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Chromosomal DNA in higher plants

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There is an astonishing variation in the amount of chromosomal DNA among species of higher plants. Much of this variation is due to the amplification of base sequences within the chromosomes. As a result, the amount of DNA in the nuclei of many species is very great. In particular, the chromosomes are rich in repetitive DNA, which may comprise 70% or more of the total. This fraction contains at most only a few genes that code for proteins. What, then, is its functional significance? There is evidence that DNA amount *per se* affects cell size and the duration of cell divisions. The results of recent assays also provide evidence that particular repetitive sequences have specific effects upon the phenotype. While these effects may be small in themselves they may nevertheless be important in Nature and in plant breeding.

1. Introduction

Among species of higher plants there is an astonishing variation in nuclear DNA amount. Much of it is due to polyploidy, but there is a widespread and substantial variation resulting from the amplification and, perhaps, deletion of DNA segments within the chromosomes. The extent of such variation is revealed by the survey of Bennett & Smith (1976). Among diploid flowering plants we have, at one extreme, species like *Lotus corniculatus* with less than 1 pg of DNA in $2C$ nuclei. At the other extreme there are species of the genus *Fritillaria* with nuclear DNA amounts of 180 pg. The range is 300-fold. Investigations of species within a number of genera and families of flowering plants provide some of the answers that bear upon the following problems.

(1) What is the structural basis of the DNA variation within chromosomes?

(2) What functional significance, if any, has the DNA that results from the amplification of base sequences that often accompanies the divergence and evolution of species?

We have answers to account for many aspects of the structural changes associated with the variation in chromosomal DNA. The question of function with respect to the DNA variation remains largely unresolved. This is true not only for higher plants but for eukaryotes in general. In short, the C-value paradox has yet to be explained. The question of function is of concern not only from the standpoint of natural selection and adaptation. Now that methods have been developed by which DNA fragments may be excised and cloned in alien species it is imperative to find out which of the chromosomal DNA fractions in eukaryotes may be of use to the applied scientist, the plant breeder and others, for the improvement of plant and animal products.

2. DNA variation in *Lathyrus*

(a) *Quantitative change*

The results of our own investigations among species within the genus *Lathyrus* display many of the features characteristic of the quantitative nuclear DNA variation associated with the

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divergence and evolution of flowering plant species. Table 1 and figure 1*a* show the distribution of nuclear DNA amounts in *Lathyrus* species. All are diploids with 14 chromosomes. Two observations may be made.

(*a*) Despite their close taxonomic affinity there is, roughly, a threefold variation among species. It is tempting, on the face of it, to argue that much of the chromosomal DNA must be redundant or inert. The more specialized inbreeding annual species, have, on average, a significantly lower DNA content than the outbreeders. Since inbreeders must be derived from outbreeding ancestors we have to conclude that the evolution of *Lathyrus* species may have been accompanied by a massive DNA diminution (Rees & Hazarika 1969).

(*b*) There is an element of discontinuity associated with the DNA distribution. The discontinuity is, moreover, of a surprisingly consistent pattern. There is an interval of approximately 3 pg between each group. The variation, in other words, is achieved by a series of

