Studies in the Cytology of Pteridophyta

IX. On the Nature of the "Border-brim".¹⁾

By

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In Pteridophyta the spermatogenesis has been studied by BELAJEFF (1898) in *Gymnogramme* and *Equisetum*, by ARNOLDI (1910) in *Salvinia*, by SHAW (1898) in *Onoclea* and *Marsilia*, by CAMPBELL (1907) in *Ophioglossum*, by YAMANOUCHI (1908) in *Nephrodium*, by SHARP (1912, 1914) in *Equisetum* and *Marsilia* and by ALLEN (1914) in *Adiantum* and *Aspidium*. According to them a spherical blepharoplast appears in the spermatid at first (laying aside the question of the homology of centrosome and blepharoplast), elongates along the surface of the nucleus and at last coalesces with the nucleus, bearing many cilia.

In various plants it has been recognized by many authors that the blepharoplast originates from the centrosome. In Pteridophyta the homology of the blepharoplast and the centrosome has been asserted by BELAJEFF (1898) in *Gymnogramme sulphurea* and *Equisetum arvense* and by SHARP (1912, 1914) in *Equisetum arvense* and *Marsilia quadrifolia*, but the origin of the centrosome has not been made clear. IKENO, however, has stated in 1903 that in the spermatogenesis of *Marchantia polymorpha* the centrosome comes out of the nucleus of the spermatogenous cell. On the other hand, in *Oedogonium*, *Vaucheria* and *Cladophora* (STRASBURGER 1892), *Chara* (BELAJEFF 1894, MOTTIER 1894) and *Isoetes* (SHIBATA 1902) the blepharoplast has been thought to differentiate from the cytoplasm of the spermatid, while in *Dioon* (CHAMBERLAIN 1909), *Derbesia* (DAVIS 1908), *Polytrichum* (J. & W. VAN LEEWEN-REIJNVAAN 1907), *Mnium* (J. & W. VAN LEEWEN-REIJNVAAN), *Anthroceros* (BAGHEE 1924), *Mnium* (WILSON 1911) and *Atrichum* (WILSON 1911) it has been supposed to originate from the nucleolus (according to the classification of YAZAWA 1931).

As mentioned above, in Eufilicineae, Marsiliaceae and Equisetaceae the blepharoplast was thought to have a centrosomal origin, while in *Isoetes* a

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cytoplasmic. In Salviniaceae, Ophioglossales, Lycopodiales and Psilotales the origin of the blepharoplast has not yet been clarified.

The writer (1932, 1933a, 1933b, 1933c, 1934, 1935), however, has observed that in Pteridophyta the true cilia-bearing portion is not the so-called blepharoplast which has long been thought to be the cilia-bearer, but the "cilia-bearing band" (Figs. 1, 2, 4, *cb*) ["cilia-bearing portion" (Fig. 3, *cbp*) in the case of *Isoetes japonica* AL. BR.]. The same structure of the spermatozoid has been also shown by ROGERS (1926/27), MÜHLDOERF (1930), and DRACINSCHI (1930, 1931). The completed blepharoplast which was thought to be the cilia-bearing portion by various authors has been designated as the "border-brim" (Figs. 1, 2, 3, 4, *b*) by the writer (1932). According to the writer's opinion (1934) all of the "border-brim", "cilia-bearing band" and "lateral bar" (Fig. 1, *l*) develop from the spherical blepharoplast of the early stage and they must be generally designated

Figs. 1-4. The spermatozooids of Pteridophyta, somewhat schematically drawn.

Fig. 1. A species of Eufilicineae. Fig. 2. *Equisetum arvense* L.

Fig. 3. *Isoetes japonica* AL. BR. Fig. 4. *Salvinia natans* HOFFM.

b, border-brim; *c*, cilium; *cb*, cilia-bearing band; *cbp*, cilia-bearing portion; *n*, nucleus; *l*, lateral bar; *p*, cytoplasmic band.

as the blepharoplast. The blepharoplast means the body which develops the cilia, but usually the completed cilia-bearer is also included in this term.

