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Nuclear DNA Variation, Chromosome Numbers and Polyploidy in the Endemic and Indigenous Grass Flora of New Zealand

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• *Background and Aims* Little information is available on DNA C-values for the New Zealand flora. Nearly 85 % of the named species of the native vascular flora are endemic, including 157 species of Poaceae, the second most species-rich plant family in New Zealand. Few C-values have been published for New Zealand native grasses, and chromosome numbers have previously been reported for fewer than half of the species. The aim of this research was to determine C-values and chromosome numbers for most of the endemic and indigenous Poaceae from New Zealand.
• *Scope* To analyse DNA C-values from 155 species and chromosome numbers from 55 species of the endemic and indigenous grass flora of New Zealand.
• *Key Results* The new C-values increase significantly the number of such measurements for Poaceae worldwide. New chromosome numbers were determined from 55 species. Variation in C-value and percentage polyploidy were analysed in relation to plant distribution. No clear relationship could be demonstrated between these variables.
• *Conclusions* A wide range of C-values was found in the New Zealand endemic and indigenous grasses. This variation can be related to the phylogenetic position of the genera, plants in the BOP (Bambusoideae, Oryzoideae, Pooideae) clade in general having higher C-values than those in the PACC (Panicoideae, Arundinoideae, Chloridoideae + Centothecoideae) clade. Within genera, polyploids typically have smaller genome sizes (C-value divided by ploidy level) than diploids and there is commonly a progressive decrease with increasing ploidy level. The high frequency of polyploidy in the New Zealand grasses was confirmed by our additional counts, with only approximately 10 % being diploid. No clear relationship between C-value, polyploidy and rarity was evident.

Key words: Chromosome number, C-value, distribution, New Zealand, Poaceae, polyploidy, rarity, taxonomy.

INTRODUCTION

Variation in the amount of nuclear DNA of the entire chromosome complement or holoploid genome size, here called C-value following Greilhuber *et al*. (2005), together with changes in chromosome number and structure, has been implicated in the diversification and speciation of flowering plants (Levin, 2002). This variation is clearly an important component of adaptation, as variation in C-value affects key parameters of plant growth such as the duration of the cell size, cell cycle and life form (Bennett, 1972, 1987; Gregory, 2005). Correlations have also been made between C-value and plant distribution, with the common observation that plants of more tropical latitudes appear to have lower values than those of temperate latitudes (Bennett, 1976; Levin and Funderburg, 1979). However, other studies, e.g. Grime and Mowforth (1982), report the reverse correlation between C-value and latitude. In a large-scale study on the Californian flora, Knight and Ackerly (2002) demonstrated that species with low C-values predominate in all environments and that in extreme environments species with the highest C-values are poorly represented. There has also recently been the suggestion that high C-values may be maladaptive and lead to plant extinction (Vinogradov, 2003). There also appears to be a clear phylogenetic pattern to C-value variation in the Poaceae, with higher values being most common among the members of the Bambusoideae, Oryzoideae, Pooideae (BOP) clade (Kellogg and Bennetzen, 2004).

Polyploidy is clearly one possible contributor to C-value variation, but the relationship between C-value and ploidy is far from clear-cut (Leitch and Bennett, 2004). In their 'all angiosperms' and monocot samples Leitch and Bennett (2004) have shown that the mean amount of 1C DNA does not increase in direct proportion to ploidy and that mean genome size (C-value divided by ploidy level) shows a clear decrease. Nevertheless, polyploidy affects many other genetic and phenotypic characters, and in New Zealand, where 63 % of angiosperms are reported to be polyploid (Hair, 1966), it appears to have played an important role in the evolution of the flora. Polyploids also frequently, though not always, have different distributions to their diploid progenitors, and polyploids are often over-represented among colonizer species (Stebbins, 1971; Thompson and Lumaret, 1992; Levin, 2002; Brochmann *et al*., 2004).

One of the key recommendations that arose from the first Angiosperm Genome Size workshop and conference held at the Royal Botanic Gardens, Kew, in September, 1997 [*Annals of Botany* **82** (Supplement A)] was the need to obtain an improved representation of the world's flora in the DNA C-value database (http://www.rbgkew.org.uk/cval/homepage.html). One under-represented area is Australasia. There have been few studies of Australasian plants, with only 19 out of 465 first authors of papers listed

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in the database coming from the region (Bennett and Leitch, 2005). Only three studies have been published that include New Zealand native angiosperms (Murray *et al.*, 1992, 2003; Hanson *et al.*, 2003). Thus, it is timely to produce a survey of a large family, the Poaceae, from New Zealand.

Grasses are a significant component of the New Zealand endemic and indigenous flora. Poaceae are the second largest family in terms of species and in the recent flora (Edgar and Connor, 2000) 157 species are described as endemic and 31 as indigenous. The New Zealand flora is significant for its high level of species endemicity, in that approximately 85 % of the approximately 2300 vascular plant species are endemic (Cockayne, 1967; Wardle, 1991; de Lange and Norton, 1997; Wilton and Breitwieser, 2000); in the grasses 87·6 % are considered to be endemic (Wilton and Breitwieser, 2000). Recent studies have shown that many of the species-rich genera are the results of recent speciation following long-distance dispersal, mainly from Australia, Malesia and South America (Wardle, 1978; Pole, 1994, 2001; McGlone *et al.*, 2001; Winkworth *et al.*, 2002).

Grasses occupy a wide variety of habitats in a landmass composed of islands of varying sizes that span almost 25° of latitude and rise to over 3000 m. These plants also show a variety of distribution patterns from species that are widespread to those that are restricted to a small local area. Two recent publications have provided an excellent framework for further investigation of the New Zealand grasses, a new grass flora (Edgar and Connor, 2000) and an updated catalogue of threatened indigenous New Zealand plants (de Lange *et al.*, 2004). A recent review (Murray, 2005) has suggested that intraspecific C-value variation can be an indicator of taxonomic heterogeneity. With a number of grass genera for which species delimitation is unclear (Edgar and Connor, 2000), C-value data could provide useful indicators.

In this paper we report on the C-values of 155 species (plus a further six subspecific taxa) and new chromosome numbers for 55 taxa of grasses from the New Zealand endemic and indigenous flora.

MATERIALS AND METHODS

Plant material

The plant material used in this study is listed in Table 1. The majority of species have been studied from single individuals as many of them are rare, confined to restricted areas or found in remote parts of the New Zealand botanical region. Figure 1 shows the botanical provincial boundaries of the North and South Islands of New Zealand and the principal offshore islands mentioned in Table 1 so that the origin of the plant samples can be located. All plants were collected from natural populations and grown either in the experimental garden or in a glasshouse at the University of Auckland. The plants studied are listed in Table 1. The recent grass flora (Edgar and Connor, 2000) includes 157 endemic and 31 indigenous species. Here we include a further two species, *Paspalum orbiculare* and *Bromus arenarius*, as indigenous although they were treated as naturalized by Edgar and Connor (2000). Voucher specimens of all of the plants used in this study have been deposited either in the herbarium of Auckland Museum (AK), the Allan Herbarium, Landcare Research (CHR) or the Otago University Herbarium (OTA). Chromosome numbers were determined either from root tip meristems or from pollen mother cells using standard preparation techniques. In a small number of species we did not determine the chromosome numbers ourselves but for completeness when determining various statistics additional chromosome numbers were obtained from Dawson (2000) and de Lange and Murray (2002).

Plant classification and determination of conservation status

Grasses have been grouped into two major clades, BOP (Bambusoideae, Oryzoideae, Pooideae) and PACC (Panicoideae, Arundinoideae, Chloridoideae + Centothecoideae) by the GPWG (2000). Within this framework we have grouped the New Zealand genera into tribes and subfamilies following Edgar and Connor (2000).

The conservation status of species was obtained from the most recent classification of threatened plants in New Zealand (de Lange *et al.*, 2004). That paper, which uses the New Zealand Threat Classification System (see Molloy *et al.*, 2002), recognizes nine species as 'Acutely Threatened', four as 'Chronically Threatened' and 58 as 'At Risk'. Globally all of these taxa fall within the IUCN category of 'Threatened' (IUCN, 2000).

