Cytological and Morphological Studies on the Gametophytes of Ferns XI. The Influences of Neutral Salts and Hydrogen Ion on the Duration of Life of the Fern-Spermatozoid

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伊倉伊三美*: シダ類の配偶体に関する細胞学的並びに形態学的研究 XI.
シダ類精子の生存時間に及ぼす中性塩及び水素イオンの影響

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The chemotaxis of spermatozoids was studied by Shibata (1902, 1905, 1911) in *Salvinia* and some ferns, and recently by Rothschild (1952) in *Pteridium*. Fujii and Asahina (1952) investigated the discharges of the *Isoetes*-spermatozoids caused through the influences of ethyl ether and other chemicals. According to Du Buy and Nuernbergk (1938), several authors (Buller 1900, Lidforss 1905, Bruchmann 1909) had studied the chemotaxis of fern-spermatozoid caused by the different chemicals, especially the salts of organic acids.

Concerning the influences of neutral salts or hydrogen ion concentrations, Yamaha (1946) carried out the experiments on the vital staining of various cells of several plants and Igura (1954) on the vital staining of fern-prothallia. However, their influences on the duration of life of the spermatozoids are presumed to be significant and important in the physiology of fertilization in plants. From this viewpoint the present work was undertaken.

Materials and Methods

The spermatozoids of the following ferns were used as materials: *Athyrium melanolepis* Christ, *Equisetum arvense* L., *Osmunda japonica* Thunberg, *Plagiogyria Matsumureana* Makino, *Athyrium pycnosorum* Christ, *Matteuccia struthiopteris* Todaro, and *Osmunda cinnamomea* L. var. *asiatica* Fernald. The former four species were mainly applied to the test of the neutral salts and the latter three to those of the hydrogen ions. For the purpose of confirming the effects of cations on the duration of life of the spermatozoid, the aqueous solutions of chlorides such as KCl, NaCl, BaCl₂, CaCl₂, MgCl₂, SrCl₂, AlCl₃, FeCl₃ were used as the media, whose concentrations were made in the various grades from 5×10^{-3} to 0.08 M chiefly at the intervals of 0.02 M. The buffer-solutions which were held in the hard glass bottles,

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were prepared according to McIlvaine's or Kolthoff's table and their pH-values were from 2.2 to 9.0 at the intervals of 0.2. The concentrations of the buffer-solution were made to be various grades (the ions contained in these buffer-solutions were considered to be neglected). The redistilled water was used in all the cases. The microscopic observation was made under the illumination of the light source whose candle power was about 1200 LX, and the temperatures of room and water in the experiment were kept to be 20° and 18° respectively.

The paraffin was set at the both sides of a drop of medium on the slide glass, in which the prothallia were dipped and the cover glass was put on the drop and the paraffin and the border of cover glass was sealed with paraffin. According to this method, the concentration of the medium on the slide glass was able to be kept constantly for a long time during the observation. Thus the preparation was observed under the phase contrast microscope. In the solutions used as the media, the duration of movement of the spermatozoid which was extruded out of the spermatid was measured by a stop watch and the duration of movement was regarded as the life-time of spermatozoid (Yuasa 1933, 1938, Igura 1950)¹⁾. Namely, three to five minutes after the spermatozooids swam out of the spermatids, the prothallia were removed and the mean value of the longest time was obtained, during which the spermatozooids began to swim and all spermatozooids stopped their movements.

Results

It is not easy, in fact, to ascertain the stop of movement by tracing only one spermatozoid and it is doubtful whether the stop of movement means the death of spermatozoid or not. That is, even after the stop of movement, the spermatozoid may continue to live. As stated before, however, it was regarded that the duration of movement means the duration of life of the spermatozoid in the present study. On the other hand, in the study of the duration of life of the spermatozoid, the various conditions of the medium, in which the spermatozoid lives, e. g. acidity, osmotic pressure, concentration of ions, contents of various gases, temperature, light and others, must be considered (Yuasa 1937). Besides these habitat factors, the individual variation of the duration of life is also important, even in the same habitat-factors.

The results of observations concerning the duration of life of the spermatozoid are given in the following Table 1.

In all the media, the more the concentration of the solution increases, the more the duration of life decreases. Thus, the limit concentrations of solutions used as the media, in which the spermatozooids are able to live, are summarised as Table 2.

1) As stated later, it is difficult to determine precisely the duration of life, but usually the duration of movement means the duration of life.