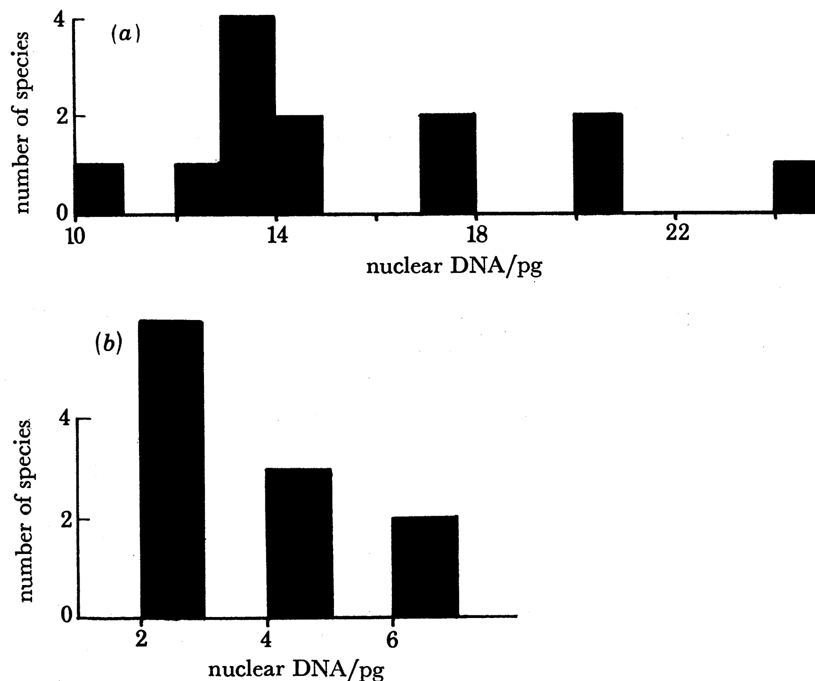

Figure 1. The distribution of nuclear DNA amounts among (*a*) *Lathyrus* species and (*b*) species of the genus *Clarkia*.

Table 1. Nuclear DNA amounts (picograms) in 2*C* nuclei of *Lathyrus* species

	total DNA	DNA in heterochromatin	DNA in euchromatin	non-repetitive DNA	repetitive DNA
L. angulatus	10.90	1.60	9.30	4.36	6.54
L. articulatus	12.45	1.90	10.55	5.48	6.97
L. nissolia	13.20	2.77	10.43	5.41	7.79
L. clymenum	13.75	2.91	10.84	5.23	8.52
L. ochrus	13.95	3.48	10.47	5.58	8.37
L. aphaca	13.97	—	—	5.17	8.90
L. cicera	14.18	3.36	10.82	5.96	8.22
L. sphaericus	14.18	—	—	—	—
L. sativus	17.15	4.50	12.65	5.83	11.32
L. odoratus	17.16	—	—	5.67	11.49
L. hirsutus	20.27	7.49	12.78	6.17	14.20
L. tingitanus	22.18	7.39	14.79	7.90	14.28
L. sylvestris	24.26	8.70	15.56	7.27	16.99

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'quantum jumps'. It appears as if changes involving only particular DNA packages were tolerable during speciation. This could be interpreted as an adaptive constraint upon the quantitative DNA variation. If so, the variation must be of functional significance. Similar 'quantum jumps' have been reported by Rothfels *et al.* (1966) in the Anemonae and by Martin & Shanks (1966) in *Vicia*. We have recently found a particularly striking example in the genus *Clarkia* (figure 1*b*).

(*b*) *The distribution of quantitative DNA change*

Comparisons between the chromosomes of species with low and high nuclear DNA amounts indicate that the quantitative DNA changes implicate all chromosomes within the complement (Rees & Hazarika 1969). The same is true for other genera, e.g. *Allium* (Jones & Rees 1968) and *Lolium* (Rees & Jones 1967). However, different chromosome components are affected to different degrees.

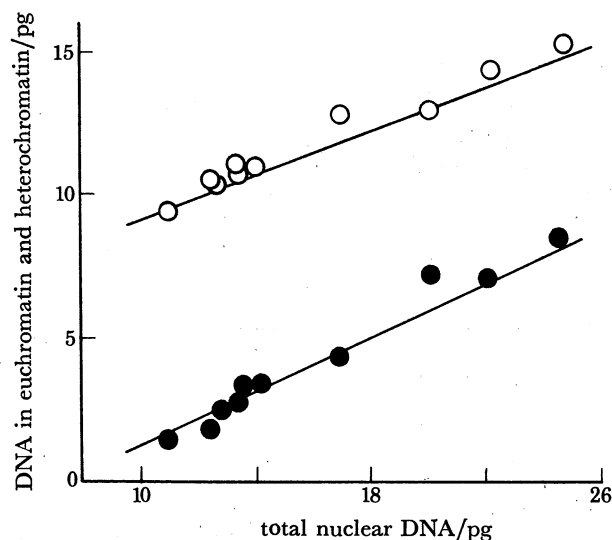

Figure 2. The amount of DNA located in euchromatin (○) and in heterochromatin (●) plotted against the total nuclear DNA amount in *Lathyrus* species.

