Investigations on the differentiation of developmental stages of the fern, *Matteucia struthiopteris* (L.) Tod.

Badania nad różnicowaniem się stadiów rozwojowych paproci. *Matteucia struthiopteris* (L.) Tod.

J. PIETRYKOWSKA

INTRODUCTION

The fern prothallia were described for the first time in 1828 by B i s c h o f f, who considered them to be organs corresponding to cotyledons of higher plants. The real discoverer and interpreter of fern prothallia was L e s z c z y c - S u m i ń s k i (1848) who observed and made drawings of all the developmental stages of the prothallium. He also succeeded in observing the antheridia and the liberation of sperms (a process noticed already some years earlier by N ä g e l i). He discovered and described the archegonium — which thus far — was considered to be an early developmental stage of the antheridium, he also observed the penetration of sperms in the archegonia and the formation of the sporophytic embryo. Later investigations on the developmental cycle of ferns — specially those of H o f m e i s t e r (1851) — solved the problem of sexual reproduction of *Pteridophytes* and explained the role played by the prothallium in development of these plants. The normal course of development of the gametophyte (the prothallium) — is according to D ö p p's (1950) scheme as follows: a spore, unbranched filament, unilayered thallus, formation of a multilayered central part of the prothallium and of reproductive organs.

Germination leads to the formation of the first cell of the filament and of the first rhizoid (K a r p o w i c z 1927). Almost in all species successive transversal (anticlinal) divisions of the first cell lead to the formation of the filamental stage. A later developmental stage of the prothallium is the growth of an unbranched filament into a flat prothallium. Three main types of divisions leading to the formation of a flattened prothallium (K a r p o w i c z 1927) can be distinguished: 1. peri-

clinal division in the apical cell, parallel to the filamental axis, 2) periclinal division occuring however only in the second and third cell below the apical one, 3) oblique division occuring mostly in the second, less frequently in the third cell, below the apical one.

Cells formed during later intensive divisions are arranged on the same plane and form a thallus like prothallium. Meristematic activity causes the formation of two lobes and a multilayered central part resulting in a heart shaped prothallium, characteristic of the fern prothallia of the *Polypodiaceae* family. On a prothallium developed in such a way reproductive organs are already formed; archegonia appear during the formation of the two lobes of the prothallium and of the multilayered central part, antheridia may develop already at an earlier stage. It is assumed that bisexuality of prothallia is a normal phenomenon in the development of ferns, whereas, dioecism is considered to be result of the influence of external conditions.

The morphogenetic changes of fern prothallia and their further development depend on such factors as composition of the nutrient solution (N a g a i 1914), humidity, temperature (C o n w a y 1949), light (L i f e 1907, N a g a i 1914, D ö p p 1937, C o n w a y 1949, M o h r 1956), and chemical factors (M o h r 1956).

The purpose of this work was to carry out observations on the developmental course of the fern gametophyte and to examine the influence of external factors on the process of morphogenesis of the fern prothallia with special attention to light as one of the most important factors.

The following factors were considered: A) spore sowing density, B) volume of the nutrient solution, C) light intensity, D) length of the daily light period.

MATERIAL AND METHOD

Matteucia struthiopteris (L.) Tod. was chosen as the experimental plant material because of the great amount of spores produced by this species and their prompt and regular germination and growth. Sporophylls of *Matteucia struthiopteris* (L.) Tod. from plants grown in the Botanical Garden in Cracow were collected in November 1959 and kept between paper sheets. Spore were obtained by shaking sporophylls over a white sheet of paper.

For investigations on the influence of the sowing density on the subsequent development of the prothallia the following densities of spore

suspensions (obtained as described previously — P i e t r y k o w s k a 1962) were used:

Weight in mg for 25 ml nutrient solution	50	20	10	5	2	1	0.1
Relative concentration	1	0.4	0.2	0.1	0.04	0.02	0.002

For investigations on the morphological changes shown by the prothallia developing on various areas 20 mg, and in other experiments 2 mg of spores were sown out in every flask. In experiments on the influence of the culture areas on the development of different morphological stages, flasks of various size were used. The areas of the nutrient solutions were: 12,56; 28,26; 58,06 and 113,04 cm^2.