Table 1. The duration (minutes) of life of the spermatozoid in the media of the neutral salts. (Room temperature, 20°; water temperature, 18°)

<div>M \ Salt</div>	KCl [*]	NaCl [^]	BaCl ₂ ^Δ	CaCl ₂ [○]	MgCl ₂ ^Δ	SrCl ₂ ^Δ	AlCl ₃ ^Δ	FeCl ₃ ^Δ
5 × 10 ⁻³	31-36	30-35	6	53-58	40-43	35-40	50-55	4-5
0.01	30-35	22-27	×	25-30	28-32	29-32	37-42	×
0.02	18-20	11-16	×	9	6	8	8-13	×
0.04	15-20	5-10	×	2	3	4	×	×
0.06	10-15	×	×	×	×	×	×	×
0.08	×	×	×	×	×	×	×	×
Exp.	I [*]	II ^Δ	III ^Δ	IV [○]	V ^Δ	VI ^Δ	VII ^Δ	VIII ^Δ
Redistilled H ₂ O	92-97	66-70	68-73	122-127	108-110	95-100	74-78	65-70

^{*}, *Equisetum arvense* L.; ○, *Plagiogyria Matsumureana* Makino; Δ, *Athyrium melanolepis* Christ; ×, the spermatozoids are immobile; Exp., experiment.

Considering the influences of the neutral salts on the duration of life of the spermatozoid shown in Table 2, it is found that the limit concentration of solution is highest in the case of KCl and lowest in that of FeCl₃, and the more the ion-value of the solution becomes high, the less the limit of concentration; i. e. the series of ion (cation) is as follows, K⁺, Na⁺>Ca⁺⁺, Mg⁺⁺, Sr⁺⁺, >Al⁺⁺⁺>Fe⁺⁺⁺.

The manners of the movements of spermatozoids in the solutions whose concentrations are lower than the limit one are same in the cases of the observations by Yuasa (1933) and Igura (1950) which were made in the tap water. When the concentration of solution becomes higher than the limit one, the spermatids tend to discharge out of the antheridium as small masses. Though they scatter gradually, the spermatozoids do not swim out of the spermatids. Even if the spermatozoid swims out of the spermatid in the solution whose concentration is high, its number is small, its movement is slow in general, and the duration of movement is short. Even when the movement is

Table 2. The limit concentrations of the solutions of neutral salts within which the spermatozoids are able to live (*Athyrium melanolepis* Christ). (Room temperature, 20°; water temperature 18°)

—, the spermatozoid is immobile; ±, the small number of spermatozoid move for a short time; +, the spermatozoids move.

active, it suddenly loses its velocity and stops, and sometimes it makes a rotatory movement in a right-handed direction or often in a left-handed.

As to the influences of the hydrogen ion on the duration of life of the spermatozoid, the results shown in the following Table 3 are obtained.

Table 3. The influence of the hydrogen ion concentration on the duration of life of the spermatozoid. (Room temperature, 20°; water temperature, 18°).

The figures (except those of pH-values) in the table represent the duration-times (minutes) within which the spermatozoids are able to live.

<div>pH-value \ M</div>	McIlvaine-buffer: <i>Matteuccia struthiopteris</i> Todaro						Kolthoff-buffer: <i>Osmunda cinnamomea</i> L. var. <i>asiatica</i> Fernald					
	0.1	0.05	0.025	0.01	5×10^{-3}	1×10^{-3}	0.1	0.05	0.025	0.01	5×10^{-3}	1×10^{-3}
9.0	×△	×△	▲10	15	20	30	×△	×△	×	10	15	20
8.2	×	×	15	20	40	50	×	×	10	20	35	50
7.2	×	×	15	30	30	45	×	▲10	15	20	35	40
6.0	×	×	▲10	20	20	35	×	×	▲15	20	20	40
5.2	×△	×	▲10	15	15	30	×△	×	▲10	10	20	25
4.2	×△	×△	▲10	15	15	20	×△	×△	×△	▲10	15	20
3.0	×△	×△	×△	▲10	▲10	△10	×△	×△	×△	▲10	▲10	▲15
2.2	×△	×△	×△	×△	×△	▲10	×△	×△	×△	▲10	▲10	▲10

×, the antheridium discharges but the spermatozoid does not swim out of the spermatid; △, the number of the discharged antheridia is small; ▲, the number of spermatozoids which move is small.

