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Nucleic acid content and Chromosome morphology in *Chrysanthemum*

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1. INTRODUCTION

The relationship between the gene and deoxyribose nucleic acid (DNA) is well established and it has been suggested that, if the genotype is determined mainly by the genetic information contained in the nuclear DNA, differences between genotypes would be directly reflected in differences in nuclear DNA constitution (Rees & Walters, 1965). Such differences in nuclear DNA could arise in a number of different ways and might be either qualitative or quantitative. Conversely, however, differences in DNA content would reflect differences in the genotype only if all the DNA was genotypically active. If a proportion of the DNA was inactive then species with similar numbers of genes and similar genotypes could contain widely different total amounts of DNA.

There have, in recent years, been a number of reports of quantitative variation in the nuclear DNA content in angiosperm genera and species. Sunderland & McLeish (1961) reported a fourfold difference in DNA per nucleus between three genera of the Papilionaceae and Rees, Cameron, Hazarika & Jones (1966) have shown differences of a similar magnitude between diploid species of *Lathyrus*, a sevenfold difference between diploid *Vicia* species and a similar but smaller variation in *Lolium*. More recently, Baetcke, Sparrow, Nauman & Schwemmer (1967) reported similar differences in a large number of genera. Rees *et al.* suggested that large changes in the amount of nuclear DNA could occur quite independently of change in chromosome number and that these could result in qualitative differences in the nuclear genetic material of genera which do not display polyploidy. Because such variation was found to occur in genetically closely related species it was further suggested that much of the nuclear DNA might be 'uninformative'.

In the present study the DNA contents of various diploid and polyploid *Chrysanthemum* species have been determined and the results are related to chromosome number and morphology. *Chrysanthemum* is a particularly suitable genus for such studies as it contains not only a large number of species but also degrees of polyploidy ranging from $2x$ to $22x$ ($x = 9$). There are also differences in chromosome size between species with the same chromosome number, and it is therefore possible to ascertain whether in such species these differences are correlated with variation in DNA content. The relationship between DNA content, chromosome number,

chromosome size and gene content will be considered and evidence presented to show that there is wide variation in nuclear DNA between species.

2. MATERIALS AND METHODS

DNA measurements

Photometric measurements of DNA content in 2C root-tip nuclei at telophase or early interphase were made using a Barr & Stroud integrating microdensitometer. Nuclei were stained by the Feulgen reaction and the technique used was essentially that of McLeish & Sunderland (1961). Since the results to be presented are largely comparative and involve a number of different species, and because the technique necessarily includes a number of potential sources of error, some additional details of the experimental procedure are given.

Root-tip segments 2 mm long were fixed in acetic alcohol (1:3) for 1 h and subsequently washed in 95% alcohol and passed through an alcohol series to water.

Table 1. *Analysis of variance of DNA content in roots of a single plant*

Source of variation	D.F.	M.S.	*F*
Between roots	9	18·32	3·85***
Between nuclei within roots	140	4·76	—

Analysis of variance of DNA content of ten different plants of Chrysanthemum boreale

Source of variation	D.F.	M.S.	*F*
Between plants	9	123·6	8·8***
Between roots within plants	10	14·08	2·53**
Within roots	280	5·57	—

Significance levels in this and subsequent tables: ** $P < 1\%$; *** $P < 0{\cdot}1\%$.

Hydrolysis was in N-HCl at 60 °C and the optimum hydrolysis time, i.e. that giving maximum staining, was calculated for each species. There was variation between species, and times between 15 and 20 min were used.

To determine whether plant age could influence the DNA estimates, measurements were obtained from nuclei of roots of a single plant taken at intervals of 2 months. The variation in DNA content in this experiment was found to be no greater than that between roots of a single plant taken at any one time.

The nuclei of many of the species used varied in size and intensity of staining and it was necessary to select appropriate optical conditions for each set of measurements. The diploid species *C. boreale* $2n = 18$ was used as a standard reference or control species. Roots from it were taken for DNA measurements along with those from other species being studied. The DNA contents of fifteen early interphase nuclei of *C. boreale* were measured under optical conditions most suitable for each of the different species and the means of each of these sets of measurements used as a reference in subsequent DNA determinations.

An estimate of the variation in DNA content within individual plants was obtained by taking segments from ten different roots of a single plant of *C. boreale*. These were fixed, hydrolysed and stained and the mean DNA content of fifteen early interphase nuclei of each root was calculated. The mean DNA contents of two roots of each of ten different plants of *C. boreale* were also measured and the variation between roots determined in the same way. The analyses of these experiments are shown in Table 1. There were significant differences between the mean DNA values of both roots and plants.

