J. Cell Sci. **63**, 173–179 (1983)
Printed in Great Britain © *The Company of Biologists Limited 1983*

DNA DENSITY IN MITOTIC AND MEIOTIC METAPHASE CHROMOSOMES OF PLANTS AND ANIMALS

M. D. BENNETT, J. S. HESLOP-HARRISON, J. B. SMITH AND J. P. WARD

Plant Breeding Institute, Maris Lane, Trumpington, Cambridge CB2 2LQ, U.K.

SUMMARY

Studies of chromosome disposition at metaphase using serial thin-sectioning and three-dimensional reconstruction techniques have produced accurate estimates of the total volume of chromosomes per cell in 15 plant and two animal species. Comparing this character with the 4*C* DNA amount showed no indication of systematic differences in DNA density between *either* organisms with widely different (>200-fold) *C* values *or* different groups or organisms. For example, there was no significant difference between the density of DNA in somatic metaphase chromosomes of man (0·141 pg/μm^3) and its mean in 14 angiosperm plant species (0·182 pg/μm^3), or between four dicotyledons (0·180 pg/μm^3) and 10 monocotyledons (0·182 pg/μm^3). However, evidence was found showing that DNA density can vary significantly within a species. Thus, although the total chromosome volume per cell was closely correlated ($r > 0·97$) with 4*C* DNA amount in somatic and meiotic cells, the density of DNA in metaphase chromosomes was significantly lower in meiocytes (0·131 pg/μm^3) than in somatic metaphase cells (0·179 pg/μm^3).

INTRODUCTION

Studies of nuclei using serial thin-sectioning and three-dimensional reconstruction have produced important new information about chromosome structure (Holm & Rasmussen, 1977; Bennett, Smith, Ward & Jenkins, 1981), disposition (Bennett, 1982; Heslop-Harrison & Bennett, 1983*a*,*b*) and meiotic pairing behaviour (Rasmussen, 1976; Wallace & Jones, 1978; Hobolth, 1981). Chromosome volume can be estimated very accurately in serial photomicrographs of reconstructed nuclei (Bennett, Smith, Ward & Finch, 1982). A by-product of our work on chromosome structure and disposition has been the accumulation of estimates of the total volume of chromatin in the full complement of metaphase chromosomes in more than 200 somatic or meiotic cells of several higher plant and animal species and hybrids. These results are of considerable interest in their own right. The purpose of this paper is: first, to describe comparisons of the results for different species and developmental stages; and second, to compare the present results with others previously obtained using light microscope techniques.

MATERIALS AND METHODS

Human fibroblasts from a karyotypically normal XY_1 cell line were cultured using standard techniques. Cells not exposed to pretreatment with trypsin (in the final passage) or colchicine were

collected and fixed in cacodylate-buffered gluteraldehyde before osmication, dehydration and embedding in Spurr's resin. Embedded cells were obtained through Dr A. C. Chandley (MRC Clinical and Population Cytogenetics Unit, Western General Hospital, Edinburgh).

To obtain root-tip metaphases in plants, seeds were germinated (unless otherwise stated) on moist Whatman's no. 1 filter paper in Petri dishes at 23 °C in the dark for about 3 days. Thereafter, dicotyledon seedlings were transferred to pots filled with moist vermiculite in a growth chamber at about 23 °C for about 1 week. First lateral root tips from seedlings about 10 days old were sampled. Root tips were taken from 1–2 cm long roots of seedlings of monocotyledons of about 3 days old, except for *Hordeum bulbosum* and *H. chilense* when they were taken from 'bulbs' and 6-month-old plants, respectively, and grown hydroponically in aerated mineral solution as described previously (Finch, Smith & Bennett, 1981). Excised root tips were either fixed directly for electron microscopy, or treated in ice-water (1 °C) for 24 h prior to fixation to accumulate metaphases. While prolonged exposure to such a low temperature may induce large changes in chromosome volume, treatment at 1 °C for 24 h does not have this effect – the mean total volumes of chromosomes per metaphase root-tip cell in 10 ice-water-treated (102·1 μm^3) and 10 untreated (106·8 μm^3) cells of *Hordeum vulgare* cv. Tuleen 346 were not significantly different.

