

# CONTRIBUTIONS TO THE CYTOLOGY OF AOSPORY IN FERNS.

## I. A CASE OF INDUCED AOSPORY IN *OSMUNDA REGALIS* L.

BY IRENE MANTON, PH.D.

*Lecturer in Botany in the University of Manchester.*

(With Four Text-figures.)

### INTRODUCTION.

IN 1924 Prof. Lang described and figured<sup>1</sup> some instances of "induced apospory" in *Osmunda regalis*. Normal sporeling plants, grown under unanalysable but unfavourable cultural conditions, or after wounding, were seen on several occasions to produce prothalli from the tips of leaves. Only leaves of the juvenile type were thus affected and the tendency ceased to be evident when the sporelings grew older. When such leaves were severed and laid on soil the prothalli grew into apparently normal gametophytes bearing sex organs and producing numerous young plants.

Induced apospory had previously been obtained by Goebel (1907) in detached sporeling leaves of a number of different species of ferns including *Osmunda regalis*<sup>2</sup>. Since the forms experimented upon had been chosen at random and were known to be usually normal in life history, the opinion was expressed that such morphological plasticity was probably widespread among ferns, and comparison was made with protonemal regeneration from the sporophyte in mosses. In no case however were the induced prothalli kept alive nor were any plants obtained from them. An additional value therefore attaches to the newer data.

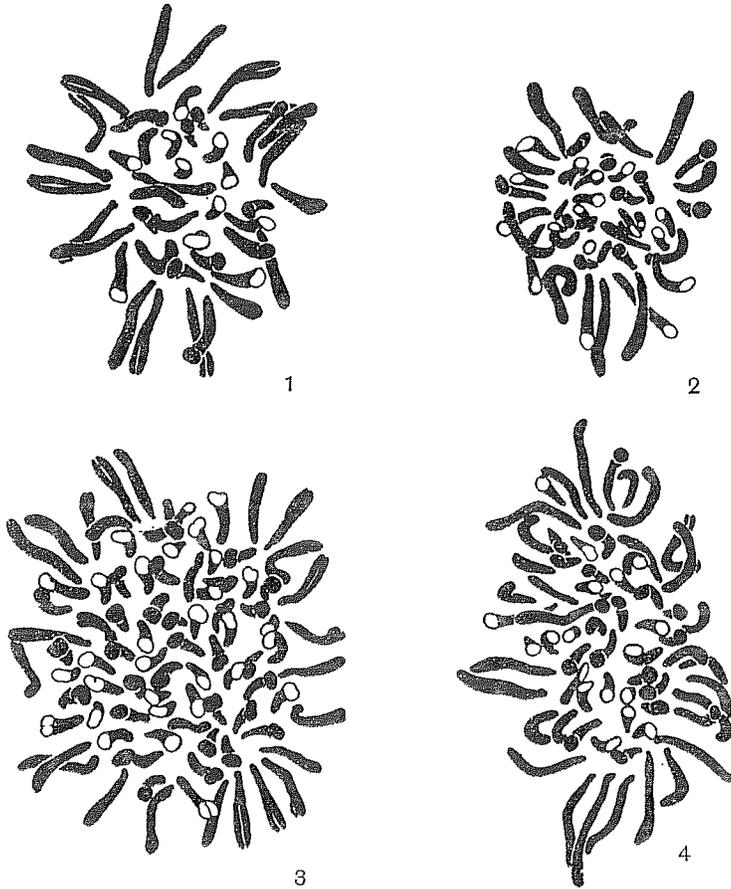
No instance of induced apospory in ferns has so far been subjected to cytological analysis, though evidence has been accumulated concerning the nuclear phenomena in cases of direct (*i.e.* obligate) apospory. For this reason and since the full investigation of the material will require work through several growing seasons, the interest of some cytological facts already obtained seem to justify publication in the present form.

<sup>1</sup> *Mem. and Proc. of the Manchester Lit. and Phil. Soc.* LXVIII, 53.