Flow cytometry

Determinations of nuclear DNA C-values were made using flow cytometry. In most cases only a single plant was available for analysis, but where several accessions were available values were measured on different days. All gave consistent results with little day-to-day variation. Nuclei were extracted by chopping fresh young leaves with a pair of single-edged razor blades into a final volume of 10 mL of ice-cold Galbraith's buffer (Galbraith *et al.*, 1983), containing 3 % (w/v) polyvinylpyrrolidone. The chopped material was filtered through a 32-μm steel mesh filter and centrifuged at 300 *g* for 4 min to obtain a pellet of nuclei. The pellet was resuspended in 300 μL Galbraith's buffer containing100 μg mL^{-1} propidium iodide. In our laboratory we have found that RNase treatment has no effect on C-value so we routinely omit this step from our procedure. To obtain stable and repeatable results in *Cenchrus* it was necessary to wash the pellet of nuclei in 15 mL Galbraith's buffer and re-centrifuge before adding the propidium iodide. After staining on ice for at least 60 min, samples were analysed using an EPICS Elite ESP flow cytometer (Beckman-Coulter, Hialeah, FL, USA) using the air-cooled argon laser emitting light at 488 nm. Excitation of the probe propidium iodide was at 488 nm with fluorescence emitted measured using a 610 ± 10-nm band-pass filter. The instrument was aligned daily with flow check beads (Beckman-Coulter) that are labelled with a defined fluorescence intensity. Three replicates of each sample were

TABLE 1. *Chromosome number (numbers in bold taken from Dawson, 2000), ploidy level, DNA C-value (pg per 2C nucleus), place of origin of the plants and location of voucher specimens of plants used in the present study*

Species	*n*	2*n*	Ploidy	2C	Place of origin	Voucher
Achnatherum petriei (Buchanan) S.W.L.Jacobs & J.Everett		42	6*x*	1·77	South I., Otago, Awahokomo	AK 286416
Agrostis dyeri Petrie		42	6*x*	10·81	North I., Wellington, Tararua Ranges	AK 282090
A. imbecilla Zotov		42	16*x*	10·78	South I., Canterbury, Old Man Range	AK 286444
A. magellanica Lam.		84	12*x*	21·23	Auckland Is., Enderby I.	AK 281998
		84	12*x*	21·77	Campbell I.	AK 281989
A. muelleriana Vickery		42	6*x*	11·26	South I., Canterbury, Crimea Range	AK 281984
		42	6*x*	–	South I., Southland, Garvie Mountains	AK 282063
A. muscosa Kirk		42	6*x*	10·58	South I., Otago, Ohau Downs	AK 286818
		42	6*x*	–	North I., Taranaki, Egmont National Park	AK 282074
		42	6*x*	–	North I., Taranaki, Puketapu Road	AK 282074
		42	6*x*	–	South I., Canterbury, Lake Tekapo	AK 286744
A. pallescens Cheeseman		42	6*x*	10·88	South I., Southland, Te Anau Downs	AK 286499
A. personata Edgar		42	6*x*	10·64	South I., Canterbury, Crimea Range	AK 281042
A. petriei Hack.		42	6*x*	10·85	South I., Otago, Glenmore Station	AK 286748
Amphibromus fluitans Kirk		42	6*x*	7·97	North I., Wellington, Lake Wairarapa	AK 282157
Anemanthele lessoniana (Steud.) Veldkamp		**40–44**	4*x*	1·89	North I., Wellington, Haurangi Range	AK 281149
Australopyrum calcis Connor & Molloy subsp. *calcis*		14	2*x*	11·27	South I., Marlborough, Leatham Valley	AK 296501
A. calcis subsp. *optatum*		14	2*x*	11·60	South I., Canterbury, Castle Hill	AK 286443
Connor & Molloy		14	2*x*	11·57	South I., Canterbury, Tengawai	AK 281153
Austrofestuca littoralis (Labill.) E.B.Alexeev.		**28**	4*x*	7·42	Great Barrier I., Kaitoke Beach	AK 281576
Austrostipa stipoides (Hook.f.) S.W.L. Jacobs & J. Everett		44	4*x*	3·15	North I., North Auckland, Ambury Park	AK 280019
Bromus arenarius Labill.		28	4*x*	16·45	North I., North Auckland, Ponui I.	AK 283726
Cenchrus caliculatus Cav.		102	6*x*	11·12	Kermadec Is., Raoul I.	AK 282949
Chionochloa antarctica (Hook.f.) Zotov		42	6*x*	5·31	Campbell I.	AK 281160
C. australis (Buchanan) Zotov		42	6*x*	4·43	South I., Canterbury, Lake Tennyson	AK 281047
C. bromoides (Hook.f.) Zotov		42	6*x*	6·09	North I., North Auckland, Mokohinau Is.	AK 283728
C. cheesemanii (Hack.) Zotov		42	6*x*	5·47	North I., Wellington, Tararua Ranges	AK 281546
		42	6*x*	–	South I., Marlborough, D'Urville I.	AK 282156
C. conspicua subsp. *cunninghamii* (Hook. f.) Zotov		42	6*x*	6·33	North I., South Auckland, Karangahake Gorge	AK 286731
C. crassiuscula (Kirk) Zotov subsp. *crassiuscula*		42	6*x*	5·00	Stewart I., Table Hill	OTA 57968
C. crassiuscula subsp. *directa* Connor		42	6*x*	–	South I., Fiordland, Fiordland National Park	OTA 57599
C. crassiuscula subsp. *torta* Connor		42	6*x*	–	South I., Fiordland, Takahe Valley	OTA 57597
C. defracta Connor		42	6*x*	–	South I., Nelson, Mt Dun, Windy Point	OTA 57937
C. flavescens subsp. *brevis* Connor		42	6*x*	5·17	South I., Nelson, Mt Maling	AK 281046
C. flavescens subsp. *hirta* Connor		42	6*x*	–	South I., Westland, Mt Ryall	OTA 57964
C. flavescens subsp. *lupeola* Connor		42	6*x*	5·32	South I., Nelson, Mt Owen	OTA 57964
C. flavicans Zotov f. *flavicans*		42	6*x*	7·57	North I., South Auckland, Hahei	AK 286419
C. flavicans f. *temata* Connor		42	6*x*	7·77	North I., Hawke's Bay, Te Mata Peak	AK 286444
C. juncea Zotov		42	6*x*	5·38	South I., Nelson, Mt Augustus	AK 286772
C. lanea Connor		42	6*x*	5·06	Stewart I., Table Hill	AK 286646
C. macra Zotov		42	6*x*	5·01	South I., Otago, Mt St Bathans	AK 286726
		42	6*x*	–	South I., Otago, Cromwell	AK 286729
C. nivifera Connor & K.M.Lloyd		42	6*x*	–	South I., Southland, Fiordland National Park, Mt Burns	No voucher
C. pallens Zotov subsp. *pallens*		42	6*x*	5·13	North I., Wellington, Tararua Ranges	AK 281982
C. pallens subsp. *pilosa* Connor		42	6*x*	–	South I., Marlborough, Mt Fyffe	OTA 57595
C. rigida (Raoul) Zotov subsp. *rigida*		42	6*x*	5·07	South I., Otago, Lake Mahinerangi	AK 286730
C. rigida subsp. *amara* Connor		42	6*x*	–	South I., Southland, Takahe Valley	OTA 57963
C. rubra Zotov subsp. *rubra* var. *rubra*		42	6*x*	5·14	North I., Wellington, Rangipo Desert	AK 286452
C. rubra subsp. *rubra* var. *inermis* Connor		42	6*x*	5·36	North I., Taranaki, Egmont National Park	AK 286647
C. rubra subsp. *cuprea* Connor		42	6*x*	–	South I., Canterbury, Lake Tennyson	AK 286451
C. rubra subsp. *occulta* Connor		42	6*x*	–	South I., Westland, Paparoa Range	OTA 57962
		42	6*x*	–	South I., Canterbury, Lake Tennyson	AK 286462
C. spiralis Zotov		42	6*x*	5·11	South I., Southland, Mt Luxmore	AK 286781
C. vireta Connor		42	6*x*	4·97	South I., Westland, Haast Pass	OTA 57585
Cortaderia fulvida (Buchanan) Zotov		90	10*x*	8·30	North I., South Auckland, Lake Rotorua	AK 281166
		90	10*x*	8·36	North I., North Auckland, Kerikeri River	AK 208542
C. richardii (Endl.) Zotov		90	10*x*	7·85	South I., Otago, Awahokomo Creek	AK 256111