To obtain male meiosis, plants were grown in a growth chamber at 20 °C with continuous light for at least 10 days before the onset of meiosis. Florets containing anthers with first metaphase of meiosis were identified by light microscopic examination of a squash preparation of a single anther. The remaining two anthers from such florets were taken for electron microscopy.

Root tips and anthers were fixed and prepared for electron microscopy and, as with the human cells, serially sectioned as previously described (Bennett, Smith, Simpson & Wells, 1979).

Adults of the N2 strain of the nematode *Caenorhabditis elegans* (var. Bristol), maintained as described by Brenner (1974), were prepared for electron microscopy as described by Ward, Thomson, White & Brenner (1975), but fixed in 2·5 % gluteraldehyde and post-fixed in 1 % osmium tetroxide. Photomicrographs of 0·05 μm thick sections of three reconstructed male (XO) meiocytes at first metaphase were kindly lent to us by Dr D. G. Albertson (MRC, Laboratory of Molecular Biology, Cambridge).

A detailed description of the methods used to estimate chromosome volume in reconstructed metaphase cells has also been published (Bennett *et al.* 1982). Briefly, the method depends on tracing the area of chromatin on micrographs of each section of a nucleus using a digitizer linked to a microcomputer. The magnification of the micrograph and section thickness are known, so the volume of chromatin in each section, and by addition in the nucleus, can be measured. The method gives more accurate estimates of chromosome volume (Bennett *et al.* 1982) than are possible by light microscopy (Bennett & Rees, 1969), since it does not involve the assumption, usually made in deriving estimates of chromosome volume by light microscopy, that chromatids are perfect cylinders.

4*C* DNA amounts for angiosperms were from Bennett & Smith (1976), or in the cases of *Sorghum* sp. and *Zea mays* cv. Seneca 60 were measured by Feulgen photometry as previously described, using *H. vulgare* cv. Sultan (4*C* = 22·2 pg) as a standard. 4*C* DNA values for man and *C. elegans* were from Vendrely (1955) and Sulston & Brenner (1973), respectively.

RESULTS AND DISCUSSION

Comparison of chromosome volumes estimated by two methods

Estimates of total chromosome volume per cell in 17 species are shown in Table 1. It is interesting to compare these estimates obtained using reconstructed nuclei with those obtained previously by one of the present authors using light microscopy of squash preparations of colchicine-treated material. The present result for *Vicia faba* (321·2 μm^3) is within the range noted previously for *V. faba* (303·2–637·7 μm^3; Bennett, 1970), and is close to that estimated for a first lateral root (398·8 μm^3). Similarly, the present result for *Secale cereale* (200·4 μm^3) is within the range noted for roots of that species grown in 'normal' conditions (138·4–244·4 μm^3; Bennett &

Table 1. *Amount of DNA per nucleus, the total volume per cell of metaphase chromosomes, and the DNA per unit volume of metaphase chromosomes at mitosis and meiosis in several plant and animal species*

Material and taxon	4C DNA amount (pg)	Total volume per cell of metaphase chromosomes (μm^3)	No. of cells reconstructed	DNA per unit volume of chromosomes (pg/μm^3)
Somatic mitosis				
Dicotyledon (root tip)				
Vicia sativa	9·0	43·9	3	0·205
Pisum sativum cv. Minerva Maple	19·5	100·8	1	0·193
V. narbonensis	25·4	162·1	1	0·157
V. faba (PBI, inbred line 6)	53·3	321·3	2	0·166
Monocotyledon (root tip)				
Sorghum sp. line 275 (CIMMYT)	3·0	23·5	2	0·128
Zea mays cv. Seneca 60	10·9	75·2	10	0·145
Aegilops umbellulata PBI accession "A"	20·2	128·0	10	0·157
Hordeum chilense	21·8	80·6	2	0·270
H. bulbosum clones J1 and L6	22·0	99·7	2	0·221
H. vulgare cv. Tuleen 346	22·2	104·5	20	0·211
Secale africanum PBI line R102	29·7	169·5	2	0·175
S. cereale cv. King II	33·1	200·4	5	0·165
Triticum durum cv. Cocorit	49·1	307·8	2	0·160
T. aestivum cv. Chinese Spring	69·3	366·8	2	0·189
Mammalian cell culture (fibroblast)				
Homo sapiens	12·0	85·3	2	0·141
Male meiosis (first metaphase)				
Monocotyledonous plants				
H. vulgare cv. Tuleen 346	22·0	136·9	4	0·162
T. monococcum	24·9	204·4	2	0·122
S. cereale cv. Petkus Spring	33·1	247·1	1	0·134
T. aestivum cv. Chinese Spring	69·3	565·9	5	0·122
Animal				
Caenorhabditis elegans	0·332	2·9	3	0·144

