Cytological Study on Induced Apospory in Ferns

Chihiro Takahashi

Biological Laboratory, Department of General Education, Nagoya University,
Mizuho-ku, Nagoya, Japan

Received September 20, 1961

Introduction

The life cycle of the pteridophyte is composed of two generations, the sexual generation and the asexual generation, which are separated on different individuals called respectively the gametophyte and the sporophyte which succeed each other in the regular order. In brief there can be seen the typical alternation of generations in the pteridophyte. The sporophyte is a diploid organism producing the spore, the starting cell of the gametophyte, by the meiosis and is larger in size, more complicated and advanced in form and structure. On the other hand the gametophyte is a haploid organism producing the gametes which by the syngamy give rise to the zygote, the starting cell of the sporophyte and is smaller in size, simpler and more reduced in form and structure but is autotrophic like the sporophyte. How different in form, structure and function are the two generations which develop from an initial single cell, the spore or the zygote, having the identical hereditary material apart from a set of chromosomes or two sets of those! How does the difference between the two generations come to be as it is? The alternation of generations is usually accompanied by that of the nuclear phases. Does the former result necessarily from the latter? In other words, is the haploidy caused by the meiosis responsible for the gametophytic characters and is the diploidy caused by the syngamy responsible for the sporophytic characters? It seems that they are not definitely responsible at least because it is well known that there are the short cuts in the life cycle named the apospory and the apogamy which occur spontaneously or are experimentally induced. The apospory is the phenomenon by which the gametophyte takes place from the sporophyte without the intervention of normally formed spore. The apogamy is the phenomenon by which the sporophyte takes place from the gametophyte without the intervention of a sexual process. Thus the alternation of generations by the apogamy or the apospory is not accompanied by that of the nuclear phases.

It is expected that these deviations from the normal life cycle provide an important clue to studying the alternation of generations not only in the pteridophyte but also in plants in general. The pteridophyte is a good material for extending the work along this line. Its haploid, sexual generation is inconspicuous but free-living. So the culture is easily and abundantly

carried out in the laboratory. The diploid, asexual generation also is easy to be obtained from the gametophytic culture and to be reared at the younger stage in the laboratory. The study was undertaken in order to bring about a better understanding of the life cycle of the pteridophyte. The cytological and morphological sides of such study by means of induced apospory will be reported in the present paper. Since apospory was found, many data about this interesting phenomenon have been accumulated. Nevertheless it seems that more detailed and comprehensive study is necessary. It will deal with problems as follows: what method is favourable for inducing apospory?; what organs, tissues and cells are involved in the apospory?; by what process is the apospory induced?; how does the aposporous prothallium behave about producing the sporophyte?; how are the morphology and the physiology of the aposporous prothallium and the tetraploid sporophyte as compared with those of the normal prothallium and sporophyte?

Material and methods

Two species of ferns, *Pteridium aquilinum* (L.) Kuhn var. *latiusculum* (Desv.) Und. and *Dryopteris erythrosora* (Eat.) O. Ktze. were used in this study. By the present writer the apospory was successfully induced in both species. But the extensive study was carried out exclusively on the former, *Pteridium aquilinum* var. *latiusculum*. The reasons were as follows: the former grows more rapidly; the abundant aposporous outgrowth occurs more easily.

The spore of *Pteridium aquilinum* var. *latiusculum* reaches its maturity in September and that of *Dryopteris erythrosora* in July in the vicinity of Nagoya. The leaves with the mature sporangia were cut in the habitat and brought into the laboratory where they were dried on the paper. The sporangia soon caused the dehiscence and released the spores. The spores were collected, sifted from the impurities and stored in the desiccator until they were taken out of it and sown on the culture medium.

The spores were sown in the Petri-dishes of six centimeters in diameter on the culture medium which contained 20 per cent dilute Knop's solution and was solidified with 1 to 2 per cent agar. The pH was 5.2 or so. The conditions under which prothallia were reared were either the room temperature and the dispersed light in the laboratory or the continuous illumination of the fluorescent lamp and the temperature adjusted at 20° to 25°C in the incubator. The culture brought about the same results except the growth duration under both conditions. The culture was discarded, when it was badly damaged by the contamination of algae, fungi or bacteria.

