Some Cytological Observations on Parthenogenesis in *Daphnia pulex* (de Geer)

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(*With 3 Plates*)

Parthenogenesis is a very common phenomenon widely known in many forms of animals. Soumalainen (1950) classified parthenogenesis into the following three groups based on cytological conditions:

A. Generative or haploid parthenogenesis.
B. Somatic parthenogenesis.
   1, automictic parthenogenesis, or parthenogamy.
   2, apomictic parthenogenesis.

Apomictic parthenogenesis is the most common type of parthenogenesis. It is found in several species of the Cladocera (Crustacea). In this type the meiotic phnomena are lacking, wholly or in part. No chromosome reduction takes place in the eggs, and the somatic chromosome number is thus maintained.

Knowledge about the chromosome behavior and other cytological phenomena in parthenogenesis of the Cladocera has remained incomplete, although several papers have been contributed to this field by earlier investigators, such as Weismann & Ishikawa (1891), Kühn (1908), Fanghaut (1921), Chambers (1913), Taylor (1915), Schrader (1926), Allen & Banta (1928, '29), Mortimer (1935) and von Dehn (1948). In reviewing the reported cases, it becomes apparent that there are many doubtful and conflicting points in previous cytological studies of *Daphnia*, and this induced the present author to make a new investigation of *Daphnia pulex*. The present article reports a part of studies on some cytological phenomena in parthenogenesis.

The author wishes to express his gratefulness to Professor Sajiro Makino for revising the manuscript and valuable criticism. Further thanks are also due to Dr. Y. Matsui, Director of the Nippon Institute for Scientific Research on Pearl, for his keen interest in this subject.

Material and methods

An ample number of living specimens of *Daphnia pulex* were supplied as material for the study. The times suitable for observations were determined

by consulting their life-history. Many events concerning reproduction were easily observable macroscopically in parthenogenetic mother animals which were cultured at 20–22°C (Figs. 1–10). For convenience in cennection with fixation, the author divided the course of parthenogenesis as follows :

1), the time when the mother discharges the young from the brood chamber.

2), the time of the molting of the mother animal, within 10 minutes before the laying of parthenogenetic eggs.

3), the time of attainment of full maturity of the ovary, immediately before egg-laying (Figs. 1–3).

4), the time when, by 10 minutes after molting, the parthenogenetic eggs migrate from the ovary to the brood chamber. This process is finished within 3 to 4 minutes (Figs. 3–10).

5), the time during which the parthenogenetic eggs in the brood chamber develop gradually into young animals.

6), the time while pigmentation occurs in the eyes of young in the brood chamber.

7), the time by which pigmentation in young eyes seems to be completed about 4 to 5 hours before molting (Fig. 1).

The mother animals were preserved with fixatives in the seven groups as mentioned above. The most satisfactory results were obtained by a combination method of fixation with Ohlmacher's fluid and Allen-Bouin's solution. The animals were put whole into the mixture of the two solutions at room temperature for 15–30 minutes, and then transferred to another sample of the same mixture kept at 37–38°C. Sections were made from the material thus treated according to the usal paraffin method, and colored with Heidenhain's iron-haematoxylin with counterstaining of light green.

Observations

Before the young are discharged from the brood chamber, each of the ovaries contains germ cells forming two layers. At this stage the embryonal eyes develop pigments. There is a germ "anlage" in the posterior part of each ovary. The oogonia occurring in the "anlage" are tiny cells characterized by a small amount of cytoplasm and a well-marked nucleus which contains prominent nucleoli (Fig. 21). Before the molting of the mother animal, the mitotic proliferation of oogonia has taken place in the ovary. After molting, oogonial division is infrequent. The oocytes are distinguishable from the oogonia only by their larger size and by the presence of small ovoid cytoplasmic bodies (Figs. 23–24). They show a densely stained nucleolus (Figs. 22–25). The growth in size of the nucleus is accompanied by an increase in volume of distinct bodies which look as if they were composed of the nucleolar substance (Figs. 26–27). There is a vacuolized mass in the nucleolus occupying its the whole area (Figs. 26–27). The cytosome of the oocyte increases in volume with time and shows vacuoles. The

vacuoles seem to be filled with a substance that shows a weak affinity to acidic dye. Then, the vacuolized nucleolar mass breaks down into small aggregates of granular appearance (Figs. 28–29). In the meantime, the nucleus gradually migrates to one side in the cytoplasm. During this migration the nucleus seems to extrude fine granules in the cytoplasm. The granules are of nucleolar origin, variable in size and shape, and take the same stain as the chromatin. It seems very probable that these granules are connected with the production of the yolk granules of the parthenogenetic egg (Fig. 29). By this time, the cells generally assume a pear-shape (Figs. 27, 29).

