5	2	t		3	52	34	6
	6	11	18		7	21	18	15
	7	19	11		7	24	9	11
	8	4	4		5	19	57	6
	9	6	6	× 34	8	12	31	t
C flavicans	1	4	5		9	67	3	2
C frigida	1	t	t		6	37	43	3
C macra	1	6	5		—	9	62	11
	2	4	4		3	11	50	20
	3	8	6		5	10	48	8
C pallens	1	t	t		1	9	73	10
	2	t	t		2	25	49	9
	3	3	3		4	30	35	16
	4	—	4		3	14	66	3
	5	t	t		2	17	49	28
C rigida	1	t	1		—	75	12	9
	2	t	t		—	58	18	18
	3	t	1		—	51	28	16
	4	t	2		4	39	43	8
	5	1	3		1	13	76	4
C rubra	1	t	1		—	20	66	9
	2	1	2		2	22	51	9
	3	t	1		2	15	61	11
	4	3	5		7	29	41	8
	5	t	4		8	22	31	17
	6	9	2		8	42	30	4
C spiralis	1	t	5		7	17	61	8
C teretifolia	1	—	2		1	4	65	16

Footnotes as Table 1

Previous work [9] has shown that it is difficult to characterize, or fingerprint, species solely by the composition of their epicuticular waxes as in some species composition varies with locality and time This work demonstrates, however, that within a particular locality, species can be identified by their grouping sequence as determined from the carbon chain lengths of the lipid classes of the epicuticular wax. It is, therefore, possible to identify species of *Chionochloa* at a particular locality utilising the carbon chain lengths of the lipid constituents of the wax—there is site-species specificity.

From the analysis of the chain lengths of the acids, alcohols and alkanes, it is also clear that the major fatty acyl chains are C_{30} and/or C_{32} with the decarboxylation enzymes producing C_{29} and/or C_{31} alkanes. It has previously been suggested that this is the simplest situation with enzymes optimized for C_{30} and C_{32} chain lengths [9] but there are notable exceptions, *viz* (i) *C. acicularis* which has a possible C_{26} and C_{34} fatty acyl pathway. (ii) *C beddiei* synthesizing the lower carbon chain lengths of C_{26} and C_{28}. (iii) *C. cheesemanii*, *C. conspicua* [1, 3], *C. crassiuscula*, *C. macra* [2], *C. pallens* [5] and *C rigida*

Table 3 Homologue composition (wt %) of aldehydes in epicuticular waxes of species of *Chionochloa*

		Carbon chain length						
Species	No	24	26	28	30	D	e	32
C acicularies	1	t	1	2	42	-	2	3
C antarctica	1	25	26	15	12		2	6
C australis	1	7	23	13	12	5	1	23
	2	2	4	7	8	58	4	6
C beddiei	1	2	50	25	7		t	3
C bromoides	1	t	1	3	16			68
C cheesemanii	1	t	t	t	1	48	—	2
C conspicua	1	13	12	9	10	t	t	20
	2	13	15	12	20		2	18
	3	7	14	14	12		-	27
C crassiuscula	1	t	t	6	73			13
C defracta	1	4	7	12	20	—	1	35
C elata	1	t	4	10	21		t	24
C flavencens	1		14	34	44	-		4
	2	9	15	24	25	—	t	2
	3	2	11	25	51	—	2	2
	4	2	10	29	40	9	1	3
	5	3	9	10	13	57	-	4
	6	20	24	26	17		t	8
	7	6	4	1				75
	8	24	12	5	13	30		4
	9	23	19	8	16	-	12	5
C flavicans	1	13	18	17	33		4	9
C frigida	1		2	6	42	-	1	23
C macra	1	-	39	16	12			28
	2	41	25	10	5	1	t	9
	3	50	24	8	4		-	5
C pallens	1	t	3	5	53	—	3	32
	2	t	1	4	29	-	32	28
	3	t	4	23	20		—	14
	4	2	1	1	89			—
	5	12	3	3	20		5	35
C rigida	1	-	6	29	51			4
	2		6	20	50	—	-	8
	3	-	10	24	42		-	16
	4	3	17	19	28	11		13
	5	1	6	8	45	10	t	14
C rubra	1		4	5	10		66	13
	2	-	-		77			2
	3	1	7	11	29		7	36
	4	9	20	23	34		t	6
	5	2	7	14	7	19	t	11
	6	55	14	14	7		t	3
C spiralis	1		11	13	16	14	-	33
C teretifolia	1	—	19	13	11		-	48

