

## Correlations Between Nuclear Volume, Cell Volume and DNA Content in Meristematic Cells of Herbaceous Angiosperms

Many investigations over the past 80 years suggest that a general relationship may exist between nuclear volume and cell volume and more recently between nuclear volume and the amount of DNA per cell. As early as 1893, STRASBURGER<sup>1</sup> reported evidence for a constant ratio between the average diameter of a plant meristematic cell and that of its nucleus. Later observations on diploid plant cells from species characterized by differences in chromosome size suggested that total chromosome mass may be correlated with nuclear and cell size<sup>2,3</sup>. This idea was further supported by the many reports of larger nuclei and cells in polyploid than in closely related diploid plants<sup>4-13</sup>, and by the increase in nuclear and cell volume resulting from the presence of B-chromosomes<sup>14,15</sup>. From a study of 13 angiosperm species, MARTIN<sup>16</sup> reported that the fresh weight of meristematic cells is correlated with DNA content. A nearly direct relation exists between DNA content and cell size among unicellular algal species<sup>17</sup> and in a polyploid series, 1  $\mu$  to 8  $\mu$ , of yeast<sup>18</sup>.

The herbaceous angiosperms have the widest range of DNA content of any vascular plant group<sup>19</sup>. BAETCKE et al.<sup>20</sup> in this laboratory made parallel quantitative measurements of DNA content and nuclear volume from root meristematic cells of 30 angiosperm species representing a 36-fold range in DNA content per cell. Their results indicated that the average nuclear volumes of both root and shoot meristem cells were directly proportional to DNA content, and the data plotted on a double logarithmic scale fitted a regression line of slope = +1.

**Materials and methods.** To test the hypothesis that a quantitative relationship exists between meristematic nuclear volume, cell volume and DNA content, we have determined nuclear and cell volumes in 14 species of herbaceous angiosperms (Table) representing an evolutionarily diverse array of families. Nuclear volumes of the cells in the outer cell layer of actively growing shoot meristems were determined from longitudinal sections using previously described techniques<sup>21,27</sup>. For each nucleus measured the corresponding cell was measured in

2 dimensions and the volume calculated using the formula, volume = length  $\times$  (width)<sup>2</sup>, assuming the depth to be equal to the width. Measurements of depth of cells in transverse sections of 5 of these species showed that variation between depth and width did not exceed 10%. At least 10 sets of nuclear volume and cell volume measurements were obtained for each species.

**Results and discussion.** The average nuclear volume, cell volume, and nuclear volume as percent of cell volume (NV/CV) for each species studied are given in the Table. When values in column 5 are averaged, the nucleus is seen to occupy 18% of the total cell volume, a cyto-nuclear ratio of 5.6:1. However, an average of all individual NV/CV values (170 cells) shows a ratio of 5.3:1 ( $19 \pm 1\%$ ).

