On the Spermatozoid of *Ginkgo biloba* *

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(With 2 Plates and 4 Text-figures)

It was in 1896 that Hirase for the first time observed living spermatozoids of *Ginkgo* coming out from the pollen tube. The study of living *Ginkgo* spermatozoids has been carried out in detail by Fujii (1898, 1899, 1900), Ikeno (1899), Miyake (1898, 1902) and Bessey (1901).

In his second paper, in 1899, Fujii gave a most valuable description of living spermatozoids and the methods by which they emerge from the pollen tubes in sugar solutions.

Forty years have already elapsed since the spermatozoids of *Ginkgo* were discovered, yet to the knowledge of the writer the references in the literature to the living spermatozoid of *Ginkgo* still remain those given above. It is the object of the present account to reexamine the morphology of spermatozoids and to describe some additional facts in regard to fertilization.

Description

Two mature spermatozoids before they start to swim are lying side by side within the common wall of the mother cell. In the present case the mother cell means Hirase's "Körperzelle" i.e. body cell, because the whole contents of the body cell is transformed into two spermatozoids (Text-fig. 1). The presence of this cell wall can be observed more definitely after the spermatozoids come out in the pollen tube from the mother cell as in Text-fig. 2. Fujii (1899) and Miyake (1898) have already described this cell wall. Miyake (1902) states "It is interesting to note that this wall have not been found either in *Cycas* or *Zamia*".

In *Dioon edule* and *Ceratozamia*, Chamberlain (1909, 1912) observes that two sperms are formed within the two sperm mother cells, resulting from the division of the body cell. In the case of *Ginkgo* there is no wall between the spermatozoids. This fact affords a proof that two spermatozoids are developed in a common old cell membrane of the mother cell.

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The nucleus which occupies the greater part of the whole body is more hyaline than the surrounding cytoplasmic portion which is granular in appearance. The spiral band makes the indentation which forms a deep helicoid furrow on the outside of the spermatozoid body and moreover the spiral band as a whole is largely pressing on the nucleus, showing that the latter is of a fluid nature (Text-fig. 1).

On a section of fixed and stained spermatozoid in the mother cell a similar observation may be clearly made (Pl. 13, Fig. 5). Hirase (1898) explains the indentation with his fixed material as follows "trois tours de spire qui sont séparés les uns des autres par des canaux". Thus the cytoplasmic sheath of the spiral band takes a compressed form when the spermatozoids are enclosed in the mother cell (Text-fig. 4). So

Text-figs. 1 and 2. The pollen tube of *Ginkgo* in 10% cane sugar solution: 1. Living spermatozoids in the mother cell. $\times 300$. 2. Spermatozoids swimming freely in the pollen tube. $\times 250$.

long as the two spermatozoids are still enclosed in the mother cell they can, by gently vibrating their cilia and moving the parts of their bodies, change their relative positions inside the mother cell.

When the spermatozoids come out into the wider chamber of the pollen tube, breaking the wall of the mother cell, all of the cilia are soon vibrating so rapidly that they can hardly be seen.

They rotate on the long axis of their body (around an axis passing through the apex of the helicoid spiral) move forward with the spiral band leading, pull apart from each other and twist up (Text-fig. 2, Pl. 13, Fig. 1). In Text-fig. 2 the spiral bands of two spermatozoids are shown turning toward the observer. Close to the spermatozoid on the left hand side a little spherical body is seen. That is a part of cytoplasm which was separated from the spermatozoid as a result of injury. Such a cytoplasmic portion

usually takes on a spherical shape showing that it is of a liquid nature. Fujii (1900) noticed that the injured spermatozoid left the

Text-fig. 3. Mature spermatozoid while swimming freely. $\times 600$.

cytoplasmic portion, and that then such an injured spermatozoid continued to swim as if it had been uninjured. He compared this phenomenon with the case of the lower type of organism which has an undifferentiated organ, or with the spermatozooids of ferns which often leave the cytoplasmic fragment. In the present case a similar phenomenon is indicated, for the injured spermatozoid was able to swim for nearly half an hour.