Differences in absorption could be due not only to true DNA differences but to non-proportionality between the measured amount of stain and the actual DNA content. Such non-proportionality could result from many causes, and in a complex technique, such as is used here, errors might occur. However, the estimate of variation between plants was larger than that between roots and it would seem probable that there are real differences in DNA content between plants. It is therefore necessary, when comparing the DNA contents of different species, to obtain measurements of several plants of each species.

Chromosome size was determined from the means of measurements made on the total chromosome complement of ten cells of each species. In an attempt to reduce possible variation between plants, two cells of each of five different plants were measured in all the species studied. The lengths, cross-sectional areas (πr^2) and volumes ($\pi r^2 l$) were calculated from magnified photomicrographs taken from colchicine-pretreated roots. A variable degree of contraction results from such pretreatment but analysis of chromosome length and cross-sectional areas suggests that these estimates enable comparative studies to be made.

3. RESULTS

The DNA content found in the nuclei of twelve different diploid species and hybrids is shown in Table 2. It can be seen, and the analysis of variance confirmed, that there are highly significant differences between different diploid species. There was similar variation in the triploid and tetraploid species (Table 3). The nuclei of the three hexaploid species studied contained identical DNA contents.

Some apparently unrelated species, of varing degrees of ploidy, were found to contain similar amounts of DNA, e.g. *C. viscosum* $2n = 18$, *C. leucanthemum* $2n = 36$, and *C. indicum* $2n = 54$.

The DNA content of euploid and aneuploid forms of *C. viscosum* ($2n = 34, 36, 37$) and *C. leucanthemum* ($2n = 25, 26, 27$) showed increase in DNA content with increase in chromosome number. As was to be expected, the DNA contents of colchicine-induced autotetraploid forms of *C. viscosum* (106·7) and *C. segetum* (99·2) were approximately twice that of the diploids (55·7, 46·2 respectively). Similarly, the DNA contents of the diploid and triploid forms of *C. parthenium* showed a ratio of 1 : 1·61, approximately that to be expected. However, the natural polyploid forms of *C. argenteum* and *C. leucanthemum* differed significantly from the expected values, the triploid form of *C. argenteum* having only 14 per cent

more DNA than the diploid. The parent plants of the triploid forms of these two species were not available for study and it is possible that they contained different DNA contents from the diploid and tetraploid forms of the same species used in this study.

Table 2. *DNA content in diploid species*

Species	DNA content†	Mean total chromosome length per nucleus†	Mean chromosome cross-sectional area	Mean chromosome volume per nucleus	DNA content per unit length	Ratio of DNA content to DNA of *C. parthenium*
C. argenteum	43·1	39·23	0·0983	3·8563	1·099	2·76
C. balsamita	35·8	25·19	0·0725	1·8263	1·421	2·29
C. boreale	36·2	27·76	0·0845	2·3457	1·304	2·32
C. carinatum	50·9	—	—	—	—	3·26
C. coronarium	45·7	44·03	0·0973	4·2841	1·038	2·93
C. frutescens	47·2	—	—	—	—	3·03
C. myconis	38·2	33·86	0·0907	3·0711	1·128	2·45
C. parthenium	15·6	23·71	0·0615	1·4580	0·658	1·00
C. praeteritum	31·0	34·79	0·1005	3·4964	0·891	1·99
C. segetum	46·2	38·45	0·1168	4·4910	1·202	2·96
C. viscosum	55·7	45·34	0·1824	8·2700	1·228	3·57
Hybrid (*C. segetum* × *C. coronarium*)	45·4	36·85	0·1294	4·2841	1·232	2·91

Analysis of variance of DNA content of diploid species

Source of variation	D.F.	M.S.	*F*
Between species	11	320·590	87·52***
Between plants within species	24	3·663	

† All DNA and chromosome measurements are in arbitrary units.

The DNA contents of two interspecific hybrids and their parents were investigated. The diploid hybrid between *C. segetum* × *C. coronarium* had the expected intermediate DNA content. The triploid hybrid obtained by crossing the diploid forms of *C. segetum* × *C. viscosum* probably arose after the production of an unreduced gamete from one of the parental species. A comparison of the DNA content of the parents and hybrids, together with study of chromosome morphology, suggests that *C. segetum* is more likely to have produced the unreduced gamete. A triploid form of *C. segetum*, produced after hybridization between the diploid and autotetraploid forms, had the expected intermediate DNA value (Table 3).

