

PROTHALLI OF *CERATOPTERIS PTERIDOIDES* (HOOK.) HIERON.⁽¹⁾

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Abstract: Spores of *C. pteridoides* are destined to produce male, hermaphroditic, and female prothalli, like those of *C. thalictroides*, under culture conditions. The sexuality of the prothalli is related to their vegetative growth.

INTRODUCTION

The prothalli of *C. thalictroides* have been studied by many, i.e. Kny (1875), Goebel (1908), Yabe and Yasui (1913), Mahabálé (1948), Javalgekar (1960), Pal and Pal (1962), Chiang (1963), Momose (1964), Chiang and Chiang (1967), Nayar and Kaur (1969), Klekowski (1970), and Schedlbauer and Klekowski (1972). There are, however, two opposite opinions concerning their sexuality; some workers believe that they are monoecious, but others not. None of the workers except Pal and Pal (1963), Chiang (1963) and Chiang and Chiang (1967) have ever mentioned seeing the female prothalli of this fern. The present study dealt with the morphology of the prothalli of a related member, *C. pteridoides* with special reference to their sexuality. So far this species has not been studied in this aspect.

MATERIALS AND METHODS

The prothalli of *Ceratopteris pteridoides* (Hook.) Hieron. investigated were raised from spores which were collected on Oct. 30, 1972 in the greenhouse of the Department of Botany, National Taiwan University where the plants (collected from Taipei Zoo in 1960 by Dr. C. E. DeVol) had been kept growing in water-tanks. The spores were sieved through lens paper which retained the debris of the sporangial wall and the spores were stored in paper bags and then in PVC bags for future use. The spores were sown on Feb. 12, 1973 on a layer of filter paper wetted with 1/2 Hoagland's solution in petri dishes and kept under continuous illumination of two 40W white fluorescent lamps plus one 60W incandescent lamp plus natural diffuse light from the north window of the laboratory (approximately 2,500 lux) at room temperature. Observations were made within one month after the sowing of the spores.

OBSERVATIONS

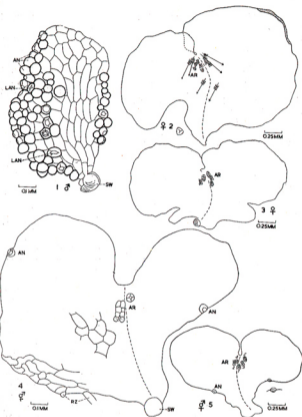
As in *C. thalictroides* (Pal and Pal, 1963; Chiang and Chiang, 1967), the spores of *C. pteridoides* are destined to produce both unisexual and bisexual prothalli, i.e. male, female and hermaphroditic prothalli under culture conditions.

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Male prothalli. The male prothallus is typically spatulate at maturity, bearing only antheridia (Fig. 1). It is very much smaller than the female prothallus, being about 0.5 mm wide and 1 mm long. It is only one cell thick throughout the whole prothallus. The male prothallus is neither bilaterally nor radially symmetrical, i.e. one lateral side is slightly larger than the other side. More antheridia occur on the larger side of the prothallus. On one specimen as shown in Fig. 1, 64 antheridia were found, 52 of them were formed on the larger side of the prothallus, while only 12 were borne on the other side. The ratio of the number of antheridia on the two sides in this example is approximately 4:1. This asymmetric occurrence of the antheridia on the male prothallus is correlated with the asymmetric growth of the prothallus. The central region of this prothallus from the posterior end (spore) to the apex remains sterile or vegetative. The number of the vegetative cells which are seen in the ventral view was 64, and was accidentally equal to that of antheridia, in this example (Fig. 1). Two types of vegetative cells are recognizable: in one type they are larger and more or less elongated cells located in the mid-region of the prothallus, with their long axis parallel to the long axis of the prothallus; and in the other type they are isodiametric marginal cells borne on the tip of the prothallus. The antheridia produced on the male prothalli are more or less globose, projecting from either the lateral margin or the undersurface (ventral side) near the lateral margin of the prothalli (Fig. 1).