The form of spermatozooids when swimming free in cane sugar solution (10%) is somewhat like the shape of the fruit of an egg-plant or the mature *Ginkgo* ovule. In Text-fig. 3 there is shown a shape which is

more spherical than that in the figures of the previous investigation. According to Miyake "the size seems to vary from about $110\ \mu$ to $80\ \mu$ in length, and from $50\ \mu$ to $85\ \mu$ in width". That of Text-fig. 3 is about $90\ \mu \times 72\ \mu$ in size. The spiral band which is compressed on the apex of the body when it is enclosed in the mother cell wall, now expands showing a somewhat cone-like shape. The width of the spiral band is distinctly broadest at the apical end and gradually decreases, finally fading out entirely at the open end (Text-figs. 3, 4). Viewed from the head or apex of the spiral, the direction of the turns of the spiral band is left to right that is to say in the direction of the hands of a clock (Pl. 13, Figs. 1, 4). A total of two and a half turns seems to be a correct estimate. On the external margin of the spiral band there exists a well stained

Text-fig. 4. Diagrams of spiral band of the spermatozoid. a. Compressed form which is seen while the spermatozoid is enclosed in the mother cell wall. b. Expanded spiral band which is seen when the spermatozoid is swimming freely.

line (fixed and stained with Heidenhain's iron-haematoxylin), from which numerous cilia are developed (Pl. 13, Figs. 4, 5, 6).

The nucleus of the swimming spermatozoid is transparent and occupies a larger portion of the whole body. The surrounding cytoplasmic part is more granular and contains an oil-like globule of unknown nature. With regards to this globule Hirase (1898 p. 130) states this is a nutritive substance which will be brought into the egg cell. Herzfeld (1927 p. 826) names it as „extranukleären Nukleolen“ stating „Die ausgestossenen extranukleären Nukleolen sowie ihre Umgebung scheinen Exkretionen des spermatogenen Kerns zu sein, die dem Untergang geweiht sind“ Schnarf (1933) throws doubt upon the reason as to why two globules appear, occupying a strictly symmetrical position on both sides of the nucleus of the body cell, and why after the nuclear division each of them is transferred into a spermatozoid. The function of this globule is as yet unknown.

With the exertion of a slight pressure, the body of the spermatozoid may change its form, but being soft and very elastic, it recovers its shape once the pressure is removed. Fujii (1899) gives the following description as regards the methods by which the spermatozooids get out of the pollen tube, namely; in general there are two methods, (a) gradual egress, and (b) sudden egress and the factor chiefly responsible for the occurrence of one method or the other is the density of the surrounding fluid.

(a) Mode of gradual egress. In Fujii's Fig. 4 an example is shown, where the head of the spermatozoid appeared a little way out of the pollen tube and according to his description the spermatozoid emerged entirely from the tube after five minutes and recovered its normal shape.

Exactly similar behavior of the spermatozoid has fortunately been observed by the writer. In Fig. 2 the whole of the spiral band has already resumed its normal form, and the cilia are vibrating very vigorously, while the main part of the body still remains inside the tube so that the whole body takes on an appearance like that of a silkworm cocoon. Once the body was definitely escaped it soon takes on a normal shape, the constriction in the middle part of the body being no longer visible (Fig. 3). From this observation it can be seen how soft and elastic the body is. Another remarkable example of a gradual egress of the spermatozoid is demonstrated with photographs in Pl. 14, Figs. 10–15. In this case the base or the side of the spermatozoid body stretches considerably so that it slips through the narrow opening out of the tube. It is noteworthy that under such conditions the spiral band as a whole gives the impression of being solid, retaining its normal shape, while the rest of the body takes on

the appearance of a liquid apparently enclosed in a thin plasma membrane. The cilia vibrate regardless of however the body of the spermatozoid may be deformed (Fig. 10). It may in any case be recognized that the cytoplasmic sheath which constitutes the spiral band has different physical properties, if one compares it with the cytoplasm surrounding the nucleus. One side of the spiral band turns toward the outside of the tube, and the spherical part of the body increases in size (Fig. 11). Then the spiral band as a whole appears out of the tube extending somewhat while retaining its original form, and one end still remains in the tube while the other side contacts with the spherical shaped body. By comparing such a deformed spermatozoid with another spermatozoid swimming in the pollen tube, it can be easily understood how flexible the whole body is and how eminently it is fitted for its difficult task of finding and swimming through the narrow passage between the neck cells of the archegonia (Fig. 12). When the spiral band comes away from the wall of the pollen tube, the spermatozoid completely recovers its normal form while the vibration of the cilia continues very actively (Fig. 13). By continuous rotation of the body at a constant speed the spermatozooids roll around, first here, then there like a large infusoria (Figs. 14, 15).

(b) Mode of sudden egress. According to Fujii (1899) in this case the membrane of the mother cell containing the two spermatozooids is suddenly thrust out of the pollen tube. At first the shape of the spermatozoid is very irregular because of the surrounding pressure produced by sudden protrusion, but gradually it separates from the mother cell and finally takes on its complete form, like the fruit of an egg plant. Meanwhile it gently begins to swim at a definite speed. This fact also shows how soft and elastic it is. The writer has twice been able to observe such a mode of escape, where the spermatozoid which is irregularly shaped soon recovers its complete form as described by Fujii.

