On the Abnormal Spermatogenesis in an Indian Dragonfly, *Ictinus rapax* (RAMBUR)*

(With 20 figures)

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Many years have already elapsed since the spermatogenesis of insects was brought to our knowledge, and probably, nearly every field in investigation of this class of animals has been touched upon to some extent, although there remains some room for more knolwedge, especially in particular fields. In the present paper the writer wishes to bring forth to light the results of his study concerning abnormal sperms in the testicular follicle. Fortunately we have at hand the dragonfly, which allows sufficient investigation without great technical difficulties. The persistent existence of the cyst-wall throughout the whole period of the spermatogenesis offers many advantages for the study of the quantitative data, such as the total number of abnormal gonad-cells in the cyst.

The preparation of *Ictinus rapax* (RAMBUR) for this study is one of the specimens collected in Western India, and used by Asana and Makino (1) for a comparative study of the chromosomes in the Indian dragonflies, and thereafter employed further by the present author (2) in an investigation of the spermatogenesis. Material was fixed in Flemming's strong solution. Sections were cut into eight micra in thickness and subjected to Heidenhain's iron haematoxylin method of staining, using light green as the counter-stain.

The writer is much indebted to Professor Sajiro Makino of the Institute of Zoology, Hokkaido University, for his sincere supervision during the course of the present study, and also for first introducing the writer to the study of the very interesting order, Odonata.

Observation

Examining precisely the developmental changes of the gonad-cells in the cyst there is scarcely found any abnormal one throughout the whole spermatogonial period except only one or two examples. However, at the periods of the spermatocyte and the spermatid, abnormal cells increase suddenly in number, especially at the latter. The first example found at the early spermatogonial period is a cyst containing three cells which are supposed to be derived from the abnormal cell-division or from fusion of two cells occurring on the way of multiplication-division from the 2-cell to the 4-cell stage (Fig. 1). In the present case, one of the three nuclei is ovoid, and distinctly larger in