TABLE 1. *Continued*

Species	*n*	2*n*	Ploidy	2C	Place of origin	Voucher
C. splendens Connor		90	10*x*	7·89	North I., North Auckland, Whatipu	AK 283784
		90	10*x*	7·82	North I., South Auckland, Kawhia	AK 281157
		90	10*x*	7·71	North I., North Auckland, Surville Cliffs	AK 281158
		90	10*x*	8·49	North I., North Auckland, Kawau I.	AK 287115
		90	10*x*	8·27	North I., South Auckland, Mercury Is.	AK 287117
C. toetoe Zotov		90	10*x*	7·94	North I., South Auckland, Rotorua	AK 281165
C. turbaria Connor		90	10*x*	9·58	Chatham Is., Rekohu I.	AK 251864
Deschampsia cespitosa (L.) P.Beauv.		**26**	2*x*	10·43	Chatham Is., Rekohu I.	AK 282160
D. chapmanii Petrie		26	2*x*	11·05	South I., Southland, Garvie Mountains	AK 282066
		26	2*x*	–	Campbell I.	AK 281995
D. gracillima Kirk		26	2*x*	–	Campbell I.	AK 281991
D. tenella Petrie		26	2*x*	10·07	South I., Southland, Garvie Mountains	AK 282080
Deyeuxia aucklandica (Hook.f.) Zotov		56	8*x*	–	North I., Hawke's Bay, Kaweka Range	AK 253147
		56	8*x*	14·06	South I., Southland, Garvie Mountains	AK 282067
		56	8*x*	13·98	South I., Canterbury, Lake Tennyson	AK 288429
D. avenoides (Hook.f.) Buchanan		70	10*x*	–	North I., North Auckland, Auckland City	AK 281152
		70	10*x*	13·53	South I., Canterbury, Lake Tennyson	AK 255953
		70	10*x*	15·52	South I., Canterbury, Maitland Forest	AK 282062
D. lacustris Edgar & Connor		28	4*x*	16·13	South I., Canterbury, Lake Tennyson	AK 281049
D. quadriseta (Labill.) Benth.		56	8*x*	15·36	North I., North Auckland, Waikumete Cemetery	AK 250793
D. youngii (Hook.f.) Buchanan		28	4*x*	9·87	South I., Canterbury, Maitland River	CHR 562376
D. aff. *quadriseta*		56	8*x*	13·20	North I., Wellington, Waimarino Plains	AK 286819
Dichelachne crinita (L.f.) Hook.f.		70	10*x*	16·36	Chatham Is., Rekohu I.	AK 282152
		70	10*x*	–	South I., Otago, Alexandra	CHR 569774
D. inaequiglumis (Hack.) Edgar & Connor		70	10*x*	16·40	North I., North Auckland, Waikumete Cemetery	AK 250805
D. lautumia Edgar & Connor		70	10*x*	17·67	South I., Marlborough, Flaxbourne River	AK 286907
D. micrantha (Cav.) Domin		70	10*x*	16·85	North I., Gisborne, Torere Point	AK 286503
Echinopogon ovatus (G.Forst.) P.Beauv.	$\mathbf{21_{II}}$		6*x*	10·85	North I., North Auckland, Hunua Range	AK 286820
Elymus apricus Á.Löve & Connor		**42**	6*x*	28·20	South I., Otago, Matangi	AK 286417
E. enysii (Kirk) Á.Löve & Connor		**28**	4*x*	20·54	South I., Canterbury, Porters Pass	AK 286502
E. falcis Connor		42	6*x*	27·57	South I., Canterbury, Waitaki Valley	AK 286908
E. multiflorus (Hook.f.) Á.Löve & Connor		**42**	6*x*	25·16	North I., North Auckland, Anawhata	AK 282082
E. sacandros Connor		42	6*x*	27·74	South I., Marlborough, Flaxbourne River	AK 286643
E. solandri (Steud.) Connor		42	6*x*	28·56	North I., Wellington, Sinclair Head	AK 281159
		42	6*x*	28·40	South I., Marlborough, Stephens I.	AK 282151
		42	6*x*	26·59	South I., Marlborough, Upper Wairau River	AK 284756
E. tenuis (Buchanan) Á.Löve & Connor		56	8*x*	32·09	South I., Canterbury, Sawdon Run	AK 282069
Festuca actae Connor		42	6*x*	16·52	South I., Canterbury, Banks Peninsula	AK 281513
F. coxii (Petrie) Hack.		56	8*x*	21·50	Chatham Is., Rekohu I.	CHR 569775
F. deflexa Connor		42	6*x*	16·02	South I., Nelson, Mt Owen	OTA 57588
F. luciarum Connor		56	8*x*	19·85	North I., Hawke's Bay, Mangaharuru Range	OTA 57621
F. matthewsii (Hack.) Cheeseman subsp. *matthewsii*		42	6*x*	16·26	South I., Southland, Fiordland National Park	OTA 57938
F. matthewsii subsp. *aquilonia* Connor		42	6*x*	–	South I., Canterbury, Mt Fyffe	OTA 57936
F. matthewsii subsp. *pisamontis* Connor		42	6*x*	–	South I., Otago, Pisa Range	OTA 57945
F. novae–zelandiae (Hack.) Cockayne		42	6*x*	16·83	South I., Canterbury, Cass	AK 287121
		42	6*x*	–	South I., Otago, Awahokomo	AK 252541
F. ultramafica Connor		56	8*x*	20·62	South I., Nelson, Mt Dun	OTA 57629
Hierochloe brunonis Hook.f.		84	12*x*	27·81	Campbell I.	AK 281993
H. equiseta Zotov		42	6*x*	18·10	South I., Canterbury, Porters Pass	CH 562182
H. fusca Zotov		84	12*x*	29·68	North I., Wellington, Kapiti I.	AK 286448
		84	12*x*	27·55	Chatham Is., Rekohu I.	CHR 562185
H. novae–zelandiae Gand.		28	4*x*	12·54	South I., Canterbury, Tennyson Tarns	AK 287053
H. redolens (Vahl) Roem. & Schult.		84	12*x*	29·97	South I., Canterbury, Rakaia River	CH 562181
Imperata cheesemanii Hack.		20	2*x*	1·45	Kermadec Is., Raoul I.	AK 253146
Isachne globosa (Thunb.) Kuntze	30_{II}		6*x*	3·64	North I., North Auckland, Western Springs	AK 256108
Koeleria cheesemanii (Hack.) Petrie		28	4*x*	9·95	South I., Canterbury, Crimea Range	AK 281043
K. novozelandica Domin sens. str.		28	4*x*	–	South I., Canterbury, Balmoral	CHR549886
		28	4*x*	9·82	South I., Canterbury, Porters Pass	CHR 569776
K. riguorum Edgar & Gibb		28	4*x*	–	South I., Nelson, Douglas Range	AK 286472
K. aff. *cheesemanii*		28	4*x*	13·23	South I., Canterbury, Porters Pass	CHR 569776
K. aff. *novozelandica*		28	4*x*	10·41	South I., Otago, Awahokomo	AK 282149
Lachnagrostis ammobia Edgar		98	14*x*	25·48	Stewart I., Mason Bay	AK 247127
L. billardierei (R.Br.) Trin.		56	8*x*	–	Chatham Is., Rekohu I.	AK 282153
		56	8*x*	–	Chatham Is., Rekohu I.	AK 229942
		56	8*x*	–	North I., North Auckland, Kaitarakihi	AK 250913

