

Variation in DNA C-value and haploid genome size in New Zealand native grasses

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Abstract Flow cytometry has been used to measure the C-values of 29 species in 13 genera of New Zealand native grasses. An almost 18-fold variation was found between the lowest value, 0.78 pg for *Zoysia pauciflora*, and the highest value, 13.70 pg for *Pyrrhanthera exigua*. Some of this variation can be attributed to differences in chromosome number or ploidy level but groups of related species with the same chromosome number also show big interspecific differences. A distinction was found in general, between species in genera of tropical origin and those of temperate origin. In several cases there are examples of apparent intraspecific variation that may indicate taxonomic heterogeneity.

Keywords C-value variation; flow cytometry; grasses; ploidy level

INTRODUCTION

Nuclear DNA C-values in angiosperms show an approximately 1000-fold variation from about 0.1 pg to about 125 pg (Bennett et al. 2000) and this variation can be shown to be correlated with a number of fundamental characteristics of the plant such as the duration of mitotic and meiotic cell cycles, the size of pollen and stomatal guard cells, and minimum generation time (Bennett 1987). Correlations have also been found between nuclear DNA content and different environmental variables, such as maximum and minimum temperature in different seasons, precipitation, and altitude, but the relationships are often complex (see Knight & Ackerly 2002 for a review). There is significant variation between plant families in this key parameter with the Gramineae (*nom. alt.* Poaceae) showing the greatest range of variation, 104-fold between the lowest and highest values, compared with the approximately 22-fold range found in the related Cyperaceae (Leitch et al. 1998). The observation has also been made that tropical plants often appear to have smaller genomes than do temperate plants (Levin & Funderburg 1979), and Bennett (1976) has shown that the C-values of cultivated cereals, pasture grasses, and pulses show clinal variation; species with larger C-values being found in more northern latitudes with a progressive decline in genome size in species occurring closer and closer to the equator.

The Gramineae is the second most speciose family, after the Compositae (*nom. alt.* Asteraceae), in the New Zealand native flora (Wilton & Breitwieser 2000) and contains elements of both tropical and temperate origin (Wardle 1991; Edgar & Connor 2000). Although chromosome numbers are now known for 117 of the 188 indigenous grass taxa (Edgar & Connor 2000; de Lange & Murray 2002; B. G. Murray & P. J. de Lange unpubl. data) there is no published information on DNA C-values for these taxa. We have chosen species from 13 genera that are either predominantly tropical (*Imperata*, *Isachne*, *Paspalum*, and *Zoysia*) or

temperate (*Cortaderia*, *Deyeuxia*, *Festuca*, *Koeleria*, *Lachnagrostis*, *Poa*, *Pyrranthera*, *Rytidosperma*, and *Trisetum*) in their distribution. In this paper we report C-values obtained by flow cytometric analysis of isolated nuclei in 29 species from these genera of native grasses.

MATERIALS AND METHODS

The plant material used in this study is listed in Table 1. For flow cytometry, nuclei were obtained by chopping fresh young leaves with two 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, which was then filtered through a 32 µm steel mesh filter and centrifuged at 300 g for 4 min to obtain a pellet of nuclei, thereby removing any potential interfering compounds. The pellet was resuspended in 300 µl Galbraith's buffer containing 100 µg ml⁻¹ propidium iodide. After staining for about 45 min the sample was analysed using an EPICS Elite ESP flow cytometer (Beckman-Coulter, Hialeah, Florida, USA) using the air-cooled argon laser emitting light at 488 nm. Excitation of the probe propidium iodide was at 488 nm with fluorescence measured using a 610 nm ± 10 nm bandpass filter. The instrument was aligned daily with flow check beads (Beckman-Coulter) that are labelled with a defined fluorescence intensity.