4. CHROMOSOME SIZE

In all *Chrysanthemum* species there is a single basic chromosome number, $x = 9$. These nine chromosomes show differences in length but they are otherwise structurally similar and for the purposes of this study it has been assumed that the individual chromosomes are cylindrical in shape.

There were significant differences in both chromosome length and volume between many of the sixteen *Chrysanthemum* species studied (Tables 2, 3). Increase in the degree of ploidy did not necessarily result in change in chromosome size. Thus the ratio of chromosome lengths in the diploid and autotetraploid forms of *C. viscosum* was 1 : 1·93 while chromosome volume gave a ratio of 1 : 1·99. A similar result was found in the different forms of *C. segetum* and *C. parthenium*, although the ratio for volume in *C. segetum* was somewhat high. The total chromosome lengths of the hybrids of the two crosses *C. segetum* × *C. coronarium* and *C. segetum* × *C. viscosum* were not significantly different from those expected.

Table 3. *DNA content in polyploid species*

Species	DNA content	Mean total chromosome length per nucleus	Mean chromosome cross-sectional area	Mean chromosome volume per nucleus	DNA content per unit length
Triploid species					
C. argenteum	48·8	38·98	0·0697	2·7170	1·252
C. parthenium	26·1	38·53	0·0804	3·0980	0·677
Hybrid (*C. segetum* 2*x* × *C. viscosum* 2*x*)	69·2	59·80	0·1099	6·5720	1·157
C. leucanthemum					
2*n* = 25	41·6	—	—	—	—
2*n* = 26	45·8	—	—	—	—
2*n* = 27	46·3	—	—	—	—
C. segetum†	73·8	—	—	—	—
Tetraploid species					
C. segetum	99·2	77·45	0·1777	13·7629	1·281
C. viscosum 2*n* = 36	106·7	87·34	0·1884	16·4549	1·222
C. viscosum 2*n* = 34	98·7	—	—	—	—
C. viscosum 2*n* = 37	115·9	—	—	—	—
C. corymbosum	65·7	—	—	—	—
C. leucanthemum	57·5	—	—	—	—
Hexaploid species					
C. indicum	57·8	61·55	0·794	4·8871	0·939
C. koreanum	56·0	—	—	—	—
C. rubellum	55·4	—	—	—	—

† Obtained after hybridization between diploid and tetraploid forms of *C. segetum*.

5. DNA CONTENT AND CHROMOSOME SIZE

The relationship between DNA content and chromosome length, cross-sectional area and volume is shown in Fig. 1 and Table 4. It will be seen that there is close correlation between them: as DNA content increases chromosome size increases. There is almost direct proportionality between DNA content and both length and volume. The relationship with cross-sectional areas is less clear. This latter character plays a relatively small part in determining total chromosome volume in these species and consequently chromosome length gives a reliable indication of DNA content. However, since chromosome length is correlated with cross-sectional

area and because there is no evidence that chromosomes with larger cross-sections have relatively less DNA, it cannot be expected that DNA content should be exactly proportional to chromosome length. In *Chrysanthemum* species there is little visible heterochromatin and presumably little variation in the degree of coiling along the chromosomes, since otherwise this close relationship between DNA content and chromosome length would not be expected.

Analysis of the data contained in Fig. 1 shows that all four regressions are highly

