

malige Teile des Organs darstellen. Das erstere erscheint als das wahrscheinlichere.

Wenn sich, wovon ich überzeugt bin, die Mycetomnatur der Pyrosomenleuchtorgane bestätigt, wird damit der Symbiontenforschung ein neues interessantes Gebiet eröffnet, das eine Anzahl weiterer Fragen im Gefolge hat.

5. Note on the Number of Chromosomes in the male *Daphnia pulex*.

By Monica Taylor S. N. D.

(College of Notre Dame, Downanhill, Glasgow.)

(With 9 figures.)

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The determination of the number of chromosomes in male Cladocera is of great interest in view of the methods of reproduction obtaining in this group. As is well known from Weismann's¹ researches, the winter eggs, which require fertilization, produce two polar bodies, while the eggs which develop parthenogenetically produce but one. In a recent investigation Kühn² showed that the latter eggs contain, as was to be expected, the somatic number of chromosomes. Owing to the great preponderance of females over males in this group, however, it is probable that the eggs examined were destined to develop into females, and so the occurrence of special cytological processes in the maturation of the parthenogenetic eggs destined to develop into males is not excluded. The most important thing to establish at the outset is whether the male has a diploid number of chromosomes or a haploid (as in male Hymenoptera produced parthenogenetically).

With a view to determining this question, Dr. Agar collected over the course of a few years, males of various species of Cladocera, a preliminary examination of the somatic and spermatogonial mitoses of which had shown the probability that the number is diploid. This material Dr. Agar handed over to me for more detailed examination.

The only part which proved suitable for detailed work was *Daphnia pulex*, of which the collection contained a number of males of recorded ages, from 24 hours to 6 days and upwards. The majority of these specimens had been fixed in Gilson-Petrunkevitch, cut into transverse and sagittal sections of thicknesses from 4 to 7 μ , and stained in Ehrlich's and Heidenhain's haematoxylin, prolonged staining being

¹ Weismann A., 1886, Richtungskörper bei parthenogenetischen Eiern. Zool. Anz. Nr. 9.

² Kühn, Alfred, Die Entwicklung der Keimzellen in den parthenogenetischen Generationen der Cladoceren *D. pulex* De Geer und *Polyphemus pediculus* De Geer. Archiv für Zellforschung 1. Band. S. 538.

necessary in each case. A few were fixed with Flemming's solution, but it was found difficult to stain satisfactorily after the use of this fixative.

The small size of the cellular elements in the testis prevents *Daphnia pulex* from being a favourable subject for a detailed study of the early prophase of the meiotic division and of syndesis, so the work was chiefly confined to a determination of the number of chromosomes in different tissues, and in the various generations of cells in the testis. The somatic number of chromosomes in this species is given by Kühn as 7—10, probably 8.

The gonad of the male *Daphnia pulex* is paired, and lies right and left of the alimentary canal. In the younger specimens each testis is solid, and consists of the relatively large primordial germ cells, the nuclei of which are commonly in the resting condition. Examples of mitoses among these cells are not easy to obtain — the gonad becoming very active only when the individual is three days old. *Daphnia* of from 4 to 5 days old are most useful for showing all stages in the spermatogenesis. In such specimens each testis consists of an elongated, lobulated sac pointed at the anterior end, and passes insensibly into the vas deferens. The sac is incompletely and irregularly subdivided into compartments by the presence, in the walls of the gonad at irregular intervals, of large cells probably somatic in character. The developing spermatogonial elements are found in clusters in the peripheral parts of the sac; in the gradually appearing central lumen are ripe spermatozoa. In a creature 6 days old the whole gonad is filled with ripe spermatozoa which lie in a matrix which stains deeply with eosin, the walls of the sac being by this time exceedingly thin, and the large somatic cells having almost entirely disappeared. The chromosomes are most easily counted in prophase, when it is quite possible to show that the number of chromosomes in the spermatogonial cells is the same as in those of the tissues of the female, and lies between 8 and 10, fig. 1, *a. a'*.

In the metaphase of the spermatogonial and meiotic divisions the chromosomes are so close together that, though equatorial plates are exceedingly numerous in the sections, and the spindle apparatus and centrosome are well organised and quite typical, yet these do not form favourable objects for counting chromosomes, fig. 1, *b*. (Cf. equatorial plate from a cell in the intestine, *c*).