(*c*) *Heterochromatin and euchromatin*

Table 1 and figure 2 show the amounts of DNA located in euchromatin and in heterochromatin in 10 *Lathyrus* species differing in total nuclear DNA amount. With increasing total DNA it will be seen that, while there is an increase in both euchromatin and heterochromatin, the rate of increase is greater within heterochromatin than within euchromatin. We emphasize, in particular, the linearity of the regressions in figure 2. It signifies that the extra, supplementary DNA is of a consistent composition throughout the genus. The addition of each picogram of DNA located within euchromatin is accompanied by an additional 1.2 pg located within heterochromatin. This constancy, we again suggest, implies a rigid constraint upon the kind of DNA variation tolerable during divergence and speciation (Rees & Narayan 1977).

(*d*) *Repetitive and non-repetitive DNA*

Table 1 and figure 3 show the amounts of non-repetitive and repetitive DNA in *Lathyrus* species with different DNA amounts. An increase in the total DNA amount is due to an increase in both non-repetitive and repetitive components, but preponderantly the latter. We

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emphasize again the linearity of the regression lines, showing that the composition of the supplementary DNA is constant throughout the genus. For every picogram of non-repetitive DNA there is an increase of 4.2 pg of repetitive DNA. The constancy, like that which applies to DNA in euchromatin relative to heterochromatin, suggests yet again a rigid constraint upon the nature of the DNA changes associated with divergence and evolution. The constraints are at least an indication that the variation may be of adaptive and functional significance (Hutchinson *et al.* 1980).

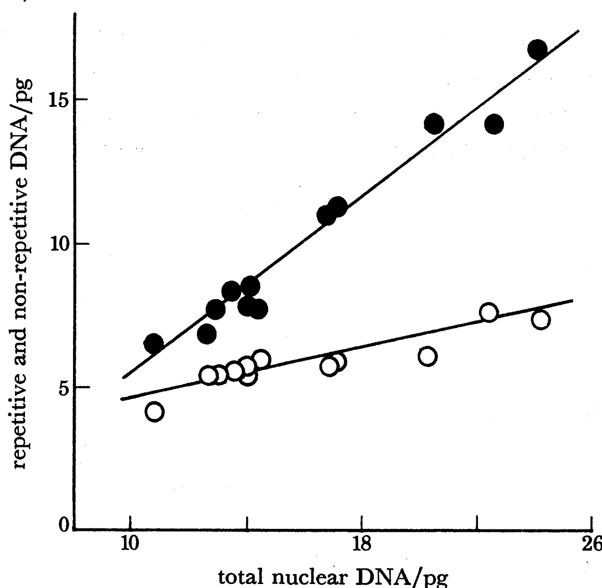

FIGURE 3. The amount of repetitive (●) and of non-repetitive (○) DNA plotted against the total nuclear DNA amount in *Lathyrus* species.

(*e*) *Base sequence composition*

Variation in the amount of both repetitive and non-repetitive DNA is accompanied by changes in base sequence composition (Narayan & Rees 1977), for example a heavy satellite is readily distinguished when the DNA of *L. tingitanus* is bound to actinomycin D and centrifuged in a caesium chloride gradient (figure 4). *In situ* DNA–DNA hybridization shows that the repeated sequence that makes up this satellite is located in heterochromatic blocks adjacent to the centromeres of *L. tingitanus* chromosomes. The satellite DNA does not 'hybridize' to the DNA in chromosomes of other species, e.g. *L. hirsutus*. The base sequence is either rare or absent from this species.