TABLE 1. *Continued*

Species	*n*	2*n*	Ploidy	2C	Place of origin	Voucher
		56	8*x*	–	North I., North Auckland, Taitomo I.	AK 288306
		56	8*x*	18·25	North I., North Auckland, Kauri Point	AK 288137
L. elata Edgar		98	14*x*	26·81	North I., South Auckland, Pureora	AK 283507
		98	14*x*	–	North I., Wellington, Tongariro National Park	AK 288307
L. filiformis (G.Forst.) Trin.		56	8*x*	12·57	North I., North Auckland, Auckland City	AK 281150
L. leptostachys (Hook.f.) Zotov		84	12*x*	25·21	Auckland Is., Enderby I.	AK 281999
		84	12*x*	24·43	Campbell I.	AK 281988
L. littoralis (Hack.) Edgar subsp. *littoralis*		56	8*x*	13·51	Great Barrier I., Medlands Beach	AK 282162
		56	8*x*	15·32	North I., North Auckland, Cornwallis	AK 288077
		56	8*x*	13·47	South I., Westland, Punakaiki	AK 253019
L. littoralis subsp. *salaria* Edgar		56	8*x*	16·72	South I., Canterbury, Christchurch	AK 282150
		56	8*x*	16·49	South I., Canterbury, Brooklands	AK 282144
L. lyallii (Hook.f.) Zotov		98	14*x*	17·09	South I., Canterbury, Lake Tekapo	AK 288430
		98	14*x*	23·47	South I., Canterbury, Lake Tennyson	AK 286721
		98	14*x*	22·78	North I., Wellington, Tongariro National Park	AK 252979
L. pilosa (Buchanan) Edgar subsp. *pilosa*	49_{II}	98	14*x*	23·78	South I., Marlborough, Isolation Creek	AK 256032
		98	14*x*	24·66	Chatham Is., Rekohu I.	CHR 562182
L. uda Edgar		98	14*x*	25·50	South I., Southland, Garvie Mountains	AK 282068
Microlaena avenacea (Raoul) Hook.f.		**48**	4*x*	3·40	North I., North Auckland, Waitakere Ranges	AK 282158
M. carsei Cheeseman		**48**	4*x*	3·25	North I., North Auckland, Kerikeri River	AK 281154
M. polynoda (Hook.f.) Hook.f.		**48**	4*x*	2·37	North I., North Auckland, Waitakere Ranges	AK 286418
M. stipoides (Labill.) R.Br.		48	4*x*	1·80	North I., North Auckland, Auckland City	AK 286645
		48	4*x*	1·80	Chatham Is., Rekohu I., Otoi Creek	AK 286445
		48	4*x*	1·83	North I., Taranaki, New Plymouth	AK 288314
Oplismenus hirtellus subsp.		54	6*x*	5·21	North I., North Auckland, Waitakere Ranges	AK 286447
imbecillis (R.Br.) U.Scholz		54	6*x*	5·27	Kermadec Is., Raoul I.	AK 286724
Paspalum orbiculare G.Forst		63	6*x*	3·13	North I., North Auckland, Cornwallis Park	AK 252543
Poa acicularifolia Buchanan subsp. *acicularifolia*		28	4*x*	5·35	South I., Canterbury, Castle Hill	AK 286743
Poa acicularifolia subsp. *ophitalis* Edgar		28	4*x*	6·15	South I., Nelson, Mt Dun	AK 286644
P. anceps G.Forst subsp. *anceps*		28	4*x*	6·00	North I., North Auckland, Kaitarakihi Bay	AK 289038
		28	4*x*	6·01	North I., North Auckland, Kauri Point	AK 288074
P. anceps subsp. *polyphylla* (Hack.) Edgar		28	4*x*	5·45	Kermadec Is., Raoul I.	AK 282943
P. antipoda Petrie		28	4*x*	–	Antipodes Is.	AK 286714
P. astonii Petrie		28	4*x*	5·98	South I., Otago, Dunedin	AK 281038
P. breviglumis Hook.f.		28	4*x*	4·24	North I., Taranaki, Mt Taranaki	AK 288317
		28	4*x*	4·38	South I., Canterbury, Pineleugh	CHR 569777
		28	4*x*	4·55	North I., Wellington, Tararua Ranges	AK 281888
P. buchananii Zotov		28	4*x*	5·66	South I., Canterbury, Crimea Range	AK 281045
P. chathamica Petrie		112	16*x*	21·30	Chatham Is., Rekohu I.	CHR 562183
		112	16*x*	20·96	Chatham Is., Rekohu I.	AK 286905
P. cita Edgar		**84**	12*x*	14·43	North I., Wellington, Tararua Ranges	AK 282070
		84	12*x*	14·99	South I., Nelson, Charleston	AK 287016
P. colensoi Hook.f.		28	4*x*	5·61	North I., Wellington, Tongariro National Park	AK 253027
		28	4*x*	5·26	North I., Taranaki, Egmont National Park	AK 282076
		28	4*x*	5·40	South I., Canterbury, Lake Tennyson	AK 286711
		28	4*x*	5·16	South I., Otago, Rastus Burn	CHR 569779
		28	4*x*	5·49	South I., Otago, Alexandra	AK 256110
		28	4*x*	5·32	South I., Canterbury, Old Man Range	AK 256156
		28	4*x*	5·47	South I., Otago, Awahokomo Bluffs	CHR 549885
		28	4*x*	5·22	South I., Otago, Trotters Gorge	CHR 569778
		28	4*x*	5·52	South I., Otago, Rastus Burn	AK 286708
		28	4*x*	5·65	South I., Otago, Blue Creek	AK 286775
P. dipsacea Petrie		**28**	4*x*	5·59	South I., Marlborough, Bert's Creek	AK 285251
P. foliosa (Hook.f.) Hook.f.		28	4*x*	5·99	Auckland Is., Enderby I.	AK 286739
P. imbecilla Spreng.		28	4*x*	4·26	South I., Canterbury, Christchurch, Hagley Park	AK 286463
P. incrassata Petrie		28	4*x*	5·60	South I., Otago, Rastus Burn	AK 286790
P. intrusa Edgar		28	4*x*	6·01	South I., Southland, Routeburn	AK 286789
P. kirkii Buchanan		28	4*x*	4·89	South I., Canterbury, Lake Tennyson	AK 282065
P. lindsayi Hook.f.		28	4*x*	4·56	South I., Canterbury, Tekapo Tarns	AK 282072
		28	4*x*	4·51	South I., Canterbury, Spider Lakes	AK 286497
P. litorosa Cheeseman		**263–266**	38*x*	32·24	Auckland Is., Enderby I.	AK 281151
		263–266	38*x*	32·56	Campbell I.	AK 281987
P. maniototo Petrie		28	4*x*	5·49	South I., Otago, Awahokomo Bluffs	AK 259138
P. matthewsii Petrie		28	4*x*	5·49	South I., Canterbury, Castle Hill	AK 286717