The spore germination takes place several days after sowing. Then a protonema, a filamentous prothallium, is formed as a result of the one-dimensional growth. An apical portion of the protonema begins growing two-dimensionally to turn into a spatulate prothallium where a meristematic region

is established and forms an apical notch. Now the gametophyte takes the form of a cordate prothallium and after this, of a bow-like prothallium. They have a three-dimensionally built midrib and only there the archegonia are formed. The antheridium can be formed at any developmental stage of the gametophyte, even on the basal cell without the protonema formation just after the germination (Takahashi, unpublished). The successive form change of the prothallium with its growth is attributed to the establishment and the activity of the meristematic region by which new cells are produced mainly perpendicularly to an apico-basal axis. Accordingly the value of a ratio of the prothallium width to the prothallium length decreases which results in the form change of the prothallium. If there is no syngamy on the cordate or the bow-like prothallium, the apical notch will usually change into the multicell-layered, somewhat flattened cylindrical projection which has not the wings, or has the narrow and rippled ones and continues to grow at the tip. The projection bears a large number of archegonia and rhizoids on the ventral surface and sometimes on the dorsal surface too. This structure resembles that reported by Lang (1898) and Mottier (1927, 1931). They observed that the apogamous phenomena occurred in connection with the projecting growth, but the present writer did not. The embryo arises sexually on the cordate and the bow-like prothallia and on such projecting prothallia as well. The sporophyte thrives normally under the present condition. No apogamy was found through the course of this study.

The young sporophyte with leaves and roots is established a month or two after the beginning of the spore culture. Such early leaves as the first, second and third leaves were usually used in this study, with the exception of the case for the special purpose as mentioned later. The leaves were detached near their base and cultured in the Petri-dishes with 20 per cent dilute Knop's solution. A large number of aposporous outgrowths have been got by this method. Since the aposporous prothallium was induced and grown in the Petri-dish with a lid, there was no possibility of contamination by fern spores of the same and other species. In order to avoid the excessive contamination of algae, fungi or bacteria it is better to use the liquid medium than to use the agar medium. It was proved that the aposporous outgrowth occurred also in the aseptic culture. But it was not necessary to culture the sporophytic organ under the sterile condition so far as the present study was concerned. The culture was kept either in the incubator adjusted at 20° to 25°C of which the walls were made of glass through which the dispersed light streamed in, or in the laboratory under the room temperature and the dispersed light. Some months after the beginning of the culture the aposporous outgrowth can be noticed on most of the detached leaves.

The aposporous prothallium was cultured as it was attached to, or detached from, the sporophytic organ. In either case the aposporous pro-

thallium gave rise sexually to a new sporophyte. Such a sporophyte was planted in a sphagnum-packed pot, when the writer intended to develop it further.

Results

Attempts were made to induce apospory in *Pteridium aquilinum* var. *latiusculum* by methods, such as : injuring or damaging with tweezers the undeveloped leaves as they were attached to the sporophyte, which later gave rise to various types of the malformed leaves ; subjecting the very young sporophyte to starvation by providing water without nutrition under the weak light, resulted in the failure of developing the adult type of leaves ; culturing the detached leaves or roots in the nutrient solution. It was revealed that only the last method was successful in the early leaves and roots. The sporophyte usually raises its leaves freely in the air, but it happened that the leaves, especially the first ones, grew diving into the agar or the solution, or in contact with the agar surface. However in these cases apospory did not occur. In view of above mentioned results it is evident that in order to induce apospory it is necessary to detach from a whole sporophyte a given organ where apospory may occur.

In order to know the ability of inducing apospory on the leaf in relation with the age of the sporophyte, the leaves were detached from the sporophytes which were in various stages of development from the very young sporophytes still attached to the prothallia and bearing one or a few small leaves of the juvenile type, to the old ones growing for many years and bearing many large leaves of the adult type. It was revealed that the leaf to be detached for inducing apospory must be the early one on the very young sporophyte. The earlier the leaf was, the easier the induction of apospory was. The leaves up to the seventh could exhibit this phenomenon but the eighth or the later leaves could not so far as the present study was concerned. As for the age of the leaf, either a young, folded leaf in which cell divisions were actively proceeding or a mature, fully expanded one in which there could not be shown any mitotic figures, showed the ability of inducing apospory. The young, folded leaf usually sank in solution and the mature, expanded leaf was usually on the float. However, whether the leaf was sinking or floating, or which surface was in contact with the solution did not matter.

The aposporous outgrowth occurred on any part of the leaf, such as the lamina, the stipe and the rachis (Figs. 9-12). The whole leaf was not necessary for inducing apospory. The leaf was cut into some pieces of the lamina and the stipe. Each of them could give rise to the aposporous outgrowth. The outgrowth was of the epidermis, the mesophyll or the cortex origin (Figs. 1-7). On the young, folded leaf it was exclusively of the epidermis origin. On the mature, expanded leaf the outgrowth of the epidermis origin, the mesophyll origin and the cortex origin could be seen.

