Cytological and Morphological Studies on the Gametophytes of Ferns XII. Suction Force of the Wall Cell of Antheridium and of the Spermatid

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伊倉伊三美*: シダ類の配偶体に関する細胞学的ならびに形態学的研究 XII.
造精器壁細胞および精細胞の吸水力

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Introduction

As regards the mechanism of the dehiscence of antheridium, many investigations were hitherto reported¹⁾. They were made principally from the morphological viewpoint, though some of them from more or less taxonomic standpoint. Fujii and Asahina²⁾ studied the discharges of the *Isoetes*-spermatozoids caused by chemicals. The dehiscence of antheridium of fern has, however, not yet been analysed cytomorphologically. Hiraoka³⁾ studied the suction force of the spore mother cell of *Cyrtomium* in meiosis and reported that an increase in suction force at the onset of the bouquet stage and a decrease in the pachytene stage. This decreased suction force is maintained till later stages. Recently Holden⁴⁾ studied the factor of dehiscence of flax-fruit, and found that the diffusion pressure deficit (DPD) shows a slightly greater value for the dehiscent variety than for the semidehiscent variety and the degree of imbibition has an effect on the amount of dehiscence. In order to understand the mechanism of the dehiscence of antheridium, it is important to measure the osmotic values of wall cells of the adult antheridium and of the spermatid, which in turn will give information about the magnitudes of their suction forces.

Materials and Methods

As experimental materials, the antheridia produced on the prothallia of the following ferns belonging to Eufilicineae were employed: *Leptogramma totta* J. Smith, *Matteuccia struthiopteris* Todaro, and *Osmunda cinnamomea* L. var. *asiatica* Fernald. Of these, however, the first one was chiefly used.

As for the determination of suction force, the principles and the methods of "Gleichgewichtsmethode" of Ursprung and Blum⁵⁾, Ursprung⁶⁾, and Hiraoka³⁾ were followed. The prothallia, on which the antheridia were produced, were dipped in a drop of liquid paraffin, and the minor diameters of the wall cells, i. e. the cap cell,

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the ring cell, and the basal ring cell, were measured by means of a micrometer (L). Then, the liquid paraffin was replaced by a series of aqueous solutions of sucrose whose concentrations were made from 0.50M to 0.80M with intervals from 0.01M to 0.02M. And as in the case of the liquid paraffin, the minor diameters of the wall cells were measured (L'). The immature antheridia, where the spermatoteleoses were going on, did not extrude the spermatids in the solution. Therefore, the mature antheridia which had finished the spermatoteleoses were selected for observation. As the form of wall cell is not suitable for the measurement of its volume, the diameter of the wall cell was regarded as the criterion showing the osmotic pressure of the wall cell.

When L was equal to L' at a certain concentration (C_1) of the aqueous solution of sucrose, viz. the wall cell did not alter its form (volume) and no extrusion of the spermatids out of the antheridium occurred, the osmotic value C_1 was regarded as the suction force of the wall cell. The temperature of the solution was kept at $20 \pm 2^\circ$. For the purpose of investigating the suction force, the experiment was conducted merely by using the solution of sucrose, and glucose, urea, KCl, CaCl_2 and AlCl_3 were used in order to obtain complementary data concerning this problem. The concentrations of these aqueous solutions were made up from 0.50M to 1.00M with intervals from 0.01M to 0.02M.

Now that the forms of the spermatids are almost spheric, the changes of their volumes are available to be measured by a method which is identical with that in the case of the wall cell. The volume of the spermatid within the central cavity of antheridium which was dipped in a drop of liquid paraffin on the slide glass, was measured by means of a micrometer (V). Next, the liquid paraffin was replaced by a series of aqueous solutions of sucrose of graded concentrations as media, and the volume of the spermatid extruded out of the antheridium or in the central cavity was measured (V'). When V and V' are the same at a certain concentration (C_2), the osmotic value represented by this concentration of the sucrose solution was regarded as the suction force of the spermatid. According to the same measurement, the volumes of spermatids contained in the central cavity of the antheridium were also represented as v and v' , respectively.