TABLE 1. *Continued*

Species	n	2n	Ploidy	2C	Place of origin	Voucher
P. novae–zelandiae Hack.		28	4x	6·32	South I., Canterbury, Crimea Range	AK 282179
		28	4x	6·24	South I., Otago, Richardson Mountains	AK 281040
P. pusilla Berggr.		28	4x	5·50	North I., Taranaki, Egmont National Park	AK 286498
P. ramosissima Hook.f.		28	4x	5·69	Auckland Is., Enderby I.	AK 282001
P. schistacea Edgar & Connor		28	4x	11·02	South I., Otago, Hector Mountains, Two-Mile Valley	AK 287133
P. spania Edgar & Molloy		28	4x	4·81	South I., Otago, Awahokomo	AK 282077
P. sublimis Edgar		28	4x	5·02	South I., Canterbury, Arthur's Pass	AK 288308
P. sudicola Edgar		28	4x	6·49	South I., Nelson, Pike Peak	AK 286397
		28	4x	–	South I., Nelson, Pike Peak	AK 282001
P. xenica Edgar & Connor		28	4x	6·67	South I., Nelson, South Branch Riwaka River	AK 286909
P. aff. cita(a)		112	16x	17·44	South I., Marlborough, Stephens I.	AK 286773
P. aff. cita(b)		112	16x	17·40	South I., Nelson, Golden Bay	AK 286771
P. aff. colensoi		28	4x	6·05	North I., South Auckland, Te Moehau Range	AK 286641
Puccinellia stricta (Hook.f.) Blom		14	2x	3·55	South I., Canterbury, Christchurch	AK 282179
P. walkeri subsp. *chathamica* (Cheeseman) Edgar		42	6x	9·61	Chatham Is., Rekohu I.	AK 282171
		42	6x	–	Chatham Is., Rekohu I.	AK 287133
		42	6x	–	Auckland Is., Enderby I.	AK 282000
Pyrrhanthera exigua (Kirk) Zotov		approx.156	26x	21·51	South I., Canterbury, Sawdon Run	AK 253685
Rytidosperma biannulare (Zotov) Connor & Edgar		48	4x	8·06	North I., North Auckland, Waikumete Cemetery	AK 255954
R. buchananii (Hook.f.) Connor & Edgar		48	4x	7·07	South I., Canterbury, Porters Pass	AK 285603
R. clavatum (Zotov) Connor & Edgar		24	2x	3·32	South I., Otago, Ranfurly	AK 256106
		24	2x	–	South I., Canterbury, Waimakariri River	AK 255952
R. corinum Connor & Edgar		48	4x	7·19	South I., Canterbury, Hakataramea Pass	AK 286053
R. gracile (Hook.f.) Connor & Edgar		24	2x	2·48	South I., Otago, Old Man Range	AK 256105
R. horrens Connor & Molloy		24	2x	3·66	South I., Canterbury, Maitland River	AK 286640
R. maculatum (Zotov) Connor & Edgar		48	4x	6·04	South I., Canterbury, Waimakariri River	AK 256107
R. petrosum Connor & Edgar		48	4x	8·26	North I., Wellington, Cape Palliser	AK 250800
R. pulchrum (Zotov) Connor & Edgar		24	2x	–	North I., Wellington, Ruahine Range, Toka	AK 286446
R. pumilum (Kirk) Connor & Edgar		24	2x	3·24	South I., Canterbury, Porters Pass	CHR 562374
R. setifolium (Hook.f.) Connor & Edgar		24	2x	4·96	North I., South Auckland, Te Moehau Range	AK 286450
		24	2x	4·54	North I., Wellington, Tongariro National Park	AK 253000
		24	2x	4·77	North I., South Auckland, Mt Pirongia	AK 281156
R. telmaticum Connor & Molloy		24	2x	3·43	South I., Canterbury, Lake Tekapo	CHR 562184
		24	2x	2·83	South I., Canterbury, Hakatere	AK 286487
R. thomsonii (Buchanan) Connor & Edgar		48	4x	6·27	South I., Canterbury, Tekapo Tarns	AK 286745
		D*	?	6·63	South I., Otago, Lake Hawea	AK 286747
		24	2x	3·51	South I., Canterbury, Glenmore	AK 286746
R. unarede (Raoul) Connor & Edgar		48	4x	–	North I., Gisborne, Hicks Bay	AK 256109
		48	4x	–	South I., Otago, Flat Top Hill	AK 256013
R. viride (Zotov) Connor & Edgar		24	2x	2·76	North I., Wellington, Rangipo Desert	AK 286500
Simplicia buchananii (Zotov) Zotov		28	4x	11·07	South I., Nelson, Oparara, Honeycomb Cave	AK 252968
S. laxa Kirk		28	4x	10·24	South I., Otago, Ngatapa	AK 285424
Spinifex sericeus R.Br.		18	2x	5·41	North I., North Auckland, Muriwai Beach	AK 286774
Stenostachys deceptorix Connor		28	4x	19·42	South I., Nelson, Matiri Plateau	AK 286396
S. gracilis (Hook.f.) Connor		28	4x	17·91	South I., Canterbury, Maitland Forest	AK 282084
S. laevis (Petrie) Connor		28	4x	18·83	South I., Canterbury, Lake Tennyson	AK 281985
Trisetum arduanum Edgar & A.P.Druce		28	4x	11·07	North I., South Auckland, Kawhia, Awaroa Reserve	AK 246714
T. drucei Edgar		28	4x	11·37	North I., Wellington, Mangaweka	AK 252495
T. lepidum Edgar & A.P.Druce		28	4x	16·70	South I., Canterbury, Lake Tennyson	AK 281986
T. serpentinum Edgar & A.P.Druce		28	4x	10·81	South I., Nelson, Hackett Creek	AK 252504
T. spicatum (L.) K.Richt.		28	4x	9·82	South I., Canterbury, Crimea Range	AK 282071
		28	4x	–	South I., Canterbury, Porters Pass	CHR 562377
		D*	?	19·48	Campbell I.	AK 281997
T. tenellum (Petrie) A.W.Hill		28	4x	10·29	South I., Canterbury, Maitland River	CHR 562375
T. youngii Hook.f.		28	4x	–	South I., Nelson, Lockett Range	AK 256029
		28	4x	10·13	South I., Canterbury, Lake Tennyson	AK 281048
T. aff. *lepidum*		28	4x	17·02	South I., Otago, Awahokomo	AK 251835
Zotovia colensoi (Hook.f.) Edgar & Connor		**48**	4x	2·93	North I., Wellington, Tararua Ranges	AK 281551
Z. thomsonii (Petrie) Edgar & Connor		**approx. 48**	4x	2·74	Stewart I., Mt Rakeahua	AK 283814
Zoysia minima (Colenso) Zotov		40	4x	0·99	North I., North Auckland, Piha Beach	AK 282083
		40	4x	–	South I., Canterbury, Kaitorete Spit	AK 256104
Z. pauciflora Mez		40	4x	0·97	Great Barrier I., Whangapoua Beach	AK 252969

* D, plant died before a chromosome count could be made.

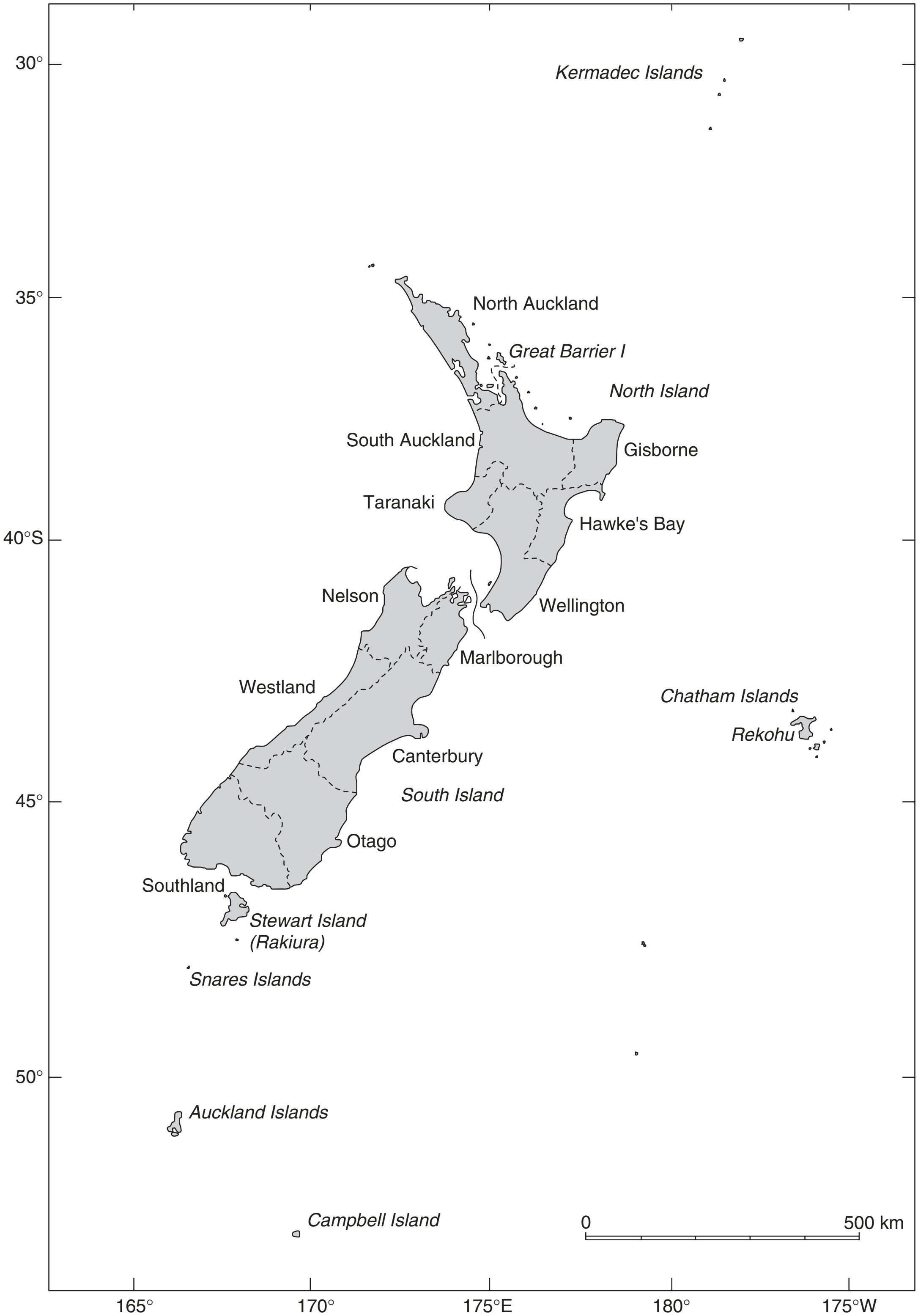

FIG. 1. Map of New Zealand showing the main islands and the traditional botanical provinces.

prepared and at least 5000 nuclei were measured from each replicate.