D = unknowns following the even chain aldehydes, e = unknowns between the even chain aldehydes
Footnotes as Table 1

[2, 3] with high concentrations of a C_{33} alkane, indicative of a C_{34} fatty acyl pathway. (iv) *C flavescens* [6] and to a lesser degree [7] from Westland with no dominant chain length and (v) *C flavescens* [9] with an odd-chained C_{27} pathway. The Australian species *C frigida* had a C_{32} elongation decarboxylation pathway

Despite these exceptions, the variation in the chain lengths in the lipid fractions may still be explained by enzyme specificity. For example, in the majority of species, the specificity of the enzyme synthesizing the acid, alcohol and aldehyde were directed towards C_{32} and C_{30} and for the alkanes to C_{31} and C_{29}. Chain lengths of 24, 26 and 28 were found in many of the acid, alcohol and aldehyde fractions but the corresponding C_{23}, C_{25} and C_{27} alkanes did not occur to the same extent A possible explanation is that the decarboxylation enzymes are less specific for 24, 26 and 28 carbon chain lengths or alternatively the enzymes responsible for utilising the 24, 26

Table 4 Homologue composition (wt%) of primary alcohols in epicuticular waxes of species of *Chionochloa*

Species	No	Carbon chain length					
		24	26	28	30	U	32
C acicularis	1	4	2	2	5	16	64
C antarctica	1	43	7	4	2	t	10
C. australis	1	2	1	4	7	24	28
	2	2	3	5	2	22	41
C. beddiei	1	t	17	42	26	—	11
C. bromoides	1	—	8	18	39	2	18
C cheesemanii	1	1	t	1	10	—	71
C. conspicua	1	3	2	1	t	4	17
	2	10	7	5	5	—	46
	3	5	2	3	7	—	49
C crassiuscula	1	1	2	20	24	5	44
C defracta	1	21	8	4	5	1	59
C elata	1	t	5	5	21	—	62
C flavescens	1	23	5	17	37	2	14
	2	31	4	19	39	2	4
	3	16	5	17	46	3	9
	4	20	3	17	47	3	9
	5	41	11	13	14	2	15
	6	53	8	11	6	t	7
	7	62	6	4	3	2	4
	8	73	4	1	1	3	4
	9	2	24	4	3	8	40
C. flavicans	1	57	7	3	8	—	9
C. frigida	1	t	1	4	11	—	45
C. macra	1	17	10	3	t	24	43
	2	8	2	3	2	—	23
	3	19	7	2	2	12	19
C pallens	1	2	12	3	8	27	46
	2	1	3	2	8	5	72
	3	7	2	8	7	1	66
	4	1	2	3	18	—	62
	5	—	—	—	3	5	90
C. rigida	1	21	4	12	30	8	18
	2	48	3	9	12	12	16
	3	25	4	13	16	9	26
	4	40	4	7	9	9	14
	5	4	7	7	5	24	45
C. rubra	1	17	3	4	17	2	56
	2	29	3	2	9	—	49
	3	29	5	3	5	2	42
	4	15	6	10	5	8	41
	5	2	2	13	6	—	62
	6	66	3	4	2	2	10
C spiralis	1	1	4	4	4	—	81
C. teretifolia	1	t	3	1	5	—	18

U, unknown; t, Trace $< 0.5\%$

and 28 carbon acid and alcohols act faster than the decarboxylation enzymes. Both possibilities would result in a reduction in the C_{23}, C_{25} and C_{27} alkanes [9].

