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*A RELATIONSHIP BETWEEN DNA CONTENT, NUCLEAR VOLUME, AND MINIMUM MITOTIC CYCLE TIME**

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Mitosis is the culmination of many processes integrated in such a manner that two almost exact replicas of a nucleus or cell can be produced time and time again, each replica containing the necessary genetic information to repeat the process. The term mitotic cycle has been applied to those events which occur between the same stage in two successive divisions.[1] The biochemical changes that take place between two successive divisions have been the subject of recent reviews.[2-4] Although much information can be obtained by studying the processes in cells of a single species or a single cell type within an individual, equally useful information can be obtained from investigating similar cells from different species. The purpose of these studies was to determine whether or not a relationship existed between three cellular characteristics, namely, DNA content per cell, nuclear volume, and the minimum mitotic cycle time.

Material and Methods.—Minimum mitotic cycle time measurements: The full details of the technique used to measure minimum mitotic cycle time are published elsewhere.[5, 6] The technique involves the production of tetraploid cells by treating the meristems with colchicine for short periods of time which varied with different species. The number of tetraploid cells produced during the treatment depends upon (1) the number of cells that entered metaphase while colchicine was effective, and (2) the concentration of colchicine used. The affected cells undergo karyokinesis but no cytokinesis, and enter interphase as usual. The next time these affected cells divide they will be tetraploid and hence distinguishable from normal diploid cells. The period of time between the colchicine treatment and the initial appearance of tetraploid cells in division is the minimum mitotic cycle time. All cycle time measurements were performed at 23 ± 1.0°C.

Nuclear volume measurements: Collections for nuclear volume studies were made from growing plants before floral transition. Root meristems were killed, fixed in Craf III, dehydrated, and infiltrated with paraffin by the use of a tertiary butyl alcohol series. Sections were cut at 10 μ and stained with safranin-fast green. The diameters of interphase nuclei of meristematic cells just above the root cap were measured with a Zeiss ocular micrometer. Ten nuclei on each of two slides were measured for each species, and average nuclear volumes were calculated.

DNA measurements: The deoxyribonucleic acid (DNA) was extracted from root-tip material with sodium chloride, a modification of the Schmidt-Thannhauser procedure,[7] and the amount of DNA was estimated by the diphenylamine reaction.[8]

Cell counts: The number of cells per meristem was determined by excising the terminal 2 mm of 40 meristems and randomly selecting 10 of these excised segments. Cell separation was accomplished by hydrolyzing the segments with 1 *N* HCl until the tissue was soft. Following hydrolysis the segments were mascerated and suspended in 1 cc of 1 *N* HCl. Four 0.05 cc aliquots were removed from this suspension for cell counts with a hemocytometer.

Results.—Figures 1*A* and 1*B* show the time at which the tetraploid cells appeared

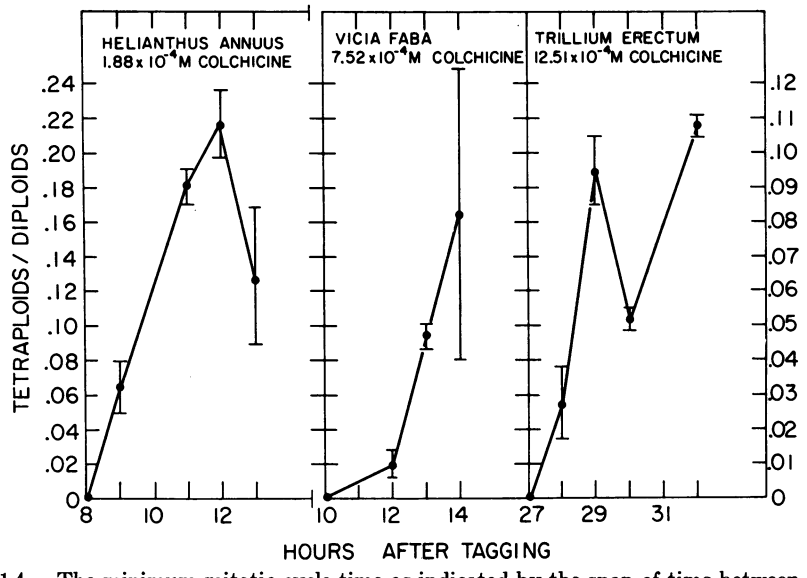

FIG. 1A.—The minimum mitotic cycle time as indicated by the span of time between the production of tetraploid cells with colchicine and their initial appearance in the subsequent division.