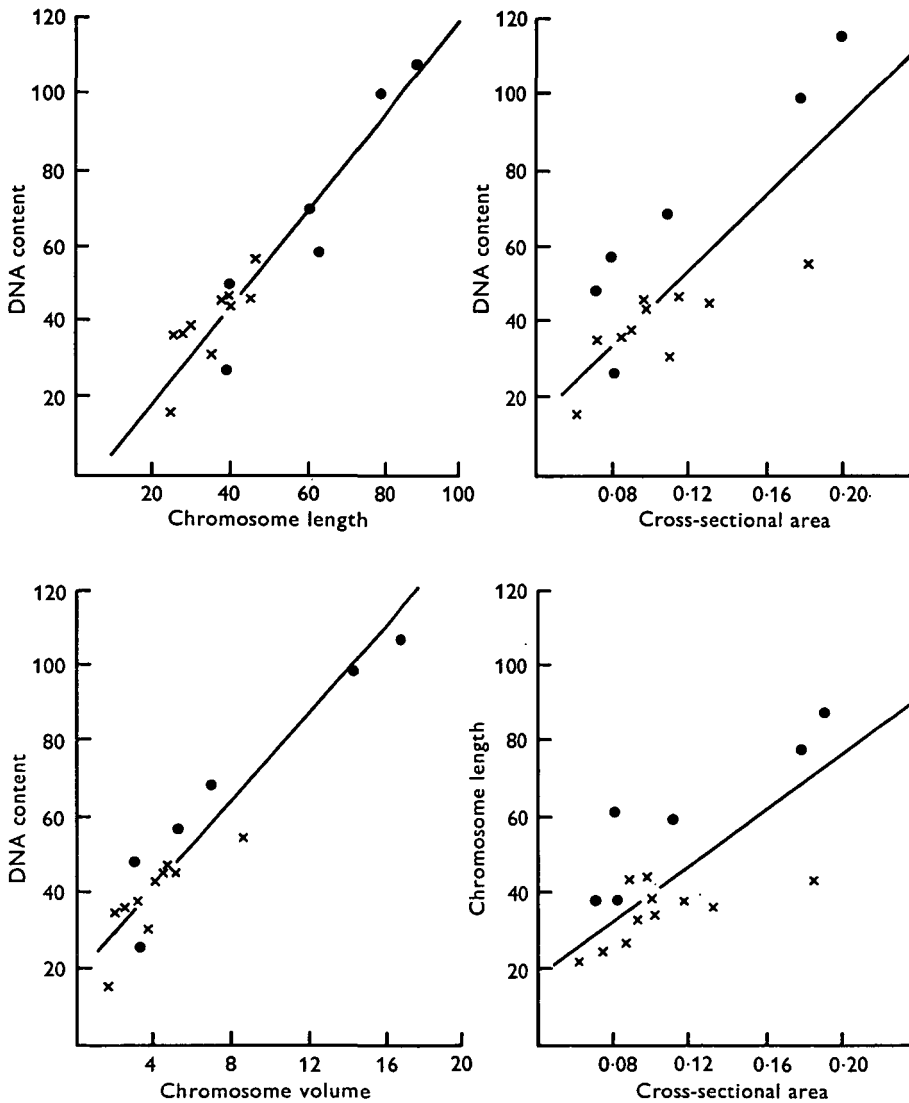

Fig. 1. The relationship between DNA content and chromosome size calculated from the data contained in Tables 2 and 3. Linear regressions have been calculated and are analysed in Table 4 (diploid species are represented by crosses and polyploid species by dots).

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significant (Table 4). It was to be expected that these regressions would not differ significantly from proportionality, i.e. from passing through the point of origin. Analysis shows that while three do not differ, the fourth, i.e. the regression of DNA content on chromosome volume, differs significantly. This suggests that there may be a curvilinear, rather than a straight-line, relationship between these two characters.

Table 4. *Analysis of regressions in Fig.* 1

	DNA × chromosome length $y = 1{\cdot}27x - 6{\cdot}58$			DNA × chromosome cross-sectional area $y = 495{\cdot}8x - 3{\cdot}9$		
Source of variation	D.F.	M.S.	F	D.F.	M.S.	F
Regression	1	7946·1	123·3***	1	6145·7	32·0***
Residual	14	64·4	—	14	192·6	—
S.E. of intercept = 5·44				S.E. of intercept = 30·58		
t = 1·04				t = 0·13		

	DNA × volume $y = 19{\cdot}05 + 5{\cdot}8x$			Chromosome length × cross-sectional area $y = 4{\cdot}22 + 371{\cdot}03x$		
Source of variation	D.F.	M.S.	F	D.F.	M.S.	F
Regression	1	7930·1	121·1***	1	3441·6	34·4***
Residual	14	65·5	—	14	106·2	—
S.E. of intercept = 3·28				S.E. of intercept = 17·5		
t = 5·8***				t = 0·241		

These results confirm reports of a similar correlation found between DNA content and interphase nuclear volume (Baetcke *et al.* 1967). These workers showed, however, that a number of species did not follow this trend; for example *Helianthus annuus* and *Gladiolus* sp. have the same nuclear volume but different DNA contents while *Allium cepa* and *Tradescantia paludosa* have similar amounts of DNA but differ in nuclear volume. They suggest that these exceptions may reflect limitations of the technique used to obtain the DNA values, which were measured using the diphenylamine reaction.

Apparent exceptions have also been noted in the present study. Thus *C. parthenium* and *C. balsamita* have similar chromosome volumes but differ markedly in DNA content, while *C. balsamita* and *C. myconis* show the converse relationship. These four species have the lowest chromosome volumes of all the species studied and it may be that they reflect limitations in the experimental technique here employed. However, when DNA content per unit length is considered, other species give abnormally high or low values, e.g. the triploid form of *C. argenteum* and both the diploid and triploid forms of *C. parthenium* respectively. The possibility of real differences in the organization of the DNA within the chromosomes cannot, as yet, be completely discounted.