The behavior of the chromosomes at about and following this stage was very difficult to trace, because of the presence of the densely stained nucleolus in the nucleus. Sometimes, the nucleus of the nurse cell grows as large as the oocyte, but does not show the breaking down of the nucleolus. In earlier stages, the development of both oocytes and nurse cells proceeds in a similar manner, but the process of the yolk formation takes place in oocytes only. With the passage of time, the yolk granules increase in number in the oocytes. At a later stage, the nucleus of every oocyte is filled with the yolk granules densely aggregated (Fig. 32).

Immediately before egg-laying, a distinct body makes its appearance in the area of the germinal vesicle. It stains with haematoxylin, and contains a spherical body surrounded by small spherules. After the eggs are laid, this body migrates to the opposite side of the egg (Fig. 38). Then it breaks down into a few masses of irregular shape. It can be detected in the cytoplasm of the cleaving egg. The destiny and function of this body remain unknown at present.

Following the breakdown of the nuclear vesicle, the chromosomes, very tiny in size, move to the peripheral part of the egg and form the polar spindle. The polar spindle is observed at a variety of distances from the egg surface before the egg is laid. The spindle seems to move in some degree in the peripheral region of the egg, due probably to the movement of yolk granules. The migrations of eggs from the ovary to the brood chamber is completed within 3 to 4 minutes (Figs. 3–10). At this stage the polar spindle is very prominent because of showing distinct fibers; it is surrounded by a small amount of cytoplasm with a deep deposition of pigment which is countinuous to a dense accumulation of heavy yolk granules (Figs. 12, 13, 34 and 35). Under high power magnification, the polar spindle appears as a minute light spot, imbedded in yolk granules at the periphery of the egg (Fig. 13, 35). The chromosomes are seen forming the equatoiral plate. But they are so extremely small in size as not to permit a detailed study to determine their number (Fig. 43). Though not exactly counted, there are more than fifteen elements in the metaphase plate. In another paper, the author reported 20 chromosomes in the diploid set of the spermatogonium, and 10 elements as the haploid complex in both primary and secondary spermatocytes (Ojima 1954). It is highly probable therefore that, the polar spindle possesses a diploid

chromosome set. Three minutes after egg-laying, the division advanced to anaphase. Each chromosome separates into two halves which go to opposite poles (Fig. 136). After the separation of the chromosomes the outer pole of the spindle is lifted above the egg surface exhibiting a disc (Figs. 14, 37). Meanwhile, the spindle fibers become gradually indistinct and assume the appearance of a granular structure. After the polar body is completely extruded, either in the polar body or within the egg, the chromosomes are seen fused together into condensed and irregular masses (Fig. 37).

All the process of the polar division takes place during 8 to 10 minutes when the eggs have come into the blood chamber. After the extrusion of the polar body is completed, the sister chromosomes left in the egg converge and tend gradually to join into a compact mass of irregular outline. There is not even the slightest evidence for the formaltion of the second polar body. Meanwhile, the chromatic bodies in the egg lose much of their capacity to take stain, and the nuclear membrane makes its appearance to enclose the bodies. The pronucleus is thus produced. It begins to move towards the deeper part of the egg. At the same time it grows considerably, and its form becomes spherical.

Usually the first cleavage spindle is found in the process of division in eggs fixed 15 minutes after laying (Figs. 15, 16, 39 and 40). Once every 15 minutes the cleavage increases (Figs. 16–19). The blastmeres move to the egg periphery, and finally the egg exhibits a typical superficial cleavage (Fig. 20).

Discussion

With regard to the behavior of the nucleolar substance in the Cladocera, there is a little discrepancy between the reports published by different authors. Allen and Banta (1929), and v. Dehn (1948) observed in *Moina macrocopa* and *M. rectirostris* that the nucleolar substance breaks down into smaller elements, gradually lose their affinity to nuclear stains, and then fill the nucleus. In *Daphnia pulex*, the nucleolar substance continues as a somewhat vacuolated mass until it has reached a later stage of the growth period. Then it breaks down as occurred in *Moina*, and seems to be used in yolk formation. After the yolk has formed, the remaining nucleolar substance seems to aggregate until it forms a mass outside the germinal vesicle. That mass disappears either in the full grown egg or in the egg after ovulation; it seems probable that it may be used for reorganization of the nucleolar substance. At the stage just prior to egg-laying, the mass may be extruded into the ooplasm. In the egg after ovulation it migrates to the opposite pole of the egg, and lies close to the egg membrane. The corresponding body was observed in *Moina macrocopa* by Allen and Banta (1929); they called it a degenerated body. But, nothing has been made clear about the origin of this interesting body. It is noticeable that the results of the present investigation suggest a role of the nucleolar substance in the formation of the yolk. Kühn (1908) seems to be the only author to report the behavior of chromosomes in the growing oocyte. He