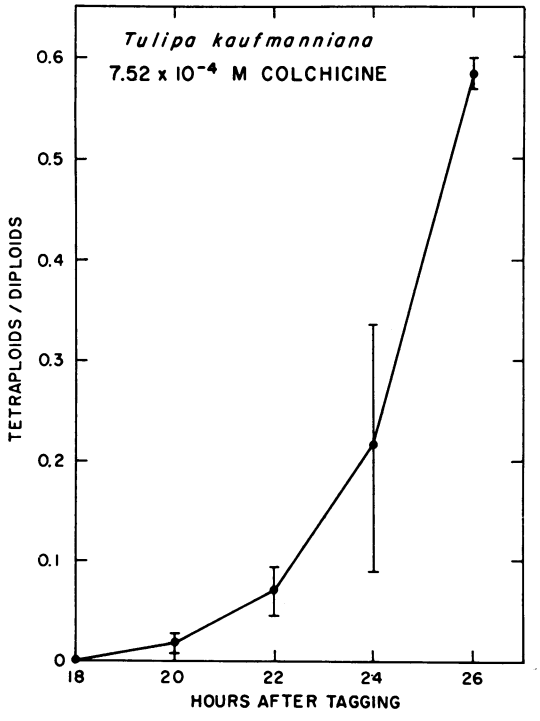

FIG. 1B.—The minimum mitotic cycle time as indicated by the span of time between the production of tetraploid cells with colchicine and their initial appearance in the subsequent division.

in mitosis after tagging or marking with colchicine. All colchicine treatments were of 1 hr duration except that of *Helianthus annuus* which was 30 min. The data are expressed as the ratio of tetraploid to diploid dividing cells (prophase through late anaphase) versus time for two reasons. First, this ratio is the most sensitive measurement for indicating the appearance of the tetraploid or tagged cells; and second, this ratio is independent of large fluctuations in the flow of cells through metaphase. Consequently, the influence of natural synchronization, if any, is negated.

The minimum cycle time was arbitrarily set to be the time at which the ratio of tetraploid to diploid dividing cells reached a value of approximately 0.1. The minimum cycle time for *Pisum* and *Tradescantia* was obtained from the literature.[5, 9] These and the values determined from Figures 1*A* and 1*B* are summarized in Table 1. These data indicate that the minimum cycle time of the cells from various species ranged from 9–29 hr and were different.

Interphase nuclear volumes varied from 1,175 μ^3 for *Trillium erectum* to 195 μ^3 for *Helianthus annuus* and were directly related to the minimum cycle time (Fig. 2).