Results

In the aqueous solution of sucrose 32 antheridia were examined in every solution and the wall cells of antheridia showed increases in their volumes. That is to say, the wall cells of antheridia swelled in some hypotonic solution (below 0.96M) (Fig. 1), though the swelling grades were not the same. As to the swelling grade, that of the basal ring cell is the greatest in many cases, and it may be assumed, in general, that the swelling grade of the basal ring cell is the greatest, that of the cap cell the smallest, and that of the ring cell intermediate (Table 1). This phenomenon is also observed in the experiments concerning glucose and urea. The results in the

case of *Leptogramma totta* were shown in Table 1. However, other species used in the present experiment showed also fundamentally the same results. In the case of sucrose, 0.74M aqueous solution makes the volumes of the wall cells increase and these cells swell. Then, the extrusion of the spermatids occurs. On the other hand, in the case of 0.98M solution, the wall cells swell and the spermatids extrude rarely out of the antheridium, but in many cases the wall cells do not change their volumes, namely they do not show their turgor pressures, consequently the extrusion of the spermatid does not arise. The wall cells show the convex plasmolyses and, of course, the spermatids do not extrude out of the antheridium in the case of 1.00M solution. Some representative results are given in Table 1.

From these results it is presumed that the suction force of the wall cell corresponds to the osmotic value of 0.98M aqueous solution of sucrose, which is calculated to be 33.6 atmospheres in *Leptogramma totta*. According to the same presumption, the suction force is supposed to correspond to the osmotic value of 0.85M and 0.92M aqueous solution of glucose and urea, respectively. These osmotic values represented as suction forces of the wall cells are generally greater than those of the prothallial cells which are vegetative. For instance, in the case of sucrose, the greatest osmotic value of the prothallial cell in VI region (apical region) was 0.82M aqueous solution⁷⁾. This fact is considered to be worthy of notice.

Concerning KCl, CaCl₂, and AlCl₃, some representative results are shown in Table 2.

From Table 2, the suction force of the wall cell is supposed to correspond to the osmotic value of 0.76M aqueous solution of KCl in *Leptogramma totta*. It is similarly found that, in CaCl₂ and AlCl₃ solutions, the osmotic values of 0.61M and 0.58M aqueous solutions were regarded as the suction force of the wall cell, respectively. These values are greater than the osmotic values of the prothallial cells as in the case of

Fig. 1

Fig. 2

Fig. 1. The wall cells of antheridium of *Matteuccia struthiopteris* Todaro which are increasing their volumes in 0.96M aqueous solution of sucrose (Phase contrast microscope, negative high).

a, cap cell; r, ring cell; b, basal ring cell; c, central cavity in which the spermatids are included.

Fig. 2. The spermatids extruded out of the central cavity of antheridium in *Osmunda cinnamomea* L. var. *asiatica* Fernald in redistilled water (Phase contrast microscope, negative high).

a, detached cap cell; s, spermatids out of which the spermatozoids will be extruded in a moment.

Table 1. Increase of the diameter of the wall cells of the antheridium in *Leptogramma totta* J. Smith. (1).

Solution	Concentration (M)	Increase of the diameter of the wall cell ($L' - L$)/ $L \times 100$			Extrusion of spermatids out of the antheridium
		Cap cell	Ring cell	Basal ring cell	
Sucrose	0.96	150.0	166.6	166.6	Extrusion
		166.6	200.0	208.3	"
		207.7	258.3	233.3	"
		215.3	275.0	280.0	"
	0.98	130.0	146.9	150.0	"
		0.0	0.0	0.0	No extrusion
	1.00	0.0	0.0	0.0	"
		Convex plasmolysis	Convex plasmolysis	Convex plasmolysis	"
	0.83	113.3	169.2	169.2	Extrusion
		207.0	258.3	269.2	"
Glucose	0.85	84.6	100.0	100.0	No extrusion
		0.0	0.0	0.0	"
	0.87	0.0	0.0	0.0	"
		Convex plasmolysis	Convex plasmolysis	Convex plasmolysis	"
	0.90	130.7	169.2	176.9	Extrusion
		215.3	246.1	275.0	"
Urea	0.92	50.0	53.3	53.3	No extrusion
		0.0	0.0	0.0	"
	0.94	Convex plasmolysis	Convex plasmolysis	Convex plasmolysis	"