Similar investigations on the influence of various volumes of the nutrient solution were carried out in gauges with flat bottom in which the upper area of the solution was 7.07 cm^2. Other experiments were performed in 100 ml Erlenmajer flasks containing always 25 ml nutrient solution. Since in the previous work optimal growth was observed on Hoagland's solution (B o n n e r and G a l s t o n — 1952) this solution was used in all experiments. Volumes 5, 10, 15 and 25 ml were used for investigations on the influence of various volumes of the nutrient solution on the morphogenesis of the prothallium.

Cultures were kept in a dark and in a light thermostat provided with fluorescent tubes giving light intensities ranging from 2800 to 3000 lux. In both thermostats the temperature was 26—28°C. In experiments on the action of the length of the light period — the cultures were daily supplied with light for 1, 6 and 12 hours. Other experiments were carried out in continuous light. Neutral filtres were applied to reduce the light intensity which was measured by means of a luxmeter equipped with Lange's photo-cell.

In all these experiments the cultures were grown for 36—40 days — whereas observations on the influence of light were performed for 24 days. Only exceptionnally observations of the whole developmental cycle of the gametophyte were carried out for about 2 months until fertilization took place. Measurements were made after 12, 24—27, 36—40 days after sowing. For microscopical observations samples were taken from cultures by means of a needle. The progress of differentiation was determined by calculating the frequencies of the following developmental stages: 1) filamentous prothallia, fig. 1a; 2) flattened prothallia, fig. 1b; 3) heart shaped prothallia, fig. 1c. Microscopical observations were made with objectives 5 × and 10 ×, and an eyepiece 15 ×. For

more detailed observations an 40 × objective was used. In filamentous prothallia measurements of cell length and width were performed in investigations on the influence of various densities of spore sowing on
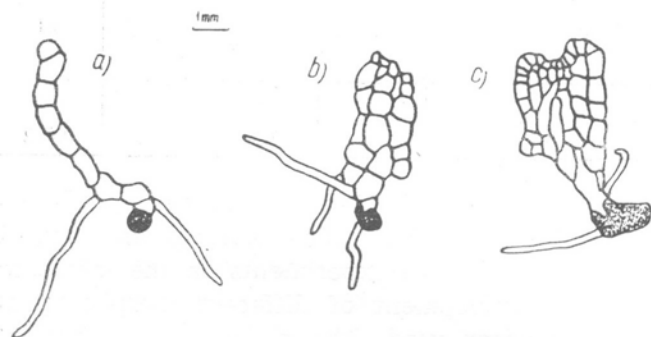

Fig. 1. Main morphological types of fern prothallia (*Matteucia struthiopteris*): *a* — filamentous prothallium, *b* — flattened prothallium, *c* — heart shaped prothallium

the morphogenesis of prothallia. Measurements were performed by means of an eyepiece 8 × provided with scale and an 5 × objective. The cell volume was calculated according to the formula for the volume of a cylinder.

RESULTS

A. *Spore sowing density*

It was shown in a previous investigation (1962) that the density of sowing spores of *Matteucia struthiopteris* has a marked influence on their germination. The following experiments were performed in order to establish whether the sowing density influences the further development of the prothallia. The formerly used densities of the spore suspensions were also applied in these experiments. The curves in fig. 2A and 2B illustrate the frequencies (in percent) of the different morphological stages attained by the prothallia after 12 and 27 days of growth in continuous light (3000 lux).

From the obtained results and those given in the previous paper it may be seen that the sowing density influences much more the further development of the prothallia than the process of germination.

With the highest sowing densities (1 and 0,4) even after 40 days of growth no differentiation of prothallia was observed beyond the filamentous stage. Prothallia remain in the form of filaments composed of few elongated cells. With the decrease of the sowing density the percentage of flattened prothallia and completely developed heart shaped forms increases. After an initial small inhibition it reaches the maximum for the lowest sowing density (0.002).

A modification of the above experiment consisted in sowing the same amounts of spores (20 mg) on nutrient solutions having the same volume (25 ml) but presenting increasing free surfaces. In this way various sowing densities were obtained.