Detailed information about the composition, variation and the distribution of satellite DNA in flowering plants, particularly cereals, are given by Flavell *et al.* (1980) and Gerlach & Peacock (1980). For the present it will suffice to emphasize that a change in the amount of DNA during speciation is accompanied by a change in base composition.

We would expect, of course, to find some base sequences to be conserved, particularly those associated with structural genes. We need to bear in mind, however, that the amount of chromosomal DNA that contributes to the structural genes is small. Estimates of the number of structural genes in the chromosomes of eukaryotes range from 10000 to 30000 (see Cavalier-Smith 1978), which would implicate less than 5% of the DNA in *Lathyrus* and many other species of flowering plants. It is the function of the remaining 95% or more of the chromosomal DNA, composed of both repetitive and non-repetitive base sequences, that remains an enigma. We shall now consider the results of some experiments that bear upon this problem.

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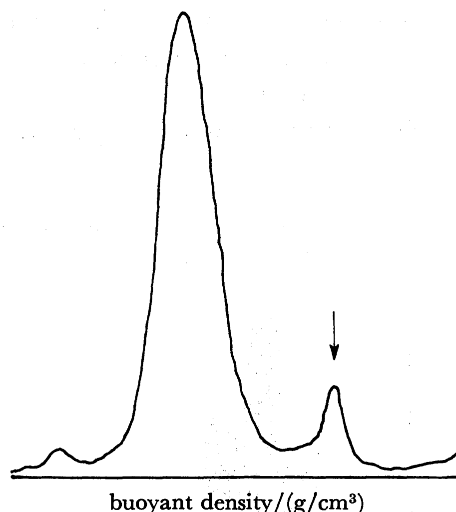

FIGURE 4. A 'heavy' DNA satellite (arrowed) in *Lathyrus tingitanus* revealed after binding with actinomycin D and ultracentrifugation in a CsCl gradient.

3. The consequences of variation in DNA amount

(*a*) *Cell size and cell division*

There is a large body of evidence showing that the duration of mitotic cycles in root meristems of flowering plants increases with increasing nuclear DNA amount (Van't Hof 1965; Evans *et al.* 1972). In root meristems, also, the cell size and mass increase with increasing DNA amount (Martin 1966). The dependence of these important components of growth on the DNA amount might well be expected to have important adaptive implications. In this connection, Bennett (1972) has pointed out that short-lived, ephemeral plants have, in general, lower nuclear DNA amounts than long-lived perennials. There is, however, a puzzling element in this relation. At 20 °C a change of 1 h in the duration of mitotic cycles in root meristems of dicotyledons is achieved by loss or gain of 3 pg of nuclear DNA (Evans *et al.* 1972). Are we, in the light of this fact, to conclude that control over the duration of mitotic cycles and, for that matter, of cell size is achieved by variation in nuclear DNA? If so, the control invokes massive alterations in chromosome material; on the face of it a crude and, materially, expensive strategy. Even so, a powerful argument to this effect has, indeed, been presented by Cavalier-Smith (1978).

(*b*) *An assay of DNA effects in* Lolium

Leaving aside the consequences of DNA variation upon cell cycles and cell size, what other effects can we attribute to the DNA that is amplified, or deleted, in conjunction with speciation in flowering plants? The nuclei of inbreeding species of *Lolium* have 40 % more DNA than those of outbreeders. All are diploids with 14 chromosomes. Despite this large DNA difference, the species produce viable F_1 hybrids, and fertile hybrids at that.

Viability

Figure 5 shows estimates of DNA amounts in *L. perenne* (an outbreeder, *L. temulentum* (an inbreeder), their F_1 hybrid and their backcross progenies. The DNA values in the backcross follow a normal distribution, ranging from that of the 'low' DNA parent to the mid-parent value. We conclude that the segregation of the supplementary DNA fraction, that which distinguishes *L. perenne* from *L. temulentum*, has no effect upon the viability of gametes or of zygotes.

