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# Observational and Experimental Studies of Meiosis with Special Reference to the Bouquet Stage
## VII. Viscosity change of cytoplasm in spore mother cells undergoing meiosis

By

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The viscosity of cytoplasm was studied in intact spore mother cells of some ferns by an observation of Brownian movement exhibited by minute fat granules in the cytoplasm. The main purpose of the study was to get some knowledge on the following two points; 1) a periodical change in magnitude of cytoplasmic viscosity during meiotic prophase, and 2) a local difference in magnitude of cytoplasmic viscosity in the spore mother cells in the bouquet stage. The results obtained will be reported below.

## Material and Method

Intact spore mother cells of *Cyrtomium Fortunei*, *Polystichum polyblepharum* and *Rumohra dimorphophylla*, lying *in situ* in the sporangia, were used as the material (cf. Hiraoka, 1952).* They contain minute fat granules of different sizes. Among these granules, the granules of a definite size, which did not lie very near the cell structures (nucleus, plastids, cell membrane and other neighbouring fat granules etc.), were favourable for an observation of Brownian movement. They may be called the "indicator granules". The image of an indicator granule was plotted on a section paper every 5 seconds for 30 seconds with the aid of a Zeiss' camera lucida, and half the maximum distance between any two plotted dots out of 7 was assumed as an arbitrary measure showing the amplitude of the Brownian movement exhibited by the granule (Weber, 1924). The plotting was made on several indicator granules found in various regions of the cytoplasm in the spore mother cells undergoing meiosis. The magnitude of Brownian movement was graded into 5

* Liquid paraffin of E. Merck, washed several times with di-distilled water, was used as the mounting medium. In the preparations mounted with liquid paraffin, the spore mother cells are kept intact and they undergo the process of nuclear division normally at least for about 24 hours.

classes, "very slight", "slight", "medium", "vigorous" and "very vigorous", which have relative amplitudes of 1.0, 1.3, 2.0, 2.7 and 3.3 respectively. On the ground that the amplitude of Brownian movement is inversely proportional to cytoplasmic viscosity (Weber, 1924, cf. Svedberg, 1928), the relative viscosity values of cytoplasm shown by the 5 classes in magnitude of Brownian movement are 1.00, 0.77, 0.50, 0.37 and 0.30 respectively.*

The observations of Brownian movement were made at a room temperature of 20.0–22.3°C, and the temperature was practically constant during each observation.

## Observations

Changes in cytoplasmic viscosity in the spore mother cells undergoing meiosis are essentially the same in all three species studied, and only the results obtained in the spore mother cells of *Cyrtomium* are described unless otherwise stated.

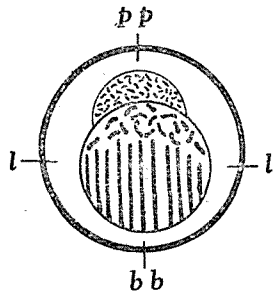
Stages from interphase preceding meiosis to leptotene: In these stages, the nucleus takes the central position in the cell (Figs. 3–5 in the previous paper, Hiraoka, 1952). The indicator granules, which are found here and there in the cytoplasm, are motionless showing a high cytoplasmic viscosity in these stages.** At the end of the leptotene stage, the indicator granules come to show a "very slight" Brownian movement or a vibratory one.

The bouquet stage: In this stage, the nucleus is found displaced from the central position to an eccentric one in the cell. Faintly green colored plastids are localized to form a group in the broader region of the cytoplasm produced by the nuclear displacement, and indicate the region of the "plastid pole". The chromosome threads show a regular bouquet arrangement in the nucleus. The base of the bouquet and the "plastid pole" take diametrically opposite positions across the nucleus in the cell (Figs. 6 and 7 in the previous paper, Hiraoka, 1952). The indicator granules are abundantly found in the region of the cytoplasm confronting the lateral sides of the bouquet figure (Text-fig. 1, "l") and in the region of the cytoplasm near the plastid group at the "plastid pole" (Text-fig. 1, "pp"), but it is rather difficult to find the indicator granules in the region of the cytoplasm confronting the bouquet base (Text-fig. 1, "bb").