Rees, 1969). However, the present value for *H. vulgare* roots (104·9 μm^3) is lower than noted previously (126·4–185·1 μm^3; Bennett, 1971). These comparisons show that the two methods produce similar results, but suggest that the light microscopic method may tend to overestimate chromosome volume. This might be expected, since squashed chromatids are assumed to be perfect cylinders (Bennett & Rees, 1969; Bennett, 1970) in estimation of chromosome volume by the latter method. The present method does not make this assumption but reveals its limitations – in serial electron micrographs, there are often considerable variations in shape and area between cross-sections of a chromatid.

DNA density and variation in C *value*

Fig. 1A shows the positive relationship between the mean total chromosome volume (*c.v.*) per somatic metaphase cell, and the 4*C* DNA amount for 15 species ($P < 0{\cdot}001$; $r = 0{\cdot}98$). This result confirms previous comparisons using estimates of *c.v.* obtained by light microscopy (Rees, Cameron, Hazarika & Jones, 1966). Fig. 1B shows a similar relationship between *c.v.* per male meiocyte at first metaphase, and the 4*C* DNA amount for five species ($P < 0{\cdot}001$; $r = 0{\cdot}99$). This correlation, while not unexpected, has not been reported previously for meiotic metaphase chromosomes, as far as we know.

For biological relationships it is striking how close the points for different species in Fig. 1A and B are to the regression lines; indeed, no single point departs significantly from the regression line for the population of points in either graph.

The present results show no indication of systematic differences in DNA density between organisms with widely different DNA *C* values. Interphase nuclei have been subdivided into four types on the basis of their gross appearance (Delay, 1948), and this classification has been shown to correlate closely with the gross differences in species DNA *C* value (Barlow, 1977). The appearance of chromatin at interphase in electron micrographs also differs between species with low and high DNA *C* values

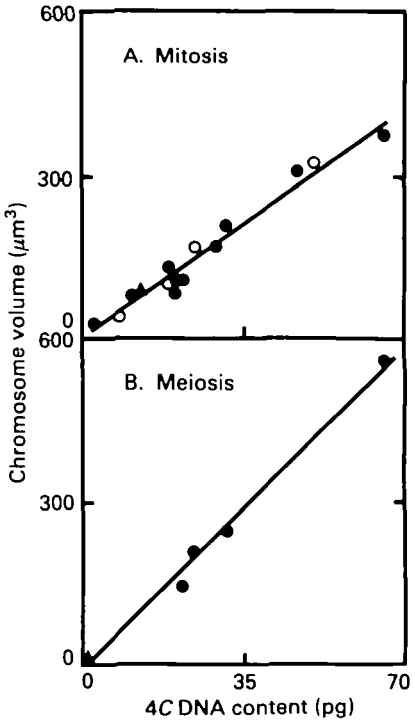

Fig. 1. The relationship between 4*C* DNA amount (pg) and total somatic chromosome volume (μm^3) at mitotic metaphase (A) and total meiotic bivalent volume (μm^3) at metaphase (B). (●) Monocotyledonous plants; (○) dicotyledonous plants; (▲) animals.

(Bennett *et al.* 1981). Nevertheless, the present results suggest that, whatever the basis of the above differences at interphase, large interspecific differences in *C* value may have no systematic effect on the degree of condensation of chromatin (and hence the appearance of chromosomes) at metaphase of mitosis or meiosis. Thus, a nematode with one of the smallest DNA amounts known for an animal (0·332 pg) and bread wheat with a high DNA amount (69·3 pg) both have similar densities of DNA in their meiotic metaphase chromosomes (0·114 and 0·122 pg/μm^3, respectively). Similarly, in the sample of 14 angiosperm plant species with a 23-fold range of 4*C* DNA amounts (Table 1), the mean density of DNA in somatic metaphase chromosomes was the same (0·178 pg/μm^3) for the two species with the smallest DNA *C* values (*Sorghum* sp. 3·0 pg and *Vicia sativa* 9·0 pg) as for the two species with the highest DNA *C* values (*V. faba* 53·5 pg and *Triticum aestivum* 69·3 pg).