An initial pilot study to determine the overall range of C-values for the taxa studied used *Hordeum vulgare* 'Sultan' (2C = 11·12 pg DNA/2C nucleus) as an external standard. Once this range was established, we used three different internal standards, *H. vulgare* 'Sultan', *Secale cereale* 'Petkus Spring' (2C = 16·57 pg) and the indigenous

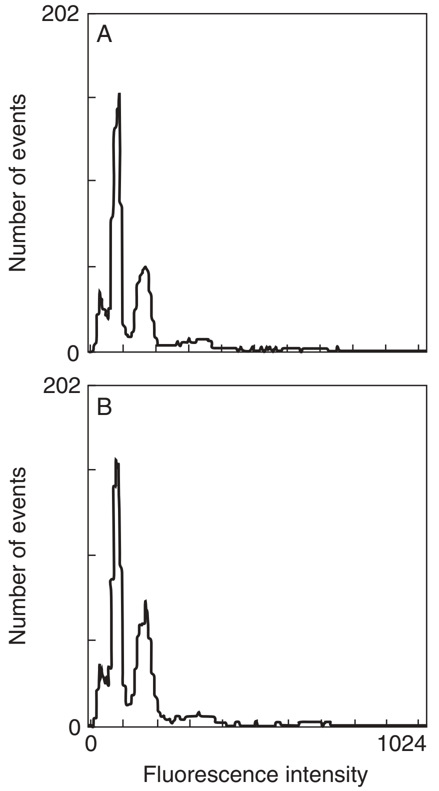

FIG. 2. Flow cytometric profiles for two independent runs (A and B) of *Poa anceps* subsp. *polyphylla* (left peak) and *Hordeum vulgare* 'Sultan' (right peak) carried out for the calibration of the former as a new standard.

Poa anceps subsp. *polyphylla* (2C = 5·45 pg), which were co-chopped with the taxa to be determined. *Poa anceps* subsp. *polyphylla* was calibrated against *H. vulgare* to provide a grass standard that was closer to the lower values that we obtained in our preliminary study. The flow profiles of two independent runs of *P. anceps* subsp. *polyphylla* and *H. vulgare* are shown in Fig. 2A, B. Neither *Zea mays* W64A (2C = 5·47 pg) nor *Sorghum bicolor* 'Pioneer' 8695 (2C = 1·74 pg), both grasses and recommended standards (Johnston *et al*., 1999; Bennett *et al*., 2000), was available in New Zealand. C-values were reported previously for 26 of the species reported here using *Actinidia chinensis* as an external standard (Murray *et al*., 2003). On repeating the analyses with the grass internal standards we have found that these earlier values showed the same ranking but were approximately 30 % lower than those reported here.

Following Leitch and Bennett (2004) we have calculated mean 2C genome size as the amount of 2C DNA in picograms divided by the ploidy level of the species.

Chromosome number determination

Somatic chromosome numbers were determined from root tips that were pretreated with a saturated solution of paradichlorobenzene for 18 h at 4 °C, fixed in 3 : 1. ethanol/acetic acid and stained with FLP-orcein (Jackson, 1973). Meiotic chromosomes were observed in pollen mother cells that were fixed and stained as outlined above.

RESULTS

C-value variation

C-values were determined for 155 species (161 taxa because in some species two subspecies or forms of a species were measured; Table 1). A wide range of C-values was observed, from 0·97 pg per 2C in *Zoysia pauciflora* to 32·40 pg per 2C in *Poa litorosa* (Table 1), representing a 33·4-fold variation. The spread of absolute values within genera varied considerably: some, such as *Chionochloa*, *Cortaderia* and *Microlaena*, showed a narrow range of values whereas others, such as *Lachnagrostis* and *Poa*, showed a wide range. When the ranges were expressed in relative terms, the highest over the lowest, *Poa* with a value of 7·4 was clearly different to the others, such as *Chionochloa* (1·75), *Cortaderia* (1·2), *Microlaena* (1·88) and *Lachnagrostis* (2·13). There was little evidence of any grouping of values that would result in discontinuities in C-values within genera. At the genus level, measurements were available for ten genera present in both New Zealand and the rest of the world (Table 2). It is difficult to see any clear trends from these comparisons as in some cases, e.g. *Elymus*, *Festuca* and *Paspalum*, the mean genome sizes are remarkably similar in both geographical areas, but in others, e.g. *Deschampsia*, *Imperata* and *Trisetum*, there are large differences. Similarly, comparisons of minimum and maximum C-values for the genera (Table 2) show different patterns. In *Agrostis*, *Festuca*, *Poa* and *Trisetum* the maximum values for the New Zealand representatives are higher than those from elsewhere but in *Bromus*, *Deschampsia* and *Imperata* they are lower. In *Imperata* and *Koeleria* the New Zealand species show lower minimum values than the non-New Zealand samples but the reverse is the case in *Deschampsia* and *Trisetum*. However, the limited sizes of the samples must be borne in mind when such comparisons are made.

C-value and phylogeny

In Table 3 the 35 genera are grouped into the two major clades, BOP and PACC, and then subfamilies and tribes, and the mean 2C value and mean genome size for each genus has been calculated. Many of the genera in the PACC clade had relatively small mean C-values and mean genome sizes. However, there were some interesting exceptions. *Pyrrhanthera* in Arundinoideae (PACC) had the third largest C-value observed (21·51 pg per 2C) but the high ploidy level in this monotypic genus (26*x*) means it had a small mean genome size. The genera in Ehrhartoideae and the tribe Stipeae in Pooideae, in the BOP clade, have low values for both of these measurements.

C-value and ploidy level

The mean, standard deviation and range of C-values for different ploidy levels are given in Table 4. There is a progressive increase in mean C-value with increasing ploidy level, with the exception of the 10*x* category, but it is clear that there is a large range of values at each level and that there no defined incremental increase with increasing ploidy. Nine of the New Zealand genera show a range of different ploidy levels and in seven of these, *Deyeuxia*,

TABLE 2. *A comparison of the mean genome size (amount of 2C DNA divided by ploidy level) and minimum and maximum 2C-values (pg) for genera common to both New Zealand and the rest of the world. Data for the rest of the world comparisons were obtained from www.rbgkew.org.uk/cval/homepage.html (C-values) and www.mobot.org (Index to Plant Chromosome Numbers)*

	New Zealand				Rest of World			
Genus	Number of species	Mean genome size	Min. 2C-value	Max. 2C-value	Number of species	Mean genome size	Min. 2C-value	Max. 2C-value
Agrostis	8	1·80	10·58	21·50	7	1·96	3·35	10·30
Bromus	1	4·11	16·45	16·45	46	3·90	3·75	32·65
Deschampsia	3	5·26	10·07	11·05	3	4·07	9·95	18·00
Elymus	7	4·58	20·54	32·09	12	4·55	5·85	30·30
Festuca	7	2·67	16·02	21·50	18	2·71	3·35	15·15
Imperata	1	0·73	1·45	1·45	1	2·71	10·85	10·85
Koeleria	4	2·72	9·82	13·23	1	4·60	9·20	9·20
Paspalum	1	0·52	3·13	3·13	3	0·57	1·20	3·05
Poa	29	1·39	4·26	32·40	6	1·52	2·35	10·75
Trisetum	8	2·98	9·82	19·48	1	1·28	5·10	5·10

Elymus, *Festuca*, *Hierachloe*, *Lachnagrostis*, *Poa* and *Puccinellia*, there is a progressive decrease, to different degrees, in genome size with increasing ploidy (Table 5). In *Rytidosperma* the tetraploids have a slightly higher genome size than the diploids and in *Agrostis* the hexaploids and 12-ploids have almost identical genome sizes (Table 5).

C-value and rarity

We obtained C-values for a significant proportion of the plants in the various conservation categories and the results are shown in Table 6. We also examined the relationship between rarity and C-value at generic level. Because the numbers of species within genera are much smaller at this level we have grouped the plants classified into the three categories outlined above as a single category 'Threatened' and compared their values with those of the non-threatened members of four genera, *Chionochloa*, *Poa*, *Rytidosperma* and *Trisetum*, for which the chromosome numbers of the plants analysed are the same. In *Chionochloa* the comparison between threatened and non-threatened is 5·56 (n = 9) to 5·46 (n = 10), in *Poa* 5·80 (n = 9) to 5·75 (n = 17), in *Rytidosperma* 8·26 (n = 1) to 6·96 (n = 5) and in *Trisetum* 11·09 (n = 2) to 11·60 (n = 5).

Intraspecific C-value variation

Deyeuxia avenoides, *Lachnagrostis littoralis*, *L. lyallii* and *Rytidosperma thomsonii* all appear to show intraspecific C-value variation. In the first three species, the plants all had the same chromosome number but in *R. thomsonii* two different chromosome numbers were obtained (Table 1). In *D. avenoides*, the lower C-value was 87 % of the higher value, in *L. littoralis* it was 88 % and in *L. lyallii* it was 74 %. In *R. thomsonii* diploid and tetraploid plants were counted and the tetraploids had 1·84 times the DNA C-value of the diploid.