The outgrowth grew out from both surfaces of the lamina no matter which surface was in contact with the solution. It seemed that some hairs on the stipe grew into the prothallia as well as the epidermis cell did. The gametophytic tissues were observed below the epidermis of both the lamina and the stipe (Figs. 4-6). They were not always distinguished from the

Figs. 1-8. The origin of the gametophytic outgrowth induced aposporously in *Pteridium aquilinum* var. *latiusculum*. The stippled cell shows the gametophytic cell. 1, the aposporous prothallium originating from the stipe epidermis. h, hair. 2-3, the aposporous prothallium originating from the marginal cells (fig. 2) and the epidermis cell of the lamina (fig. 3). 4, the aposporous prothallium occurring in the stipe cortex. v, vascular bundle. 5, ten days after fig. 4 was drawn. It grows further below the epidermis and a part of it comes forth on the surface breaking the epidermis. v, vascular bundle. 6, the aposporous prothallium with antheridia originating from the mesophyll cell. As a result of growth it breaks the epidermis and comes to grow outside. r, undergrown rhizoid. 7, both epidermis cells and mesophyll cells are involved in this case. 8, the aposporous prothallium originating from the root epidermis. There also can be seen other inceptions of the gametophytic outgrowth.

sporophytic tissue around them by their greener colour and cell shape. They sometimes formed in the mesophyll the undergrown rhizoids, and the antheridia which showed the atypical shape caused by the confinement in the small intercellular space but had the normal spermatozooids (Fig. 6). Growing under the epidermis, they eventually emerged out breaking the epidermis. There was also the outgrowth in which process both epidermis cells and mesophyll ones were involved together (Fig. 7). The outgrowth occurred everywhere on the surfaces of the lamina and the stipe, on the leaf margin, in the mesophyll and in the stipe cortex (Figs. 1-7, 9-12). It may be closely approximated to, or independent of, the vascular tissue. It occurred on the apex and the basal part of the lamina. It grew out anywhere without definite positional relation to a severed portion. In short there was no relation between the occurrence of the outgrowth and the leaf structure. But the outgrowth from a cut surface of the stipe was very rare. It was not, however, observed that the outgrowth originated from the guard cell and the vascular tissue. It originated from one to several sporophytic cells. Many outgrowths could occur on one and the same leaf. Usually the occurrence of the gametophytic tissue was discernible first of all as the greener cells as compared with the sporophytic tissue around them. The former was growing while the latter was decaying. More and larger chloroplasts were in the former cells (Fig. 12). In some cases, especially in the case of the younger leaf, the inception of the outgrowth could not be distinguished from the sporophytic tissue surrounding it. Because the cells of the epidermis or the mesophyll which originally had many, large chloroplasts, remained green and alive even after the outgrowth fully developed. The outgrowth was often full of extremely large starch grains in its basal part. It grew attaching to the sporophytic organ but it could grow isolated from the sporophytic organ.

It was also confirmed that the aposporous outgrowth could occur on the detached root (Figs. 8, 13-14). It originated from the epidermis at or rather near the growing point. It had not been seen as yet that the outgrowth originated in the tissue below the root epidermis. It seems necessary that the root to be detached should be the young, short, growing and early one. To induce apospory was much more difficult on roots than on leaves. In this case also the first indication of the aposporous outgrowth was that the incipient cells turned green developing many, large chloroplasts, while the other cells remained white or turned slightly brown. Several, large chloroplasts were originally in the cells of the root cap but rapidly degenerated after the root was cut off. In some cases there were the green, short root hairs which grew out after the root was cut off. They looked like the inception of apospory. It was, however, not ascertained whether such root hairs could grow into the aposporous outgrowth.

It was evident that the outgrowth was a true gametophyte. But neither the sporophytic outgrowth nor the intermediate outgrowth which bore both

Figs. 9-14. Some examples of apospory in *Pteridium aquilinum* var. *latiusculum*. 9, an aposporous prothallium originating from a marginal cell of the first leaf. $\times 25$. 10, several cordate prothallia aposporously produced on the lamina of the first leaf. A filamentous prothallium can also be seen on the leaf margin. $\times 6$. 11, some aposporous prothallia originating from the stipe epidermis of the first leaf. $\times 25$. 12, two gametophytic cells of the stipe epidermis with many chloroplasts. The epidermis cells which were originally normal sporophytic changed into them. $\times 200$. 13, many aposporous prothallia originating from the root epidermis. $\times 25$. 14, many gametophytic cells with chloroplasts into which the epidermis cells of the root changed. $\times 50$. Figs. 15-18. The comparative gametophytic characters of *Pteridium aquilinum* var. *latiusculum*. 15-16, the comparative sizes of spermatozoids in the normal haploid (fig. 15) and the aposporous diploid (fig. 16) prothallia. $\times 500$. 17-18, the comparative sizes of antheridia in the normal haploid (fig. 17) and the aposporous diploid (fig. 18) prothallia. $\times 200$.