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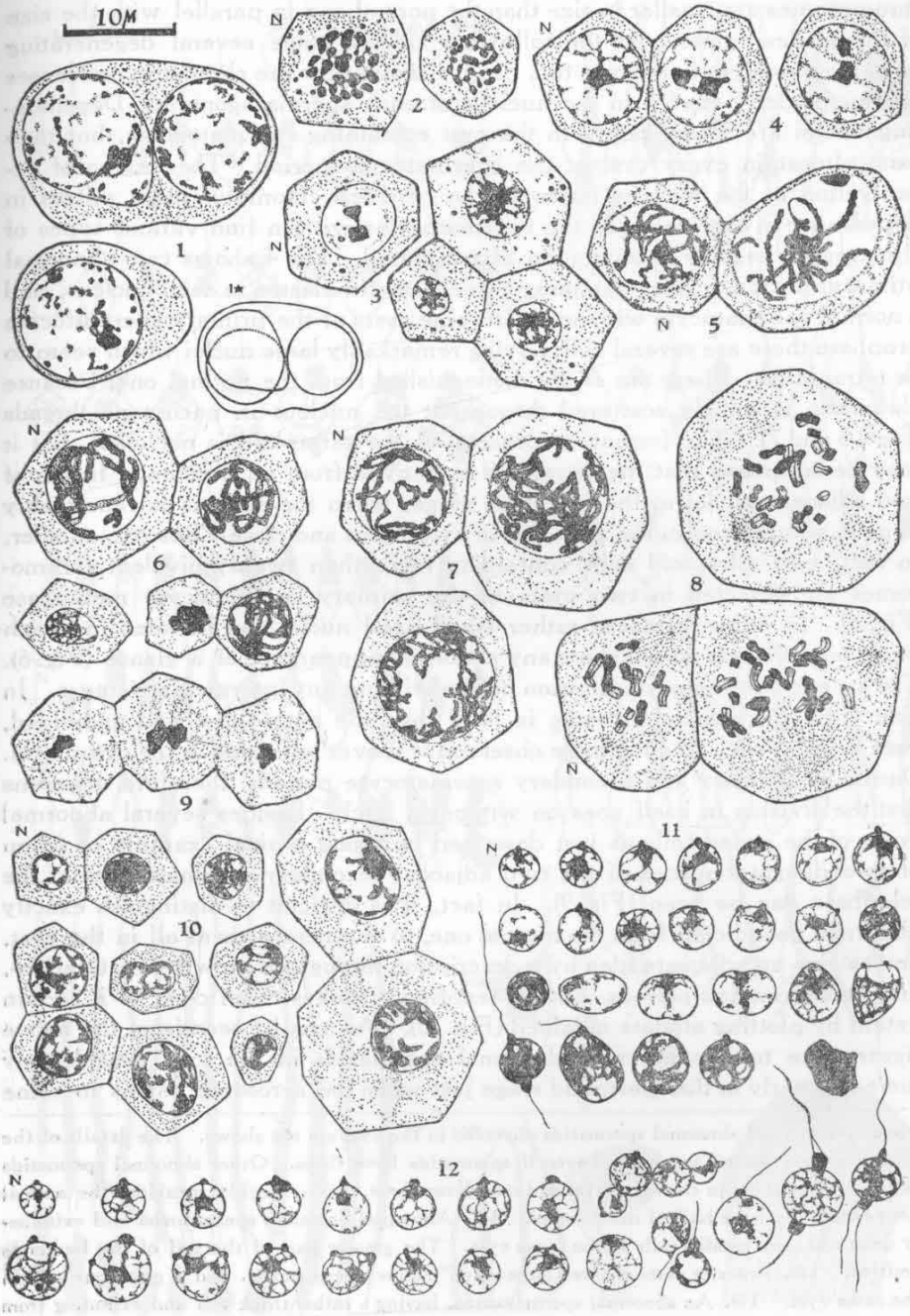
volume than the other spherical two, but its chromatin network does not show any abnormal aspect in its resting stage. In the following stages, therefore, the number of cells in the cyst will repeat in duplicate in consequence of their subsequent cell-divisions. If this assumption is correct, a remarkable disagreement between the prospective value and that based on the actual survey in respect of the total number of the cells in the cyst at the later developmental stages may be interpreted as a result of such an abnormal multiplication occurring in the early spermatogonial period. The earliest stage when was discovered a small number of "degenerating" nuclei is the 32-cell stage. In this case abnormal three nuclei are easily distinguished from other normal ones by their strong capacity for staining and comparatively small size. They contain chromatin materials, which are arranged in large, deeply staining, closely crowded, irregular masses. These masses entirely differ in appearance from those formed at any stage of the multiplication-division. They apparently remain undivided throughout the following spermatogonial period. The exact behavior of these degenerating nuclei or cells is difficult to make out in details, but it seems probable that they do not finally degenerate and disappear in the cyst in the strict sense. At the 64-cell stage, as an extremely rare example, a small-sized nuclear plate, which is approximately 4/5 in diameter as long as the normal one and clearly distinguishable from the others in the polar view of the metaphase group of chromosomes is observed (Fig. 2). This spermatogonium possesses twenty-three well-defined chromosomes, which are arranged on the equatorial plane in quite similar

Figs. 1 – 19. All figures, except 1*, were drawn by aid of camera lucida. Abbreviation: N, normal gonad-cells. The developmental stages of these figures correspond respectively with those of the figures given in the previous paper (2) as shown in the following table, viz.,

No. of present figs.	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19
No. of previous figs.	4b	8c	9b	13d	13h		13i	13j	14b	15	16		19	22	23	24	28	30	33