Tetraploid *Actinidia chinensis*, which has been calibrated against several of the standards proposed by Bennett & Smith (1976) and has a 2C DNA content of 2.56 pg, was used as an external standard (Ferguson et al. 1997). Between 5000 and 10 000 nuclei were analysed from each sample. The coefficient of variation of the 2C peaks used to estimate C-value was, in all samples, below 6%. Examples of the flow profiles of three species of grass and the *Actinidia* standard are shown in Fig. 1. In most cases only a single plant was studied, but multiple accessions of some species were available for investigation. Similar results were obtained for all accessions of *Festuca novae-zelandiae* (mean 9.65, standard deviation (SD) 0.21) *Paspalum orbiculare* (mean 1.97, SD 0.13), and *Rytidosperma clavatum* (mean 2.45, SD 0.05). In contrast, in *Poa colensoi*, *Lachnagrostis pilosa* ssp. *pilosa*, and *Deyeuxia quadriseta*, where several plants were examined, appreciable differences were obtained between accessions (see below).

RESULTS

A wide range of DNA C-values was observed in our samples of grasses (Table 1; Fig. 2A). There was an approximately 18-fold range between the highest (13.70 pg DNA/2C nucleus in *Pyrranthera exigua*) and lowest values (0.78 pg DNA/2C nucleus in *Zoysia pauciflora*). The lowest C-values were obtained from the species in the genera that are primarily distributed in the tropics (*Imperata*, *Zoysia*, *Paspalum*, and *Isachne*); they had an overall mean value of 1.63 pg/2C nucleus and a range of 0.78 to 2.50. The highest value (13.70 pg DNA/2C nucleus) was obtained for the endemic *Pyrranthera exigua*, a species with $2n = 26x = c. 156$, and the mean C-value for all species in the temperate genera was 7.37 pg/2C nucleus with a range of 1.90 to 13.70.

DNA amounts per haploid genome (2C-DNA amount divided by ploidy level) also showed variation but the range was smaller, 12-fold, than that seen for C-value. Again there was a clear difference between the tropical and temperate genera; the mean value for the former was 0.38 pg (range 0.20 to 0.50) and 1.03 pg (range 0.47 to 2.43) for the latter (Table 1; Fig. 2B). In the four genera where we had species with different ploidy levels available for analysis (*Deyeuxia*, *Festuca*, *Lachnagrostis*, and *Rytidosperma*) there was either a small, though progressive, reduction in mean haploid genome size with increasing ploidy level or no difference between ploidy levels (Table 2).

Apparent intraspecific variation in C-values and haploid genome size was seen in three taxa, *Deyeuxia quadriseta*, *Lachnagrostis pilosa* ssp. *pilosa*, and *Poa colensoi*, all of which are highly variable morphologically. In *L. pilosa* ssp. *pilosa* this variation is correlated with differences in both chromosome number and the morphology of the plants (cf. Edgar & Connor 2000). In the case of *Deyeuxia quadriseta*, the plants studied relate to the gumland (Waikumete) and montane (Tongariro) variants discussed by Edgar (1995).

DISCUSSION

The range in C-value observed in our analysis of 29 of the 188 species of grass that are classified as endemic or indigenous to New Zealand (Edgar & Connor 2000) shows that there is considerable interspecific variation, though this is nowhere near the 100-fold variation reported for the family worldwide (Leitch et al. 1998). Although there is no

Table 1 Grass species, arranged alphabetically by tribe, investigated with their ploidy level, 2C nuclear DNA C-values (pg), DNA amount per haploid genome (pg), locality, and herbarium voucher number. * indicates predominantly tropical species.