the gametophytic character, such as the sex organ, and the sporophytic character, such as the stoma or the vascular element, was observed. The transition from the sporophyte to the gametophytic outgrowth was not a gradual change. So the writer could clearly recognize the demarcation between them. The evidences that the outgrowth was a prothallium were as follows: 1) The vascular element and the stoma, which were the typical characters of the sporophyte, were absent. 2) The typical prothallial shape, such as filamentous, cordate or bow-like, was assumed. The development was in the same way as that of the prothallium starting from a spore. In some cases the outgrowth became more or less three-dimensionally built at the beginning of its growth, especially the outgrowth originating from the cortex or the mesophyll cells usually assumed a spherical shape. The flat prothallium derived from such a structure. 3) The cell shape was not similar to that in the sporophytic tissue from which the outgrowth originated but was the same as that in the gametophytic one. The cell shape of the epidermis and the mesophyll of the mature lamina was irregular but that of the outgrowth was somewhat rectangular. The cell shape of the stipe epidermis was slender and was regularly arranged but that of the outgrowth was not so slender and was not so regularly arranged. 4) The rhizoid was present. 5) As the critical evidence the sex organ and the gamete were produced. On the cordate or the bow-like prothallium the archegonia were formed and the fertilization occurred giving rise to a sporophyte which had many interesting characters as compared with a normal sporophyte produced on a normal prothallium as mentioned later in this report. The writer has got hundreds of tetraploid plants these years. It is necessary to mention that no apogamy occurred on the aposporous prothallium as well as on the normal prothallium developed from a spore.

The tetraploid plants were also capable of giving rise aposporously to the probably tetraploid prothallium in the same way as in the case of the diploid prothallium. The antheridia-bearing prothallia were obtained but not the archegonia-bearing or the cordate ones so far. Since the obtained prothallium was too small in number to describe in detail, the present report does not contain the further mention of it.

There was no difference in appearance between the normal haploid prothallium and the aposporous diploid prothallium. Such characters of the haploid prothallium as the nucleus, the spermatozoid, the antheridium and the rhizoid were compared with those of the diploid prothallium under the microscope. It was revealed that there was the considerable difference between them. The characters of the diploid prothallium were generally larger than those of the haploid prothallium as shown in Table 1 and in Figs. 15-18. The diameter of the fixed spherical nuclei at the resting stage was measured in the marginal cells near the meristematic region. The resting nuclei took various forms such as spherical, ellipsoid and spindly ones in relation to the

Figs. 19-23. Some examples of two-year-old sporophytes of *Pteridium aquilinum* var. *latrusculum*. All pots have the same size of six centimeters in diameter. 19, a normal diploid plant. 20, a tetraploid plant. It is almost normal in appearance and hardly distinguishable from the normal diploid plant. But this plant has a little thicker leaves. 21, a dwarf tetraploid plant. 22-23, two tetraploid plants with the irregularly shaped leaves, The leaves are also thick, dark green and cold-hardy. The plant in fig. 23 is hairy.

cell age. The diameter of the fully grown, alive rhizoids was measured at the middle of their length. The fixed spermatozoids were measured at the widest part of their body. The diploid spermatozoids were normally shaped and actively motile. The antheridia on one and the same prothallium exhibited various sizes. Their diameter increased in proportion to the number

Table 1. The comparative measurements of some characters in the normal haploid gametophyte and the aposporous diploid gametophyte

Object		Mean	Ratio	
Antheridium, fixed Diameter (μ)	n	{	No. of spermato- zoid contained	100
			16	123
			32	150
			64	120
	2n	{	16	150
			32	173
			64	202
			81	over 100
Rhizoid, alive	n	11	100	
Diameter (μ)	2n	20	182	
Nucleus, fixed	n	8.5	100	
Diameter (μ)	2n	10.0	118	
Spermatozoid, fixed	n	2.3	100	
Width (μ)	2n	3.3	143	

of spermatozoids formed in them. The antheridia on the haploid prothallium usually contained 32 or 64 spermatozoids, but sometimes 16. The antheridia on the diploid prothallium usually contained 32 or 64, too, but sometimes 16 or over 100.

In the tetraploid sporophytes there were many deviations from the normal morphology and physiology of the diploid sporophytes. They were the irregularly shaped leaf, the thick leaf, the uneven leaf surface, the dark green, the hairiness, the dwarfness, the anomalous stomata and the cold-hardiness. But each tetraploid sporophyte may take a different combination of such deviations (Figs. 19–23). Some of the tetraploid sporophytes were almost normal in appearance (Fig. 20). Many tetraploid sporophytes showed the more or less abnormal leaf shape (Figs. 21–24). The diploid sporophyte had the regularly and symmetrically pinnated leaves but many of the tetraploid sporophytes had irregularly and asymmetrically pinnated leaves. These malformed leaves were remarkably thick. Their surface was irregularly uneven, while that of the diploid plant was not so but was elevated only along the leaf vein. There were very often the tetraploid plants with the dark green leaves. The reason may be attributed partly to the larger number of chloroplasts per volume and partly to the thicker leaf. They may remain green longer in winter. There were often a considerable number of stomata even on the leaf margin and the rachis. There were more and larger hairs on

some of those plants. A few of the tetraploid plants were dwarf on which the normal adult type of the leaf failed to develop (Fig. 21). On one of the dwarf plants all its small and abnormal leaves up to the twenty-fifth consisted almost of the stipe with the poor lamina. Two types of the anomalous stomata, the epidermized stoma and the poreless multicellular stoma, were found on the tetraploid plants as reported in another paper (Takahashi, in press).