sucrose. For example, in CaCl_2 solution the greatest value of the prothallial cell in VI region (apical region) of *Leptogramma totta* was 0.44M aqueous solution.

The representative results with respect to the spermatids shown in Table 3 were obtained.

As the materials, 132 spermatids produced in 37 antheridia were employed and the spermatids were observed to be osmotically in equilibrium with 0.74M aqueous solution of sucrose used as a medium (Table 3). That is, this concentration of the sucrose solution shows the osmotic value which corresponds to the suction force and equals to 23.1 atmospheres in *Leptogramma totta*. This value is considerably high. A possible consideration is able to be advanced below from the results of Table 3. When the antheridium was immersed in the water, the increases of the volumes of the spermatids contained in the central cavity of the antheridium occur more or less owing to their suction forces. After the extrusion of the spermatids out of the antheridium, the spermatids gradually increase their volume (Fig. 2) and reach the maximum of increasing, and then the spermatozooids come out of the spermatids. This high suction force is considered to be an important factor which plays a funda-

Table 2. Increase of the diameter of the wall cell of antheridium in *Leptogramma totta* J. Smith. (2).

Salt	Concentration (M)	Increase of the diameter of the wall cell ($L' - L$)/ $L \times 100$			Extrusion of spermatids out of the antheridium
		Cap cell	Ring cell	Basal ring cell	
KCl	0.74	100.0	130.7	130.7	Extrusion
		166.6	207.2	208.0	"
		208.8	284.6	284.6	"
	0.76	33.3	44.6	33.3	No extrusion
		0.0	0.0	0.0	"
CaCl ₂	0.78	Convex plasmolysis	Convex plasmolysis	Convex plasmolysis	"
	0.60	100.0	113.3	114.2	Extrusion
		207.7	233.3	246.1	"
	0.61	17.8	19.2	20.0	No extrusion
		0.0	0.0	0.0	"
AlCl ₃	0.62	0.0	0.0	0.0	"
		Convex plasmolysis	Convex plasmolysis	Convex plasmolysis	"
	0.56	115.3	130.7	140.0	Extrusion
		203.3	233.3	246.1	"
	0.58	100.0	115.3	115.3	No extrusion
		0.0	0.0	0.0	"
	0.61	0.0	0.0	0.0	"
		Convex plasmolysis	Convex plasmolysis	Convex plasmolysis	"

Table 3. Increase of the volume of spermatid in *Leptogramma totta* J. Smith.

Solution	Concentration (M)	Spermatid in the central cavity of antheridium ($v' - v$)/ $v \times 100$	Spermatid extruded out of the antheridium ($V' - V$)/ $V \times 100$	Extrusion of the spermatozoid
Sucrose	0.72	0.0	30.9	Extrusion
		12.5	45.2	"
		14.3	58.2	"
		19.0	61.7	"
		21.2	72.8	"
	0.74	0.0	27.1	"
		0.0	0.0	No extrusion
	0.76	0.0	0.0	"
		Shrinkage of the spermatid-membrane occurs slightly	Shrinkage of the spermatid-membrane occurs slightly	"
Redistilled water		0.0	40.5	Extrusion
		13.2	58.2	"
		24.9	103.4	"

mental role in the dehiscence of the antheridium and in the extrusion of the spermatozoid out of the spermatid.