When the spermatozooids are discharged in the archegonial chamber it is not hard to suppose that they swim to the neck cell and continue to gradually revolve around and around. The entire spermatozoid swims into the receptive cavity, the existence of which is maintained by Hirase (1918) and the writer (1928), passing between the ruptured neck cells. The entrance of the receptive cavity is very small and narrow compared with the size of the spermatozoid, and the latter must be greatly stretched out so that it assumes a very long, slender form, during its passage through this entrance. The spermatozoid has need of all the elasticity possible, as is indicated also in the case of gradual escape from the pollen tube. It is certain

that the entire spermatozoid is capable of passing without injury through the narrow entrance canal between the neck cells into the receptive cavity, as the spermatozoid of normal shape has been found in many instances in the receptive cavity.

Frequently two spermatozooids are found in the receptive cavity of which only one takes part in fertilization while the other may be found presenting a normal appearance or in some stage of disintegration. So far as the cases observed are concerned, the head or apical end of the spermatozoid which reaches the egg cytoplasm becomes attached to the latter. The escape of the nucleus from the body is shown in Pl. 14, Figs. 7-9. The male nucleus slips out of its cytoplasmic sheath and passes on alone through the egg cytoplasm to the female nucleus. The ciliferous band includes a well stained line, which as already stated remains near the boundary of the egg cytoplasm. Thus the function of the spiral band of the spermatozoid certainly ends with the transporting of the male nucleus from the pollen tube to the egg cell. The cilia and cytoplasm remain meanwhile as the residual substance, which ultimately disappears.

Summary

1. The whole contents of the body cell of *Ginkgo* is transformed into two spermatozooids. Between the two spermatozooids there is no cell wall when they are formed, however they are surrounded by a common wall.

2. So long as the spermatozooids are still enclosed in the mother cell the cytoplasmic sheath of the spiral band takes on a compressed form.

3. The swimming spermatozoid has an expanded spiral band, which is distinctly broadest in width at the apical end and gradually decreases finally fading out entirely.

4. Several methods by which the spermatozooids get out of the pollen tube in sugar solutions are described.

5. The body of the spermatozoid is soft and elastic, so that as a result of a slight pressure it may change its form, but it recovers its shape again after the pressure is removed.

6. The cytoplasmic sheath of the spiral band only may be of a comparatively solid nature, whereas the remaining parts of the body, which are surrounded by the plasma membrane are of a highly liquid nature.

7. The entire spermatozoid is found in the receptive cavity. This fact proves that the spermatozoid passes without injury through the narrow opening or canal between the neck cells.

8. The male nucleus slips out of its cytoplasmic sheath, then travels on alone and fuses with the egg nucleus, while the cilia bearing cytoplasmic sheath is left near the boundary of the egg cytoplasm.

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Explanation of Plates

All figures are photomicrographs taken with Agfa Isochrom plates by using Wratten B filter. For photomicrographs 1-3, 10-15 Leitz achromatic objective 4 and Zeiss comp. ocular 10 were used. For photomicrographs 4-6 Leitz oil imm. objective 1/12 and Zeiss comp. ocular 8 were used. For photomicrographs 7-9 Leitz oil imm. objective 1/12 and periplane ocular 4 were used. Figs. 4-9. The preparations were fixed with Bouin's fluid and stained with Heidenhain's iron-alum haematoxylin.

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Plate 13

- Fig. 1. Mature spermatozooids swimming in the pollen tube.
Fig. 2. A spermatozoid is just escaping through a narrow opening out of the pollen tube.
Fig. 3. The same spermatozoid as in Fig. 2. It recovers the body shape at the outside of the pollen tube.
Fig. 4. Apical end of the spiral band viewed from the head.
Figs. 5-6. Two oblique sections of the spermatozoid which is still enclosed in the mother cell, showing the compressed spiral band on the apex of the body.
Figs. 7-9. Three consecutive sections of the spermatozoid in the receptive cavity. The apex of the spermatozoid touches the egg cytoplasm. The nucleus slips out of the cytoplasmic sheath, while the spiral band is left behind.

Plate 14

- Figs. 10-15. Series of the behaviour of one spermatozoid which is escaping out of the pollen tube, showing how the spermatozoid body is stretched and deformed and how it recovers the normal shape. In Fig. 12 (a lower magnification than others) one of the two spermatozooids is seen outside and the other still inside the pollen tube.