6. DISCUSSION

Statistical analysis showed significant differences in DNA content both between roots of the same plant and between plants of the same species although, as has already been suggested, some of these differences may result from errors of the technique. The variation in DNA content between different species was, however, very much greater, and since the main conclusions reached here refer to species differences the intra-plant and intra-species variation will not be considered further.

The present study has shown that species with the same chromosome number may differ widely in DNA content. In diploids, for example, the relative amounts of DNA varied between 1·0 and 3·57. The striking differences in DNA content between diploid species are associated with differences in chromosome size. The relative lengths (1·0:1·92), cross-sectional areas (1·0:2·96) and volumes (1·0:5·66) of the same diploid species differ, and it is not always possible to relate change in DNA content directly to change in chromosome size.

There is, however, an over-all close linear relationship between DNA content and chromosome length and a probable curvilinear relationship between DNA and chromosome volume. It is suggested that the apparent exceptions to this relationship could result from limitations of the technique employed. Using nuclear volume rather than chromosome volume, other workers have obtained similar results in many different genera of both plants and animals (Baetcke *et al.* 1967; Bick & Jackson, 1967; McLeish, 1963; Rees *et al.* 1966). Nirula, Bhaskaran & Swaminathan (1961) found DNA content to be correlated with chromosome length in *Triticum* species but not in *Sorghum*. Variation in, for example, the degree of coiling could influence chromosome length, and Nirula *et al.* suggested that differences in amounts of heterochromatin in the more advanced asymmetrical karyotypes of *Sorghum* species could account for the difference between these two genera. It is possible that comparison of nuclear volume, rather than chromosome length, with DNA contents would be more meaningful in genera like *Sorghum* where there are large amounts of heterochromatin.

The reasons for these differences in DNA content between the diploid species are not fully understood. Schrader & Leuchtenberger (1949) suggested that variation in DNA content in tissues with the same chromosome number might result from polyteny. It has also been shown that duplications of segments of the chromosomes at meiosis can account for variations in DNA content (Rees & Jones 1967). The relatively large differences in DNA between the diploid *Chrysanthemum* species are, however, unlikely to have arisen as a result of duplications, since there is no cytological evidence of duplications in the interspecific hybrids studied and there are no reasons for thinking that the differences can be related to the polytene nature of the chromosome.

In *Chrysanthemum*, species with widely different chromosome numbers, e.g. *C. viscosum* $2n = 18$ and *C. indicum* $2n = 54$, may have almost identical total DNA contents. The chromosomes of such species do, however, contain almost identical quantities of DNA per unit area of the chromosome.

In *Chrysanthemum*, it would seem reasonable to expect that the phenotypically similar diploid species would contain similar numbers of genes and there is no reason to think that gene size differs between these species. If these assumptions are correct, then the wide variations in total DNA must mean that some of the nuclear DNA is genetically inactive and that some species contain relatively large quantities of this inactive DNA. It may well be that the inactive DNA is, in some genera at least, related to heterochromatin, and Stebbins (1966) suggested that a positive correlation between chromosome size and DNA content could be related to changes in amounts of heterochromatin. However, there is no evidence of large quantities of visible heterochromatin in any species of *Chrysanthemum*.

The relationship between DNA content and chromosome number was a simple one in the colchicine-produced autotetraploids. Chromosome doubling resulted in a twofold increase in DNA content without any influence on chromosome size. A similar result has been found previously in *Triticum* (Bhaskaran & Swaminathan, 1960). Some natural polyploids, however, have no more DNA than lower polyploids or diploids. Thus *C. viscosum* $2n = 18$, *C. leucanthemum* $2n = 36$, and *C. indicum* $2n = 54$ have similar amounts of DNA. It is highly improbable that these species contain similar numbers of genes and they may well contain different proportions of inactive DNA.

SUMMARY

1. The DNA contents of twenty-eight different species and forms of *Chrysanthemum* have been measured by photometry. It is shown that there are large differences in DNA content between some species with identical chromosome numbers.

2. The DNA contents of natural polyploids are frequently not those expected when comparison is made with diploid forms of the same species. The DNA contents of induced polyploids are those expected.

3. Chromosome length and volume are positively correlated with DNA content.

4. The relationship between chromosome number, chromosome size, DNA content and gene number is considered, and it is suggested that the differences in DNA content may result from the presence of differing amounts of genetically inactive DNA in the chromosomes.

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