NUCLEAR DNA CONTENT OF THREE *CREPIS* SPECIES

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1. INTRODUCTION

VARIOUS comparisons made on series of animals and plants have established correlations between the size of the genome, measured as the diploid nuclear (2C) DNA content, the combined volume of metaphase chromosomes, the interphase nuclear volume, and cell size. DNA content is also correlated to the duration of the mitotic cycle and of its period of DNA replication (S phase) in Angiosperm root-tips (Van't Hof, 1965; Evans and Rees, 1971). It is rarely pointed out that the variability of these features and the imprecision of their measurement may well permit only the vague conclusion that the correlations are all positive. *Vicia faba* provides an outrageous example of imprecision, with a 2C DNA content calculated to be either 24 or 40 pg. and various measurements of the mitotic cycle ranging from 14 to 19 hours. Under the stipulated conditions of colchicine arrest and feulgen staining, chromosome volume is closely correlated to DNA content within different Angiosperm genera (Jones and Rees, 1968; Rees and Hazarika, 1969) but the regression lines differ considerably between genera (Evans and Rees, 1971). Other examples and exceptions are cited by Lints and Jones (1969) and by Bennett and Rees (1969).

It is on the basis of such a correlation that *Crepis neglecta* and *C. fuliginosa* have been held to provide an exceptionally striking case of genomic difference between closely related species of Angiosperms. Tobgy (1943) measured the chromosomes at mitotic metaphase in these two species and their hybrid, finding that the haploid set of *fuliginosa* chromosomes was 55·5 per cent. the length, 67 per cent. the diameter and thus only 25 per cent. the volume of the *neglecta* set. Despite these differences, the two species naturally interbreed to produce viable but very infertile hybrids. Tobgy demonstrated that the three *fuliginosa* chromosomes could have arisen by means of reciprocal translocations and the loss of only one arm and centromere from the four *neglecta* chromosomes—a loss of less than 10 per cent. of the total metaphase length or volume. The *fuliginosa* chromosomes pair and form chiasmata with virtually all the *neglecta* chromosomes during meiosis in the hybrid. Tobgy (1943) concluded from these observations that the chromosome differences at metaphase were largely differences of contraction, not of material. Both Stebbins (1966) and Callan (1967), however, chose the volume difference to illustrate their theories concerning the evolution and organisation of chromosomes.

We have measured the DNA content of nuclei in primary root-tips of these two species and of *C. capillaris*, believing that even our inexperience and a temperamental microdensitometer could not prevent us from distinguishing between the predictions that *C. fuliginosa* nuclei contain either 25 per cent. or 90 per cent. as much DNA as *C. neglecta* nuclei.

2. MATERIAL AND METHODS

Seeds were germinated on filter paper until both the primary root and the cotyledons had emerged, and then processed according to the optimum conditions established for *Vicia faba* by Fox (1969): fixation in ethanol-acetic (3 : 1) for at least 24 hours, hydrolysis in 5N HCl for 1 hour, staining in Feulgen pH 3·6 for 2 hours followed by three 10-minute washes in 0·03M SO_2 and mounting in glycerol, all at room temperature. The absorption of green light (nominally 560 nm.) by nuclei in cubical cells of the apical mm of the root was recorded as the mean of three readings, less blank background readings, on a Barr and Stroud GN2 integrating microdensitometer.

C. capillaris roots were originally intended to serve as a standard for converting all measurements into absolute units. The 2C DNA content of *C. capillaris* has been estimated as 2·45 pg. and as 4·2 pg. Our reasons for distrusting both estimates are given in the discussion. As an alternative standard, blood smears from the toad *Xenopus laevis* were dried on slides and then processed identically to the seedlings. All *Xenopus* erythrocytes are expected to contain the 2C value of 6·3 pg. DNA (Dawid, 1965). We have tested their suitability as standards for Feulgen densitometry. One obvious source of error is that erythrocyte nuclei are relatively small but densely stained. All nuclei were crushed to reduce the known tendency of microdensitometers to underestimate the staining of condensed chromatin and chromocentres.

3. RESULTS

Initial densitometer readings of potentially meristematic cells produced a distribution of absorption classes, mostly within a two-fold range for each plant species and thus presumably including 2C, 4C and intermediate (S-phase) nuclei. Although 2C nuclei predominated in these distributions (fig. 1), the expected 2C and 4C modes could not be clearly identified. The frequency distributions do not correspond well to expectations derived from the durations of G_1, S and G_2 phases of the mitotic cycle (Langridge, O'Malley and Wallace, 1971), but do resemble the distributions reported by McLeish and Sunderland (1961) from both meristematic and differentiating zones in root-tips of several Angiosperms. In these cases there appear to be populations of non-dividing or rarely dividing 4C cells. We tested this possibility by immersing *neglecta* seedlings in 10^{-6}M FUdR (5-fluorodeoxyuridine) for 6 hours prior to fixing and processing. This treatment should inhibit DNA synthesis and arrest cells in S-phase long enough for G_2 cells to pass through mitosis into G_1 (Taylor, Haut and Tung, 1962). In terms of densitometry, that should deplete the 4C category and augment the 2C category to the extent that the sample consists of meristematic cells. The FUdR treatment was effective in abolishing mitotic figures but did not significantly alter the frequency distribution of absorption classes from that of control seedlings or from that shown in fig. 1. We conclude that there is a population of persistent 4C cells even in the apical 1 mm of these small roots.