Twelve taxa are represented by single samples: *C. acicularis*, *C. antarctica*, *C. beddiei*, *C. bromoides*, *C cheesemanii*, *C. crassiuscula*, *C. defracta*, *C. elata*, *C. flavicans*, *C. frigida*, *C. spiralis*, *C teretifolia*, which limits the interpretation of the data. It also creates in the case of *C. cheesemanii* (Q1 Fig. 1) a departure from the proposition that plants from the Nelson region display a simple C_{30} elongation-decarboxylation pathway. However, despite these limitations, Fig. 1 indicates that both *C. pallens* (at Q6) and *C. flavescens* (at Q5), from the Tararua Range, depart significantly from other members of the same species, except for the Clarke River sample. The Tararua Range plants also differ from South Island populations (5–7) in their triterpene methyl ether composition, ecological and nutritional patterns.

Table 5 Homologue composition (wt %) of fatty acids in epicuticular waxes of species of *Chionochloa*

		Carbon chain length							
	No	18	20	22	24	26	28	30	32
C acicularis	1	—		—	1	9	24	43	13
C antarctica	1	7	3	13	9	11	11	15	14
C australis	1	4	-	2	6	19	17	14	27
	2	—	9	4	11	16	17	12	16
C beddiei	1	-			5	31	11	3	29
C bromoides	1	-				3	7	31	10
C chesemanii	1	—	15	—	2	1	3	5	48
C conspicua	1	12	2	1	9	11	11	9	26
	2	14	7	2	6	5	6	9	12
	3		—-	14	5	6	9	10	25
C crassiuscula	1	33	3	-	t	2	12	14	26
C defracta	1	37	-	—	1	2	3	4	33
C flavescens	1		—	—	2	12	29	28	15
	2	27	8	--	4	7	16	12	20
	3	-	11	-	2	8	26	21	18
	4	29	7	--	2	6	21	16	12
	5	--	--	6	5	12	15	23	30
	6		—	—	14	14	18	13	11
	7	8	7	5	31	17	14	9	3
	8		5	3	26	14	5	6	25
	9	17	2	4	27	16	4	5	12
C flavicans	1	--	2	2	12	13	15	28	13
C frigida	1	29	4	2	-		2	10	14
C macra	1	—	—	-	—	48	14	9	19
	2	3	2	3	28	18	6	5	18
	3	9	7	4	33	14	7	4	10
C pallens	1	12	3	33	1	3	2	8	38
	2		2	--	1	1	7	29	29
	3	19	6	3	1	3	19	18	22
	4		—	21	1	9	8	15	13
	5	-	8	1	t	1	2	16	17
C rigida	1	—	—	—	6	9	36	31	7
	2	--		-	3	11	28	42	8
	3		--	—	8	12	30	26	11
	4	40	6	2	4	10	9	11	10
	5	22	17	3	2	7	7	5	17
C rubra	1	—	—	—	--	12	14	22	29
	2		14	1	4	4	4	19	33
	3	29	5	-	2	7	5	8	39
	4	17	2		6	16	19	12	15
	5		-	1	2	4	25	34	10
	6	—	3	4	2	9	25	34	10
C spiralis	1		-	3	9	13	12	20	18
C teretifolia	1	—	--	—	6	23	7	3	14

Footnotes as Table 1

The other taxa in group 'Q' are the two localised maritime species *C bromoides* (Northland) at Q2 and *C beddiei* (Wellington) at Q7 both of which are closely related (Zotov [1]), and *C rubra* from Mt Egmont (6) at Q3 which is atypical of the species There is no adequate explanation for the position of *C. cheesemanii* (Nelson) at Q1 or *C. flavescens* (Clarke River, 7) at Q4