1. Two serial sections of an abnormal cyst containing three spermatogonia in early prophase of the cell-division. 1*, a diagrammatic figure, showing mode of arrangement of the three nuclei in the cyst. **2.** Polar views of metaphase groups of chromosomes in the 64-cell stage. An abnormal spermatogonium with a small-sized nuclear plate. **3.** A normal spermatogonium in resting stage and three spermatogonia having "degenerating" nuclei. They are discovered altogether in the same cyst at the 128-cell stage. **4.** Two abnormal primary spermatocytes in early prophase, containing two chromatin masses in each nucleus. **5.** An abnormal primary spermatocyte with a remarkably large nucleus which seems to be a tetraploid. **6.** Prophases of the primary spermatocytes in the cyst. Cells having rather small-sized or "degenerating" nuclei. **7.** Prophases of two abnormal primary spermatocytes with large nuclei which seem to be tetraploids. **8.** Metaphases of the primary spermatocytes. Two tetraploids found in two cysts. One of them has already been shown in the previous paper (2: Fig. 13j**). **9.** A typical example of fusion of two nuclei of two adjacent secondary spermatocytes in telophase. **10 – 16** (13 – 16: on page 167). A serial arrangement of the developing stages of the spermatids, showing examples of all kinds of abnormal spermatids contained in an individual cyst


10M
N
1
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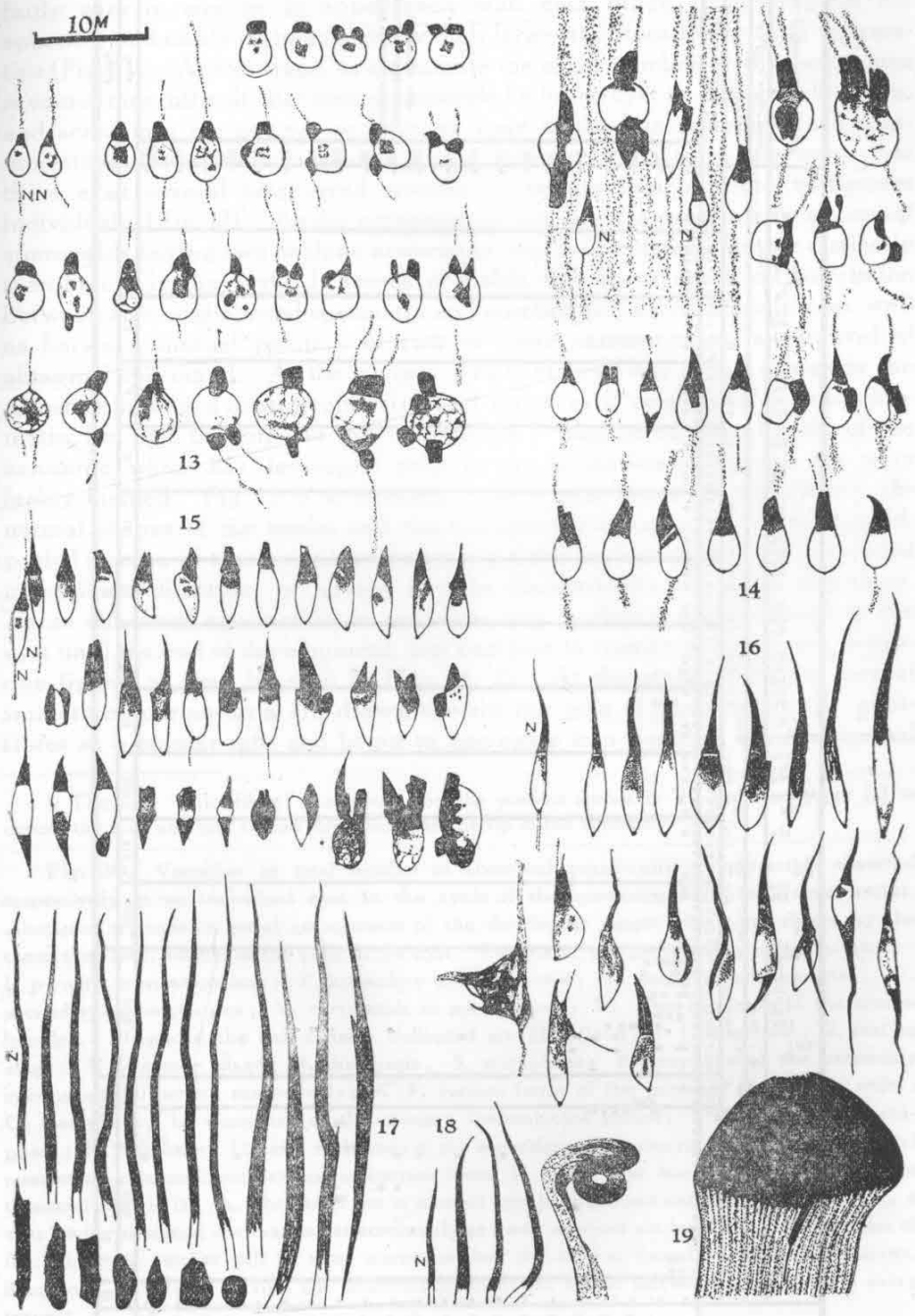
12