The dimensions of the epidermis cell and the stoma on the tetraploid plants were measured as compared with those of the diploid plant as shown in Table 2. The stomata on most tetraploid plants showed the same structure as those on the diploid ones (Fig. 25). Accordingly to the statistics the length of the stoma on the tetraploid plant was larger than that on the diploid one by 1.4 times in the mean value and showed a wider variation.

The circumstance was essentially the same with the area of the epidermis cell. The area of the epidermis cell on the tetraploid plant was larger by 1.7 times in the mean value, showed a wider variation and was not so normally distributed. The tetraploid epidermis cell and its cell wall also were thicker. The volume of the tetraploid epidermis cell as calculated was larger by 2.2 times.

Fig. 24. The early leaves of the juvenile type on the sporophyte of *Pteridium aquilinum* var. *latiusculum*. The number shows the order of the leaves formed on the plants. $\times 2$. a: a series of the normal leaves of regularly increasing complexity on a normal diploid sporophyte. b, c and d: three series of the abnormal leaves on the tetraploid sporophytes. d: a dwarf plant.

Table 2. The comparative measurements of the cells in the diploid sporophyte and the tetraploid sporophyte

Object		No. of used plant	No. of measured object	Mean	Ratio
Stoma	2n	12	3186	38.3 ± 0.08	100
Length (μ), alive	4n	11	2003	53.3 ± 0.16	139
Epidermis cell	2n	6	84	6400 ± 230	100
Area (μ^2), alive	4n	8	90	11100 ± 680	174
Epidermis cell,	2n	3	52	12.6 ± 0.39	100
Thickness (μ), alive	4n	4	58	16.2 ± 0.39	129
Epidermis cell	2n			81000	100
Volume (μ^3), calculated	4n			180000	200

Fig. 25. The comparative leaf epidermis of the normal diploid (left) and the tetraploid (right) sporophytes of *Pteridium aquilinum* var. *latiusculum*. $\times 150$.

Discussion

It has been reported that apospory occurred in many families of the Filicinae of the Pteridophyta. The phenomenon has not been observed so far in other classes of the Pteridophyta, the Psilotinae, the Lycopodiinae and the Equisetinae. In the present study two species of the Filicinae, *Pteridium aquilinum* var. *latiusculum* and *Dryopteris erythrosora* were used. Apospory was successfully induced in both species. As for the former the contradictory works on inducing apospory were reported by Lawton (1932) who was unsuccessful and by Bell and Richards (1958) who were successful. As for the latter the present writer reports the induced apospory for the first time.

It was considered impossible that the materials used in this study were genetically aposporous like the "peculiar" *Scolopendrium* sporophyte reported by Andersson-Kottö (1932). The indication of naturally occurring apospory (as reported by Farlow 1889) was not noticed in the habitat on many old sporophytes from which the spores had been collected. Hurel-Py (1950) observed the aposporous prothallia occurring on the intact sporophytes of *Gymnogramme* whose leaves dived into the agar medium. But no apospory was observed on the present material unless the sporophytic organ was detached. References have been made by many investigators to the fact that apogamy and apospory were associated. In fact there were many instances in which they were allied phenomena (Digby 1905, Wesselowska 1907, Farmer and Digby 1907, Steil 1919a, b, Heilbronn 1932, Sarbadhikari 1939, Duncan 1941, Wilkie 1956). But on the present material no apogamy occurred before and after apospory.

Numerous attempts were made to obligatorily induce apospory in ferns by methods which had been described by different investigators. Successful results were obtained by methods, such as growing the young sporophyte under the weak light (Steil 1919a), doing so under the malnutrition, damaging the leaf apex of the sporophyte (Lang 1924) and culturing the detached organs of the young sporophyte on the moist medium (Goebel 1907, Heilbronn 1928, 1932, Beyerle 1932, Lawton 1932, 1936, Hurel-Py 1950, Bell and Richards 1958). The present writer obtained the successful result by the last method.

Attempts to induce apospory from the later leaves of the old sporophytes have so far been unsuccessful (Bower 1889) with the exception of some horticultural varieties (Bower 1884, Druery 1884). These ferns were genetically abnormal and their prothallia were apogamous. On the other hand, many workers have found it possible to induce the gametophyte aposporously only from the very early leaves of the young sporophyte. The present writer also confirmed this fact in two ferns.

The possibly tetraploid prothallia were obtained but not the octoploid sporophyte as yet by Heilbronn (1932), Lawton (1932, 1936) and the present writer. How far can the highly polyploid sporophyte and gametophyte be obtained by this method? How are the morphology and the physiology of thus obtained polyplont? These are the problems in future.

