On the Spermatogenesis of *Daphnia pulex* (de Geer)

By

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(*With 17 Textfigures*)

Apomictic parthenogenesis has been known to occur in Turbellaria, Trematoda, Nematoda, Rotifera, Mollusca, Crustacea, and Insecta. There are many papers pertaining to physiological influence of environmental factors upon sex-determination in Cladocera (Crustacea). Knowledge on the cytology in relation to the alternation of generation in this group of animals is very meagre, many important problemes concerning the chromosomes having remained obscure. This is probably due to the unfavourable nature of this group of animals as material for cytological investigation because of the small size of both cells and chromosomes. In addition, there is a difficulty in determing the time suitable for study. Reference to the literature shows the cytological studies of Cladocera having been confined to the behavior of chromosomes during oogenesis and cleavage. The present paper deals with the behavior of chromosomes in spermatogenesis of *Daphnia pulex* (de Geer).

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Material and methods

The material with which the present work was done was provided with the stock culture in the author's laboratory. The male individuals produced from a single mother were fixed for observations. Following the removal of the shells, the whole bodies were put into the fixatives. For fixation, Carnoy's fluid, Allen-Bouin's mixture, Champy's weak solution, Meves' modification of Flemming's mixture, Flemming's strong solution, Flemming's weak solution, Niiyama-Flemming's solution were tested. Flemming's weak solution, Niiyama-Flemming's solution and Champy's weak solution proved excellent for the preservation of the chromosomes. Sections were made 3 to 4 μ in thickness, and colored with

Heidenhain's iron-haematoxylin with light green as a counter stain. The optic combination with a Leitz 25× periplan ocular and a Olympus 110× objective was useful for the chromosome study. All the figures are the camera-lucida drawings taken at desk level on which the microscope was set.

Observations

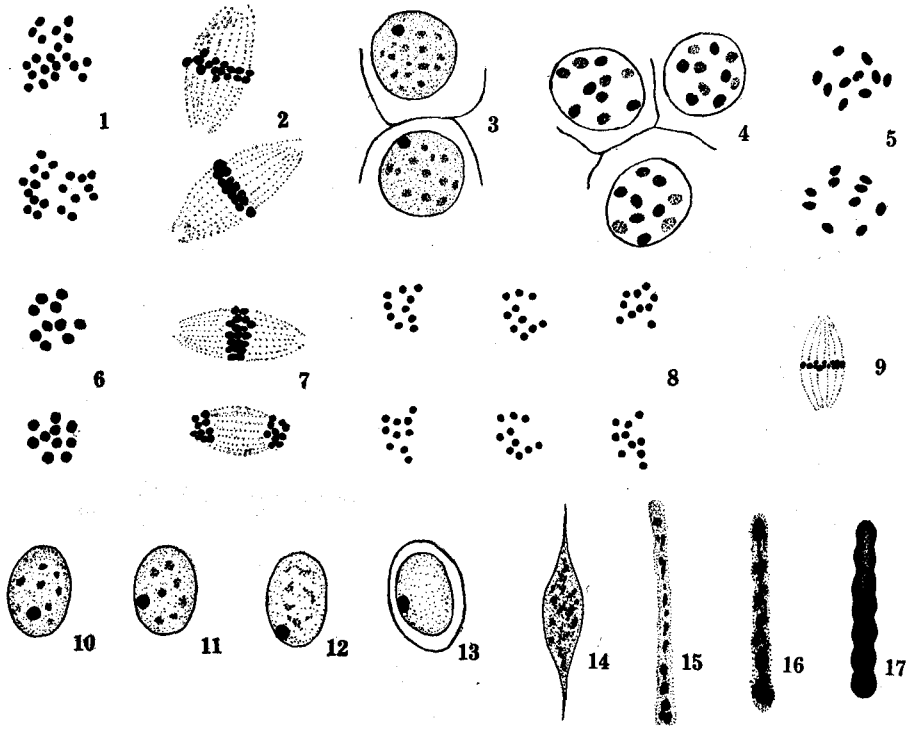
Spermatogonium : The testis are found lying along both side of the intestine by the time when the young had been discharged from the brood-chamber of the mother. By this time the spermatogonial cells were found in the stage of prophase. After the young swim out from the brood-chamber, the multiplication of spermatogonia was taking place resulting in the production of the primary spermatocytes. Generally it was a difficult task to make exactly the chromosome count in spermatogonia because the chromosomes stuck together into a clumped mass, due probably to the influence of the fixatives. In a few well-preserved metaphase plates, however, successful count of the spermatogonial chromosomes was made and the diploid number of 20 was confirmed for this species (Fig. 1). The individual chromosomes are very minute in size and show no characteristic feature available for the identification of the homologous pairs.

Primary spermatocytes : The transverse sections of the testis show germ cells in process of meiosis ; they were arranged covering the anterior part toward the posterior in order of maturating processes. The nuclei in the stage of growing period are generally large in size and in each a deeply stained nucleolus is observed. The chromosomes are found as chromonema threads of vague outline. (Fig. 3). Nearing the metaphase stage, they assume elliptical dot-shape of sharper outline and are found lying close to the inner surface of the nuclear membrane (Figs. 4–5). There are 10 chromosomal elements of bivalent structure in every nucleus at this stage. The first molting of the animal occurs 10 to 20 hours after the young were discharged from the brood-chamber of the mother. By this time the cells are in process of the first meiotic division. The chromosome count was made with indisputable clearness in the metaphase plate of the primary spermatocyte, since the chromosomes arrange themselves well-apart from one another with distinct outline. In every equatorial plate examined, 10 bivalents were observed constituting the haploid complex. Morphologically the chromosomes are all of dot-like type being uniform in both size and shape (Fig. 6). In division, all the bivalents separate symmetrically into two equal halves, and any peculiar chromosome to be regarded as the sex-chromosome failed to be observed through the course of the first division (Fig. 7).