manner as seen in the case of the normal spermatogonium in the cyst. But all chromosomes are smaller in size than the normal one in parallel with the size of the nuclear plate. At the following 128-cell stage several degenerating nuclei are found in four cysts, too. Fig. 3 shows how the chromatin condenses into a crowded network in the nucleus of each spermatogonium. Degenerating nuclei are found rarely in the cyst containing spermatogonia, but they exist almost in every cyst at the spermatocyte period. The degree of degeneration of the nucleus inclosing the crowded chromatin mass differs in appearance in each cell. In the spermatocytes we can find various types of abnormality besides degeneration above stated. Fig. 4 shows two abnormal primary spermatocytes containing two chromatin masses in each nucleus, and a normal spermatocyte with one. In some cysts at the primary spermatocyte prophase there are several cells having remarkably large nuclei which seem to be tetraploids. These are easily distinguished from the normal one, because chromatin is thickly scattered throughout the nucleus in pachytene threads (Figs. 5 and 7). The formative process of the tetraploid is not clear, but it may be supposed that the tetraploid is derived from an accidental fusion of two adjacent nuclei at the leptotene stage, when the nucleus is remarkably large in volume compared with that of cytoplasm and closely lies one another. In fact, two tetraploid cells containing more than twenty bivalent chromosomes are detected in two cysts at the primary spermatocyte metaphase (Fig. 8). In other instances rather small-sized nuclei can be seen in which pachytene threads do not show any abnormal appearance at a glance (Fig. 6). The second spermatocyte division occurs without any intermediate stages. In side view the sex-chromosome is later than the autosomes, and undivided, goes to one pole. In no spindle observed is it ever left alone in the cytoplasm. During the primary and secondary spermatocyte periods, therefore, it seems that the division in itself goes on without a hitch. Besides several abnormal types of the gonad-cells as just described only one typical example of union of two daughter nuclei of the two adjacent secondary spermatocytes at the telophase can be seen (Fig. 9). In fact, it is difficult to distinguish exactly abnormal gonad-cells from the normal one, to enumerate them all in the cyst, and to give an adequate idea by a description throughout growth-, maturation-, and transformation-periods, but the text-figure makes them clear to a certain extent by plotting all data obtained (Fig. 20). As may be seen from the above figures, the total number of abnormal spermatids in the cyst considerably increases early in the spermatid stage just when the acrosome begins to come

respectively. All abnormal spermatids observed in the cyst are not shown. The details of the pilot-fibre and the tail are omitted even if spermatids have them. Other abnormal spermatids which are found out in the same cyst and not shown here, have, roughly speaking, the normal form except the large bulk of their nuclei. **17.** Abnormal immature spermatozoa and extremely deformed ones lacking tails in the same cyst. The greater part of the tail of the former is omitted. **18.** Anterior parts of three large-sized mature spermatozoa, and a grotesque one in the same cyst. **19.** An abnormal spermatozoön, having a rather thick tail and extruding from the head-part of the sperm-bundle.