<sup>2</sup> Cf. Goebel's *Organographie*, ed. 2, I, 422. An interesting case is also quoted (p. 421) of the spontaneous proliferation of prothalli from a single leaf of an otherwise normal adult plant of *Asplenium dimorphum*.

THE CYTOLOGY OF AOSPORY IN *OSMUNDA*.

The normal chromosome number for *Osmunda regalis* is well known to be 22 in the haploid gametophyte and 44 in the diploid sporophyte<sup>1</sup>. These numbers have been confirmed here in plants of the same general strain in which the original abnormalities had been induced. Fig. 1 illustrates a somatic mitosis in a normal root.



Figs. 1-4. Chromosomes in *Osmunda regalis*.  $\times 2200$ .

1. From a root tip of a normal plant.
2. From a developing antheridium of the aposporously produced prothallus.
3. From a root tip of a tetraploid plant.
4. From a root tip of a triploid plant.

<sup>1</sup> Cf. De Litardière, 1920, p. 329.

The aposporous prothallus investigated was derived from the leaf depicted in Fig. 5 of Prof. Lang's paper. The leaf was of the juvenile palmate type with five veins and five corresponding apical meristems from each of which had developed a prothalloid growth. "This leaf was laid down on soil and the five new growths gave rise to a crop of prothalli aposporously produced that have borne numerous young plants<sup>1</sup>." This crop has since been maintained in continuous cultivation, indefinite vegetative propagation being facilitated by the massive liverwort-like habit. Morphologically the prothalli are quite normal.

A cytological preparation of developing antheridia was obtained by Prof. Lang's assistant, Mr Ashby, in 1927. In this there is one antheridium containing eight cells in full mitosis of which one presents a metaphase plate of chromosomes in face view (Fig. 2). An exact count of this plate is not possible since the origin of various fragments of chromosomes (not shown in the figure) is uncertain. There is no doubt, however, that the number is approximately 44 and that the prothallus, as was to be expected from its origin on the edge of a leaf, possesses the unreduced diploid number of chromosomes normally characteristic of the sporophyte. A cytological change other than that of the numerical relation of the chromosomes must therefore be assumed to have initiated the alteration in ontogeny of the growing points of the leaf concerned.

In the autumn of 1931 cytological preparations were made of root-tips from seven plants of the progeny from this same prothallial crop. The plants are now of considerable size, having been transferred from the culture pan into a bed in 1924.

Of these seven plants three were found to have approximately, and two exactly, 88 chromosomes. All five may therefore be regarded as tetraploids. Since organs of both sexes occur on the prothalli, it is probable that the tetraploids owe their origin to a sexual fusion of diploid gametes.

Two out of the seven plants examined, however, proved to have 66 chromosomes. They are therefore triploids. Of the genesis of these triploids nothing is at present known, but there are clearly two alternative explanations. A sexual fusion may have occurred between haploid and diploid gametes (an occurrence which is known to be possible in the mosses) by the accidental contamination of the original prothallial culture with a normal gametophyte from a self-sown spore. The only other explanation necessitates the occasional occurrence of a reduction division somewhere in the diploid prothallus. This possibility is of such great

<sup>1</sup> Lang, *loc. cit.* p. 57.

theoretical interest that accurately controlled experiments are being undertaken to investigate it further.

Apart from the problems raised by the presence of the triploids, the fact of the production of tetraploid plants from a morphologically normal but diploid prothallus is of some interest. It is unexampled among the ferns but is obviously comparable, as was surmised by Goebel, to the phenomena of apospory in the *Bryophyta* discovered by Pringsheim and Stahl and investigated cytologically by the Marchals and more recently by F. von Wettstein<sup>1</sup>. These workers have shown in many species of mosses that regeneration of protonemata may readily be induced from fragments of sporogonia. Such (aposporous) protonemata can develop sexually perfect though diploid moss plants from which tetraploid capsules arise after fertilisation. Tetraploid moss plants can be obtained in some forms by further protonemal regeneration from fragments of these tetraploid capsules, but in all pure species such tetravalent gametophytes are sterile. Higher valencies however can be reached in hybrid strains and a continuous series culminating in octoploids of both generations has been produced in this manner from a hybrid between *Physcomitrium piriforme* × *Funaria hygrometrica*<sup>2</sup>. To what extent comparable extension of the polyploid series will be possible in *Osmunda* is under experiment.