Selected nuclei from the root-tips, identified as being at late telophase to early interphase or late interphase to early prophase, were then recorded from each species and compared to erythrocyte nuclei. Fig. 2 shows one
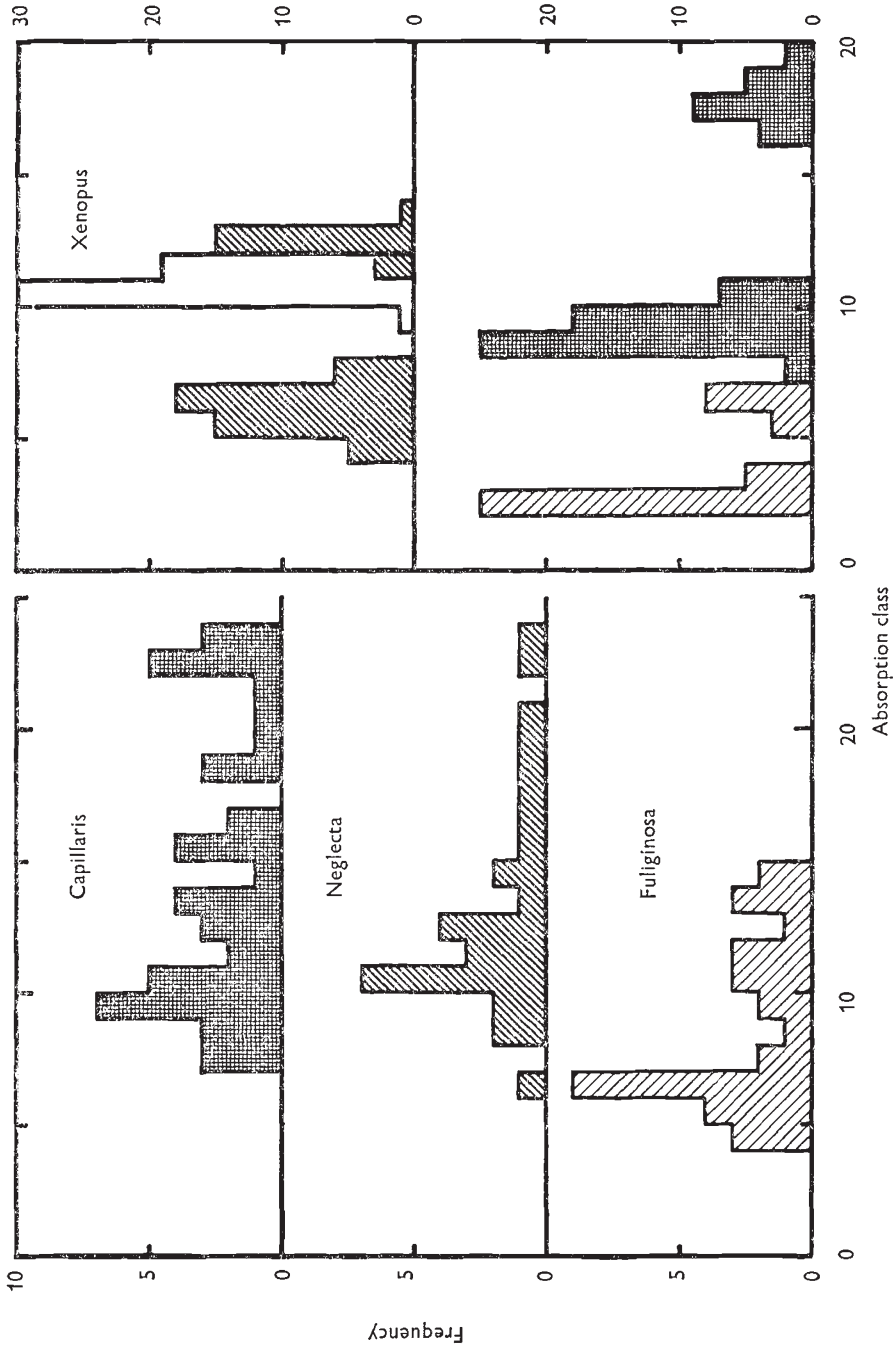
FIG. 1 (left).—Frequency of nuclei in classes of Feulgen absorption, from samples of apical root-tips of three *Crepis* species.
FIG. 2 (right).—Frequency of selected 2C and 4C nuclei of the same species (shading as in fig. 1) and of 2C erythrocytes of *Xenopus laevis*. The absorption units are arbitrary values and are not identical for the two figures.

series of readings, accumulated by two recordings for each species on consecutive days and thus perhaps exaggerating their variability. A second series of readings was made under the same conditions from similar numbers of nuclei. After adjustment for the 2C and 4C categories of nuclei, the mean values of these results provide our best estimate of the relative and absolute amounts of DNA (table 1).