10M
NN
13
15
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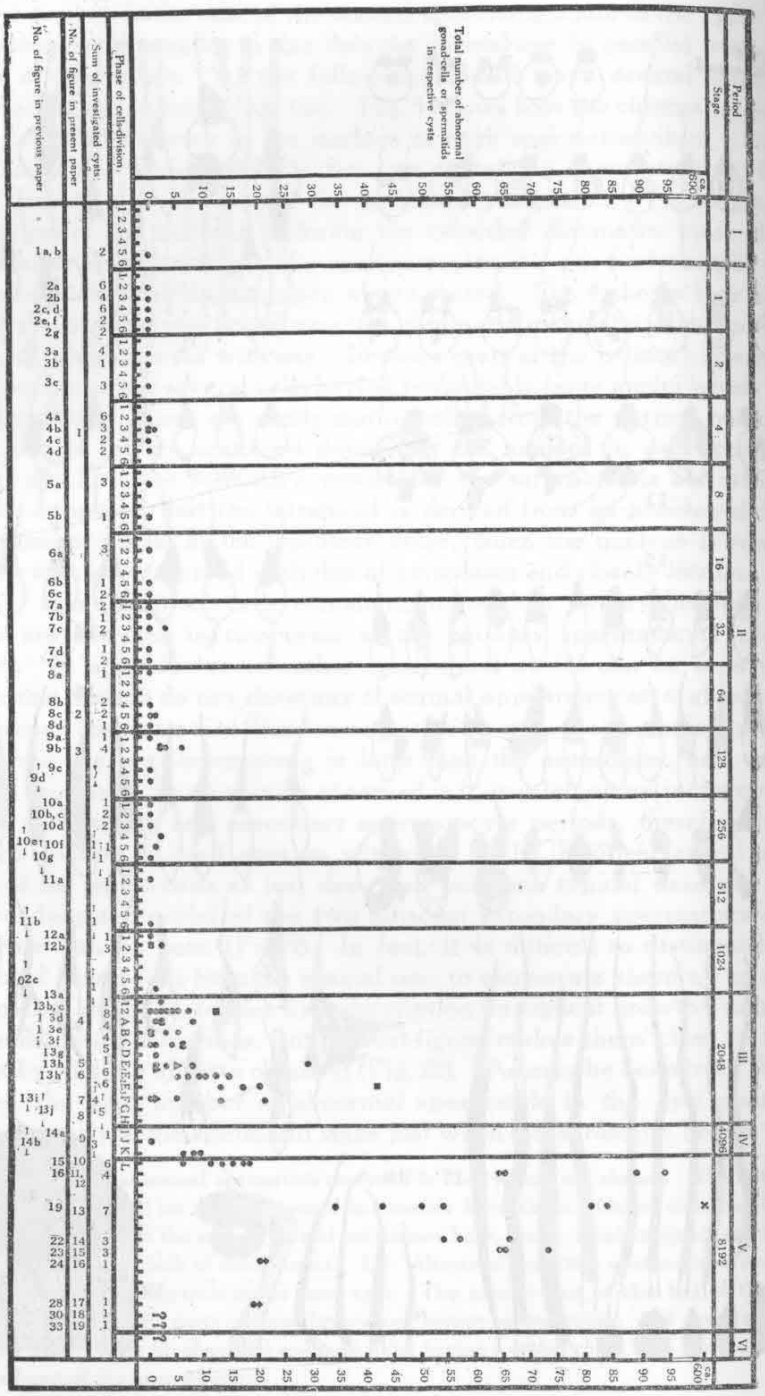
No. of figure in previous paper
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Sum of investigated cysts
Phase of cell-division
Total number of abnormal gonad-cells or spermatids in respective cysts
Period
Stage
I
II
III
IV
V
VI
1
2
4
8
16
32
64
128
256
512
1024
2048
4096
8192
ca. 600
ca. 620