On very small areas, with the highest sowing densities only very few filaments formed flattened prothallia. The highest percentage of flattened prothallia was observed on an intermediate area (58,06 cm^2). On the largest area this percentage decreased slightly, most probably because of the intervention of another factor, namely a higher concentration of the nutrient solution caused by a larger evaporation area. The conclusion to be drawn from these observations is that a too high sowing density influences harmfully the development of the fern prothallia and leads to the formation of abnormal, very elongated filamentous forms.

These abnormal forms were compared with normally developed filaments obtained from lower sowing densities.
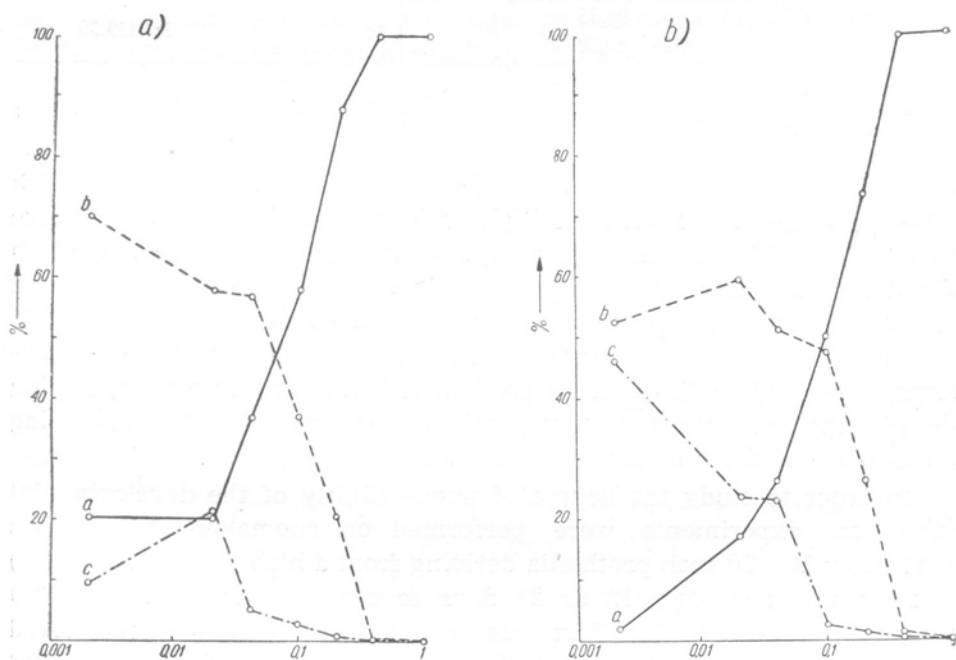

Fig. 2. The influence of the spore sowing density on the morphogenesis of the fern prothallia. x-axis — relative spore sowing density, y-axis — percentage of different morhpological stages: A. after 12 days: a — filamentous prothallia, b — flattened prothallia, c — heart shaped prothallia, B. after 27 days (a, b, c — see fig 2A)

Table 1

Dimensions and volume of cells of filamentous fern prothallia from different densities of sowing (after 12 days)

Spore sowing density mg. per 25 ml.	Dimensions of cells in μ L = length W = width	Length/width	Volume of cells in μ³
1 50 mg.	L 366.95 W 24.60	14.92	174319.59
0.4 20 mg.	L 211.15 W 32.80	6.44	178322.50
0.2 10 mg.	L 147.60 W 43.05	3.43	214634.01
0.1 5 mg.	L 98.40 W 51.25	1.92	202805.35
0.04 2 mg.	L 98.40 W 73.80	1.33	420704.28
0.02 1 mg.	L 96.35 W 77.90	1.24	458981.53
0.002 0.1 mg.	L 96.35 W 69.70	1.38	367439.39

Table 1 presents the dimensions and the corresponding volumes of the cells forming the filamentous prothallia grown for 12 days in cultures with different initial spore sowing densities. The cells which developed in cultures with the highest density sowings (1; 0,4; 0,2) are extremely elongated, narrow and characterized by small volume. With the decrease of sowing density the cells grow more and more spheric and their volumes increase more than two times.