In the leptotene bouquet stage, the majority of the indicator granules

* Owing to a small amplitude of Brownian movement exhibited by indicator granules, Pekarek's method of absolute viscosity determination (Pekarek, 1930) was not used in the present investigation.

** According to Seifriz (1920), the Brownian movement of carmine particles is exhibited only when a viscosity of the dispersion medium is lower than that of glycerin.




Text-figure 1. Schematic figure showing a *Cyrtomium* spore mother cell in the bouquet stage. Compare the figure with Figs. 6 and 7 in the previous paper (Hiraoka, 1952). Further explanation in text.

found in the "1" region of the cytoplasm exhibits a "vigorous" Brownian movement indicating a low viscosity of the cytoplasm in this region, though some granules showing a "slight" Brownian movement are also met with. The indicator granules in the "bb" region of the cytoplasm play a "slight" Brownian movement, while those in the "pp" region of the cytoplasm are motionless in the majority of cases observed and play a "very slight" Brownian movement in rather rare cases. In the zygotene bouquet and the pachytene bouquet stage, the indicator granules in the "1" region of the cytoplasm exhibit Brownian movement more actively than in the leptotene bouquet stage. Some of them exhibit a "very vigorous" and others a "vigorous" Brownian movement. The indicator granules in the "bb" region of the cytoplasm show a "slight" Brownian movement, while those in the "pp" region of the cytoplasm are motionless.

In *Rumohra* spore mother cells, the indicator granules in the "1" region of the cytoplasm show a "vigorous" or a "very vigorous" Brownian movement in the leptotene bouquet stage and a "very vigorous" movement in the zygotene bouquet and the pachytene bouquet stage; those in the "pp" region of the cytoplasm show a "slight" Brownian movement in the leptotene bouquet, the zygotene bouquet and the pachytene bouquet stage; and those in the "bb" region of the cytoplasm exhibit a "medium" movement in the zygotene bouquet and the pachytene bouquet stage. In *Polystichum* spore mother cells, the indicator granules in the "1" region of the cytoplasm play a "medium" Brownian movement in the leptotene bouquet stage, a "vigorous" movement in the zygotene bouquet stage and a "medium" movement in the pachytene bouquet stage. The indicator granules in the "bb" region of the cytoplasm play a "slight" Brownian movement in the leptotene bouquet and zygotene bouquet stages and a "very slight" movement in the pachytene bouquet stage, and those in the "pp" region of the cytoplasm show no movement throughout the leptotene bouquet, zygotene bouquet and pachytene bouquet stages. A comparative study of Brownian movement of the indicator granules in the spore mother cells of *Cyrtomium*, *Rumohra* and *Polystichum* in the bouquet stages shows that the magnitude of Brownian movement

exhibited by the granules differs in different species, and that a remarkable local difference in magnitude of cytoplasmic viscosity exists in the cells.

Stages from pachytene to diakinesis: In these stages, the nucleus takes the central position in the cell and the plastids are distributed evenly in the cytoplasm (Figs. 8–10 in the previous paper, Hiraoka, 1952). The indicator granules in the region of the cytoplasm remote from the nucleus exhibit a "slight" Brownian movement and those in the region not so remote from the nucleus are motionless (Kato, 1933).

The first metaphase: In the first metaphase, the indicator granules are found in the cytoplasm around the spindle (Figs. 11 and 12 in the previous paper, Hiraoka, 1952). The majority of them exhibits a "vigorous" Brownian movement showing a low viscosity of the cytoplasm in this region, though some of them play a "very slight" movement. The area of the spindle (atractosome) appears homogeneous and shows no visible structure at all. In rare cases, one or two granules, which are similar in size to the indicator granules, are found in the atractosome, and they are motionless during the observation. In *Polystichum* spore mother cells, the granules in the atractosome are more frequently observed than in *Cyrtomium*, and they exhibit a "slight" Brownian movement in any directions irrespective of the orientation of the spindle axis (cf. Bělař, 1930).