- <sup>1</sup> E. STRASBURGER, *Histol. Beiträge* 5, 97 (1893).
- <sup>2</sup> R. P. GREGORY, *Proc. phil. Soc. Cambridge* 15, 239 (1910).
- <sup>3</sup> F. KEEBLE, *J. Genet.* 2, 163 (1912).
- <sup>4</sup> M. NAVASHIN, *Univ. Calif. Publ. agric. Sci.* 6, 207 (1931).
- <sup>5</sup> R. R. GATES, *Arch. Zellforsch.* 3, 525 (1909).
- <sup>6</sup> S. ICHIKAWA, A. H. SPARROW, C. FRANKTON, A. F. NAUMAN, E. B. SMITH and V. POND, *Can. J. Genet. Cytol.* 13, 842 (1971).
- <sup>7</sup> E. W. LINDSTROM and L. M. HUMPHREY, *Genetics* 18, 193 (1933).
- <sup>8</sup> L. F. RANDOLPH, E. C. ABBE and J. EINSET, *J. agric. Res.* 69, 47 (1944).
- <sup>9</sup> E. W. SINNOTT, H. HOUGHTALING and A. F. BLAKESLEE, *Carnegie Inst. Wash. Publ. No. 451* (1934).
- <sup>10</sup> H. H. SMITH, *Am. J. Bot.* 30, 121 (1943).
- <sup>11</sup> W. W. TUPPER and H. H. BARTLETT, *Genetics* 7, 177 (1916).
- <sup>12</sup> W. W. TUPPER and H. H. BARTLETT, *Genetics* 3, 93 (1918).
- <sup>13</sup> H. WINKLER, *Z. Bot.* 8, 417 (1916).
- <sup>14</sup> A. MÜNTZING and S. AKDIK, *Hereditas* 34, 248 (1948).
- <sup>15</sup> L. F. RANDOLPH, *Genetics* 26, 608 (1941).
- <sup>16</sup> P. G. MARTIN, *Expl. Cell Res.* 44, 84 (1966).
- <sup>17</sup> O. HOLM-HANSEN, *Science* 163, 87 (1969).
- <sup>18</sup> N. GUNGE and Y. NAKATOMI, *Genetics* 70, 41 (1972).
- <sup>19</sup> A. H. SPARROW, H. J. PRICE and A. G. UNDERBRINK, *Brookhaven Symp. Biol.* 23, 451 (1972).
- <sup>20</sup> K. P. BAETCKE, A. H. SPARROW, C. H. NAUMAN and S. S. SCHWEMMER, *Proc. natn. Acad. Sci. USA* 58, 533 (1967).
- <sup>21</sup> A. H. SPARROW, R. C. SPARROW, K. H. THOMPSON and L. A. SCHAIRER, *Radiation Bot.* 5, Suppl. 101 (1965).

Nuclear volume and cell volume in shoot meristems of 14 species of herbaceous angiosperms, and average nuclear volume/cell volume for each species

| Species                                    | Family         | Nuclear volume<br>( $\mu\text{m}^3 \pm \text{S.E.}$ ) | Cell volume<br>( $\mu\text{m}^3 \pm \text{S.E.}$ ) | Nuclear vol./Cell vol.*<br>(% $\pm \text{S.E.}$ ) |
|--|----------------|---|--|---|
| 1 <i>Arabidopsis thaliana</i>              | Cruciferae     | 32 $\pm$ 3  | 202 $\pm$ 11                                       | 16 $\pm$ 2  |
| 2 <i>Lobularia maritima</i>                | Cruciferae     | 49 $\pm$ 5  | 321 $\pm$ 16                                       | 16 $\pm$ 2  |
| 3 <i>Hypericum virginicum</i>              | Guttiferae     | 83 $\pm$ 8  | 448 $\pm$ 45                                       | 19 $\pm$ 2  |
| 4 <i>Cicer arietinum</i>                   | Leguminosae    | 96 $\pm$ 5  | 394 $\pm$ 16                                       | 25 $\pm$ 2  |
| 5 <i>Nelumbo lutea</i>                     | Nymphaeaceae   | 139 $\pm$ 7   | 1089 $\pm$ 129                                     | 15 $\pm$ 2  |
| 6 <i>Spinacia oleracea</i>                 | Chenopodiaceae | 156 $\pm$ 7   | 711 $\pm$ 29                                       | 22 $\pm$ 1  |
| 7 <i>Cyanotis pilosa</i>                   | Commelinaceae  | 173 $\pm$ 9   | 1188 $\pm$ 124                                     | 16 $\pm$ 1  |
| 8 <i>Anemone pulsatilla</i>                | Ranunculaceae  | 435 $\pm$ 27  | 2505 $\pm$ 153                                     | 17 $\pm$ 1  |
| 9 <i>Tradescantia navicularis</i>          | Commelinaceae  | 552 $\pm$ 27  | 3558 $\pm$ 323                                     | 17 $\pm$ 1  |
| 10 <i>Convallaria majalis</i>              | Liliaceae      | 710 $\pm$ 17  | 5260 $\pm$ 508                                     | 15 $\pm$ 2  |
| 11 <i>Fritillaria lanceolata</i>           | Liliaceae      | 1466 $\pm$ 115  | 9329 $\pm$ 878                                     | 16 $\pm$ 1  |
| 12 <i>Fritillaria camtschatskensis</i>     | Liliaceae      | 1824 $\pm$ 103  | 10991 $\pm$ 1327                                   | 18 $\pm$ 2  |
| 13 <i>Lilium longiflorum</i> (4 $\times$ ) | Liliaceae      | 3273 $\pm$ 167  | 20799 $\pm$ 1568                                   | 16 $\pm$ 1  |
| 14 <i>Sprekelia formosissima</i>           | Amaryllidaceae | 4638 $\pm$ 262  | 19258 $\pm$ 1400                                   | 26 $\pm$ 1  |