Group P1 contains all the expected samples except perhaps *C spiralis* (Fiordland), which may have been expected with *C teretifolia* and *C acicularis*. Finally, the dendrogram of Fig 1, allowing for the fact that *C ovata*, *C pungens*, *C oreophila* and *C. juncea* are not included, bears little resemblance to that of Zotov [1] They are, however, constructed on different principles, the computer-generated dendrogram of this work reflects, in part, the analysis previously reported [9], and also mirrors the concept that Nelson is the biogenetic centre from which the gene-enzyme systems controlling the composition of epicuticular wax diversified [9]. Consequently, *C spiralis* (Fiordland) a species of limestone habitat, is out of place

Table 6 Assignment of each *Chionochloa* sample into groups using the major chain lengths of (a) all lipids or (b) individual lipid classes

		Assigned grouping using					
Chionochloa sample	No	(a) All lipids	(b) Individual lipid classes Acid	Alc	Ald	Ester	Alkane
C flavescens	1	1a	1	3	1	1	1
	2	1a	1	2	1	1	1
	3	1a	2	3	1	1	1
	4	1a	2	3	1	1	1
	5	1a	2	3	1	1	1
C. rigida	1	1a	2	2	1	1	1
	2	1a	2	2	1	1	1
	3	1a	2	2	1	1	1
	4	1a	1	2	1	1	1
C rubra	4	1a	1	2	1	1	1
C flavicans	1	1a	1	2	1	1	1
C spiralis	1	1b	1	1	2	1	2
C rubra	1	1b	1	1	2	1	2
	2	1b	3	1	5	1	2
	3	1b	7	1	2	1	2
C rigida	5	1*b*	1	1	2	1	2
C elata	1	1b	1	1	2	1	2
C frigida	1	1b	3	1	2	1	1
C. defracta	1	1b	7	1	2	1	2
C pallens	1	1b	7	1	2	1	2
	2	1b	3	1	2	1	2
C crassiuscula	1	1c	1	3	5	2	2
C australis	1	1c	1	1	4	2	2
	2	1c	1	1	1	4	2
C conspicua	1	1c	1	1	2	3	2
	2	1c	1	1	2	3	1
	3	1c	1	1	2	3	2
C. pallens	3	1c	1	1	1	4	1
C rubra	5	1c	2	1	1	1	1
C macra	2	2	5	1	3	1	2
	3	2	5	1	3	1	2
C. flavescens	6	2	1	2	1	1	4
	8	2	5	2	3	5	2
C antarctica	1	2	1	2	3	1	2
C pallens	4	3	1	1	5	6	2
C teretifolia	1	4	6	1	2	2	2
C macra	1	4	6	1	4	2	2
C acicularis	1	5	2	1	2	1	5
C cheesemanii	1	6	7	1	2	1	8
C bromoides	1	7	4	3	2	7	1
C rubra	6	8	5	2	3	9	3
C flavescens	7	9	5	2	2	1	7
	9	10	5	4	3	8	3
C pallens	5	11	3	1	2	10	2
C beddiei	1	12	6	5	4	1	6

in P1b with *C rubra*, *C rigida* and *C pallens*. Similarly, it is inconsistent for *C. frigida* (New South Wales) to be located in P1b.

Despite such limitations, the results have demonstrated that the major carbon chain components in the epicutricular wax of *Chionochloa* show gene–enzyme differences which allow chemical recognition of the various *Chionochloa* samples within one locality. This has not been previously demonstrated, nor has the chemotaxonomic interrelationship of the various species as illustrated by the dendrogram. Whether the interrelationship is due to climatic, geological or botanical factors has yet to be established

EXPERIMENTAL

Plant material Vegetative shoots (sheaths and blades) from large natural populations of *Chionochloa* spp were collected from sites representative of the general ecology of that area. 45 samples were taken, 25 have been reported once before [9] and 6