To make clear the origin of the blepharoplast, it is one of the important and necessary ways to know the nature of the blepharoplast (including "border-brim", "lateral bar" and "cilia-bearing band"). In the present paper the nature of the blepharoplast (especially the "border-brim") has been studied in the spermatozooids of *Matteuccia orientalis* TREVIR, *Adiantum capillus-veneris* L., *Pteris cretica* L. var. *albo-lineata* HK. and *Isoetes japonica* AL. BR.. The dried preparations were stained with various nucleus- or cytoplasm-staining dyes and tested by the microchemical method. In making the dried preparations the hanging drop containing spermatozooids were evaporated on the slide either without fixing or after fixing with absolute alcohol or over osmic fumes (for a few minutes).

**In the cases of *Matteuccia orientalis* TREVIR, *Adiantum capillus-veneris* L. and *Pteris cretica* L.
var. *albo-lineata* HK.**

When stained with the nucleus-staining dyes both the nucleus and the "border-brim" of the spermatozoid show almost the same staining reaction. They swell in a certain extent by the action of acetic acid. The result of staining with the nucleus-staining dyes are shown as follows (++++, deeply stained; +++, somewhat deeply stained; ++, lightly stained; +, very lightly stained; —, unstained):

Cytoplasm-staining dyes.	Nucleus.	Border-brim.	Cilia-bearing band.	Cilia.
Haematoxylin	++++	++++	+ or —	+ or —
Aqueous solution of gentian violet	++++	++++	+ or —	+ or —
Carm-alum	++++	++++	—	—
Safranin solved in aniline water	++++	++++	++	++
Alcoholic (95%) solution of safranin	++++	++++	++	++
Anthracenblue (chrom-alum)	++++	++++	—	—
Alizarineyanin (chrom-alum)	++++	++++	—	—
Alizarinblue (chrom-alum)	++++	++++	—	—
Aceto-carmin	++++	++++	++	++
Methylgreen solved in 45% acetic acid	++++	++++	++	++
Double staining with safranin and light green	red	red	blue	blue
FLEMMING triple staining	red	red	light yellow	light yellow
EHRlich triacid staining	blue	blue	light red	light red

With the cytoplasm-staining dyes both the nucleus and the "border-brim" as well as the "cilia-bearing band" and cilia are stained almost homogeneously.

Cytoplasm-staining dyes.	Nucleus.	Border-brim.	Cilia-bearing band.	Cilia.
Aqueous solution of fuchsin (acid)	+++	+++	++	++
Alcoholic solution of light green	++	++	++	+
Aqueous solution of orange G.	++	++	++	++
Eosin	+	+	+	+
Aqueous solution of aniline blue	+++	+++	++	++

Judging from the results of the above mentioned staining reactions the "border-brim" seems to have some resemblance in its nature to the nucleus of the spermatozoid, but the former is negative to FEULGEN's nucleal-staining reaction, while the latter is positive. Both the nucleus and the "border-brim" stain lightly red when stained with Sudan III (0.1 gr. Sudan III solved in 20 cc. of 95% alcohol). They stain black red, showing the containing of lipoids, while the cilia and the "cilia-bearing band" almost remain unstained when treated with Sudan III and haematoxylin. BULET's and xanthoproteic reactions were proved to be negative to both nucleus and "border-brim," while the MILLON's reaction was positive. From these reactions both the nucleus and the "border-brim" are thought to contain lipoids and proteins.

There is, however, a question whether the "border-brim" is homologous with any one of the starch-grain, plastid or chondriome. The starch-grain, however, is enveloped by the plastid-hull and exists in this state in the cytoplasm of the spermatid throughout the course of spermatoteleosis. So the homology between the "border-brim" and the starch grain or plastid must be denied. The "border-brim" does not solve in an alkaline solution and is not stained in vital with a solution of janus green. So the "border-brim" is thought not to be homologous with the chondriome. In fact, EMBERGER (1922) traced the chondriome during the course of spermatogenesis in *Adiantum capillus-veneris* L., showing no relation with the "border-brim".

The cilia and the "cilia-bearing band" are cytoplasmic, though they show some differences in their staining reactions. As stated in the previous paper (1934) these two portions develop secondarily from the blepharoplast.

The test was also made to the "border-brim" in the spermatozoid of *Isoetes japonica* AL. BR., which was thought to have a cytoplasmic origin.

In the case of *Isoetes japonica* AL. BR.