Tribe and species	Ploidy	2C-value	DNA/genome	Source	Voucher number
Andropogoneae					
* <i>Imperata cheesemanii</i>	2x	1.00	0.50	Kermadec Is, Raoul I.	AK 253146
Agrostidineae					
<i>Deyeuxia aucklandica</i>	6x	11.10	1.85	Hawke's Bay, Kaweka Range, Te Puke North	AK 253147
<i>D. avenoides</i>	10x	10.80	1.08	Wellington, eastern Wairarapa, Ngarata	AK 250912
<i>D. quadriseta</i>	8x	9.20	1.15	Wellington, Tongariro National Park, National Park Swamp	AK 252511
	8x	8.50	1.06	Auckland, New Lynn, Waikumete Cemetery	AK 250793
<i>Koeleria novozelandica</i>	4x	5.90	1.48	Canterbury, Mackenzie Basin, Balmoral Station	CHR 549886
<i>Lachnagrostis billardierei</i>	8x	8.40	1.05	Auckland, Centennial Park, Kaitarakihi Bay	AK 250913
<i>L. lyallii</i>	14x	6.80	0.49	Wellington, Tongariro National Park, Tukino	AK 252979
<i>L. pilosa</i> ssp. <i>pilosa</i>	8x	8.20	1.03	Marlborough, Cloudy Bay, Rarangi Rocks	AK 252989
	14x	10.00	0.71	Marlborough, upper Waima River, Isolation Creek	AK 256032
<i>L. striata</i>	12x	9.40	0.78	Wellington, Tokaanu, Tokaanu Scenic Reserve	AK 252494
<i>Trisetum antarcticum</i>	4x	7.20	1.80	Marlborough, Cloudy Bay, Rarangi Rocks	AK 252970
<i>T. arduanum</i>	4x	7.10	1.78	South Auckland, Awaroa Scenic Reserve	AK 246714
<i>T. drucei</i>	4x	8.00	2.00	Wellington, Mangaweka, Mangawharariki Stream	AK 252495
<i>T. serpentinum</i>	4x	7.20	1.80	Nelson, Bryant Range, Hackett Valley	AK 252504
Chloroideae					
* <i>Zoysia minima</i>	4x	1.30	0.33	Canterbury, Kaitorete Spit	AK 256104
* <i>Z. pauciflora</i>	4x	0.78	0.20	Great Barrier I., Whangapoua Beach	AK 252969
Danthonieae					
<i>Cortaderia richardii</i>	10x	4.70	0.47	Otago, Awahokomo Stream	AK 256111
<i>Pyrrhanthera exigua</i>	26x	13.70	0.53	Canterbury, Sawdon Run	AK 253685
<i>Rytidosperma biannulare</i>	4x	5.50	1.38	Auckland, New Lynn, Waikumete Cemetery	AK 255954
<i>R. clavatum</i>	2x	2.40	1.20	Canterbury, Waimakariri River floodplain	AK 255952
	2x	2.50	1.25	Otago, Mainototo, Ranfurly	AK 256106
<i>R. gracile</i>	2x	1.90	0.95	Otago, Old Man Range	AK 256105
<i>R. maculatum</i>	4x	4.40	1.10	Canterbury, Waimakariri River floodplain	AK 256107
<i>R. setifolium</i>	2x	3.30	1.65	Wellington, Tongariro National Park, Tukino	AK 253000
<i>R. unarede</i>	4x	5.10	1.28	Gisborne, Hicks Bay	AK 256109
Isachneae					
* <i>Isachne globosa</i>	6x	2.50	0.42	South Auckland, Rangiriri, Opuatia wetlands	AK 255951
Panicaceae					
* <i>Paspalum orbiculare</i>	"6x"	1.87	0.31	Auckland, New Lynn, Waikumete Cemetery	AK 250809
	"6x"	2.12	0.35	Great Barrier I., Kaitoke Hot Springs	AK 252544
	"6x"	1.93	0.32	Auckland, Cornwallis	AK 252543
Poeae					
<i>Festuca madida</i>	4x	9.70	2.43	Otago, Rock and Pillar Range	AK 253260
<i>F. novae-zelandiae</i>	6x	9.80	1.63	Otago, Ohau Downs	AK 253016
	6x	9.50	1.58	Otago, Waitaki Valley, Awahokomo Bluffs	AK 252541
<i>Poa colensoi</i>	4x	2.60	0.65	Otago, Alexandra	AK 256110
	4x	3.00	0.75	Otago, Old Man Range	AK 256156
	4x	3.40	0.85	Wellington, Tongariro National Park, Tukino	AK 253027
<i>P. spania</i>	4x	3.40	0.85	Otago, Waitaki Valley, Awahokomo Bluffs	AK 252538

Fig. 1 Flow cytometric profiles of **A**, *Actinidia chinensis*; **B**, *Zoysia minima*; **C**, *Poa spania*; **D**, *Lachnagrostis striata*.