It has been often described that there were three types of the outgrowth on the detached leaf or root (Goebel 1907, Steil 1919b, Beyerle 1932, Lawton 1932, 1936). They were the prothallial outgrowth, the sporophytic outgrowth and the intermediate outgrowth exhibiting both gametophytic and sporophytic nature. But only the prothallial outgrowth took place on the leaf or the root of the present material.

The question arises as to the part of the sporophyte from where the aposporous gametophyte grows out. As for the organ it has been observed that the aposporous gametophyte originated from the leaf. But there has

been so far only an investigator who proved to induce apospory on the detached root (Lawton 1932). The present writer also succeeded in inducing apospory on the root. Inducing apospory was by far more difficult on the root than on the leaf. The reason is probably that the detached roots usually decay before acquiring the regenerative ability, because the roots are neither the photosynthetic organ nor the storage organ.

As for the tissue all the previous investigators unanimously asserted that the aposporous gametophyte was of the epidermis origin. It was, however, undoubtedly revealed that the tissue below the epidermis also gave rise to the aposporous gametophyte. In fact the aposporous prothallium grew out from every part of the leaf, such as the leaf epidermis (the margin, both surfaces, the rachis and the stipe surfaces, the hair), the cortex or the mesophyll and the root epidermis possibly including the root hair with the exception of the guard cell and the vascular element. It is unknown why there has been no report that the aposporous prothallium grew out from the tissue below the epidermis as well from the epidermis. It seems more reasonable that all the sporophytic cells but those with the excessively thickened wall are able to produce the gametophytic cells aposporously according to circumstances, than that only the epidermis cell are able to do so.

Differently from the case reported by Steil (1919a) the transition of the sporophyte to the gametophytic outgrowth was so swift that the demarcation between them could be recognizable by their cell shape, arrangement and inclusions.

Up to now it was reported by a few investigators that the aposporously produced prothallium gave rise sexually to the tetraploid sporophyte (Heilbronn 1928, 1932, Manton 1932, Lawton 1932, 1936). But many other investigators failed to obtain the tetraploid sporophyte. The outgrowth obtained by the writer was undoubtedly the true prothallium in every respect and readily produced the tetraploid sporophyte by the sexual process.

The diploid prothallia were also obtained by the application of chemicals, such as colchicine and γ -lindan, to the culture of the haploid spores (Rosendahl 1941, Mehra 1952, Mehra and Loyal 1956, Yamasaki 1954, Döpp 1955). But they failed to produce the tetraploid sporophyte. They remained to be filamentous or unicell-layered and formed antheridia but not archegonia. The archegonium formation is necessarily associated with a definite morphogenetic stage, the multicell-layered midrib formation. So it is supposed that some unbalance caused in the prothallial cells by the application of those chemicals made them fail to attain to the morphogenetic stage of the archegonium formation.

The cytological study proved that the chromosome number of the normal prothallium developed from a spore, that of the normal sporophyte produced on the normal prothallium, that of the aposporous prothallium and that of the sporophyte produced on the aposporous prothallium were haploid, diploid,

diploid and tetraploid, respectively (Takahashi 1961). So the following is cytologically evident: the material used underwent normally the alternation of the nuclear phases in the usual condition; the aposporous gametophyte took place without chromosomal reduction; neither apogamy nor parthenogenesis occurred on the aposporous prothallium but it gave rise sexually to the tetraploid sporophyte. The contradictory references have been made by many botanists to the relation between apogamy and apospory. In this study the aposporous gametophyte did not produce the sporophyte apogamously but did sexually. Hence apogamy and apospory are not allied phenomena in this case.

The characters exhibited by polyploid plants often serve to distinguish them from their normal plants. The aposporous prothallium and the tetraploid sporophyte were respectively composed of larger cells than the normal prothallium and the normal sporophyte (Heilbronn 1928, Lawton 1932, 1936, Manton 1950). The same was the case with the present material. In her reports Lawton (1932, 1936) touched very briefly upon the irregularly shaped and thick leaf exhibited by the tetraploid plants of ferns. The tetraploid sporophyte obtained by the writer also exhibited various kinds of deviated characters. There could be no doubt that such abnormal characters occurred in association with the tetraploidy. But it is impossible to determine, on the basis of the present study, whether the abnormal character is related with the aneuploidy or other nuclear aberration. The most interesting abnormal characters was the poreless multicellular stoma. It was exhibited by the exceptional three tetraploid plants. They had formed none of the normal stoma with a pore. It appeared that they had grown fairly well over two years in spite of their unfavourable structure for gas exchange. The full description of the poreless multicellular stoma and the epidermized stoma, the other type of the abnormal stoma on the tetraploid plant, is given in another paper (Takahashi, in press). The tetraploid sporophyte produced on the aposporous prothallium of *Polypodium aureum* formed the viable spores which developed into the diploid prothallium (Heilbronn 1932). It is of interest whether the present tetraploid sporophytes produce the spores in future.