reported eight chromosomes in the somatic cells as well as in the parthenogenetic eggs of *Daphnia pulex*. Schrader (1926) stated that the diploid number is 24 in a pseudo-sexual egg of a species of *Daphnia*. In *Moina*, Allen and Banta (1929), and v. Dehn (1948) observed the behavior of chromosomes in some detail in oocytes, establishing 22 and 30 somatic chromosomes, respectively. The number and behavior of the chromosomes were not studied in detail in the present material, but the data presented are sufficient to justify the conclusion that only one equational maturation division takes place in the parthenogenetic egg, and that it occurs without reduction of the chromosome number.

Summary

The behavior and fate of the nucleolar substance were observed in growing oocytes of the parthenogenetic ovary of *Daphnia pulex*. A possible role of the nucleolar substance was suggested in relation to the formation of the yolk. Only one equational maturation division takes place in the parthenogenetic egg, and no reduction occurs in the chromosome number.

Literature

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Explanation of Plate I

Photomicrographs of parthenogenetic mothers, taken with the aid of a Leitz Mikas camera.

Fig. 1. A parthenogenetic mother with three youngs in the brood chamber. 40×. Fig. 2. Showing the ovary in the stage of immediately before egg-laying. 40×. Figs. 3-10. Features showing euccessive stages of ovulation of parthenogenetic eggs from the ovary into the brood chamber. 40×.

Explanation of Plate II

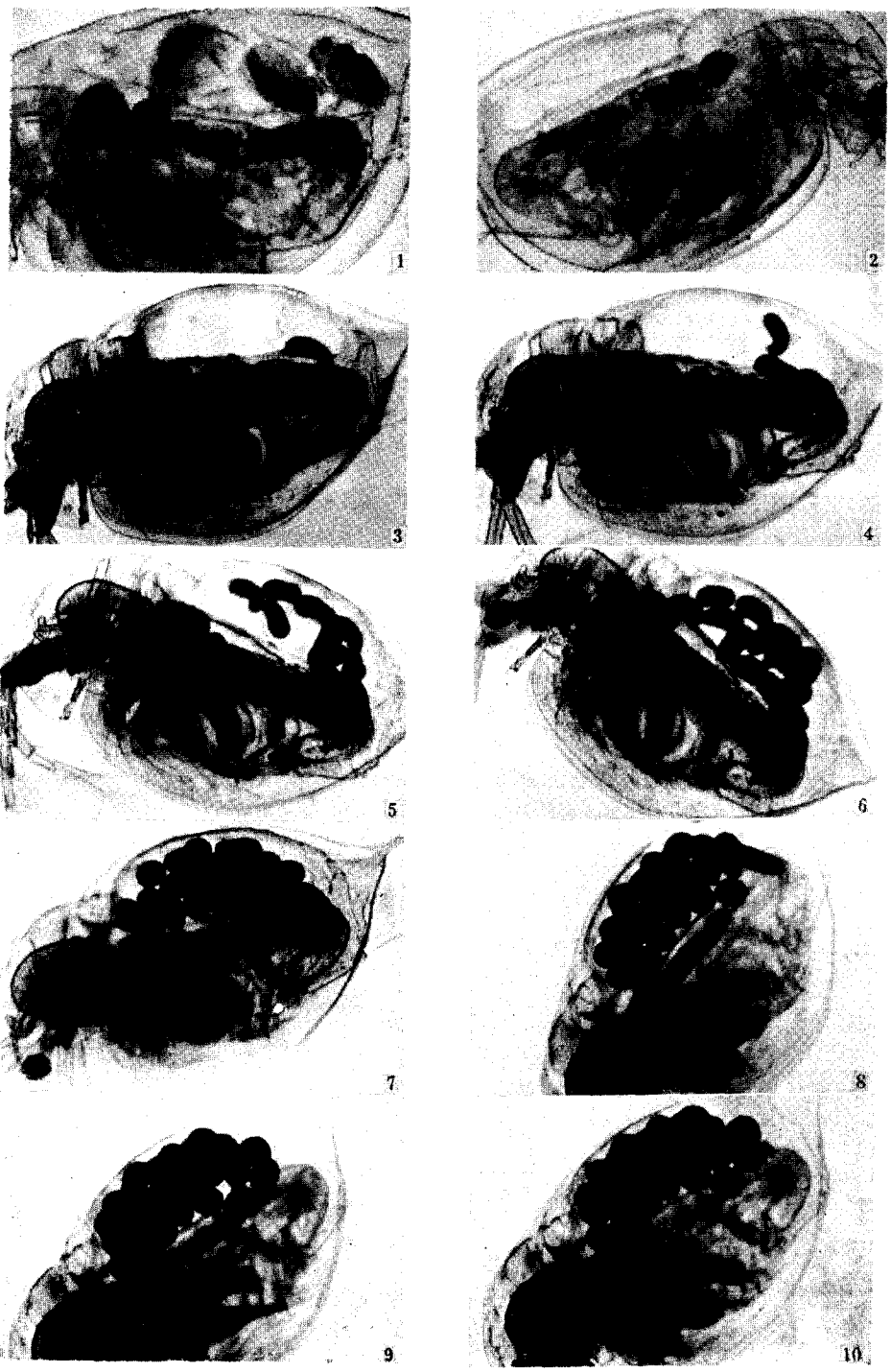
Photomicrographs of ovaries and eggs in parthenogenetic mothers, taken with the aid of a Mikas camera.