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There is one slight qualification to make. The mean DNA amount for the progeny of this and other backcrosses is marginally lower than the expected, mid-parent value. The departure from expectation is less than 1 pg. At most, therefore, the supplementary DNA derived from *L. temulentum* has a marginal effect upon the viability of gametes and zygotes (Hutchinson *et al.* 1979).

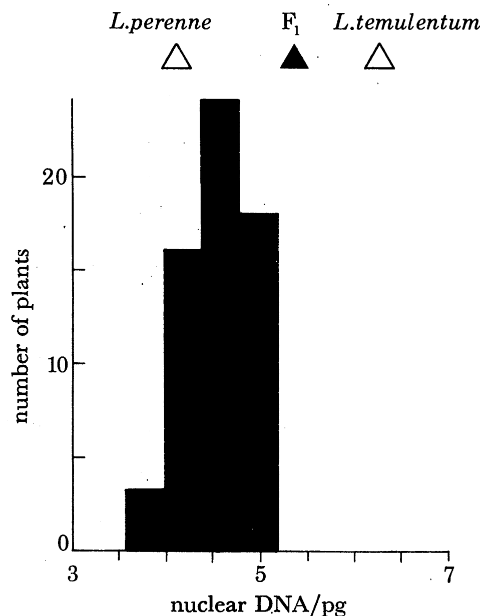

FIGURE 5. The distribution of nuclear DNA amounts in *Lolium perenne*, *L. temulentum*, their F_1 hybrid and the backcross progeny of the F_1 to *L. perenne*.

Chiasma frequencies in pollen mother cells

The extra, supplementary DNA in inbreeding *Lolium* species results from amplification of the DNA within each chromosome of the complement. This is made clear by the structure of bivalents at first metaphase of meiosis in the F_1 hybrid. All seven bivalents are asymmetrical (figure 6). The question is: how does the supplementary DNA affect homology, the capacity to pair effectively and form chiasmata at meiosis in hybrids and hybrid derivatives? Two kinds of effects on chromosome pairing and chiasma formation might be envisaged. First, there is a gross structural dissimilarity between the parental chromosomes due to the extra DNA in *L. temulentum* chromosomes. Secondly, there is the possibility that the extra DNA might carry determinants that influence chiasma formation. The mean chiasma frequencies for *L. perenne* (the outbreeder), *L. temulentum* (the inbreeder) and their F_1 hybrid are 11.6, 13.9 and 11.0 respectively (see also figure 7). The mean chiasma frequency of the F_1 hybrid is not significantly different from that of *L. perenne*, despite the structural dissimilarity of the parental chromosomes. Figure 7 shows the chiasma frequencies in plants of the progeny of the backcross, $F_1 \times$ *L. perenne*, plotted against the nuclear DNA amount. There is a wide range of chiasma frequencies. This is to be expected as the result of the segregation of parental genes controlling chiasma frequency. There is, however, no correlation between the chiasma frequencies and the DNA amounts. There is therefore no evidence that the chiasma frequency is influenced by determinants located in the supplementary DNA, nor is there any evidence that the chiasma

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frequency is influenced by the gross structural differences within bivalents that result from the supplementary DNA deriving from the *L. temulentum* parent.