The first anaphase: In the first anaphase, the indicator granules in the cytoplasm of the equatorial region of the cell exhibit a "medium" Brownian movement and those in the cytoplasm of the polar region of the cell a "slight" movement (Yamaha and Yunoki, 1936).

The first telophase: In the first telophase, the indicator granules in the cytoplasm of both the equatorial and the polar region of the cell show a "slight" Brownian movement. The plastids come to form a layer in the equatorial region of the cell.

Interkinesis: In this stage, the indicator granules in the cytoplasm of the equatorial region of the cell play a "slight" Brownian movement, and those in the cytoplasm of the polar region a "very slight" movement.

The second metaphase: In the second metaphase, the plastid layer in the equatorial region of the cell remains intact. Two spindles are formed in the positions separated from each other by the plastid layer (Fig. 13 in the previous paper, Hiraoka, 1952). The indicator granules in the cytoplasm of the equatorial region of the cell exhibit a "vigorous" or a "medium" Brownian movement. Most of the indicator granules in the cytoplasm of the polar region of the cell with respect to the spindle in the first division show a "slight" Brownian movement, while some of them a "very slight" movement.

Tetrads: In tetrad cells, the indicator granules, which are found in random positions in the cytoplasm do not show any movement at all.

Plastids: The plastids show their characteristic localizations in the spore mother cells from stage to stage as described above. They remain motionless during the period of each observation in all the stages of meiosis.

## Conclusion

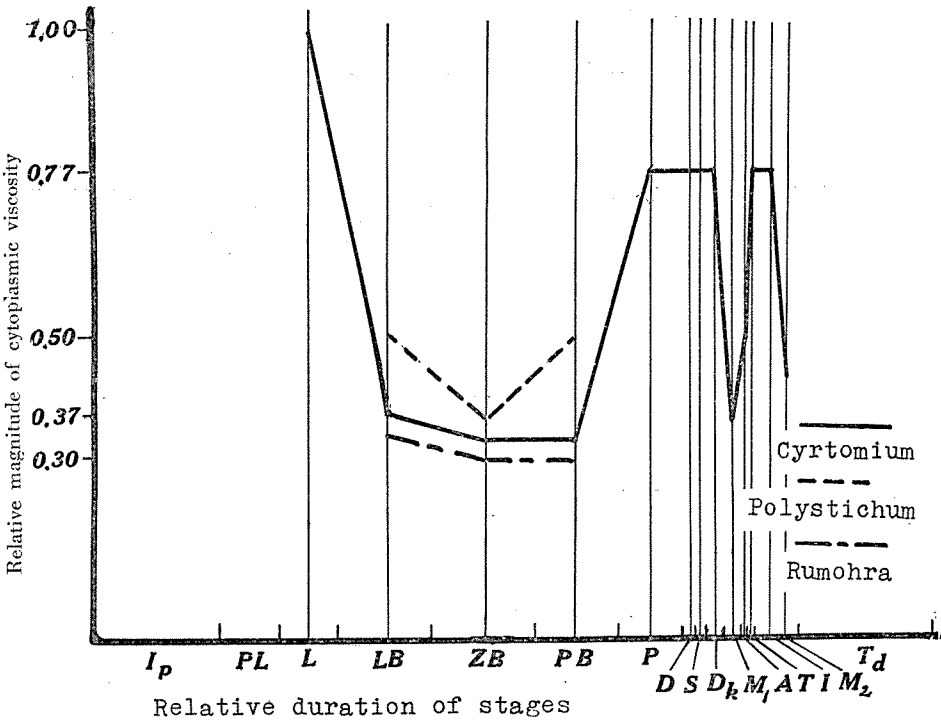
Kostoff (1930) has studied the cytoplasmic viscosity change in pollen mother cells of *Nicotiana*, and Yamaha and Yunoki (1936) in pollen mother cells of *Lilium* by means of centrifuge method,* while Kato (1933) in pollen mother cells of *Lilium* by means of Brownian movement method.** Heilbrunn (1921), and later, Fry and Parks (1934) have made centrifugation studies in *Nereis* eggs, in which the viscosity change of cytoplasm associated with the process of meiosis is partially overlapped by that associated with the process of fertilization. Unfortunately, the results obtained by these authors teach us next to nothing about the knowledge on the viscosity change of cytoplasm occurring in meiotic prophase stages, especially in the bouquet stage. The results obtained in the present investigation show that the periodical change in magnitude of cytoplasmic viscosity from stage to stage is difficult to be determined owing to the existence of local differences in magnitude of cytoplasmic viscosity in the cell, but we may say that the cytoplasmic viscosity is high in the leptotene, low in the bouquet stage and high again in the pachytene and later prophase stages (Text-fig. 2), provided that the lowest value of cytoplasmic viscosity observed in a given cell is taken as a viscosity value of the cytoplasm of the cell as a whole. The decrease in cytoplasmic viscosity in the bouquet stage seems significant when we consider the facts that in spore or pollen mother cells of 41 plant species, which are treated with a silver nitrate solution, a reduction of silver nitrate intensitively occurs in this stage (Hiraoka, 1951) and that in the spore or pollen mother cells of *Salvinia* and *Acacia*, an active streaming of karyoplasm takes place in the nucleus in this stage*** (Hiraoka, 1949).