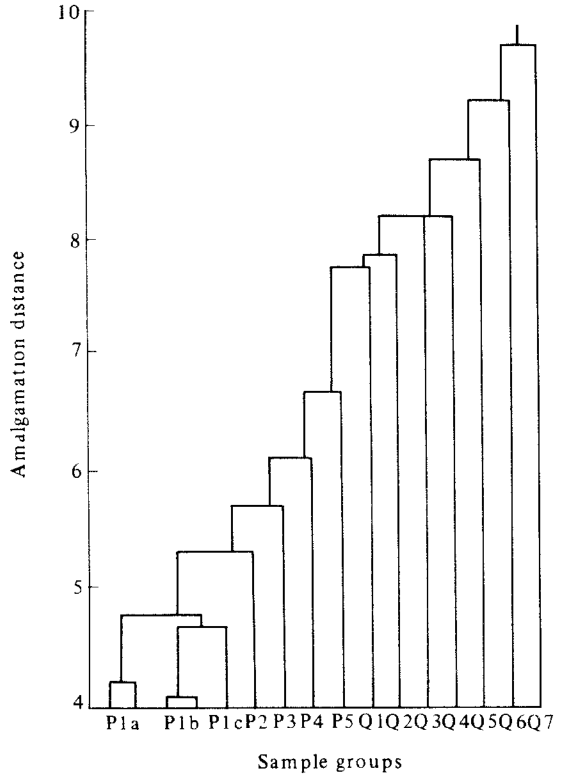

Fig 1 Dendrogram showing the relationship of *Chionochloa* species, based on carbon chain lengths Maximum amalgamation distance in the study was 9 7 units and the *Chionochloa* samples in each grouping are P1a. *C flavescens* (1 2, 3, 4, 5), *C rigida* (1, 2. 3, 4), *C rubra* (4), *C flavicans* (1) P1b, *C spiralis* (1), *C rubra* (1, 2, 3),*C rigida*(5), *C elata* (1), *C frigida* (1), *C defracta* (1), *C pallens* (1, 2) P1c, *C crassiscula* (1), *C australis* (1, 2), *C conspicua* (1, 2, 3), *C pallens* (3), *C rubra* (5) P2, *C macra* (2, 3). *C flavescens* (6, 8), *C antarctica* (1) P3, *C pallens* (4) P4, *C teretifolia* (1), *C macra* (1) P5, *C acicularis* (1) Q1, *C cheesemanii* (1) Q2, *C bromoides* (1) Q3, *C rubra* (6) Q4, *C flavescens* (7) Q5, *C flavenscens* (9) Q6, *C pallens* (5) Q7, *C beddiei* (1) See Experimental for code number details in parenthesis

twice before [9, 10] The samples are among those reported previously in refs [7, 8] The species and localities were