Chromosome numbers and ploidy levels

The chromosome numbers for 55 species are published here for the first time and, in addition, we report five new chromosome numbers in species for which chromosome numbers have been reported previously. These latter species are *Deyeuxia aucklandica*, *Lachnagrostis pilosa* subsp. *pilosa*, *Rytidosperma buchananii*, *R. thomsonii* and *Trisetum tenellum* (Table 1). With these new counts, chromosome numbers are now known for 186 species of endemic and indigenous grasses (91·6 % of the total of 203 that we have recognized in this paper; Table 7). If infraspecific ranks are included, there are 214 taxa and of these 193 (90·2 %) have been counted.

The large majority (91 %) of species in the 36 endemic and indigenous genera are polyploid; diploids are confined to six genera, *Australopyrum*, *Deschampsia*, *Imperata*, *Puccinellia*, *Rytidosperma* and *Spinifex* (Table 7). Of the species that are polyploid, 39·8 % were tetraploid and 26·3 % were hexaploid, with smaller percentages at the higher ploidy levels (Table 7). High ploidy levels were seen in *Poa*, four species were 16x and another was 38x, and the endemic, monotypic *Pyrrhanthera* was 26x. Of the genera with ten or more species, *Chionochloa* was unusual in that all 23 species were at the same ploidy level (6x) whereas the other large genera had species at a variety of ploidy levels; for example, *Festuca*, with ten species, had four ploidy levels.

Polyploidy and rarity

Information is available for eight grass taxa that are classified as 'Acutely Threatened', four that are 'Chronically Threatened', 49 that are 'At Risk' and 117 that are 'Not Threatened'. The percentage of polyploids in each category is given in Table 6. If the three threatened categories are combined then 94·8 % of these species are polyploid, slightly higher than the 89·7 % for the non-threatened category.

DISCUSSION

The present study has increased the representation of grasses in the C-value database by about 30 % and the total number of New Zealand angiosperms to 149. This

TABLE 3. *A summary of C-values and mean genome sizes in the 35 endemic or indigenous genera of New Zealand grasses arranged in clades following the most recent grass phylogeny (GPWG, 2000) and then by subfamily and tribe following Edgar and Connor (2000)*

Clade, subfamily, tribe and genus	Mean 2C DNA amount (pg)	Mean genome size (pg)
PACC—Arundinoideae		
Danthonieae		
Chionochloa	5·40	0·90
Pyrrhanthera	21·51	0·83
Rytidosperma	5·00	1·73
Cortaderiinae		
Cortaderia	8·35	0·83
PACC—Chloridoideae		
Chloroideae		
Zoysia	0·89	0·25
PACC—Panicoideae		
Paniceae		
Cenchrus	11·12	1·86
Oplismenus	5·24	0·87
Paspalum	3·13	0·52
Spinifex	5·41	2·71
Isnachneae		
Isachne	3·64	0·61
Andropogoneae		
Imperata	1·45	0·73
BOP—Ehrhartoideae		
Ehrharteae		
Microlaena	2·71	0·68
Zotovia	2·84	0·71
BOP—Pooideae		
Stipeae		
Achnatherum	1·77	0·30
Anemanthele	1·89	0·47
Austrostipa	3·15	0·79
Poeae		
Austrofestuca	7·42	1·86
Festuca	18·23	2·67
Poa	7·84	1·39
Puccinellia	6·58	1·69
Agrostideae		
Agrostis	12·16	1·80
Deyeuxia	13·95	2·09
Dichelachne	16·82	1·68
Echinopogon	10·85	1·81
Lachnagrostis	20·44	1·86
Simplicia	10·66	2·67
Amphibromus	7·97	1·33
Deschampsia	10·52	5·26
Koeleria	10·85	2·72
Trisetum	12·97	2·98
Hierochloe	23·41	2·67
Bromeae		
Bromus	16·45	4·11
Hordeae		
Australopyrum	11·43	5·72
Elymus	27·02	4·58
Stenostachys	18·72	4·68

number includes the first reports for 21 additional genera of Poaceae, five of which, *Anemanthele*, *Pyrrhanthera*, *Simplicia*, *Stenostachys* and *Zotovia*, are endemic to New Zealand. The new values show a 33·4-fold variation for the New Zealand plants (0·97–32·40 pg per 2C nucleus), well within the range of values for the family as a whole (0·50–43·25 pg per 2C nucleus). Owing to the high level of endemism in the New Zealand flora, few intraspecific comparisons between New Zealand and non-New Zealand plants can be made, but among indigenous plants some comparisons are possible. An example is *Deschampsia cespitosa*, a widespread cosmopolitan species that in New Zealand is diploid and has 10·43 pg per 2C. By comparison, Bennett *et al*. (1982), who did not count the chromosomes of the plant from South Georgia that they studied, but assumed that it was diploid, reported a C-value of 8·55 pg per 2C. Unfortunately, there is no voucher specimen associated with the measurement made by Bennett *et al*. (1982) so the identity of their plant cannot be confirmed. The value for *Imperata cheesemanii* from New Zealand is lower than the value for the one other *Imperata* species so far recorded: otherwise, the minimum values obtained for genera present in New Zealand are higher than the minimum values recorded for the same genera elsewhere in the world. In seven such genera the maximum values for New Zealand species are higher than those found elsewhere. *Poa flabellata* from South Georgia is 4*x* and has a C-value of 5·45 pg per 2C (Bennett *et al*., 1982) similar to the New Zealand 4*x* *Poa* species.

Obtaining C-values for this sample of grasses was relatively straightforward and good, symmetrical peaks with low coefficients of variation were generally obtained. Although in most cases only a single sample was available, when we did have more than one there was good agreement between measurements from different plants made on different days, usually less than 5 % difference. There are four exceptions for which there was a greater than 10 % difference between samples and these are discussed briefly below. One other unexpected result was obtained from among the *Poa* species that had $2n = 4x = 28$. *Poa schistacea* had a C-value of 11·02 pg but 22 other *Poa* species had C-values between 4·26 and 6·67 pg, with the *P. schistacea* value being approximately double the mean value of the other species.

C-values, distribution patterns and rarity

Our measurements, although admittedly limited, provide little evidence to support the contention that large C-values are maladaptive and may be a cause of extinction (Vinogradov, 2003). However, our values are relatively low compared with the global sample used by Vinogradov: the highest C-value that we obtained, 32·24 pg per 2C for *Poa litorosa*, is below the range of his 'Global concern' category but higher than the mean of his 'Local concern' category. In our sample, there are no clear differences between species that are rare or with restricted distribution and those species that are widespread. When phylogenetic constraints are reduced, by restricting the analysis to species with the same chromosome number within a single genus, there is again no large difference between the restricted and widespread species in the four genera (*Chionochloa*, *Poa*, *Rytidosperma* and *Trisetum*) for which such a comparison is possible. There also does not appear to be any correlation between polyploidy and rarity, but it must again be borne in mind that the sample sizes are not large and that the majority of New Zealand grasses are polyploid. It is also interesting

TABLE 4. *Mean, standard deviation and range of C-values at different ploidy levels in New Zealand Poaceae*

Ploidy level	2*x*	4*x*	6*x*	8*x*	10*x*	12*x*	14*x*	16*x*
No. of species analysed	15	64	41	11	10	6	5	3
Mean	5·35	8·01	10·65	18·02	12·26	24·57	24·62	18·66
Standard deviation	3·50	4·72	7·54	5·60	4·27	5·71	2·17	2·14
Range	1·45–11·50	0·97–20·54	1·77–28·56	12·57–32·09	7·85–17·67	21·50–29·97	21·11–26·81	17·40–21·13

TABLE 5. *The relationship between mean genome size (amount of 2C DNA divided by ploidy level) and ploidy level in species from nine genera of Poaceae from New Zealand that have a range of ploidy levels*

	Ploidy								
Genus	2*x*	4*x*	6*x*	8*x*	10*x*	12*x*	14*x*	16*x*	38*x*
Agrostis	–	–	1·80	–	–	1·79	–	–	–
Deyeuxia	–	3·25	–	1·75	1·45	–	–	–	–
Elymus	–	5·14	4·55	4·01	–	–	–	–	–
Festuca	–	–	2·74	2·58	–	–	–	–	–
Hierochloe	–	3·14	3·02	–	–	2·40	–	–	–
Lachnagrostis	–	–	–	1·91	–	2·07	1·76	–	–
Poa	–	1·44	–	–	–	1·23	–	1·17	0·85
Puccinellia	1·78	–	1·60	–	–	–	–	–	–
Rytidosperma	1·68	1·80	–	–	–	–	–	–	–