The staining reactions are as follows:

Nucleus-staining dyes.	Nucleus.	Border-brim.	Cilia-bearing portion.	Cytoplasmic band.	Cilia.
Haematoxylin	++++	++++	+ or —	+ or —	+ or —
Aqueous solution of gentian violet	++++	++++	+ or —	+ or —	+
Safranin in aniline water	++++	+++	++	++	++
Aqueous solution of safranin	++++	+++	++	++	++
Alcoholic (95%) solution of safranin	++++	+++	++	++	++
Anthracenblue (chrom-alum)	+++	++	—	—	—
Alizarineyanin (chrom-alum)	+++	++	—	—	—
Alizarinblue (chrom-alum)	+++	++	+ or —	+ or —	+ or —
Aceto-carmine	++++	+++	++	++	++
Methylgreen solved in 45% acetic acid	++	—	—	—	—
Double staining with safranin and light green	red	reddish blue	light blue or unstained	reddish blue	light blue
Double staining with fuchsin and iodine green	green	violet	light red or unstained	violet	light red
EHRLICH triacid staining	blue	violet	light red or unstained	violet	light red

Cytoplasm-staining dyes.	Nucleus.	Border-brim.	Cilia-bearing portion.	Cytoplasmic band.	Cilia.
Aqueous solution of fuchsin	—	+++	—	+++	+
Aqueous solution of fuchsin with drops of 45% acetic acid	++	+++	—	+++	++
Eosin	+++	+++	++	++	++
Aqueous solution of orange G.	++	++	++	+++	++
Aqueous solution of aniline blue	—	+++	—	+++	+
Alcoholic (75%) solution of light green	++	++	++	++	++

FEULGEN's nucleal-staining	Nucleus.	Border-brim.	Cilia-bearing portion.	Cytoplasmic band.	Cilia.
	+	—	—	—	—

Judging from the staining reactions, the “border-brim” in the spermatozoid of *Isoetes japonica* AL. BR. resembles in its nature the nucleus though they also show some resemblance to the cytoplasm. Stained with Sudan III the “border-brim” shows the reaction of lipoids. BUIRET's,

xanthoproteic and MILLON's reactions are negative in the case of the "border-brim".

When the spermatozoid is dried up, the cilia are destructed, and the nucleus is deformed, while the "border-brim" remains without change. With 40% NaOH most of the spermatozoid-body is destructed, while the "border-brim" remains as before. The spermatozoid-body shows no change with 33% HNO₃, but it solves completely when boiled with the latter. The "border-brim" is fixed and swells in some extent with 45% acetic acid. It is also fixed with alcohol, iodine solution, potassium iodide iodine solution, formalin (2%), osmic acid or potassium bichromate solution. When stained with ALTMANN's method the nucleus and the "border-brim" are black red, while the cilia, "cilia-bearing portion" and the "cytoplasmic band" are red. From these results the "border-brim" seems to have some resemblance in its nature to the chondriome, but it is not stained in vital with a solution of janus green.

During the spermatogenesis of *Isoetes japonica* AL. BR. only the nucleus is positive to FEULGEN's nucleal reaction and the "border-brim" is quite negative to this reaction (Figs. 5-12).

Figs. 5-12. Spermatogenesis in *Isoetes japonica* AL. BR. studied by the FEULGEN's nucleal-staining method. Only the nucleus is positive. The nuclei of spermatogenous cells and spermatids are shown to be black and those of other cells stippled.

From the results mentioned above it is to be concluded that the "border-brim" shows some resemblance in its nature to the nucleus, but

contains no thymusnucleic acid in the spermatozooids of *Matteuccia orientalis* TREVIR, *Adiantum capillus-veneris* L. and *Pteris cretica* L. var. *albo-lineata* Hk. (perhaps all the species of Eufilicineae). It may have some relation with the nucleus in its origin, but no direct relation with the chromatin. The "cilia-bearing band" and the "lateral bar" differentiate secondarily from the blebharoplast in the early stage. The cilia develop on one side of the surface of the "cilia-bearing band". In the spermatozoid of *Isoetes japonica* AL. BR. the "border-brim" shows the nuclear nature though it does not contain thymusnucleic acid, but on the other hand it is thought to have some resemblance to the cytoplasm.

In both cases the "border-brim" has the rigidity in its nature.

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