The results of DNA assays of the kind that we have described, involving many phenotypic characters in backcrosses and F_2 progenies from interspecific *Lolium* hybrids, were published by Hutchinson *et al.* (1980). The characters ranged from seedling height to flowering time. Apart from a very slight effect upon gamete or zygote viability, the assays showed that the large

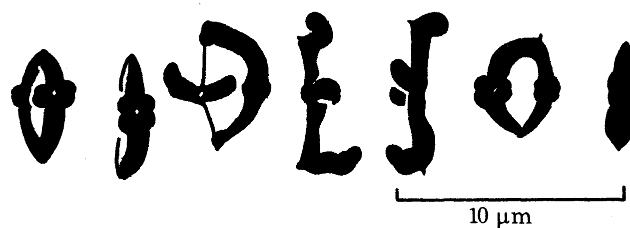

FIGURE 6. Asymmetrical bivalents at first metaphase in the hybrid *Lolium temulentum* × *L. perenne*.

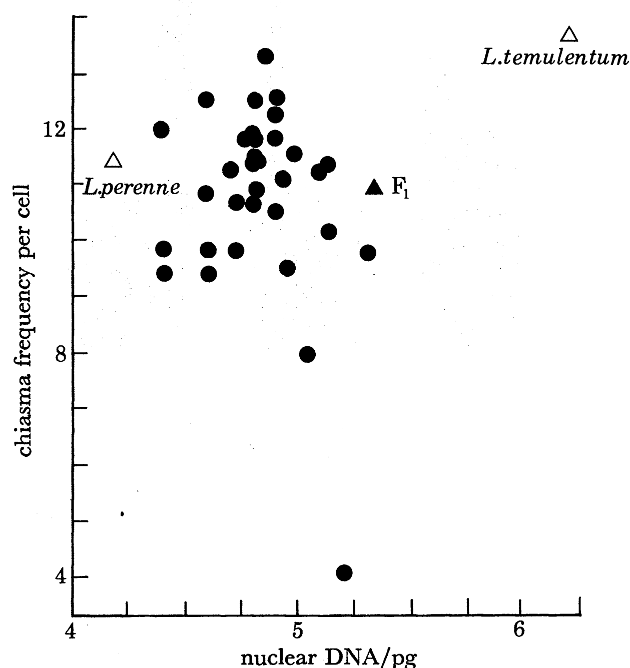

FIGURE 7. The mean chiasma frequencies of *L. perenne*, *L. temulentum*, their F_1 hybrid and the backcross progeny, plotted against the nuclear DNA amount. Data from A. Seal.

amount of supplementary DNA that distinguishes the inbreeding from outbreeding species has surprisingly little effect upon growth and development. Does this mean that much of this supplementary DNA is genetically inert, so-called selfish or parasitic DNA (Doolittle & Sapienza 1980; Orgel & Crick 1980)? Not necessarily. There are certain limitations to the assays in *Lolium*, at least for detecting relatively small effects, and small effects may be important. For detecting such effects we suggest that it would be more effective to identify and assay particular DNA base sequences rather than to assay massive amounts of chromosomal DNA as in the *Lolium* investigation.

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(*c*) *Knobbed* 10 *in maize*

A small heterochromatic knob in the long arm distinguishes the abnormal from the normal form of chromosome 10, the smallest chromosome in the maize complement. The presence of the knob on 10 has a dramatic effect on chromosome behaviour at both divisions of meiosis. The ends of the chromosomes, particularly of the long arms, display 'centromere-like' activity, i.e. they develop spindle fibres and move towards the spindle poles (Rhoades 1952). The chromosomes will respond to the presence of abnormal 10, however, only if they themselves bear heterochromatin of a particular DNA composition, namely a highly repetitive sequence of 185 base pairs (Peacock 1981). Classes of heterochromatin lacking this 185 base-pair repeat do not respond.