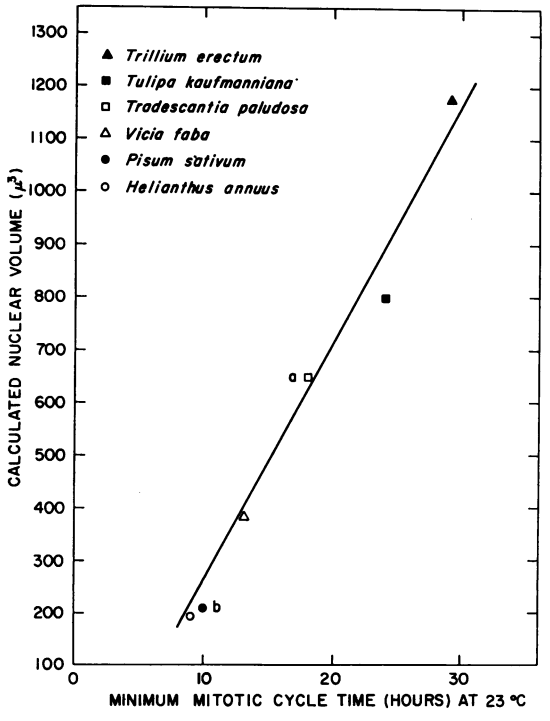

FIG. 2.—The relationship between interphase nuclear volume and the minimum mitotic cycle time of different plant species. The minimum cycle time for *a* from Wimber[9] and for *b* from Van't Hof *et al.*[5]

It has been shown recently that interphase nuclear volume and DNA content are directly related.[10] Therefore, a direct correlation between DNA content and minimum cycle time should be expected. Verification of this expectation is shown in Figure 3.

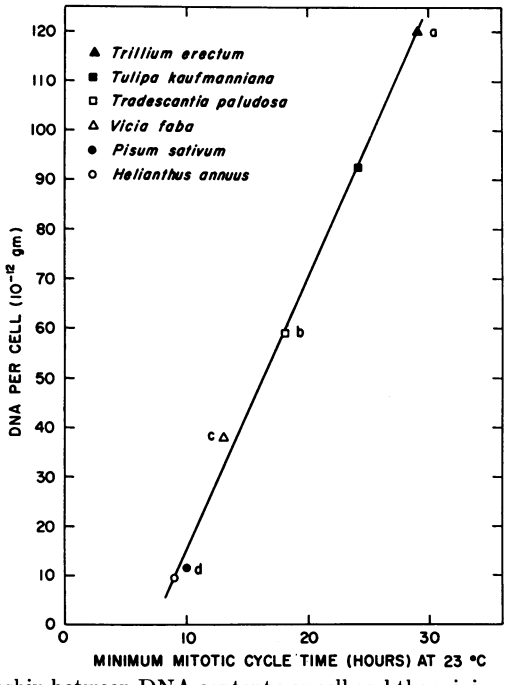

FIG. 3.—The relationship between DNA content per cell and the minimum mitotic cycle time of different plant species. The DNA content for *a* estimated graphically from Sparrow and Miksche,[10] *b* and *c*, from Sparrow and Miksche.[10] Minimum cycle time for *b* from Wimber,[9] and for *d*, from Van't Hof *et al.*[5]

TABLE 1

THE MINIMUM MITOTIC CYCLE TIME OF SEVERAL PLANT SPECIES

Plant species	Minimum mitotic cycle time (hr)	Reference
Trillium erectum	29	This paper
Tulipa kaufmanniana	23	This paper
Tradescantia paludosa	18	Wimber (1960)
Vicia faba	13	This paper
Pisum sativum	10	Van't Hof *et al.* (1960)
Helianthus annuus	9	This paper

The relationship between DNA content per cell, or interphase nuclear volume, and the minimum mitotic cycle time is generally not dependent on chromosome number or DNA per chromosome. This independence is shown in Table 2 which summarizes the experimental results.

Discussion.—The experimental results obtained from several species demonstrate that the greater the DNA content per cell the longer the mitotic cycle time characteristic of that particular species. They also show that cellular characteristics previously determined laboriously can now be estimated from a single known characteristic. However, these data are even more useful in providing some insight into the factors controlling cell proliferation. For instance, the fact that the curve in Figure 3 intercepts the abscissa at a minimum mitotic cycle time of about 6.5 hr indicates that the relationship between cycle time and DNA content is quite different for diploid cells having less than 8×10^{-12} gm. Therefore, the curve in

TABLE 2

SUMMARY OF NUCLEAR CHARACTERISTICS OF MERISTEMATIC CELLS FROM VARIOUS PLANT ROOTS

Plant species	Minimum cycle time (hr)	Calculated interphase nuclear volume (μ^3)	DNA per cell (10^{-12} gm)	Number of chromosomes	DNA per chromosome (10^{-12} gm)
Trillium erectum	29	1,175	120*	10	12
Tulipa kaufmanniana	23	800	93.7	24	3.91
Tradescantia paludosa	18 (ref. 8)	640	59.4 (ref. 10)	12	4.95
Vicia faba	13	377	38.4 (ref. 10)	12	3.2
Pisum sativum	10 (ref. 5)	200	11.67	14	0.83
Helianthus annuus	9	195	9.85	34	0.29