In the bouquet stage, the magnitude of cytoplasmic viscosity differs in different regions in the cell. The cytoplasmic viscosity is low in the region of the cytoplasm confronting the lateral sides of the bouquet figure, medium in the region confronting the bouquet base and high in the vicinity of the

* In the case where paraffin section preparations are used to examine the response of cells to centrifugation, we must take into consideration that chromosomes and cytoplasmic granules may recover from the displacement due to the centrifugation before the complete fixation of the cells (cf. Yamaha and Yunoki, 1936).

** In Brownian movement method, the cells under observation must be kept intact.

*** According to Seifriz (1920), the cytoplasmic viscosity is low when an active metabolism is carried out in the cells.




Text-fig. 2. Diagram showing the viscosity change of cytoplasm in spore mother cells undergoing meiosis of *Cyrtomium*, *Polystichum* and *Rumohra*. An existence of local differences in magnitude of cytoplasmic viscosity in the cell is neglected. The axis of ordinates denotes the relative magnitude of cytoplasmic viscosity, and the axis of abscissas the relative duration of various stages in meiosis (Hiraoka, 1952).

Abbreviations: Ip, interphase preceding meiosis; PL, preleptotene stage; L, leptotene stage; LB, leptotene bouquet stage; ZB, zygotene bouquet stage; PB, pachytene bouquet stage; P, pachytene; D, diplotene; S, strepsitene; Dk, diakinesis; $M_1$, first metaphase; A, first anaphase; T, first telophase; I, interkinesis; $M_2$, second metaphase and Td, young tetrad stage. Positions of I and $M_2$ are arbitrary.

"plastid pole". Thus the local difference in the magnitude of cytoplasmic viscosity is symmetrical with respect to the axis connecting the "plastid pole" and the bouquet base. This uniaxial symmetry in the local difference is established on the onset of the bouquet stage and has disappeared in the pachytene stage, in which stage another kind of local difference in the magnitude of cytoplasmic viscosity is found to exist between the regions remote from and close to the nucleus. The existence of the uniaxial symmetry in the local difference of cytoplasmic viscosity characterizes the spore mother cells in the bouquet stage.

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