Discussion

It has become evident that the wall cells of the antheridia in *Leptogramma totta* and other two species exhibit considerable high suction forces and are able to increase their volumes. *Leptogramma totta* shows a suction force corresponding to the osmotic value of 0.98M aqueous solution of sucrose which is equivalent to 33.6 atmospheres. Besides sucrose, the suction forces which corresponded to 0.74M, 0.61M, 0.58M, 0.92M, and 0.85M aqueous solutions of KCl, CaCl₂, AlCl₃, urea, and glucose were obtained, respectively. It can also be seen that the osmotic value of the wall cell is higher than that of the prothallial cell. It is known that the osmotic value of the seashore plant is high⁸⁾, while that of the alpine plant is, in general, low⁹⁾. In the present study the osmotic value of the wall cell of the antheridium and the spermatid have been confirmed to be higher than or almost equal to that of the seashore plant. Diannelidis¹⁰⁾ reported that the osmotic value of the guard cell of the stoma is higher than that of the epidermal cell in *Stratiotes*. The same fact has been also observed between the antheridium and the prothallial cell. The present writer considers that, as the guard cell which has the function of opening and closing has a high osmotic value, so do the wall cells of the antheridium also possess a high activity of swelling owing to their big osmotic value.

The spermatids contained in the central cavity of the antheridium increase more or less their volumes in many cases and those extruded out of the antheridium increase markedly in the water. And the suction force was supposed to correspond to the osmotic value of 0.74M aqueous solution of sucrose (23.1 atmospheres) in *Leptogramma totta*. This value is comparatively large as described above and it is considered that this magnitude of the suction force of the spermatid may be related to its extrusion out of the central cavity of the antheridium.

Therefore, confirmation of the suction force of the wall cell and that of the spermatid is significant in clarifying a factor in the dehiscence of the antheridium, i. e. the extrusion of the spermatid out of the antheridium, and the discharge of the spermatozoid out of the spermatid.

Summary

Surveying the suction forces of the wall cells, viz. the cap cell, the ring cell, and the basal ring cell of the antheridium and the suction force of the spermatid chiefly in *Leptogramma totta* J. Smith, the following facts were obtained.

1. The suction force of the wall cell is comparatively high and is equivalent to the osmotic value of 0.98M aqueous solution of sucrose which corresponds to 33.6 atmospheres.
2. This value is higher than that of the seashore plant cell known as possessing

a high osmotic value. Concerning the magnitude of the osmotic value, the relationship between the wall cells and the prothallial cells resembles the relationship between the guard cells of stomata and the epidermal cells.

3. The suction force of the spermatid is also comparatively high and corresponds to the osmotic value of 0.74M aqueous solution of sucrose (23.1 atmospheres).

4. The mechanism shown by the wall cell and the spermatid having a high suction force is considered to be an important factor in the dehiscence of the antheridium and the extrusion of the spermatozoid out of the spermatid.

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摘 要

ミゾシダ (*Leptogramma totta* J. Smith) を主な材料とした外、2 種のシダを材料として、それらの前葉体上に発育した造精器の壁細胞、すなわち帽細胞、環細胞、底部環細胞の吸水力を Ursprung and Blum などの“平衡法”を適用してしらべた結果は次のように要約できる。

1. 造精器の壁細胞の吸水力は大きく、ミゾシダでは蔗糖の 0.98 モル水溶液の浸透値、すなわち 33.6 気圧に相当する。
2. この値は高い浸透値を有する海岸植物の浸透値より高い。そして造精器の壁細胞の浸透値は前葉体細胞のそれよりも高く、この事実は開閉能ある気孔孔辺細胞の浸透値が表皮細胞のそれよりも高いという事実に似ている。
3. 精細胞の吸水力も比較的大きく、ミゾシダでは蔗糖の 0.74 モル水溶液 (23.1 気圧) に相当する吸水力がある。この値は海岸植物の浸透値とほとんど同じであるといえる。
4. 造精器の壁細胞や精細胞の吸水力の大きいことは、それら細胞の activity の高いことを示すものであり、造精器裂開や、精細胞からの精子の脱出の主要な一因子であると考える。