\* Average of individual NV/CV values for each species.

Regression analysis indicates that log cell volume is linearly related to log nuclear volume and the slope of the line ( $+0.990$ ) is not significantly different from  $+1.0$  ( $p = 0.78$ ) (Figure). The data of BAETCKE et al.<sup>20</sup> relating nuclear volume to DNA content fit a parallel regression line (Figure).

Nuclear and cell volumes were determined for 4 additional species, a gymnosperm, a subshrubby angiosperm and two pteridophytes. The same NV/CV correlation was observed, extending the data into a fourth order of magnitude for both parameters. This would suggest that the observed relationship probably holds for plant groups other than the herbaceous angiosperms.

Factors other than DNA content such as age of the meristem, location of the meristem on the plant, degree of hydration, time of year, physiological activity of the tissue, and nutritional state of the plant are known to influence chromosome size and nuclear volume<sup>20, 22-27</sup>. The cytonuclear ratio varies among tissues of the same plant<sup>28-30</sup>, may change during cell elongation and differ-

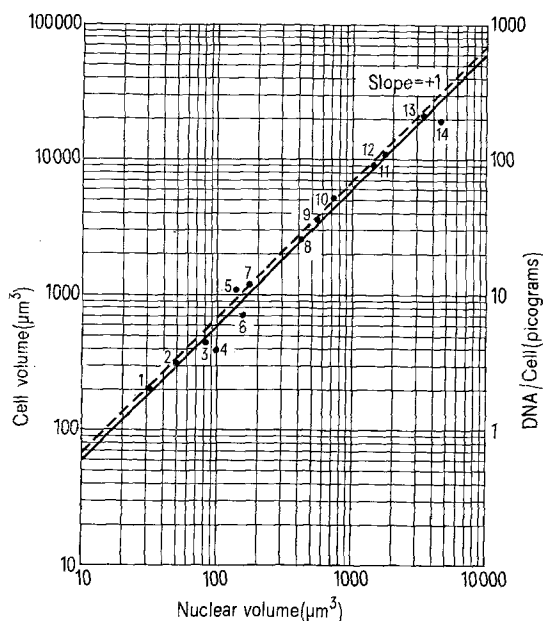
entiation<sup>28-30</sup>, and is influenced by physiological, genetic and environmental factors<sup>31</sup>. Hence the simple relationship shown above for the two size parameters and DNA content may not always hold for differentiated cells.

A linear relationship also exists in diploid plants between nuclear volume and mitotic cycle time<sup>32</sup>, between DNA content per cell and mitotic cycle time<sup>32-34</sup> and between DNA content and the duration of meiosis<sup>35</sup>. BENNET<sup>36</sup> reported a relationship between DNA content and minimum generation time in herbaceous angiosperms. Studies of nuclear parameters and cell size in relation to growth form and ecological adaptations appear to be a promising approach to a better understanding of the evolutionary significance of the wide variation in DNA content per cell among higher plants.