High densities of spore sowing are a factor inhibiting the cell divisions, especially in filamentous prothallia. With the decrease of sowing density the percentage of many celled filaments increases indicating an increase of the number of cell divisions.

In order to study the degree of irreversibility of the developmental inhibitions experiments were performed on anomalous filamentous prothallia; 10—20 such prothallia deriving from a high density sowing (1) were transferred after 12 or 27 days to separate flasks with 25 ml nutrient solution. In the first case only 2 percent of the transferred prothallia maintened their growth ability and formed heart shaped prothallia. The filamentous prothallia grown for 27 days in conditions of higher density sowings completely lost their growth ability. It results from these observations that the inhibition of the development caused by unfavourable growth conditions i.e. high sowing density is an irreversible process.

B. *Volume of the nutrient solution*

The inhibition of development of prothallia resulting from a dense spore sowing could be explained by supposing that the prothallia are able to secrete into the solution a substance inhibiting their further development. In order to test this hypothesis the following experiment was performed: constant amounts of spores (2 mg) were sown out on nutrient solutions with a constant free surface (7.07 cm^2) and a variable volume (5, 10, 15 and 25 ml). A lower percentage of differentiated prothallia was observed only in the first solution (5 ml), where the loss of water due to evaporation caused a considerable increase of its salt concentration. In cultures with greater volumes of nutrient solution no differences in the stages of differentiation were discernible.

C. *Light intensity*

The inhibited development of prothallia from high density sowings is the result of the action of several factors. One of them is doubtlessly the lack of sufficient amount of light caused by a mutual shadowing of the prothallia. When examining light influence three kinds of changes can be distinguished: those caused by intensity, quality and direction of light (D ö p p 1937).

Observations on the morphogenesis of prothallia of *Matteucia struthiopteris* grown in various light intensities (20, 60, 300, 600, 3000 lux) show that the stage differentiation does not occur in darkness. When prothallia of 2—4 cells are transferred into darkness the filaments elongate considerably but do not differentiate into later stages (L i f e 1907).

In very low light intensities (20—600 lux) the differentiation does not take place, the prothallia remain in the filamentous stage — similary as it was observed in cultures with sowings of high density. In higher

Table 2

Percent of filamentous prothallia with different number of cells grown in various light intensities

(after 12 days)

Light intensity in lux	Number of cells in filamentous prothallia							
	1	2	3	4	5	6	7	8
3000	48	10	18	11	8	8	5	2
600	46	17	20	9	8	—	—	—
300	61	22	13	4	—	—	—	—
60	92	7	1	—	—	—	—	—
20	96	4	—	—	—	—	—	—

light intensity (3000 lux) the percentage of flattened (60%) and heart shaped prothallia (20,5%) increases but the optimal light conditions are most probably beyond the highest light intensity used in our experiments.

Low light intensities do not only influence the differentiation process but also inhibit the cell divisions more obviously than high sowing densities. The frequencies of filaments with various cell numbers in 12 days old cultures in dependence on light intensity are shown in Table 2. In low light intensities (20—60 lux) only 1 or 2 cell divisions occur, whereas in 3000 lux light already filaments with 7 cells can be found.

The comparison of prothallia grown in darkness (or in low light) with prothallia developed in cultures with initial dense sowings shows certain morphological similarities which suggest that the inhibition of differentiation in dense sowings is caused, at least to a certain extent, by an insufficient supply of light.

D. *The length of the daily light period*

The well known action exercised on plants by the length of the daily light period induced us to investigate the influence of this factor on the development of prothallia. The length of the daily light periods were 1, 6, 12 and 24 hours. A control was kept in total darkness.

Figures 3A, 3B and 3C show the frequencies of the principal stages of differentiation in dependence on the length of the daily light period in 12, 24 and 36 days old cultures. A minimal light period (3000 lux) of 6 hours is necessary to initiate the process of morphogenesis. A prolongation of the illumination time induces an increase of the percent of differentiated stages which reaches its maximum in continuous light. Heart shaped prothallia are very numerous (62%) in continuously illuminated cultures. Very soon however, they lose their meristems and change into flattened prothallia. Thus, continuous illumination seems to be the optimal factor initiating the morphogenesis of prothallia, leading, however, finally to a prompt degeneration. A too short illumination period is a factor inhibiting the cell divisions, especially in filamentous prothallia. In a 12 days old dark culture only one cell division at most takes place. The lengthening of the daily light period increases the number of cell divisions which attains 7 in continuous illumination (comp. Tab. 2).