DNA density in different groups of organisms

The present results also provide no indication of a major variation in DNA density between different groups of organisms. Thus the density of DNA in human fibroblast metaphase chromosomes (0·141 pg/μm^3) is within the range of values (0·128–0·270 pg/μm^3) found for somatic metaphase chromosomes of plants (Table 1). Similarly, the density of DNA in meiotic metaphase chromosomes of a nematode (0·114 pg/μm^3) and two wheat species (0·122 pg/μm^3) was similar. A further comparison shows no significant difference between the mean DNA density of root-tip metaphase chromosomes for four dicotyledons (0·180 pg/μm^3) and 10 monocotyledons (0·182 pg/μm^3). The present results differ therefore from those of Evans & Rees (1971), who reported a significant difference between the mean DNA density in root-tip metaphase chromosomes of five dicotyledons (0·473 pg/μm^3) and six monocotyledons (0·257 pg/μm^3). They are however in agreement with Anderson, Stack & Mitchell (1982), who concluded that: "there is no consistent difference in the compaction of plant vs animal chromosomes".

DNA density of mitosis and meiosis

Further analysis of the present results (Table 1) shows that the mean density of DNA in meiotic metaphase chromosomes (0·131 pg/μm^3) was significantly lower ($P < 0·001$) than in somatic metaphase chromosomes (0·179 pg/μm^3). This result agrees with the general observation that cell, nuclear and chromosome volumes are increased in germ-line cells of many organisms as meiosis is approached. Such a change in the density of DNA may have important effects on meiotic chromosome behaviour. For example, a positive correlation between meiotic chromosome volume and recombination rates has been noted for intraspecific variation between male and female meiocytes of *Fritillaria* (Fogwill, 1958), and male meiocytes of *S. cereale* after different treatments with phosphate (Bennett & Rees, 1970).

The significantly different density of DNA in somatic and meiotic chromosomes is further evidence of intraspecific developmental variation in chromosome volume. Studies using light microscopy previously established that the volume of somatic metaphase chromosomes within a species can vary by more than 100% during

development, and considerably more in response to environmental treatments (Bennett, 1970, 1971), without detectable variation in DNA *C* value. Large increases in chromosome volume induced by simple treatments with minerals have been noted (Bennett, 1970). It seems reasonable to suppose that the variation in DNA density of somatic metaphases of different species (which ranged from 0·128 pg/μm^3 in *Sorghum* to 0·270 pg/μm^3 in *H. chilense*) is real and due to developmental or environmental causes. For example, the two highest values (*H. bulbosum* and *H. chilense*) are for *mature* plants transferred to an aerated solution (Finch *et al.* 1981), rich in minerals, while the other plant species were given no minerals.

The reason for the difference between the present results and the results of Evans & Rees (1971) noted above is unknown. However, in view of the widespread existence of the types of variation just mentioned, the difference in DNA density between monocotyledons and dicotyledons that they reported may have had an environmental or developmental basis. Given that natural and induced variation in DNA density is real, especially in response to different chemical pretreatments (Anderson *et al.* 1982), any new experiments to investigate DNA density in chromosomes of different species should, wherever possible, compare corresponding tissues from organisms grown together under identical defined and controlled environmental conditions. The present work falls short of this ideal. However, this makes the correlations displayed in Fig. 1A and B the more remarkable, and increases our confidence in accepting them.

J. S. H.-H. thanks the Agricultural Research Council for a research studentship.

REFERENCES

ANDERSON, L. K., STACK, S. M. & MITCHELL, J. B. (1982). An investigation of the basis for the lack of G-banding in plant chromosomes. *Expl Cell Res.* **138**, 433–436.

BARLOW, P. W. (1977). Determinants of nuclear chromatin structure in Angiosperms. *Annls Sci. nat.* B, **18**, 193–206.

BENNETT, M. D. (1970). Natural variation in nuclear characters of meristems in *Vicia faba*. *Chromosoma* **29**, 317–335.

BENNETT, M. D. (1971). Effects of ethirimol on cytological characters in barley. *Nature, Lond.* **230**, 406.

BENNETT, M. D. (1982). The nucleotypic basis of the spatial ordering of chromosomes in eukaryotes and the implications of the order for genome evolution and phenotypic variation. In *Genome Evolution* (ed. G. A. Dover & R. B. Flavell), pp. 239–261. London: Academic Press.