*Résumé.* Les volumes cellulaires et nucléaires de 14 espèces herbacées d'angiosperme ont été mesurés dans les méristèmes apicaux. Pour un accroissement de 100 fois, le volume cellulaire est directement proportionnel au volume nucléaire et, sur une échelle doublement logarithmique, donne une régression linéaire de pente équivalente à  $+1$ .

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Relationship between nuclear volume and cell volume in apical meristems of 14 herbaceous angiosperms. Numbered points correspond to data in the Table. Dashed line represents correlation between nuclear volume and DNA content per cell as reported by BAETCKE et al.<sup>20</sup>.

- <sup>22</sup> M. D. BENNETT, *Chromosoma* 29, 317 (1970).
- <sup>23</sup> M. D. BENNETT and H. REES, *Chromosoma* 27, 226 (1969).
- <sup>24</sup> R. BIEBL and K. HOFER, *Radiation Bot.* 6, 225 (1966).
- <sup>25</sup> H. H. SMITH, *Adv. exp. Med. Biol.* 18, 271 (1972).
- <sup>26</sup> A. H. SPARROW, in *Cellular Radiation Biology* (Williams and Wilkins, Baltimore 1965), p. 199.
- <sup>27</sup> A. H. SPARROW, A. F. ROGERS and S. S. SCHWEMMER, *Radiation Bot.* 8, 149 (1968).
- <sup>28</sup> E. W. SINNOTT and V. V. TROMBETTA, *Am. J. Bot.* 23, 602 (1936).
- <sup>29</sup> V. V. TROMBETTA, *Am. J. Bot.* 26, 519 (1939).
- <sup>30</sup> V. V. TROMBETTA, *Bot. Rev.* 8, 317 (1942).
- <sup>31</sup> E. W. SINNOTT, *Plant Morphogenesis* (McGraw-Hill, New York 1960).
- <sup>32</sup> J. VAN'T HOF and A. H. SPARROW, *Proc. natn. Acad. Sci. USA* 49, 897 (1963).
- <sup>33</sup> G. M. EVANS and H. REES, *Nature, Lond.* 233, 350 (1971).
- <sup>34</sup> J. VAN'T HOF, *Expl. Cell Res.* 39, 48 (1965).
- <sup>35</sup> M. D. BENNETT, *Proc. R. Soc. Lond. B.* 178, 277 (1971).
- <sup>36</sup> M. D. BENNETT, *Proc. R. Soc. Lond. B.* 181, 109 (1972).
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## Enhancement of Ethylemethane Sulfonate-Induced Mutation Frequency in *Drosophila* by Dimethyl Sulphoxide

Dimethyl sulphoxide (DMSO) is a dipolar, aprotic substance<sup>1</sup>, possessing a high dielectric constant<sup>2</sup> (3.9), which makes this reagent an excellent solvent for most of the inorganic and organic compounds<sup>3</sup>. JACOB et al.<sup>4, 5</sup> reported a high penetrability of DMSO through biological membranes. Other investigators also demonstrated that in biological systems DMSO is a very good carrier for drugs<sup>6-8</sup>, electrolytes as well as non-electrolytes<sup>9</sup>, polar and polarizable molecules<sup>10</sup>. Its role has been suggested to be to enhance the penetrability for dyes<sup>11</sup>, bacteriocides<sup>12</sup>, nutrients<sup>13</sup> and also to transform activity of polyoma vi-

rus<sup>14</sup>. It has been shown to be a useful carrier for chemical mutagens<sup>15, 16</sup> and colchicine<sup>17</sup> in plants. ALEXANDER<sup>18</sup> did not observe any induction of mutations in *Drosophila* by DMSO alone.

The present investigations were undertaken to study the combined treatment of DMSO and ethylemethane sulphonate (EMS) on mutation rate in *Drosophila melanogaster*. Male flies of Oregon K strains were given intra-abdominal injection of 1% DMSO, 0.15% EMS in 0.7N saline solution and 0.15% EMS solution made in 1% solution of DMSO. Flies injected with 0.7N saline solution