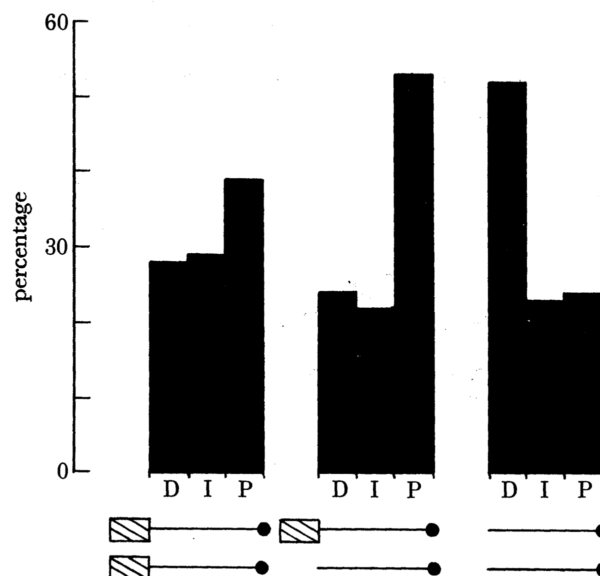

FIGURE 8. The distribution of chiasmata (in percentages) in proximal (P), interstitial (I) and distal (D) segments of medium-length chromosomes of *Chryptobothrus chrysophorus*. Hatched blocks represent heterochromatic segments, black discs represent centromeres. Data from B. John.

(*d*) *Terminal heterochromatin in grasshoppers*

The Australian grasshopper *Cryptobothrus chrysophorus* is polymorphic for large terminal blocks of heterochromatin on three of the large and six of the medium-sized chromosomes of the complement. The blocks are distal, i.e. at the chromosome ends furthest from the centromeres (figure 8). In the absence of blocks the chiasmata are located mainly in the distal euchromatin. In bivalents homozygous or heterozygous for the heterochromatic blocks, there is a dramatic shift in chiasma distribution, away from the blocks, proximally towards the centromeres (John & King 1980). It is possible that the heterochromatin interferes directly with the effective pairing of chromosomes at pachytene. If so, the repetitive DNA of which it is composed achieves its effect upon the phenotype not through transcription and translation of a product but by mechanical modifications of the chromosome architecture. The same may well be true of heterochromatic knobs in maize (Rhoades 1952). The maize story tells us, however, that such mechanical modifications may nevertheless depend on specific DNA sequences.

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4. Discussion

The results of Peacock (1981) and of John & King (1980) in §§3*c* and 3*d* are encouraging indications that assays of particular chromosome DNA sequences may provide useful information about the functional properties of that large, mainly repetitive DNA fraction of eukaryote chromosomes that, on the face of it, appears to be relatively inert. This is not to say, of course, that all of it is active. Some of it may be, as many have asserted, quite inert and 'selfish'. After all the only requirement for the spread and establishment of a selfish DNA segment is that it multiplies at a faster rate than the normal. On this score a brief reference to supernumerary, B chromosomes, which are common in higher plants, is both apposite and cautionary. B chromosomes, even when deleterious to the fitness of the organism, may spread and persist in a population because they have the capacity of increasing their number in gametic nuclei in comparison with 'ordinary' chromosomes of the complement. In this respect their DNA could qualify for the category of selfish or parasitic (Ostergren 1945). Yet this does not mean that all of the B chromosome DNA is inactive: there is ample evidence to the contrary (Jones 1975). Under certain circumstances, indeed, there is good evidence that it contributes to an increase in fitness (Rees & Hutchinson 1973). We would argue that the same may be true of much of the repetitive and other fractions of the chromosomal DNA of eukaryotes that present us with the riveting challenge of the *C*-value paradox. The argument will only gather substance, however, when we have the results of more genetic assays to analyse and evaluate.

We are grateful to Mr Alan Seal and to Professor B. John for permission to quote their experimental results.

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Discussion

P. R. Day (*Plant Breeding Institute, Cambridge, U.K.*). In figure 1, Professor Rees indicated the range of DNA amounts found in cells of diploid species. What factors restrict the upper limit to about 200 pg?

H. Rees. I can only make a guess. Cell volume is directly correlated with nuclear DNA amount. In some tissues it may be that there is an upper limit to the cell volume beyond which the diffusion of metabolites becomes inefficient. If so, there would be a constraint upon further increase in nuclear DNA.