The inhibited development of fern prothallia observed in light of low intensity and short times of exposure to light is chiefly the result of the depression of the intensity of photosynthesis. The fact, however, that prothallia grown in favourable light conditions are able to develop

flattened prothallia already in the 2—3 cells stage, but only abnormal
filamentous prothallia when cultured in unfavourable light conditions
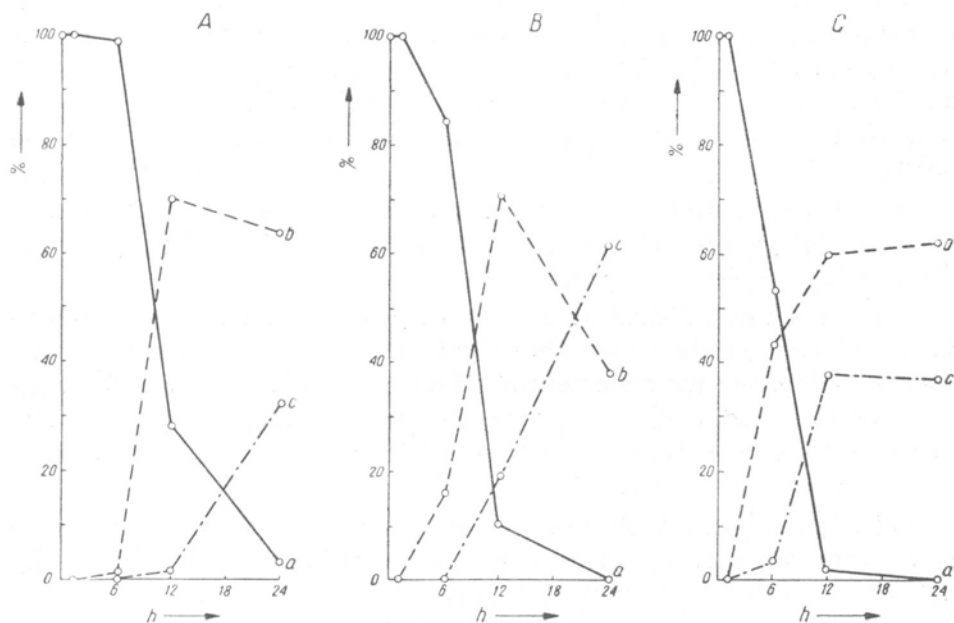and divide in one direction only producing filaments with even 20 cells,

Fig. 3. The influence of the length of the daily light period on the differentiation
of stages of fern prothallia. x-axis — daily light period in hours, y-axis as
in fig. 2. *A.* after 12 days, *B.* after 24 days, *C.* after 36 days, *a, b, c* — as
in fig. 2*A*

gives evidence that light does act not only as a trophic but also as a mor-
photic factor. It may be supposed that growth substances, which are
inactivated only by high light intensities are partially responsible for
the observed phenomena.

DISCUSSION

The results of our experiments corroborate the results of similar
investigations on the influence of light performed on other fern species.
A characteristic dependence of morphogenesis of fern prothallia on light
intensity was already given by K l e b s in 1916 (according to D ö p p
1937). In very weak light after spore germination only long filaments
with few cells were formed. In stronger illumination the filaments did
not attain such length; they were much shorter. Concomitantly with
increasing light intensity, flattened unilayered prothallia were formed,
whereas, heart shaped prothallia with a multilayered central part dif-

ferentiate in optimal light conditions. When illumination was most intensive the initiation of the growth of the prothallia was rapid, but their subsequent development was inhibited.

J. H. and P. M. Miller (1961) have found that variations in light intensity produce striking differences in the growth of the gametophyte of *Onoclea sensibilis*. With increasing light intensity the cell number also increases; this process is accompanied by a decrease in length relative to width. Increase in cell number parallels in area of the prothallium.

In the present work we obtained very similar results by cultivating the gametophytes of *Matteucia struthiopteris* in different spore sowing densities and different light intensities.