TABLE 6. *Mean C-values (± s.d.) and the percentage of polyploids (with the number of species for which data were available in parentheses) in the endemic and indigenous species of New Zealand Poaceae in relation to their conservation status (see text for explanation of conservation categories)*

Conservation status	Mean C-value	Percentage polyploids
Acutely threatened	8·95 ± 2·36 (n = 8)	87·5 (n = 8)
Chronically threatened	9·33 ± 2·99 (n = 3)	75·0 (n = 4)
At risk	12·80 ± 8·98 (n = 40)	98·0 (n = 49)
Not threatened	11·05 ± 7·38 (n = 113)	89·7 (n = 117)

that in several genera (*Agrostis*, *Festuca*, *Poa*, *Puccinellia*) the species with the highest C-values and chromosome numbers are found in the most extreme environments such as the sub-Antarctic [Auckland (Campbell) and Enderby Islands] and Chatham Islands. The genus *Elymus* has the highest mean C-value of all the grass genera in New Zealand (27·02 pg per 2C) yet it is by no means the most uncommon or threatened (de Lange *et al*., 2004). Many of the least common or seriously threatened species, such as *Poa spania*, *Amphibromus fluitans* and *Simplicia laxa*, are all within the lower half of C-values for New Zealand grasses. Differences in C-value do appear to reflect the geographical origin of the genera, with five that we have identified (following Clayton and Renvoize, 1986) (*Imperata*, *Isachne*, *Paspalum*, *Oplismenus*, *Zoysia*) as being of tropical origin all having C-values in the lowest end of the range we observed. This is in line with previous observations that tropical species of plants typically have lower C-values than temperate species (Bennett, 1976; Levin and Funderburg, 1979).

Leitch and Bennett (2004), in a survey of amounts of nuclear DNA, have pointed out that in angiosperms the mean genome size of polyploids was significantly lower than that of diploids. We have performed a similar analysis of the nine genera of New Zealand grasses that contain species with different ploidy levels and have found that most also show smaller genome sizes in polyploids compared with diploids. In some cases the differences are not great and it is possible that this reflects the recent nature of speciation/polyploidization that is commonly found in the New Zealand angiosperm flora (Wagstaff and Garnock-Jones, 1998; Heenan *et al*., 2002) and that genome diminution in some genera may reflect a longer timescale since speciation.

Taxonomic implications of C-value and chromosome variation

Four examples of putative intraspecific C-value variation have been observed. Three of these species (*Deyeuxia avenoides*, *Lachnagrostis littoralis* and *L. lyallii*) have been long recognized as being highly variable, showing differences in habit and distribution (Edgar, 1995). We also found examples of intraspecific variation in chromosome number in two species of *Rytidosperma* but were only able to measure C-values in one of them, *R. thomsonii*. This latter species is reported to have robust and small-statured races that related to the observed differences in chromosome number and C-value (B. P. J. Molloy, personal communication).

In line with previous studies (summarized by Dawson in Edgar and Connor, 2000) the majority of new chromosome counts confirm further examples of polyploidy with diploids confined to six genera, *Australopyrum*, *Deschampsia*, *Imperata*, *Puccinellia*, *Rytidosperma* and *Spinifex*. There may be some debate as to what is a diploid in some of these genera because the basic number (x) is 13 in *Deschampsia*, 10 in *Imperata* and 12 in *Rytidosperma*. We have assumed that $2n = 26$, $2n = 20$ and $2n = 24$ are diploid numbers in these three genera as these are the lowest numbers that are found and the plants are bivalent forming. In addition to the new count of $2n = 48$ for *Rytidosperma thomsonii*, we have obtained new counts for four other species that have been studied previously. In *Deyeuxia aucklandica*, de Lange and Murray (2002) reported $2n = 42$ whereas the new material had $2n = 56$. de Lange and Murray (2002) found $2n = 56$ for *Lachnagrostis pilosa* subsp. *pilosa* compared with $2n = 98$ here and they, together

TABLE 7. *The number of species at different ploidy levels in the 36 genera of Poaceae that contain species endemic or indigenous to New Zealand and the percentage of species at the different ploidy levels. This table includes the chromosome counts made here plus those taken from Dawson (2000) and de Lange and Murray (2002). This list also includes a number of undescribed taxa and chromosome races (see footnotes)*

			Ploidy level						
Genus	No. of species in NZ*	No. of species counted	2*x*	4*x*	6*x*	8*x*	10*x*	12*x*	>12*x*
Achnatherum	1	1	–	–	1	–	–	–	–
Agrostis	10	8	–	–	7	–	–	1	–
Amphibromus	1	1	–	–	1	–	–	–	–
Anemanthele	1	1	–	1	–	–	–	–	–
Australopyrum	1	1	1	–	–	–	–	–	–
Austrofestuca	1	1	–	1	–	–	–	–	–
Austrostipa	1	1	–	1	–	–	–	–	–
Bromus	1	1	–	1	–	–	–	–	–
Cenchrus	1	1	–	–	1	–	–	–	–
Chionochloa	23	23	–	–	23	–	–	–	–
Cortaderia	5	5	–	–	–	–	5	–	–
Deschampsia	5	4	4	–	–	–	–	–	–
Deyeuxia	6	6	–	2	–	3	1	–	–
Dichelachne	4	4	–	–	–	–	4	–	–
Echinopogon	1	1	–	–	1	–	–	–	–
Elymus	7	7	–	1	5	1	–	–	–
Festuca	10	10	–	1	4	4	–	–	1 × 24*x*
Hierochloe	7	5	–	1	1	–	–	3	–
Imperata	1	1	1	–	–	–	–	–	–
Isachne	1	1	–	–	1	–	–	–	–
Koeleria	5	5	–	5	–	–	–	–	–
Lachnagrostis	13	11	–	–	–	4	–	2	5 × 14*x*
Lepturus	1	0	–	–	–	–	–	–	–
Microlaena	4	4	–	4	–	–	–	–	–
Oplismenus	1	1	–	–	1	–	–	–	–
Paspalum	1	1	–	–	1	–	–	–	–
Poa	41	36	–	29	–	1	–	1	4 × 16*x*;1 × 38*x*
Puccinellia	4	4	1	1	1	1	–	–	–
Pyrrhanthera	1	1	–	–	–	–	–	–	1 × 26*x*
Rytidosperma	22	19	11	7	1	–	–	–	–
Simplicia	2	2	–	2	–	–	–	–	–
Spinifex	1	1	1	–	–	–	–	–	–
Stenostachys	3	3	–	3	–	–	–	–	–
Trisetum	11	11	–	10	–	1	–	–	–
Zotovia	3	2	–	2	–	–	–	–	–
Zoysia	2	2	–	2	–	–	–	–	–
Totals	203	186	19 (10·2 %)	74 (39·8 %)	49 (26·3 %)	15 (8·1 %)	10 (5·4 %)	7 (3·8 %)	12 (6·5 %)

* The total number of species in this table differs from that of Edgar and Connor (2000) for the following reasons. One species of *Paspalum* and one of *Bromus* have been treated as indigenous (see Materials and Methods); *Deyeuxia* includes one undescribed taxa, *D.* aff. *quadriseta*, and two taxa within *D. avenoides* differing in C-value; *Koeleria* includes two undescribed taxa, *K.* aff. *novozelandica* and *K.* aff. *cheesemanii*; *Poa* includes three undescribed taxa, *P.* aff. *cita* (a), *P.* aff. *cita* (b) and *P.* aff. *colensoi*; *Rytidosperma* includes the 2*x* and 4*x* races of *R. thomsonii* and the 4*x* and 6*x* races of *R. buchananii*; and *Trisetum* includes one undescribed taxa, *T.* aff. *lepidum*, and the 4*x* and 8*x* races of *T. tenellum*.

with Calder (1937), found $2n = 72$ for *Rytidosperma buchananii* compared with $2n = 48$ reported here. The final example is *Trisetum tenellum* with a count of $2n = 28$ obtained here compared with $2n = 56$ reported by de Lange and Murray (2002). There are relatively few examples of intraspecific chromosome number variation in the New Zealand flora; Murray *et al*. (1989) reported that only approximately 2 % of the species for which chromosome numbers were known had different chromosome races. Further investigation of these new examples is needed to ascertain whether the chromosome races and putative C-value variants are sufficiently distinct for them to be recognized as distinct taxa.

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