Fig. 11. Full grown ovary. ×120. Fig. 12. A section of ovulated eggs and oocytes. 120×. Fig. 13. Showing a polar spindle at metaphase. 4000×. Fig. 14. Lateral view of the polar body. 1500×. Fig. 15. Metaphase of the first cleavage. 1500×. Fig. 16. Anaphase of the first cleavage. 700×. Fig. 17. Telophase of the second cleavage. 700×. Fig. 18. Telophase of the second cleavage. Showing the spindle fibres and division of nuclei. 1200×. Fig. 19. Four-cell stage of a parthenogenetic egg. 250×. Fig. 20. Showing the metaphase chromosomes at the morula stage. 1500×.

Explanation of Plate III

All the figures were drawn at the level of the desk on which the microscope was set, with the aid of an Abbe's drawing apparatus.

Fig. 21. Three oogonia. 200×. Fig. 22. Early oocytes. 200×. Figs. 23-25. Oocyte at a little advanced stage, showing increase in size of the nucleolar substance. 2000×. Figs. 26-27. Showing the oocyte at further advanced stage. 2000×. Fig. 28. The nucleolar body showing degeneration. 2000×. Fig. 29. Yolk formation in the cytoplasm. 1000×. Fig. 30. The ovary at the pigmentation stage. 200×. Fig. 31. Showing the nucleolar mass in the nucleus, at the pigmentation stage. 3500×. Fig. 32. An egg prior to laying. 2000×. Fig. 33. Full grown ovary observed after molting. 200×. Fig. 34. The chromosomes in the germinal vesicle, observed before egg-laying. 2000×. Fig. 35. Metaphase spindle in the ovulated egg. 2000×. Fig. 36. Side view of the anaphase spindle in the polar division. 2000×. Fig. 37. Telophase of the polar division. 2000×. Fig. 38. Showing a germinal vesicle and a unknown body in the ovulated egg. unb, unknown body. 400×. Fig. 39. The first cleavage. 1000×. Fig. 40. The nuclei at the first cleavage telophase. 3500×.



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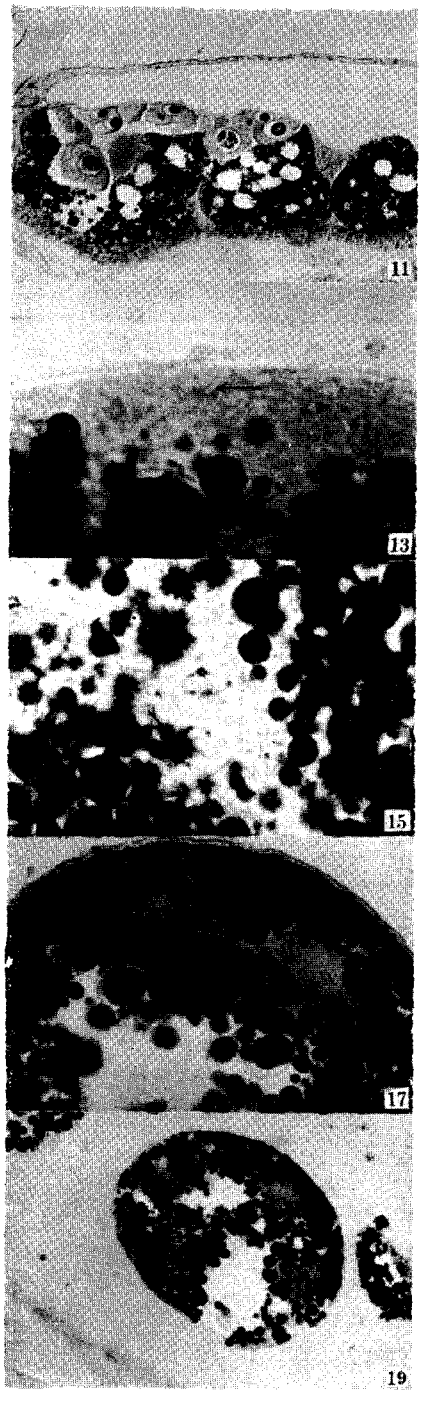

