THE LIMITING MEMBRANE OF THE CHROMOSOME

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THE accidental discovery that the chromonemata could be disorganized leaving the pellicles of the meta- and ana-phase chromosomes intact led to the suggestion that it is such a limiting membrane that gives the chromosome its morphological integrity.[1] This was a revival of the classical view of chromosome structure.[2-4] While scanning such preparations the impression obtained was that the limiting membranes of the chromosomes play a role in the formation of the nuclear membrane at telophase (*see* Photos 7–7 B, Subramaniam and Royan[1]). Mazia's[5] illustration of anaphase chromosomes having a membrane comparable in structure to the nuclear membrane (his Fig. 75) confirmed such a possibility.[6] Levan[7] records that when the two metaphase chromatids in the c-pairs of mouse ascites tumour cells were surrounded by a common pellicle, he obtained the impression that such a pellicle may get transformed directly into the nuclear membrane.

A perusal of the earlier literature reveals a lack of distinction between the pellicle and the matrix. The "sheath" described by Metz[8] presumably includes both these regions in meta- and ana-phase chromosomes. This confusion in the connotation and usage of the terms has already been discussed by others.[2,3,9] In most cases the presence of a chromosomal membrane has only been inferred[2] or deduced.[9] Actual demonstrations of such membranes are rather few.[1,4,6,10-13]

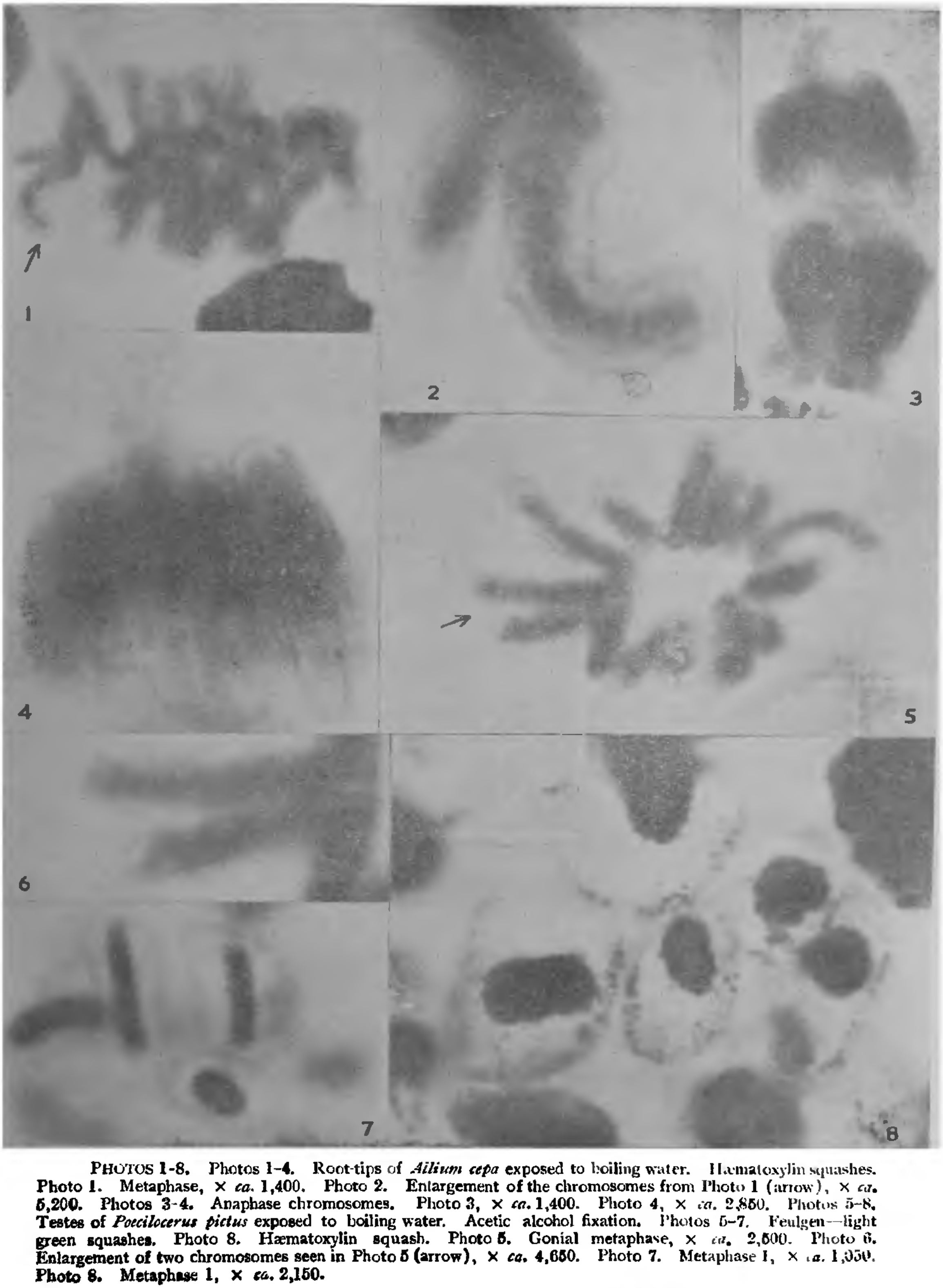
The disagreements on the question of the presence of a matrix and a pellicle stem form the differences of opinion as to the stage of division at which they could be located and studied. Theoretically, a chromosome may be expected to have a limiting membrane only after the disappearance of the nuclear membrane. In *Podisma* it is the I meta- and ana- phase chromosomes which were reported to have matrices and limiting membranes.[10] The denial of their presence[14] based on observations on meiotic prophase chromosomes does not appear, therefore, to be convincing.