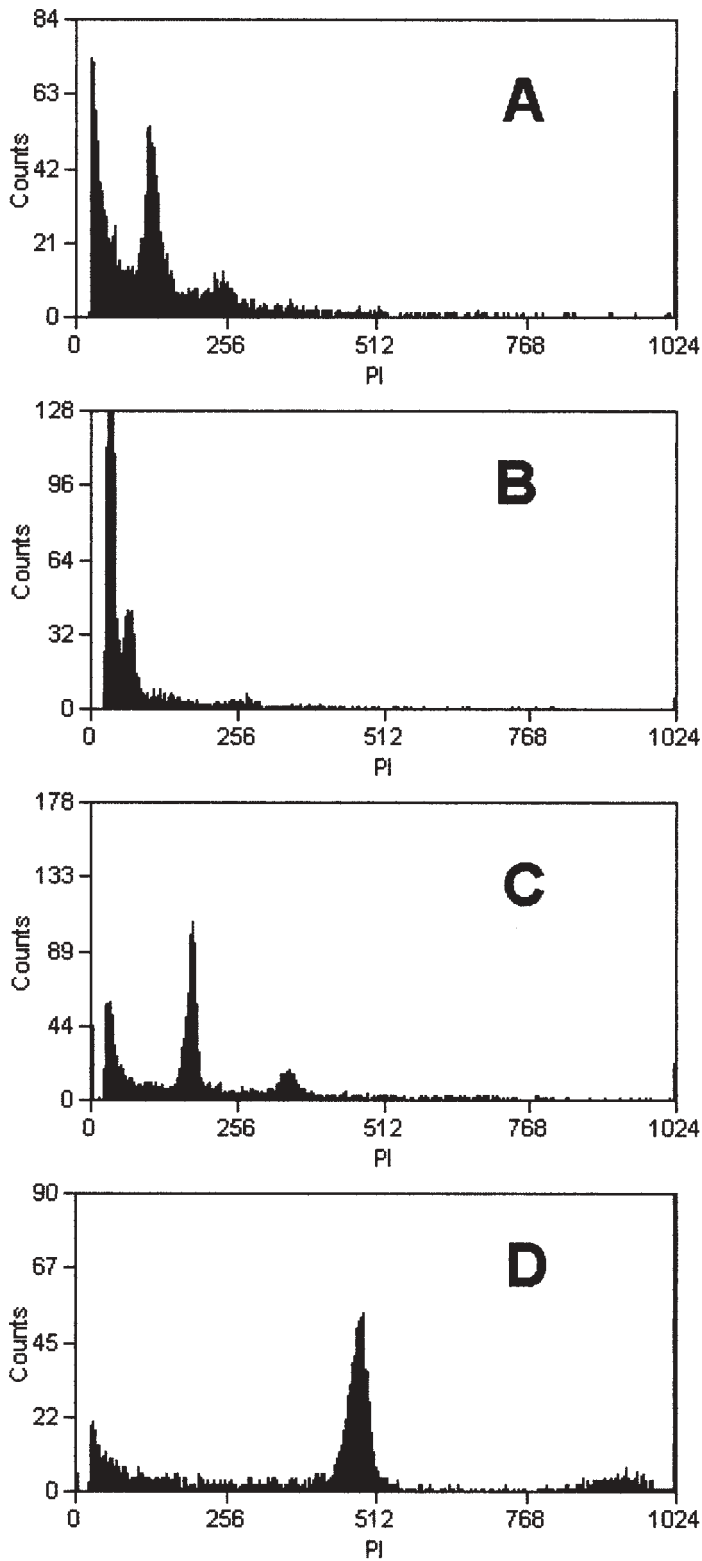


Fig. 2 DNA C-values (pg per 2C nucleus) (A) and DNA amount per haploid genome (B) for the 13 genera studied. The genera are arranged as in Table 1. Imp, *Imperata*; Dey, *Deyeuxia*; Koe, *Koeleria*; Lac, *Lachnagrostis*; Tri, *Trisetum*; Zoy, *Zoysia*; Cor, *Cortaderia*; Pyr, *Pyrrhanthera*; Ryt, *Rytidosperma*; Isa, *Isachne*; Pas, *Paspalum*; Fes, *Festuca*; Poa, *Poa*.