Secondary spermatocytes : Covering the posterior part of the testis, many secondary spermatocytes were in process of division. The chromosomes are very minute in magnitude and therefore extremely difficult for observation. By careful counting of many equatorial plates it was ascertained that the secondary spermatocytes contain invariably 10 chromosomes (Fig. 8). In division, all elements

divide synchronously and there is no evidence for the presence of any peculiar one (Fig. 9).

Spermioteleosis : With the completion of the second molting in the young, the course of spermioteleosis starts. The young show many mature spermatozoa in the testis about 40 hours after being discharged from the mother. Following the second maturation division the cells became smaller ; a nucleolus appears in



Figures 1-17. Camera-lucida drawings, × 4700. 1, spermatogonial metaphase. 2, side view of the spermatogonial spindle. 3-4, growing period. 6, metaphase of the primary spermatocyte. 7, side view of the primary spermatocyte metaphase. 8, metaphase of the secondary spermatocyte. 9, side view of the secondary spermatocyte metaphase. 10-13, formation of the spermatid. 14-17, process of spermioteleosis.

each nucleus in which the chromosomes gradually decrease visibility (Figs. 10–12). Thus the transparent spermatid nucleus is produced (Fig. 13). In the course of metamorphosis into spermatozoa the cells became elongated. The granules like chromatin made their appearance in the nucleus (Fig. 14). Then the nucleus changed as a whole into a rod-shape. The granules appeared as compact particles looking like the chromosomes (Figs. 15–16). Careful observations revealed that the particles were arranged on a line connecting each one. After the completion of metamorphosis the mature spermatozoa assume a rod-shape containing the granules which arrange themselves like the chromosomes (Fig. 17).

Discussion and conclusion

Because the small size of the chromosomes of Cladocera furnishes a difficult material for cytology, our knowledge of the chromosome morphology has been very limited in this group of animals. The chromosome numbers of this group have been summarized by Niiyama ('40) and Makino ('50).

Mortimer ('35, '36), in his work on *Daphnia magna*, concluded that both somatic and spermatogonial cells showed 20 chromosomes, while the parthenogenetic eggs contained $n=10$. In *Daphnia pulex*, Mortimer ('35) reported that there were 24 chromosomes ($2n$) in the female somatic cell, while the winter eggs contained 12 chromosomes in haploid. Kühn ('08) informed that no chromosome reduction took place during the maturation division in the parthenogenetic egg of *Daphnia pulex*. He showed that the oogonium, somatic cell and parthenogenetic egg contained uniformly 8 chromosomes in diploid. Rey ('34) investigated in *Daphnia pulex* the chromosome complex, and announced $2n=8$ in both the oogonium and somatic cell, $2n=9$ in the spermatogonium, and 4–5 chromosomes in the spermatocyte. Taylor ('15) indicated in the same species that both spermatogonium and somatic cell contained 8–10 chromosomes. Schrader ('26) reported 24 chromosomes as diploid in the oogonium, somatic cell and parthenogenetic egg.

So far as the chromosome number is concerned, the results presented by Mortimer ('35, '36) for *Daphnia magna* coincide with those of the present work on *D. pulex*. Most authors have been concerned with the chromosomes of oogenesis and somatic cells. The chromosome study in oogenesis and egg-cleavage is made in extremely difficulty, because there is rich yolk densely stained in the egg which prevents distinguishing the chromosomes. To obtain accurate and clear-cut information on the chromosomes of *Daphnia*, the study of spermatogenesis is highly needed.

The present investigation was successful in demostrating 20 chromosomes in the spermatogonial division, and 10 elements in both the primary and secondary spermatocytes. Most interesting feature here observed involves the morphological changes of the spermatid into the spermatozoon in the course of spermioteleosis. In the course of metamorphosis the nucleus of the spermatid elongates and shows

granules like the chromatin. These granules take a linear arrangement, each granule being connected in the form of beads. In general appearance the granules bear much resemblance to the chromosomes.

Résumé

The present paper contains a preliminary report on the spermatogenesis of *Daphnia pulex* (de Geer), with particular regard to the behavior and number of chromosomes. The observations established 20 chromosomes as the diploid set in the spermatogonium, and 10 elements as the haploid complex in both the primary and secondary spermatocytes. Through the course of the maturation divisions there was no evidence for the presence of any particular chromosome characteristic, either in behavior or in morphology, to the sex chromosome. The morphological changes of the spermatid into the spermatozoon was followed through the course of spermioteleosis.

Literature

Dehn, M. von. 1948. Experimentelle Untersuchungen über den Generationswechsel der Cladoceren. Chromosoma. 3.

Kühn, A. 1908. Die Entwicklung der Keimzellen in den parthenogenetischen Generationen der Cladoceren: *Daphnia pulex* und *Polyphemus pediculus*. Arch. Zellforsch. 1.

Makino, S. 1950. A Review of the Chromosome Numbers in Animals. Tokyo.

Mortimer, C. H. 1936. Experimentelle und cytologische Untersuchungen über den Generationswechsel der Cladoceren. Zool. Jahrbuch. 56.

Niiyama, H. 1940. List of the chromosome numbers in the Crustacea. Jap. Jour. Genet. 16.

Rey, P. 1934. La formule chromosomale de *Daphnia pulex*. Bull. Soc. Zool. France. 59.

Schrader, F. 1926. The cytology of a pseudosexual egg in a species of *Daphnia*. Zeits. Induk. Abst. Verer. 40.

Taylor, M. 1915. Note on the number of chromosomes in the male of *Daphnia pulex*. Zool. Anz. 45.