* Estimated graphically from Sparrow and Miksche.[10]

Figure 3 must bend and proceed through or near the origin, if the correlation applies to the lower forms of life such as bacteria, fungi, and viruses. It would be even more interesting if the curve would bend toward the origin simultaneously with a change in the genetic vehicle, i.e., the chromosomal type possessed by the higher forms of life to those still undetermined for the lower cellular forms.

The linear correlation between the DNA content of diploid dividing cells and cycle time requires still other hypotheses. First, the correlation may be coincidental, having no direct association but rather resulting from a third cell characteristic to which DNA content and cycle time are related. An alternative to this first possibility is that DNA content and cycle time are directly related in diploid plants. If the latter is true, then it follows that the limiting factor may be a reaction that proceeds at an equal rate in each species. Thus, a cell which has to replicate twice as much DNA as another cell will take twice as much time to complete the replication. Assuming that this is the case, the experimental directive derived from these data is to find an enzyme system having kinetic characteristics that are directly related to cell number only. In other words, the activity of the enzyme(s) would be the same on a per cell basis for the plant species studied. An obvious place to start investigating would be the systems associated with DNA synthesis, such as thymidine phosphorylase or DNA polymerase.

Another variable that should be considered is the length of the interphase S period. (The S period is that portion of interphase in which DNA is synthesized.) For *Tradescantia*, the S period is about 10.8 hr,[9] while in *Pisum* it is approximately 4.5 hr.[11] If the total mitotic cycle time were related to the length of the S period, the cycle time of *Tradescantia* would be expected to be at least twice that of *Pisum*. The data presented in this paper suggest that this relationship may exist, for *Tradescantia* and *Pisum* have minimum cycle times of approximately 10 and 18 hr, respectively. Assuming that the length of the S period were the contributing factor to total cycle duration, the question remains as to why the S period should vary from species to species. The answer to this question would very likely involve biochemical and biophysical phenomena associated with DNA synthesis. One such response would be thermal activation. In these experiments minimum cycle time determinations were performed at about 23°C, but it is possible that the activation energy of the processes involved in cell proliferation may differ from species to species. Thus, the optimum temperature for *Helianthus* may be different

from that of *Trillium*. The influence of temperature is a very real possibility and is presently under investigation.

In conclusion it may be said that for diploid plants a relationship does exist between the minimum mitotic cycle time, the interphase nuclear volume, and the DNA content per cell. Moreover, the relationship is such that if any one of the three cell variables is known, an estimate can be made of the remaining two.

Summary.—Experiments were performed to determine the relationship between interphase nuclear volume and DNA content per cell and the minimum mitotic cycle time in several diploid plant species. All measurements were made on meristem cells contained in the terminal 2 mm of the root. The results indicated that linear relationships exist between the interphase nuclear volume and the minimum mitotic cycle time, and between the DNA content per cell and the minimum cycle time. Linearity, however, does not exist if extrapolation is carried out to include the lower forms of life, such as bacteria and viruses. The relationships are to some extent independent of chromosome number and the amount of DNA per chromosome. The data presented enable the estimation of any two of the above three variables, if the third variable is known.

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*BAND-CENTRIFUGATION OF MACROMOLECULES AND VIRUSES IN SELF-GENERATING DENSITY GRADIENTS**

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This communication presents a new method of carrying out sedimentation velocity experiments. A thin lamella of a solution of macromolecules is layered onto a denser miscible liquid in a rotating ultracentrifuge cell. The macromolecules then sediment through the liquid in a narrow concentration distribution, or band, which is observed photographically as a function of time. The density gradients neces-