From the results of this study it is likely that a form of life as the sporophyte is gradually strengthened and unchangeably established as the sporophyte grows. It was proved that in spite of the doubled chromosome number by fertilization, the cells of the young sporophyte were not destined to be sporophytic but were able to be gametophytic depending upon the conditions. Steil (1919b) suggested that the apogamous sporophyte may have contained some gametophytic cells which developed into the prothallia. This is out of the question in the present case and many other cases in which the sporophyte used was not of the apogamous origin but developed from a fertilized egg. The present writer supposes that what makes the sporophyte

behave really as the sporophyte, exists in the material interaction among various organs. As a result of a definite development of the internal conditions in the embryo each sporophytic organ differentiates. Once the organs appear, the material interaction among them maintains and moreover, strengthens the sporophytic nature. So cutting off a given organ from its mother sporophyte is cutting off the material interaction between them and depriving it of the guarantee for being sporophytic. Differently from Heilbronn's opinion (1932), making the detached organ contact with moisture is rather keeping it alive while the sporophytic internal conditions change into the gametophytic ones, than providing it with the gametophytic milieu in order to force the prothallial growth. Various operations on the gametophyte before or after the fertilization, did not prevent the tissue developing from a zygote from attaining the morphology characteristic of the sporophyte (Ward and Wetmore 1954, Jayasekera and Bell 1959).

Summary

The study was carried out in order to bring about a better understanding of the life cycle of the pteridophyte by means of experimentally induced apospory. Inducing apospory was successful in *Pteridium aquilinum* var. *latiusculum* and *Dryopteris erythrosora*, but the former was exclusively used in the precise study because of the rapid growth of the gametophyte and the abundant induction of apospory.

The leaf or the root detached from the sporophyte produced the aposporous prothallium, but those attached to the sporophyte did not, irrespectively of the environmental conditions so far as the present study was concerned. Also the surgically damaged apex or undeveloped leaf attached to the sporophyte did not produce the prothallium. The ability of inducing apospory was limited to the early leaves or roots of the young sporophyte. The prothallial outgrowth occurred irrespectively of the leaf age and of the existence of the dividing cell. It originated from any part of the leaf without any correlation to the existing sporophytic structures, such as the leaf apex, the vascular bundle, etc. There was no polarity for the appearance of the outgrowth. Every sporophytic cell in the epidermis, the mesophyll and the cortex could produce the gametophytic cell except the guard cell and the vascular element. The outgrowth originated from one to several sporophytic cells. The first indication of the occurrence of the aposporous outgrowth was that many, large chloroplasts appeared in the sporophytic cells from which the outgrowth originated. But there were the leaves whose cells remained alive and green even after producing the aposporous outgrowth. The outgrowth on the detached root originated from the epidermis cells at or rather near the growing point. The case was essentially the same with that of the leaf.

The outgrowth was morphologically and functionally a true gametophyte

and bore the sex organs by which the tetraploid sporophyte was sexually produced. Neither the sporophytic nor the intermediate outgrowth occurred on the detached sporophytic organ.

Also the probably tetraploid prothallium was aposporously induced on the detached tetraploid leaf.

The size of some characters of the aposporous diploid prothallium, such as the nucleus, the rhizoid, the spermatozoid and the antheridium, was compared with that of the normal haploid prothallium. The former was generally larger than the latter.

The tetraploid sporophyte exhibited many deviations from the normal morphology and physiology of the diploid sporophyte. They were the irregularly shaped leaf, the thick leaf, the uneven leaf surface, the dark green, the hairiness, the dwarfness, the anomalous stomata and the cold-hardiness. Each tetraploid plant may exhibit a different combination of them. There were apparently normally shaped plants too. The dimensions of the stoma and the epidermis cell on the tetraploid leaf were compared with those of the diploid leaf. The former was larger than the latter.

The factors responsible for inducing apospory were also discussed.

The writer wishes to express his sincere thanks to Profs. Drs. Masao Kumazawa and Ichitaro Harada of Nagoya University for their kind guidance and encouragement throughout this study, and in addition, to the latter for the correction of this manuscript. His thanks are also due to Prof. Dr. Motozi Tagawa of Kyoto University for identifying the ferns very kindly.