It has been established in the present work that a normal differentiation of fern prothallia occurs only in light of high intensity. These results corroborate the statement of Mohr (1956) that only high intensities of light inducing an activation of auxins can lead in prothallia to morphogenetic changes by disturbing the polarity of cells and their three dimensional growth.

It has also been established for the prothallia of *Matteucia struthiopteris* that their normal development requires light of high intensity applied for a longer period of time (at least 6 hours per 24). These results are in agreement with the results of similar experiment carried out by Conway (1949) on the influence of the length of the day on the differentiation process in *Pteridium aquilinum*. The germinating spores exposed daily to 6 hours long illumination for 33 days form filaments slightly elongated at the top and deprived of sexual organs. With 12 hours of daily illumination, flattened and heart shaped prothallia with a certain number of archegonia appear. A daily 18 hours long illumination promotes the formation of heart shaped prothallia with numerous antheridia and archegonia.

SUMMARY

1. A study was made on the influence of the following external factors on the morphogenesis and development of the prothallia of the fern *Matteucia struthiopteris* (L.) Tod.: spore sowing density, volume of the nutrient solution, light intensity and length of the daily light period.

2. High spore sowing densities cause a notable and irreversible inhibition of the development of fern prothallia. In filamentous prothallia high sowing densities inhibit also the cell divisions.

3. Presumably no growth inhibiting substances are secreted in the nutrient solution by the growing prothallia. This is indicated by the fact that the volume of the nutrient solution is without influence on the development of the prothallia.

4. In dense sowings insufficient light supply is one of the factors which are responsible for anomalous morphogenesis of prothallia. In order to assure their normal differentiation a much higher light intensity than needed for spore germination is required.

5. The differentiation process requires light of high intensity which must be applied for a longer time. In this respect continuous light was found the most favourable.

Department of Plant Physiology *University of Cracow*   (Entered: 25.II.1962)

REFERENCES

B o n n e r J., G a l s t o n A. W., 1952, Principles of Plant Physiology, San Francisco, W. H. Freeman and Co.

C o n w a y E., 1949, The Autecology of the Bracken (*Pteridium aquilinum*). The Germination of the Spore, and the Development of the Prothallus and the Young Sporophyte, Proceedings of the Roy. Soc. Edin. 63: 325—343.

D ö p p W., 1937, Gestaltung und Organbildung innerhalb der Gametophytgeneration der Polypodiaceen unter besonderer Berücksichtigung genetischer Gesichspunkte, Beitr. Biol. d. Pflanz. 24: 201—238.

D ö p p W., 1950, Eine die Antheridienbildung bei Farnen fördernde Substanz in den Prothallien von *Pteridium aquilinum* (L.), Ber. Dtsch. Bot. Ges. 63: 139—147.

N a g a i I., 1914, Physiologische Untersuchungen über Farnprothallien, Flora 106: 281—330.

K a r p o w i c z W., 1927, Badania nad rozwojem przedrośli oraz pierwszych liści sporofitu paproci krajowych (*Polypodiaceae*), Rozprawy Wydziału Mat.--Przyrod. 68: 1—34.

L e s z c z y c - S u m i ń s k i J., 1848, Zur Entwickelungs Geschichte der Farnkräuter, Berlin, 1—22.

L i f e A. C., 1907, Effect of Light Upon the Germination of Spores and the Gametophyte of Ferns, Eighteenth Ann. Rep. Missouri Bot. Garden 109—120.

M i l l e r J. H. and M i l l e r P. M., 1961, The effect of different light conditions and sucrose on the growth and development of the gametophyte of the fern, *Onoclea sensibilis*, Amer. Jour. Bot. 48: 154—159.

M o h r H., 1956a, Die Beeinflussung der Keimung von Farnsporen durch Licht und andere Faktoren, Planta (Berl.) 46: 534—551.

M o h r H., 1956b, Die Abhängigkeit des Protonema-wachstums und der Protonema-polarität bei Farnen vom Licht, Planta (Berl.) 47: 127—158.

P i e t r y k o w s k a J., 1962, Investigations on the germination of spores of the fern *Matteucia struthiopteris* (L.) Tod., (in press).