The diploid, triploid and tetraploid *Osmunda* plants are morphologically indistinguishable on a casual inspection. Detailed comparison will however be made when the leaves have expanded in the spring. The two triploids are somewhat larger than the contemporary tetraploids and produced fertile leaves bearing spores in 1931. None of the tetraploid plants have so far developed sporangia, but since no attempt had been made to assure uniformity of growth conditions the latter facts may have no significance. It is hoped to complete the study of both the triploids and tetraploids by an investigation of meiosis and the behaviour of subsequent spore-produced generations from each.

#### COMPARABLE CYTOLOGICAL FACTS IN THE FERNS.

For comparison with *Osmunda* it may be of interest to summarise briefly the available cytological data for other cases of exceptional or aberrant life history among ferns. They include examples of both apogamy and apospory, these terms being used here in their widest sense and on cytological rather than morphological criteria. Thus by *apogamy* is im-

<sup>1</sup> Cf. Goebel's *Organographie*, ed. 2, 1, p. 421, and Wettstein, 1925.

<sup>2</sup> Cf. Wettstein, 1928, p. 125.

plied the origination of a sporophyte from any cell of a gametophyte other than the fertilised egg, but including the unfertilised egg whether haploid (cf. *Nephrodium molle*) or diploid (as in *Athyrium filix-foemina* var. *clarissima* Bolton). *Apospory* denotes the origination of a gametophyte from any cell or cells of the sporophyte other than the haploid spore but including the diploid spore (as in *Marsilia Drummondii*).

The following table summarises the facts for every case known to have been investigated cytologically.

GROUP I. *Apogamy only*. True spore formation.

- (a) Induced apogamy. Sporophyte haploid but not further investigated.  
*Nephrodium molle* Desv. Yamanouchi, 1908.
- (b) Pseudofertilisation between vegetative nuclei of prothallus.  
*Nephrodium (Lastrea) pseudo-mas*<sup>1</sup> var. *polydactyla* Wills. Farmer, Moore and Digby, 1903.  
*Nephrodium (Lastrea) pseudo-mas*<sup>1</sup> var. *polydactyla* Dadds. Farmer and Digby, 1907.
- (c) Pseudofertilisation between spore mother cells before meiosis<sup>2</sup>.  
*Nephrodium falcatum*. Allen, 1914.  
*Nephrodium hirtipes* Hk. Steil, 1919.

GROUP II. *Apogamy with apospory*.

- (a) Morphology of spores retained but a percentage of them unreduced. Both generations diploid (fate of haploid spores unknown).  
*Marsilia Drummondii* R. B. Strasburger, 1907.
- (b) Prothalli not from spores but by soral, apical or marginal apospory. Both generations diploid.  
*Athyrium filix-foemina* var. *clarissima* Jones.  
*Athyrium filix-foemina* var. *clarissima* Bolton.  
*Athyrium filix-foemina* var. *unconglomerata* Stansfield.  
<sup>3</sup>*Scolopendrium vulgare* var. *crispum Drummondiae*. Farmer and Digby, 1907.
- (c) As (b) but both generations haploid.  
*Nephrodium pseudo-mas*<sup>1</sup> var. *cristata apospora* Druery. Farmer and Digby, 1907.

GROUP III. *Apospory without apogamy*.

- Scolopendrium vulgare* "peculiar." Andersson-Kottö, 1931.  
Successive sexual fusions produce a series of increasing chromosome numbers.  
Details still uncertain.