in sight on the surface of the nuclear-wall. These abnormal spermatids fairly vary in size or in appearance with each other, most of them are spherical and easily distinguished by their large size from the normal spermatids (Fig. 11). At this stage, to enumerate the exact number of the acrosomes is sometimes difficult, because spermatids lie in the cyst in optional direction, and acrosomes are not yet so large as those at the later stages. Each common-sized spermatid has always one acrosome. It is sure, however, that there exist several large-sized spermatids having two or more acrosomes individually (Fig. 12). As the acrosome grows larger a good many abnormal spermatids having two or three acrosomes come to be detected more distinctly than before in the cyst. It seems probable that there exists no correlation between size of abnormal spermatid and number or bulk of acrosome as well as between mutual position of two or more acrosomes on an individual abnormal spermatid. As the nucleus which at first was round elongates the acrosome attains a considerable size and stains as if composed of solid chromatin, but both the tail and the "pilot-fibre"[1] elongated from the tip of the acrosome which has developed prior to the formation of the tail are very feebly stained. Fig. 13 is a drawing from a cyst showing remarkably abnormal shapes of the nuclei and the elongated acrosomes at the spermatid-period: some of these spermatids having three or four acrosomes protruded in optional direction, or having two in diametrically opposite directions. These abnormal spermatids do not show any tendency to degenerate in the cyst until the end of development, but continue to transform into more grotesque figures as may be seen in Figs. 14–16. At the stage when the normal immature spermatozoa are drawn toward the pole of the cyst by the pilot-fibres at a similar rate and begin to aggregate into bundles, some abnormal

[1] The term "pilot-fibre" is employed by the present author in the previous paper (2) to denote the long and fine thread stretched from the tip of the acrosome.

Fig. 20. Variation in total number of abnormal gonad-cells or spermatids observed respectively in an individual cyst in the cycle of the spermatogenesis of *Ictinus rapax*. Abscissae represent a serial arrangement of the developing stages which are shown by the theoretical total number of the cells in the cyst. Periods of the gonad-cells are as follows:— I, primary spermatogonium; II, secondary spermatogonia; III, primary spermatocytes; IV, secondary spermatocytes; V, spermatids or spermatozoa; VI, after expulsion of the sperm-bundles. Phases of the cell-division indicated are as follows:— 1, telophase; 2, resting stage; 3, leptotene stage; 4, diakinesis, 5, metaphase; 6, anaphase of the secondary spermatogonial period, respectively; A–F, various forms of the prophase arranged in order; G, metaphase; H, anaphase of the primary spermatocyte period; I, diakinesis, J, metaphase; K, anaphase; L, early diakinesis of the secondary spermatocyte period. ■, the total number of abnormal primary spermatocytes found in a big cyst having approximately four thousand cells in it, ▲, the same, but in a small cyst having about one thousand cells. ×, a cyst having abnormal spermatids extraordinarily as many as about six hundreds. ↑, in point of fact, the total number will be more numerous than the number based on the actual survey, because it is difficult to find out abnormal spermatozoa to the last one in the cyst in every respect.

immature spermatozoa is found on the way to the pole far behind the former. They extremely vary in size and form: a fairly thick, whip-shaped one with a thick tail proportionate to the size of the head; a deeply stained, thick stick-like one without a tail; a strongly stained conical one; and so on (Fig. 17). Toward the end of the transformation, the spermatozoa, both normal and abnormal, enormously decrease in length and aggregate into bundles in the reducing cyst. In every respect, therefore, it becomes more difficult than before to find out abnormal spermatozoa at their maturity (Figs. 18 and 19). By actual observation upon the central duct of the testis we can detect scarcely any abnormal or degenerating spermatozoön.

Conclusion

The present paper shows, first, that the "degenerating" nucleus found at the spermatogonial or spermatocyte stage may perhaps transform into an abnormal spermatid which has a deeply-stained chromatin network or mass. Second, that the acrosome autonomously grows and differentiates. Third, aside from the question concerning the cause of the extreme growth-rate of the nucleus, and of the deformation of the acrosome in these abnormal spermatids, they do not degenerate, but continue to transform as far as the terminus of the spermatogenesis. And fourth, the abnormality of the spermatid at the stage soon after the secondary maturation division is not mainly due to the fusion of the adjacent nuclei, but to the extreme growth-rate of an individual nucleus, because in either the primary or the secondary division, hardly any disturbance seems to occur as regards the behavior of the chromosomes.

Summary

1. In *Ictinus rapax* (RAMBUR) various abnormal gonad-cells are observed from the beginning of the secondary spermatogonial period to the time of expulsion of the spermatozoön from the cyst.

2. A considerable number of spermatids show extreme growth-rate of the nuclei and grotesque deformation of the acrosomes

Literature Cited

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2. Omura, T., 1952. Biol. Jour., Okayama Univ., 1, 103 – 146.