A well marked synzinesis stage occurs in the early prophases of the first meiotic division, and a well defined nucleolus, most easily seen in sections stained in Ehrlich, is present until the chromosomes are well condensed. Late prophases are characterized (fig. 1, *d*.) by the presence of the haploid number of chromosomes (i. e. 4 to 5). These are larger

than those which characterized the spermatogonial generations. On the equatorial plate they are again closely massed together (fig. 1, *e* and *f*). In side view the equatorial plates of the first meiotic division are distinguishable from those of the spermatogonial by their smaller diameter. The equatorial plates of those dividing nuclei nearest the central cavity, presumably those of the second meiotic division, are again smaller (fig. 1, *g*).

The newly formed spermatids are round (fig. 1, *h*), and the chromatin is mostly peripheral. Gradually they lengthen; the deeply staining



aa', Prophases of spermatogonial cells showing 8 and 10 chromosomes respectively; *b*, Side view of spindle of spermatogonial cell; *c*, An equatorial plate from cell in intestine; *d*, Prophase of first meiotic division, showing 4 chromosomes; *e*, Side view of spindle of first meiotic division; *f*, Equatorial plate of first meiotic division; *g*, Side view of equatorial plate of second meiotic division; *h*, Newly formed spermatids; *i*, Young spermatozoan. Magnification about 3500.

chromatin of the newly formed spermatozoa being very conspicuous (fig. 1, *i*). The spermatozoa finally contract somewhat and lose their intense staining capacity.

Spermatogenesis in male *D. pulex* is, thus, perfectly typical. The spermatogonial cells contain the diploid number of chromosomes; reduction ensues; two meiotic divisions follow, the first and second spermatocyte cells containing the haploid number. The eggs which develop into male daphnia must contain the diploid number of chromosomes, in which respect they resemble those summer eggs which have developed parthenogenetically into female daphnia.

Mitosis in somatic cells is to be found in the hind end of the alimentary canal in *Daphnias* whose ages are from 4 to 6 days. It is interesting to note that newly hatched and very young *Daphnias* are not favourable objects for the examination of somatic mitosis. The dividing cells of the alimentary canal, like those of the gonad, show a well formed spindle apparatus and a centrosome, but the massing together of the chromosomes on the equatorial plate makes it difficult to decide whether the somatic number is 8 or 10. However, the evidence from somatic mitosis confirms the results described above, namely, that the male *Daphnia* possesses the diploid number of chromosomes.

While this note was still in MS. a Paper on »The Spermatogenesis of a Daphnid-*Simocephalus vetulus*« by Robert Chambers³, published in the Biological Bulletin, Vol. 25, came into my hands. Chambers describes the degeneration of approximately half the spermatids in this species, and suggests that they are the male-producing gametes. As the specimens of *Simocephalus vetulus* in Dr. Agar's collection were too old for a complete study of spermatogenesis, and consequently for a study of degeneration, I have, since reading Chambers' Paper, re-studied all the sections of *D. pulex* used for this note, and have also made many new ones and many stained preparation of dissected gonads for the purpose of discovering whether in *D. pulex* a degeneration of spermatids takes place, such as Chambers found in *Simocephalus vetulus*.

In the foregoing note it has already been pointed out that many of the spermatocyte II cells of *D. pulex* contain 5 chromosomes, while others again show clear cases of the presence of 4 chromosomes. But, as such discrepancies occur also in spermatogonial and somatic cells, I do not think that this points to a real dimorphism of spermatozoa.

I can find no trace of degenerating spermatids (in *D. pulex*). Hence, since my observations on *D. pulex* do not accord with those of Chambers for *Simocephalus vetulus*, the question of the male and female producing spermatozoa in Cladocera generally must still be regarded as an open one.

6. Der hammerförmige Chaetognathen-Kopf.

Von Dr. Gerarda Wynhoff, Utrecht.

(Mit 2 Figuren.)

eingeg. 12. Juni 1914.

Während meines Aufenthaltes an der Zoologischen Station in Neapel wurde durch einige Sagitten mit abweichendem Kopf in einem

³ Chambers, R., The Spermatogenesis of a Daphnid *Simocephalus vetulus*. Biol. Bulletin 25. p. 134 - 140. 1913.

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