References

- Andersson-Kottö, I. 1932. Observations on the inheritance of apospory and alternation of generations. *Svensk Bot. Tidskr.* 26: 99-106.
- Bell, P. R. and Richards, B. M. 1958. Induced apospory in polypodiaceous ferns. *Nature* 182: 1748-1749.
- Beyerle, R. 1932. Untersuchungen über die Regeneration von Farnprimärblättern. *Planta* 16: 622-665.
- Bower, F. O. 1884. On apospory in ferns. *J. Linn. Soc.* 21: 360-368.
- 1889. Note on attempts to induce aposporous development in ferns. *Ann. Bot.* 4: 168-169.
- Digby, L. 1905. On the cytology of apogamy and apospory. II. Preliminary note on apospory. *Proc. Roy. Soc., B.* 76: 463-467.
- Döpp, W. 1955. Polyploidie und andere Erscheinungen bei Farnprothallien durch Wirkung von Gesarol und seinen Bestandteilen. *Naturwiss.* 42: 99-100.
- Druery, C. T. 1884. Further notes on a singular mode of reproduction in *Athyrium filix foemina* var. *clarissima*. *J. Linn. Soc.* 21: 358-360.
- Duncan, R. E. 1941. Apogamy in *Doodia caudata*. *Amer. J. Bot.* 28: 921-931.
- Farlow, W. G. 1889. Apospory in *Pteris aquilina*. *Ann. Bot.* 2: 383-386.
- Farmer, J. B. and Digby, L. 1907. Studies in apospory and apogamy in ferns. *Ann. Bot.* 21: 161-199.
- Goebel, K. 1907. Experimentell-morphologische Mitteilungen. *Sitz. math. physiol. Klasse vom 8 Juni* 119-138.

- Heilbronn, A. 1928. Über experimentell erzeugte Tetraploidie bei Farnen. Zeit. Ind. Abst. Vererb., Suppl. 2: 830-844.
- 1932. Polyploidie und Generationswechsel. Ber. Deut. Bot. Ges. 50: 289-299.
- Hurel-Py, G. 1950. Recherches préliminaires sur la culture aseptique des prothalles de filicinaées. Rev. Gen. Bot. 57: 637-734.
- Jayasekera, R. D. E. and Bell, P. R. 1959. The effect of various experimental treatments on the development of the embryo of the fern *Thelypteris palustris*. Planta 54: 1-14.
- Lang, W. H. 1898. On apogamy and the development of sporangia upon fern prothalli. Phil. Trans., B. 190: 187-238.
- 1924. On some deviations from the normal morphology of the shoot in *Osmunda regalis*. Mem. Manch. Lit. Phil. Soc. 68: 53-67.
- Lawton, E. 1932. Regeneration and induced polyploidy in ferns. Amer. J. Bot. 19: 303-333.
- 1936. Regeneration and induced polyploidy in *Osmunda regalis* and *Cystopteris fragilis*. Amer. J. Bot. 23: 107-114.
- Manton, I. 1932. Contributions to the cytology of apospory in ferns. 1. A case of induced apospory in *Osmunda regalis* L. J. Gen. 25: 423-430.
- 1950. Problems of cytology and evolution in the Pteridophyta. Cambridge Uni. Press.
- Mehra, P. N. 1952. Colchicine effect and the production of abnormal spermatozoids in the prothalli of *Dryopteris subpubescens* (Bl.) C. Chr. and *Goniopteris prolifera* Roxb. Ann. Bot., N. S. 16: 49-56.
- and Loyal, D. S. 1956. Colchicine effect on the prothalli of *Goniopteris multileneata* (Wall.) Bedd. and *G. prolifera* (Roxb.) with emphasis on abnormal spermatogenesis in polyploid prothalli. Ann. Bot., N. S. 20: 544-552.
- Mottier, D. M. 1927. Behavior of certain fern prothallia under prolonged cultivation. Bot. Gaz. 83: 244-266.
- 1931. Development of sex organs of fern prothallia under prolonged cultivation. Bot. Gaz. 92: 218-223.
- Rosendahl, G. 1941. Versuche zur Erzeugung von Polyploidie bei Farnen durch Colchicinbehandlung sowie Beobachtungen an polyploiden Farnprothallien. Planta 31: 597-637.
- Sarbadhikari, P. C. 1939. Cytology of apogamy and apospory in *Osmunda javanica* Bl. Ann. Bot., N. S. 3: 137-145.
- Steil, W. N. 1919a. A study of apogamy in *Nephrodium hirtipes* Hk. Ann. Bot. 33: 109-132.
- 1919b. Apospory in *Pteris sulcata* L. Bot. Gaz. 67: 469-482.
- Takahashi, C. 1961. Chromosome study on induced apospory in the bracken fern. Kromosomo (Tokyo) 48: 1602-1605.
- . The abnormal stomata on the diploid and the tetraploid bracken ferns. Cytologia (in press).
- Ward, M. and Wetmore, R. H. 1954. Experimental control of development in the embryo of the fern, *Phlebodium aureum*. Amer. J. Bot. 41: 428-434.
- Wesselowska, H. 1907. Apogamie und Aposporie bei einigen Farnen. Ber. Deut. Bot. Ges. 25: 85-86.
- Wilkie, D. 1956. Incompatibility in bracken. Heredity 10: 247-256.
- Yamasaki, N. 1954. Über den Einfluss von Colchicin auf Farnpflanzen. I. Die jungen Prothallien von *Polystichum craspedosorum* Diels. Cytologia 19: 249-254.