Using a micro-manipulator D'Angelo[15] demonstrated the presence of a limiting membrane for the living salivary chromosomes. Corroboration of its presence is available from fluorescence microscopy.[16] These records have to be viewed as a class apart in the context of the absence of any such membrane in lampbrush chromosomes.[17] As indicated by Metz[8] the role of the chromosomal "sheath" is to insulate it from the surrounding cytoplasm during the mitotic movements.

At a time when the chromosomal matrix was suspected to be Feulgen-positive,[3,9] McClintock[18] correlated the formation of a matrix with the dissolution of the nucleoli and suggested that a part of the nucleolar matter may take part in its formation. The above possibility finds support in the recent suggestion[19] that the metaphase chromosome may act as a vehicle for the transport of nucleolar material. Apart from the DNA, the chromosomes are known to contain RNA, phospholipids,[20] histone and non-histone proteins capable of selective removal.[21] In the above context the presence of a matrix reported recently from electron micrographs appears rather interesting.[22]

The observations presented below are from the meristematic cells of *Allium cepa* and the testes of *Pœcilocerus pictus*. The methods of handling were only slightly different. While the root-tips of the germinated bulbs of *A. cepa* were dipped in a beaker of boiling water, the testes were transferred to a tube of water kept in a beaker of boiling water. The duration of exposure was one minute for the root-tips and 5 minutes for the testes. The root-tips were processed as hæmatoxylin squashes without any fixation or hydrolysis. The testes, on the other hand, were stored in acetic alcohol (1 : 3) overnight to remove the fat present as well as harden the tissue and then processed after hydrolysis in N HCl at 60° C. for 6 minutes, either as hæmatoxylin or Feulgen squashes.

Photo 1 is of a metaphase from *A. cepa*. Each chromosome is seen to have a distinct limiting membrane. Attention is invited to the fact that the pair of chromatids are enclosed within a single pellicle (Photo 2). An anaphase is presented as Photo 3. The enlargement



PHOTOS 1-8. Photos 1–4. Root-tips of *Allium cepa* exposed to boiling water. Hæmatoxylin squashes. **Photo 1.** Metaphase, × *ca.* 1,400. **Photo 2.** Enlargement of the chromosomes from Photo 1 (arrow), × *ca.* 5,200. **Photos 3–4.** Anaphase chromosomes. **Photo 3,** × *ca.* 1,400. **Photo 4,** × *ca.* 2,850. **Photos 5–8.** Testes of *Poecilocerus pictus* exposed to boiling water. Acetic alcohol fixation. **Photos 5–7.** Feulgen—light green squashes. **Photo 8.** Hæmatoxylin squash. **Photo 5.** Gonial metaphase, × *ca.* 2,500. **Photo 6.** Enlargement of two chromosomes seen in Photo 5 (arrow), × *ca.* 4,650. **Photo 7.** Metaphase I, × *ca.* 1,350. **Photo 8.** Metaphase 1, × *ca.* 2,150.

(Photo 4) of the upper cluster in the above micrograph shows each anaphase chromosome having a well-defined limiting membrane.

The gonial chromosomes of *P. pictus* also have distinct chromosomal pellicles (Photos 5 and 6). These appeared faintly positive in Feulgen preparations (Photo 7). A possible explanation for such a reaction is that the DNA released by the denaturation of nucleoproteins may lie apposed to the pellicle. Evidence has already been presented that the granules resulting from the disorganization of the chromonemata may arrange themselves on the inner surface of the pellicle (Photos 2–2B, Subramaniam and Royan[1]). When the testes exposed to hot water and fixed in acetic alcohol were stored in 70% alcohol for 10 days and then processed as hæmatoxylin squashes, the limiting membranes of the bivalents at metaphase I enclose a matrix which is granular at its periphery (Photo 8). In the absence of such storage the matrix appears non-granular (Photo 7).

The chromonemata could be revealed by a variety of techniques.[1-4,23] The unstained spaces within the coils of the chromonemata represent the matrix.[2] Such a positional relationship is also emphasized in the recent report based on electron micrographs.[22] One of the methods used to reveal the chromonemata is exposure of cells to hot water.[24–26] This procedure produces a reorientation of the chromonemata and matrix within the pellicle.[4] In fact, such a reorientation appears necessary for a demonstration of the pellicle. The micrographs presented would emphasize that plant and animal chromosomes have distinct limiting membranes during mitotic and meiotic meta- and ana-phases.

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