As they stand, the facts recorded clearly do not cover the whole field of the phenomena with equal adequacy. Mention has already been made of the scantiness of knowledge for the induced aberrations long known morphologically from the work of Lang (1898) and Goebel (1907). The one record for induced apogamy by Yamanouchi (1908) in *Nephrodium*

<sup>1</sup> = *Dryopteris filix-mas*.

<sup>2</sup> The correctness of this interpretation of the cytological observations is not beyond question. Cf. Rosenberg, 1930, p. 24.

<sup>3</sup> Some irregularity in chromosome numbers was here reported.

*molle*, excellent though it appears to be in itself is unconfirmed (cf. Black, 1909). The very peculiar cytological behaviour attributed to the four examples of pseudofertilisation quoted in Group I would also justify confirmatory work on each of them and an extension of the investigation to any other available instance of apogamy associated with normal spore production, e.g. *Pellaea* spp. (Woronin, 1908). The evidence for the haploid nature of the sporophyte in *Nephrodium pseudo-mas* var. *crispata apospora* (as in *Nephrodium falcatum* and *hirtipes*) is suggestive rather than conclusive. Finally the work on the one form of *Scolopendrium vulgare* (in Group III) is still in progress and only available in a preliminary form (Andersson-Kottö, 1931) while the older investigation of the other (var. *crispata Drummondii* Farmer and Digby 1907 in Group II) is ambiguous.

For comparison with *Osmunda*, however, the case of the "peculiar" *Scolopendrium* is of such interest that, in spite of incompleteness, it deserves special mention. Detailed discussion is not possible until the work is more fully published, but there are three significant facts that have been specifically stated<sup>1</sup>. Firstly, the "peculiar" plants appear never to produce spores but bear sexually functional prothalli in the place of the lamina of leaves. Secondly, the aposporous habit seems to be the expression of a precise Mendelian mechanism. In both these respects *Scolopendrium* stands alone. Thirdly, the absence of apogamy leads to a multiplication of chromosomes in successive generations. This is in marked contrast to all other cases quoted above, but is clearly a strong point of comparison with *Osmunda*. The details of the chromosome series are not strictly comparable, for there seem to be complications in *Scolopendrium* which are still largely unexplained. It is noteworthy however that these appear to involve some kind of chromosome reduction in the gametophyte in connection with the organs of one sex. The problem of the origin of the triploids in *Osmunda* may therefore prove to be closely akin.

#### CONCLUSION.

The unsolved cytological problems connected with apospory in ferns are evidently many and varied. A certain orderliness appears to emerge however, in spite of diversity of detail and incompleteness of observation, if the facts are considered solely from the standpoint of the broad cytological consequences of the morphological aberration. In every case other than *Osmunda* and *Scolopendrium* apospory entails the loss of one or other

<sup>1</sup> Andersson-Kottö, 1931, pp. 273-81.

of the two normally alternating nuclear phases. Sometimes it appears to be the diploid phase which is eliminated (cf. *Nephrodium pseudo-mas* var. *cristata apospora*), more commonly it is the haploid. In either event a single chromosome number is retained unchanged throughout the whole life cycle. The virtual loss of reduction division (by the fact of apospory) is as it were *balanced* by a loss of the complementary process of sexual fusion (*i.e.* by apogamy). On the other hand, in *Scolopendrium* and *Osmunda* alone the retention of syngamy effects, as in the mosses, a *progressive* increase in the chromosomal constitution.

The difficulty of the morphological problems raised is obvious. Discussion of these, however, would here be out of place, for the cytological and morphological facts belong at present to apparently independent fields of view. These cannot be connected without the elucidation of some genetical and cytological basis for antithetic alternation of generations which will harmonise with both series of facts. At present the chromosomal data only emphasise the difficulty of applying any current conception of the physical mechanism of inheritance to the facts of life history in the higher plants.

Sincere thanks are due to Prof. Lang for the original offer of the material and for much valuable help, and also to Mr Ashby for the earlier cytological preparations and for care of the cultures.