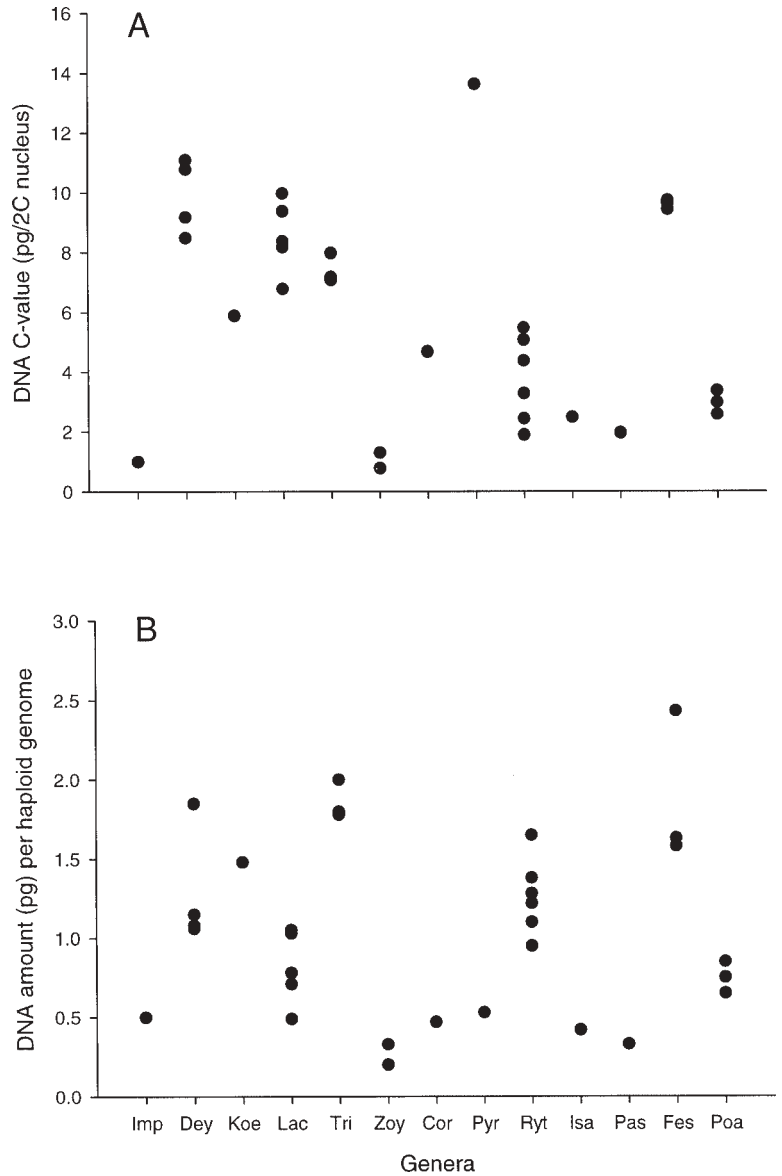


Table 2 Mean DNA amount in pg per haploid genome (2C DNA amount divided by ploidy level) in species of *Deyeuxia*, *Festuca*, *Lachnagrostis*, and *Rytidosperma* with different ploidy levels.

Genus	Ploidy level						
	2x	4x	6x	8x	10x	12x	14x
<i>Deyeuxia</i>	—	—	1.85	1.11	1.08	—	—
<i>Festuca</i>	—	2.43	1.61	—	—	—	—
<i>Lachnagrostis</i>	—	—	—	1.04	—	0.78	0.60
<i>Rytidosperma</i>	1.27	1.25	—	—	—	—	—

absolute correlation between C-value and chromosome number, the New Zealand grass flora includes *Poa litorosa*, which has the highest chromosome number, $2n = c. 266$ (Hair & Beuzenberg 1961), observed in the grasses, and its inclusion could possibly increase the range of C-values for the New Zealand endemic grasses. The four species of *Poa* that we examined, all of which were tetraploid, had a mean 2C DNA amount per haploid genome of 0.77 pg. If *Poa litorosa*, a 19-ploid, has a similar DNA amount per haploid genome as the four tetraploid species, it could have a 2C value of approximately 30 pg.