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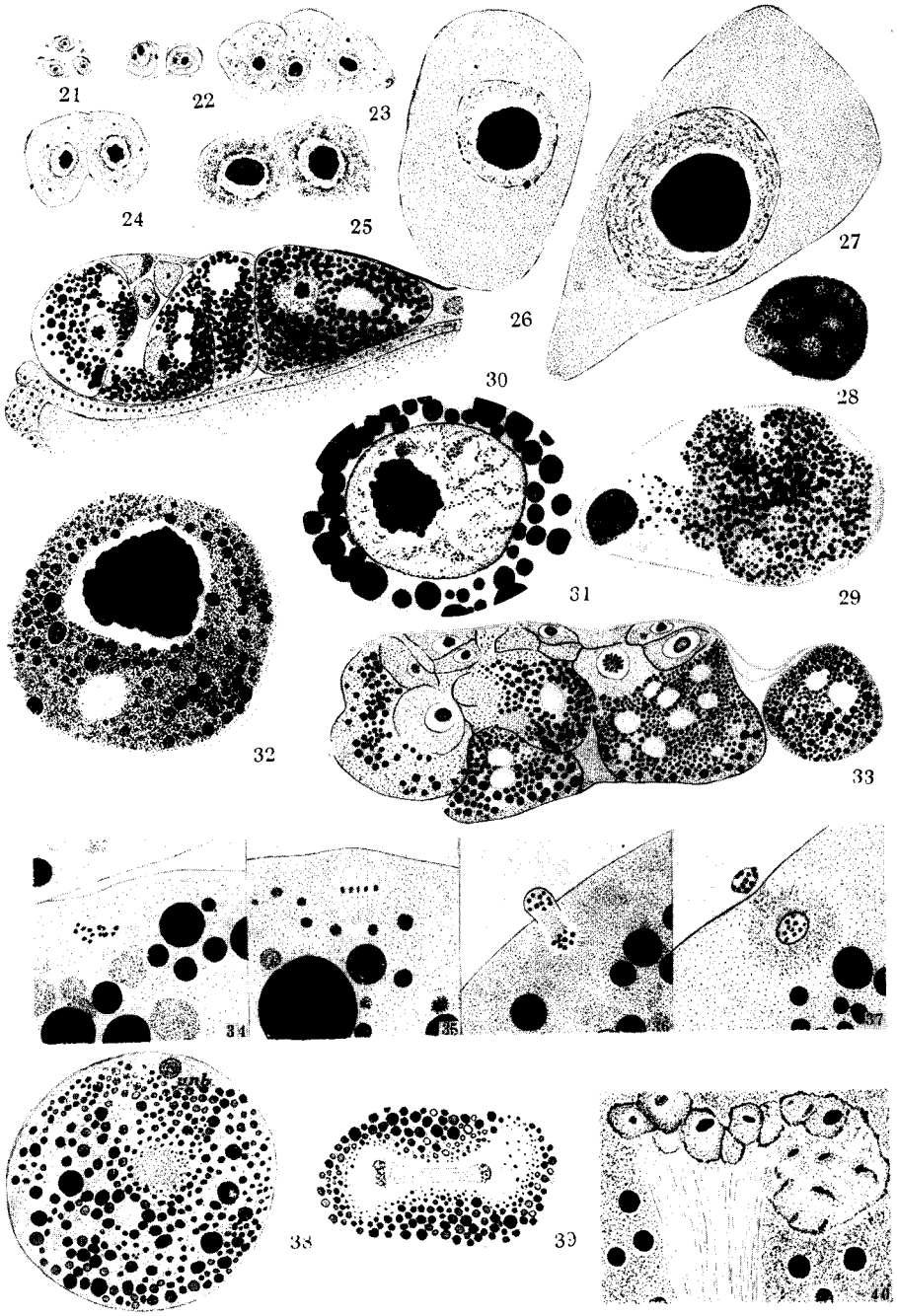

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