C acicularis Zotov	1	Fowler Pass, Fiordland, 1070 m,
C antarctica (Hook f) Zotov	1	Auckland Islands,
C australis (Buchanan) Zotov	1	St James Range, Canterbury, 1520 m,
	2	Mt Iron, Nelson, 1520 m,
C beddiei Zotov	1	Cape Palliser Wellington, 30 m,
C bromoides (Hook f) Zotov	1	Whangaruru, North Auckland, 30 m,
C cheesemanii (Hack) Zotov	1	Upper Takaka, Nelson, 460 m,
C conspicua subsp *conspicua* (Forst. f) Zotov	1	Mt Hutt, Canterbury, 850 m,
	2	Lake Sylvester, Nelson, 850 m,
	3	Mt Fox, Westland, 910 m,
C crassiuscula (Kirk) Zotov	1	Garnet peak, Canterbury, 1680 m,
C defracta Connor	1	Upper Takaka, Nelson, 300 m,
C elata (Petrie) Connor	1	Sealy Range, Canterbury, 1460 m,
C flavescens Zotov	+1*	Mt Barrosa, Canterbury, 1200 m,
	2*	Mt Hutt, Canterbury, 880 m,
	3*	St James Range, Canterbury, 850 m,
	4*	Jacks Pass, Canterbury, 980 m,
	5*	Peel Range, Nelson, 1520 m,
	6*	Mt Fox, Westland, 1130 m,
	7*	Clarke River, Westland, 980 m,
	8*	Rimutata Range, 760 m,
	9*	Mt Holdsworth, Tararua Range, 1220 m,
C flavicans Zotov	1	Lake Waikaremoana, 700 m,
C frigida (Vickery) Conert	1	Mt Kosciusko, Australia,
C macra Zotov	1†	Mt Barrosa, Canterbury, 1220 m;
	2	Jacks Pass, Canterbury, 3220 m,
	3	Mt Hutt, Canterbury, 1730 m,
C pallens Zotov	1*	Mt Hutt, Canterbury, 1280 m,
	2*	St James Range, Canterbury, 1520 m,
	3*	Lake Sylvester, Nelson, 1340 m,
	4*	Clarke River, Westland, 1000 m,
	5*	Mt Holdsworth, Tcrarua Range, 1200 m,
C rigida (Raoul) Zotov	+1*	Mt Barrosa, Canterbury, 910 m,
	+2*	Ashburton River, Canterbury, 670 m,
	+3*	Potts River, Canterbury, 640 m,
	4*	Mt Hutt, Canterbury, 760 m,
	5*	Mt Fox, Westland, 1,000 m,
C rubra Zotov	+1*	Harpers Knob, Canterbury, 640 m,
	2*	Mt Hutt, Canterbury, 1100 m,
	3*	Clarence River, Marlborough, 850 m,
	4*	Lake Sylvester, Nelson 1200 m,
	5*	Clarke River, Westland, 950 m,
	6*	Mt Egmont, 1500 m,
C spiralis Zotov	1	Takahe Valley, Fiordland, 950 m,
*C teretifolia**(Petrie) Zotov	1	Tower Peak, Southland, 1070 m

---

* Reported by Cowlishaw, Bickerstaffe and Connor [9]

† Reported by Cowlishaw, Bickerstaffe and Young [10]

Analytical methods Surface waxes were extd with petrol, sepd into five lipid classes, acids, alcohols, aldehydes, esters and alkanes, by TLC, as described previously [10] The constituents in each lipid class were identified by GC-MS and quantitated by GC The distribution of the carbon chain lengths of any one fraction indicated the degrees of similarity or difference between the samples and was determined mathematically by BMDP2M 'Clustering of Cases' Biomedical Computer Program

REFERENCES

1 Zotov, V D (1963) *N Z J Botany* **1**, 78

2 Connor, H E, Bailey, R W and O'Connor, K F (1970) *N Z J Agric Res* **13**, 534

3. Bailey, R. W. and Connor, H. E. (1972) *N. Z. J. Botany* **10**, 533
4. Connor, H. E. and Bailey, R. W. (1972) *N. Z. J. Botany* **10**, 515
5. Williams, P. A., Grigg, J. L., Nes, P. and O'Connor, K. F. (1978) *N. Z. J. Botany* **16**, 235
6. Williams, P. A., Mugambi, S., Nes, P. and O'Connor, K. F. (1978) *N. Z. J. Botany* **16**, 479.
7. Connor, H. E. and Purdie, A. W. (1976) *N. Z. J. Botany* **14**, 315
8. Connor, H. E. and Purdie, A. W. (1981) *N. Z. J. Botany* **19**, 161
9. Cowlishaw, M. G., Bickerstaffe, R. and Connor, H. E. (1983) *Biochem. Syst. Ecol.* **11**, 247.
10. Cowlishaw, M. G., Bickerstaffe, R. and Young, H. (1983) *Phytochemistry* **22**, 119