Although some of the variation in nuclear DNA content that we have observed can be attributed to differences in chromosome number, as for example in *Lachnagrostis pilosa* ssp. *pilosa*, in most cases the variation is independent of chromosome number. Amongst the diploid species of *Rytidosperma* C-values ranged from a low of 1.90 pg/2C nucleus in *R. gracile* to 3.30 pg/2C nucleus in *R. setifolium*, an almost 2-fold difference.

Both Bennett (1976) and Levin & Funderburg (1979) have reported on a correlation between C-value and the geographical origin of angiosperms: tropical species have smaller values than do temperate species. We have used Clayton & Renvoize (1986) to classify our genera as tropical or temperate and have found that, in general, the tropical genera have lower C-values and smaller genome sizes than the temperate ones. The temperate species show a much greater range of values than the tropical ones and only a few species of *Rytidosperma* and *Poa* can be included in the range of the tropical genera (Fig. 2A). The DNA amounts/haploid genome show a similar pattern (Fig. 2B) with a much wider range of values being seen in the temperate species than in the tropical ones. Thus, the trend that we have observed amongst the New Zealand grasses is similar to that seen by Bennett (1976) and Levin & Funderburg (1979).

In *Festuca* and *Lachnagrostis* there is a clear reduction in DNA per haploid genome with increasing ploidy level, whereas in *Deyeuxia* and *Rytidosperma* the reduction is very small and probably not significant (Table 2). In *Rytidosperma* it is unclear at present whether the plants with the lowest known chromosome number for the genus, $2n = 24$, are diploid or tetraploid. Clayton & Renvoize (1986) gave the basic number for the Arundineae as $x = 12$, but they reported that six species have $2n = 12$. In Table 2 and Fig. 2B we have assumed that the $2n = 24$ plants are diploid. The tendency for genome

size to decrease with increasing ploidy level in some genera is in line with the observations of Bennett & Leitch (2001) who analysed 1794 diploid and 658 polyploid angiosperms.

C-value variation can also clearly be seen to have value as an indicator of taxonomic uncertainty. In *Deyeuxia quadriseta*, *Lachnagrostis pilosa* ssp. *pilosa*, and *Poa colensoi* we have found examples of intraspecific variation in C-value. Although this is not unknown in other groups of plants (Bennett & Leitch 1995), most species usually show a remarkable lack of intraspecific variation. In *D. quadriseta*, Edgar (1995) indicated that there are two distinct variants of the species, one confined to montane sub-alpine zones and the other typical of coastal and scrubland vegetation, though she refrained from distinguishing them formally one from the other. Our montane accession from Tongariro National Park had a 2C value of 9.20 pg whereas the scrubland plant, from Waikumete Cemetery, had 8.50 pg/2C nucleus. Similarly, different C-values obtained for a small coastal variant (Rarangi Rocks, Marlborough) and the large upland form (Isolation Creek, Marlborough) of *L. pilosa* ssp. *pilosa* clearly warrant further taxonomic investigation (cf. Edgar 1995). It has long been acknowledged that *P. colensoi* is morphologically variable (Edgar 1986; Edgar & Connor 2000; B. P. J. Molloy pers. comm.), and our observations on C-value indicate that there is a difference between variants of the species even from the same region. For example, a robust variant from Alexandra (2.60 pg/2C nucleus) is quite distinct from plants that grow nearby but at higher altitudes on the Old Man Range (3.0 pg/2C nucleus) (B. P. J. Molloy pers. comm.). The status of the monotypic *Pyrrhanthera* has been questioned by Linder & Verboom (1996) and they transferred it to *Rytidosperma*, but Edgar & Connor (2000) did not adopt this for the recent flora treatment. The C-value obtained here (13.70 pg/2C nucleus), together with its chromosome number of $2n = c. 156$ (P. J. de Lange & B. G. Murray unpubl. data), along with the characters used by Zotov (1963) to define the genus, seem, in our view, ample reason to maintain *Pyrrhanthera* as distinct from *Rytidosperma*.

This survey confirms that the use of flow cytometry to determine plant C-values can produce interesting results that, in addition to quantifying an important biodiversity measure, can give insights into genome evolution and the existence of possible taxonomic heterogeneity in species of New Zealand plants.

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