#### SUMMARY.

1. Some preliminary cytological observations are recorded for a case of induced apospory previously described by Lang (1924).
2. The chromosome number reported for the normal sporophyte by other workers has been confirmed ( $2n = 44$ ).
3. One aposporously produced prothallus was shown to be diploid.
4. Five plants from the progeny of the same prothallial crop were found to be tetraploids ( $4n = 88$ ). These are assumed to be the product of sexual fusion between diploid gametes.
5. Two plants were found to be triploids ( $3n = 66$ ). The genesis of these is still unexplained.
6. Comparison is made with the mosses (cf. Wettstein) and tentatively with *Scolopendrium* (cf. Andersson-Kottö) on the ground that all three show a "progressive" increase in the chromosome complement as a result of apospory.
7. Cytological facts for all other cases of abnormal life cycle known to have been investigated among ferns are summarised. In every instance

430 *Contributions to the Cytology of Apospory in Ferns*

other than *Osmunda* and *Scolopendrium* apospory appears to have been "balanced" by apogamy with the establishment of a constant nuclear state throughout both morphological generations.

REFERENCES.

- ALLEN, R. F. (1914). "Studies in spermatogenesis and apogamy in ferns." *Trans. of the Wisconsin Acad. of Sciences, Arts and Letters*, xvii, 1.
- ANDERSSON-KOTTÖ, I. (1931). "The genetics of ferns." *Bibliographia Genetica*, viii, 269.
- BLACK, C. A. (1909). "The development of the imbedded antheridium in *Dryopteris stipularis* (Willd.) Maxon and '*Nephrodium molle*'." *Bull. Tor. Bot. Club*, xxxvi, 557.
- DE LITARDIÈRE, R. (1920). "Recherches sur l'élément chromosomique dans la caryocinèse somatique dans Filicinées." *La Cellule*, xxxi, 255.
- FARMER, J. B. and DIGBY, L. (1907). "Studies in apospory and apogamy in ferns." *Annals of Botany*, xxi, 161.
- FARMER, J. B., MOORE, J. E. S. and DIGBY, L. (1903). "On the cytology of apogamy and apospory. I. Preliminary note on apogamy." *Proc. Roy. Soc.* lxxi, 453.
- GOEBEL, K. (1907). "Experimentell-morphologische Mitteilung." *Sitzungsber. k. Bayer. Acad. der Wissenschaften, Math.-Phys. Klasse*, xxxvii, 119.
- (1913). *Organographie der Pflanzen*, ed. 2, vol. i.
- LANG, W. H. (1898). "Apogamy and the development of sporangia upon fern prothalli." *Phil. Trans. Roy. Soc. B*, cxc, 187.
- (1924). "On some deviations from the normal morphology of the shoot in *Osmunda regalis*." *Mem. and Proc. of the Manchester Lit. and Phil. Soc.* lxxviii, 53.
- ROSENBERG, O. (1930). "Apogamie und Parthenogenese bei Pflanzen." Berlin.
- STELL, W. N. (1919). "A study of apogamy in *Nephrodium hirtipes* Hk." *Annals of Botany*, xxxiii, 109.
- STRASBURGER, E. (1907). "Apogamie bei *Marsilia*." *Flora*, xcvi, 123.
- WETTSTEIN, F. VON. (1925). "Genetische Untersuchungen an Moosen." *Bibliographia Genetica*, i, 1.
- (1928). "Morphologie und Physiologie des Formwechsels der Moose auf genetischer Grundlage II." *Bibliotheca Genetica*, x.
- WORONIN, H. (1908). "Apogamie und Aposporie bei einigen Farnen." *Flora*, xcvi, 101.
- YAMANOUCHI, S. (1907). "Apogamy in *Nephrodium*" (preliminary summary of results). *Bot. Gaz.* xlv, 142.
- 1908. "Apogamy in *Nephrodium*." *Bot. Gaz.* xlv, 289.