The comparisons made in table 1 assume that Feulgen staining has the same stoichiometric relationship to nuclear DNA in blood smears and whole root-tips. In fact, Feulgen densitometry has only been justified in terms of yielding appropriate recordings in polyploid series for chromatin of a particular species, consistent results between either plant species or animal species, and with identical preparations such as isolated nuclei. In our comparison, blood smears might well reach saturation and lose excess stain more rapidly than the root-tips, whose bulk and cell walls should impede the diffusion of stain equally in both directions. Varying the time of staining shows that both roots and blood smears reach saturation within the 2 hours used in the standard procedure (fig. 3). The saturation curves are so similar that relative staining seems proportional relative DNA content at all times: there is no evidence of a diffusion effect. Only unbound reagent is lost

TABLE 1

Relative and absolute DNA contents of 2C nuclei. (Mean values from n *nuclei in 2 series of recordings)*

Species	n	Relative absorption		pg. DNA	
C. capillaris	72	9·38	100%	5·3	} 5·2
	50	9·12	100%	5·1	
C. neglecta	63	6·51	69%	3·7	} 3·6
	38	6·30	69%	3·5	
C. fuliginosa	41	3·25	35%	1·8	} 1·8
	59	3·06	34%	1·7	
X. laevis	50	11·22	120%	6·3	} standard
	80	11·35	124%	6·3	

during the SO_2 washes, while any that remains quickly reaches equilibrium throughout the glycerol mounting medium. As a consequence, the recorded difference between a nucleus and an equal area of blank background measures only bound stain, and is identical after 1 minute and the standard 30-minute SO_2 treatment (fig. 4). Blood smears do not lose stain for several hours in distilled water but can give perceptibly lower recordings after storage overnight in water. In our standard procedure, blood smears were mounted in glycerol within a few minutes after washing. We have tested several other variations of the staining procedure and found the same responses and optimal conditions described by Fox (1969) for *Vicia faba* root-tips. Blood smears and seedlings can be stored at 4° C. for several weeks in the fixative, or for at least a week when mounted in glycerol, and still yield consistent densitometer readings. Blood smears and root-tips stain maximally after 60 minutes' hydrolysis at 20°-22° C. Since the material responded identically to these variations in the staining procedure, and all preparations were crushed sufficiently to give constant densitometer readings, we conclude that nuclear DNA contents of plant roots and animal erythrocytes can be validly compared.
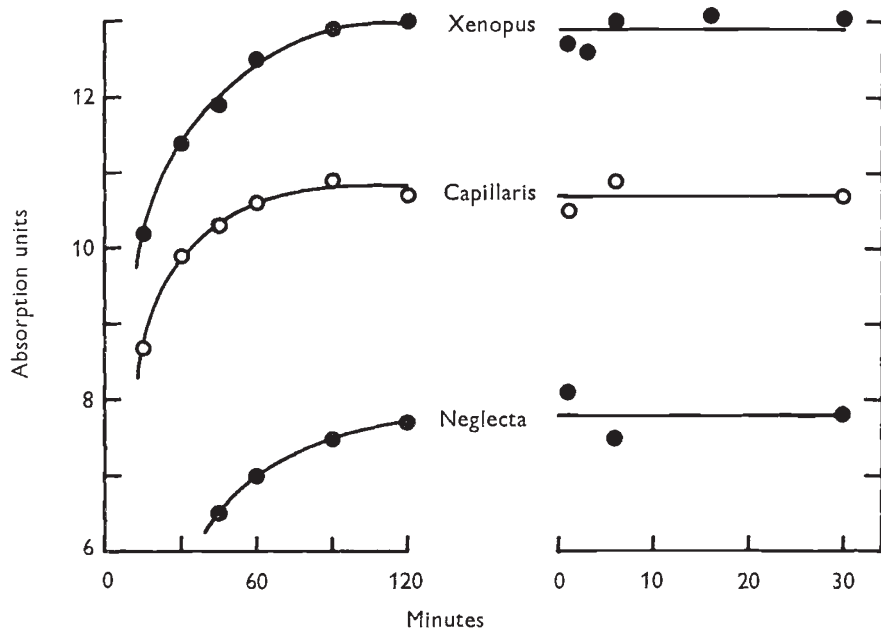
FIG. 3 (left).—Saturation curves for Feulgen staining. The mean absorption of ten 2C nuclei is plotted against time of staining, in each case followed by 30 minutes in SO_2.