BENNETT, M. D. & REES, H. (1969). Induced and developmental variation in chromosomes of meristematic cells. *Chromosoma* **27**, 226–244.

BENNETT, M. D. & REES, H. (1970). Induced variation in chiasma frequency in rye in response to phosphate treatments. *Genet. Res.* **16**, 325–332.

BENNETT, M. D. & SMITH, J. B. (1976). Nuclear DNA amounts in angiosperms. *Proc. R. Soc. Lond.* B, **274**, 227–274.

BENNETT, M. D., SMITH, J. B., SIMPSON, S. & WELLS, B. (1979). Intranuclear fibrillar material in cereal pollen mother cells. *Chromosoma* **71**, 289–332.

BENNETT, M. D., SMITH, J. B., WARD, J. P. & FINCH, R. A. (1982). The relationship between chromosome volume and DNA content in unsquashed metaphase cells of barley, *Hordeum vulgare* cv. Tuleen 346. *J. Cell Sci.* **56**, 101–111.

BENNETT, M. D., SMITH, J. B., WARD, J. & JENKINS, G. (1981). The relationship between nuclear DNA content and centromere volume in higher plants. *J. Cell Sci.* **47**, 97–115.

BRENNER, S. (1974). The genetics of *Caenorhabditis elegans*. *Genetics* **77**, 71–94.

DELAY, C. (1948). Récherches sur la structure des noyaux quiescents chêz les Phanérogames. *Rev. Cytol. Cytophsiol. veg.* **10**, 103–228.

EVANS, G. M. & REES, H. (1971). Mitotic cycles in dicotyledons and monocotyledons. *Nature, Lond.* **233**, 350–351.

FINCH, R. A., SMITH, J. B. & BENNETT, M. D. (1981). *Hordeum* and *Secale* mitotic genomes lie apart in a hybrid. *J. Cell Sci.* **52**, 391–403.

FOGWILL, M. (1958). Differences in crossing-over and chromosome size in the sex cells of *Lilium* and *Fritillaria*. *Chromosoma* **9**, 493–504.

HESLOP-HARRISON, J. S. & BENNETT, M. D. (1983*a*). The spatial order of chromosomes in root tip metaphases of *Aegilops umbellulata*. *Proc. R. Soc. Lond.* B (in press).

HESLOP-HARRISON, J. S. & BENNETT, M. D. (1983*b*). Prediction and analysis of spatial order in haploid chromosome complements. *Proc. R. Soc. Lond.* B (in press).

HOBOLTH, P. (1981). Chromosome pairing in allohexapoid wheat var. Chinese Spring. Transformation of multivalents into bivalents, a mechanism for exclusive bivalent formation. *Carlsberg Res. Commun.* **46**, 129–173.

HOLM, P. B. & RASMUSSEN, S. W. (1977). Human meiosis I. The human pachytene karyotype analysed by three dimensional reconstruction of the synaptonemal complex. *Carlsberg Res. Commun.* **42**, 283–323.

RASMUSSEN, S. W. (1976). The meiotic prophase in *Bombyx mori* females analysed by three-dimensional reconstruction of synaptonemal complexes. *Chromosoma* **54**, 145–193.

REES, H., CAMERON, F. M., HAZARIKA, M. H. & JONES, G. H. (1966). Nuclear variation between diploid Angiosperms. *Nature, Lond.* **211**, 828–830.

SULSTON, J. E. & BRENNER, S. (1974). The DNA of *Caenorhabditis elegans*. *Genetics* **77**, 95–104.

VENDRELY, R. (1955). The deoxyribonucleic acid content of the nucleus. In *The Nuclei Acids II* (ed. E. Chargaff & J. N. Davidson), pp. 155–180. New York: Academic Press.

WALLACE, B. M. N. & JONES, G. H. (1978). Incomplete chromosome pairing and its relation to chiasma localisation in *Stethophyma grossum* spermatocytes. *Heredity* **40**, 385–396.

WARD, S., THOMSON, N., WHITE, J. G. & BRENNER, S. (1975). Electron microscopical reconstruction of the anterior sensory anatomy of the nematode *Caenorhabditis elegans*. *J. comp. Neurol.* **160**, 313–338.

(Received 21 February 1983–Accepted 18 March 1983)