Fig. 1. The antheridia and spermatozooids of *Matteuccia struthiopteris* Todaro under the negative high objective of the phase contrast microscope.

A. Antheridia in which the spermatozooids (a) are included (Upper view). B. Spermatozooids in the solution of 1×10^{-3} M McIlvaine-buffer whose pH-value is 8.2; 45 minutes elapsed after they extruded out of the antheridia; some of them swam and some stopped their movements.

The duration of life of the spermatozoid is longest in both 1×10^{-3} M McIlvaine's and Kolthoff's buffer-solutions whose pH-values are 8.2 in both cases, and the mean value is 49 minutes (Fig. 1). The test in the tap water which was carried out at the same time showed that the duration of life is 39 minutes which is shorter than that of the former. The duration of life is fairly longer in 1×10^{-3} M buffer-solu-

tion whose pH-value is 5.2-9.0, but longer at pH 6.0-8.2. It is found that the near value of pH 8.2, that is, the alkaline side is optimum for the life of spermatozoid,

The smaller the pH-value becomes, the shorter the duration of life becomes gradually, and the number of spermatozooids which are able to move is small. In other words, the hydrogen ion is presumed to have an injurious effect on the life of spermatozoid. The limit concentration of the buffer-solution related to the movement of spermatozoid was found to be in the near value of 0.025 M. The smaller the concentration becomes than this value, the more suitable it becomes for the spermatozoid to live. The state of the movement of spermatozoid resembles the case of the neutral salts.

The duration of life of the spermatozoid is influenced, as above-mentioned, by the habitat-factors, but even in the same factors, it shows the individual variation. The results obtained

Fig. 2. The individual variation of the duration of life in 234 spermatozooids of *Athyrium pycnosorum*, Christ (Room temperature, 20°; water temperature, 18°; 1×10^{-3} M Kolthoff-buffer, pH 8.2).

Table 2. The limit concentrations of the solutions of neutral salts within which the spermatozooids are able to live (*Athyrium melanolepis* Christ).

(Room temperature, 20°; water temperature, 18°)															
Duration (minutes)	15	23	35	38	40	43	48	53	68	75	83	88	93	96	Total
Frequency	2	2	4	9	10	15	21	55	42	30	22	14	6	2	234

in regard to the variation in the duration of life of the spermatozoid (*Athyrium pycnosorum* Christ) in the solution of 1×10^{-3} M Kolthoff-buffer (pH 8.2) which showed the longest duration, are given in Fig. 2.

Considerations

The life of spermatozoid is influenced by the ion-value (cation) in the neutral salts in inverse proportion to the largeness of the ion-value, and the hydrogen ion disturbs the life of spermatozoid, therefore, the spermatozoid is able to continue its life for a longer time in the medium whose pH-value is large, namely whose acidity is in the alkaline side. The studies concerning the chemotaxis of spermatozoid by Shibata (1902, 1905, 1911), Rothschild (1952) and other authors were carried out in consideration of the hydrogen ion in the medium, by which the taxis occurs. In writer's opinion, however, when the spermatozooids extrude out of the spermatids and swim about in the medium on the prothallia, it is favorable that the ion-value of cation in the medium is small and its acidity is alkaline. Moreover, when the spermatozoid arrives at the archegonium through the medium and approaches to the egg-cell, the same conditions must be kept. In this way, it is

significant and important from a standpoint of the physiology of fertilization to know the influences of the neutral salts and the hydrogen ion on the duration of life of the spermatozoid.

Summary

Studying the influences of the aqueous solution of KCl and other seven kinds of the neutral salts and the hydrogen ion on the duration of life of the spermatozoid of *Athyrium melanolepis* Christ and other six species of Pteridophyta, the following facts were known.

1. The duration of life of the spermatozoid is effected by the cation and the more the ion-value of cation increases, the more the limit concentration of the solution in which the spermatozoid lives decrease gradually.

2. The hydrogen ion disturbs the duration of life of the spermatozoid. The medium whose acidity is alkaline (pH 8.2) is most favorable for the duration of life of the spermatozoid.

3. In fertilization, when the spermatozoids approach to the archegonium and enter in it and at last reach the egg-cell, it is favorable that the ion-value of cation in the medium is small and the acidity of medium is alkaline, in which the spermatozoids move on. This fact is significant and important from the viewpoint of the physiology of fertilization.

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