FIG. 4 (right).—The mean absorption of ten 2C nuclei is not affected by altering the time of SO_2 treatment, in preparations stained for 2 hours.

4. DISCUSSION

Previous estimates of the DNA content of plant cells are derived from chemical determinations and cell counts of root-tips, which are sometimes naïvely considered to represent a 3C value. Van't Hof (1965) derived a more sophisticated estimate of 2C by assuming the frequencies of 2C, intermediate, and 4C cells were proportionate to the relative durations of the similar compartments of the mitotic cycle: G_1, S and G_2+M, which should be true for the meristem. Thus he corrected his determination for *C. capillaris* from 3·82 pg./cell to a 2C value of 2·45 pg. He also adjusted a previous determination of 54·3 pg./cell for *Allium cepa* to a 2C value of 33·55 pg. The latter estimate has been used as a standard for Feulgen densitometry by Evans and Rees (1971), who thus indirectly estimated the 2C DNA content of *C. capillaris* as 4·2 pg. McLeish and Sunderland (1961) had previously obtained a quite different result, 78·7 pg./cell, for the DNA of *A. cepa* roots. Only a fault in extraction or measurement can explain the discrepancy between 78·7 and 54·3 pg./cell. The histograms of densitometer readings shown by McLeish and Sunderland (1961) and confirmed here invalidate the assumption required for Van't Hof's (1965) correction factor. This would only alter the estimated 2C value for *A. cepa* by about a picogram, but could well be larger for other species.

It seems that the errors and uncertainties reviewed in the last paragraph have prevented attempts to obtain reliable estimates of the 2C DNA content of Angiosperms. In contrast to this, we can vouch for the reliability of

Dawid's (1965) conclusion, from diphenylamine determinations and cell counts, that *Xenopus laevis* erythrocytes contain $6\cdot3 \pm 0\cdot6$ pg. DNA. By the less accurate method of absorption at 265 nm., blood from three adult females yielded $6\cdot0$, $6\cdot3$ and $6\cdot5$ pg. (Wallace, unpublished). Circulating erythrocytes are apparently incapable of DNA synthesis, and yield relatively uniform densitometer readings (fig. 2). Considering the specificity of the Feulgen reaction and the similar effects on erythrocytes and root-tips of varying the staining procedure, we can fairly claim that we have obtained good relative measurements of nuclear DNA, conforming to assumptions which are generally accepted for Feulgen densitometry. Furthermore, erythrocytes provide a much more reliable standard than has been used previously for estimating the DNA content of Angiosperm nuclei. This conclusion suggests a general technique of standardising densitometer readings, for virtually any eukaryotic cell could be compared to erythrocytes of an appropriate fish, amphibian, reptile or bird—covering the range of 1-168 pg./nucleus (see Mirsky and Osawa, 1961). Bachmann (1972) has anticipated this suggestion by standardising his measurements of DNA for several mammals against amphibian erythrocytes.

The possibility of error is greatly reduced when considering only the relative densitometer recordings from root-tips of related plants. We find that *C. fuliginosa* nuclei contain 50 per cent. as much DNA as the corresponding nuclei of *C. neglecta*. The rather greater amount of heterochromatin in *neglecta* nuclei could only introduce a trivial bias into this result, which does not confirm to either prediction (25 or 90 per cent.) mentioned in the introduction. We should conclude from our results that such predictions were completely unreliable, except that Jones and Rees (1968) find a good consistency between DNA content and both total metaphase chromosome volume and pachytene pairing on an *Allium* hybrid. Although the relative DNA content of *fuliginosa* and *neglecta* corresponds reasonably well with their relative chromosome lengths at mitotic metaphase, $55\cdot5$ per cent. (Tobgy, 1943), we suppose that must be a coincidence. Metaphase length seems to be a much poorer measure of DNA content than metaphase volume (Jones and Rees, 1968) and is obviously affected by the speed of division or type of cellular differentiation, as shown by the extreme contraction of chromosomes after prolonged colchicine treatment and the unusual lengths of chromosomes in embryonic divisions (cf. Navashin, 1934). It seems that several of the correlations cited in the introduction may be too vague to be useful for any general predictive purpose. Any insight into cell metabolism gained from such correlations is also suspect on account of the errors involved.

5. Summary

1. The relative DNA content of diploid nuclei has been estimated by feulgen densitometry for *Crepis capillaris*, *C. neglecta* and *C. fuliginosa*.

2. These relative measurements have been converted to absolute units by use of *Xenopus laevis* erythrocytes as a standard.

3. It is claimed that this technique is an improvement on previously unreliable estimates of the nuclear DNA content of Angiosperms.

6. References

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