Female prothalli. The female prothalli are larger than the male, bearing only archegonia (Figs. 2, 3). Typically, they are broadly cordate in outline, with meristematic cells lying in the anterior notch. They have two wing-like lateral lobes of approximately equal size (Fig. 3), but at times, of unequal size (Fig. 2). The formation of lateral wings of two more or less equal size indicates that the single meristematic notch produces approximately equal numbers of derivatives on both sides of the prothallus. Occasionally an additional small basal lobe formed at the base of each lateral lobe is found (Fig. 3). The female prothallus is one cell thick, except for the slightly thicker median region which is several cells thick and extends from the notch to the posterior end of the prothallus. Archegonia are produced successively from the meristematic notch on this cushion. In some prothalli, the archegonia occur alternately on both sides near the mid-line of the prothallus (Fig. 5), but in others this regularity is not distinct (Figs. 2, 3). The alternate occurrence of archegonia seems to be related to the possible presence of a prothallial apical cell which exhibits regular bilateral segmentation. Pal and Pal (1963) reported that no prothallial apical cell was found in their materials, whereas Kny (1875) and Javalekar (1960) believed that an apical cell was formed during development of the prothalli. As in *C. thalictroides*, archegonia always occur in a group behind the notch of the prothallus. It is reasonable to think that the occurrence of archegonia in one region is advantageous in intensifying the attractive force of the archegonia for spermatozooids. There is a sterile region of about 1 mm in length between the posterior end (spore) of the prothallus and the first archegonium, indicating that the prothallus needs a certain period of vegetative growth before the production of archegonium. It was observed in the present study that the long axes of the young archegonia change their orientation slightly as the prothallus grows. As shown in Fig. 2, the young archegonia occurring near the notch, have their necks obliquely inclined outwards and downwards. As the archegonia grow older, they turn gradually inwards and finally become parallel to the median line of the prothallus.



Figs. 1-5. Prothalli of *C. peridoides*. All illustrations are from the ventral sides of the prothalli. 1. A mature male prothallus with 64 antheridia (indicated by thick lines) which are borne not only on the margin but also beyond the margin of the prothallus. The number of antheridia found on the left and right sides of the prothallus is respectively 32 and 12. 2. A female prothallus with 13 archegonia. Note that the right wing of the prothallus is larger than the left, and the orientation of the archegonial necks. The dotted area indicates the withered area. 3. A female prothallus with basal lobes and 5 archegonia. 4. A monoecious prothallus with 2 archegonia and 2 marginal antheridia. 5. A monoecious prothallus with female tendency, i.e. with 5 archegonia and 2 antheridia. Note that the archegonia occur alternately on each side of the prothallial wings. The number indicates the relative age of the archegonium, i.e. no. 1 is the oldest archegonium. Figs. 1 and 4, 24 days after sowing of the spores. Figs. 2, 3 and 5, 31 days after sowing of the spores. Abbreviations: AN, antheridium; AR, archegonium; LAN, liberated antheridium; RZ, rhizoid; SW, spore wall.

Hermaphroditic prothalli. The hermaphroditic prothallus is cordate, and thus it closely resembles the female prothallus rather than the male in shape (Fig. 4, 5). However, the two following morphological features indicate that it is an intermediate prothallus between the male and female prothalli. Firstly, the size of the hermaphroditic prothallus is typically larger than the male but smaller than the female. Secondly, the apical meristem of the hermaphroditic prothallus loses its vigorous activity earlier than that of the female prothallus but lasts longer than that of the male. In the hermaphroditic prothallus, the growing apex is more or less open, resulting in a U-shaped depression (Fig. 4), indicating that the meristem is less active than that of the female prothallus in which the notch is closed or overlapped (Fig. 2). In the male prothallus, as the meristematic activity of the apex disappears in the early stage of development of the prothallus, the notch is undeveloped (Fig. 1). In *C. thalictroides*, Pal and Pal (1963) reported that in size and shape the female prothalli appeared to be intermediate between the male and bisexual ones.

Fertilization. A mature archegonium needs external water for fertilization. Five minutes after contact with water, the archegonium excretes a mass of dense protoplasmic materials (transformed from the ventral canal cell and neck canal cell) from the mouth of the archegonial neck, attracting spermatozooids. The period of attraction of a mature archegonium for spermatozooids lasts about one hour in water. Not all archegonia mature at the same time. In one specimen (Fig. 5) there were five archegonia; the first three archegonia were found actively attracting the spermatozooids, whereas the 4th and 5th, the younger, immature archegonia, attracted no spermatozooids. In this specimen, the mouth of the 4th archegonium was open whereas that of the 5th archegonium was still closed. In *C. thalictroides*, two sporophylls (one is slightly larger than the other) have been found occurring on the same prothallus (Chiang and Chiang, 1967), indicating that fertilization may occur in two archegonia of the same prothallus. In fertilization, several spermatozooids (about four) enter into the archegonium through the funnel-shaped mouth of its neck. A mature antheridium liberates spermatozooids in about three minutes after contact with water. They swim towards archegonia by moving their flagella in water. The movement of flagella continues for about twenty minutes after entering into the venter. The spermatozoid loses its mobility in a film of water between the cover slip and slide in about half an hour. The movement of spermatozooids was found retarded in this thin film of water between two glass plates.

Vegetative reproduction of the prothalli. In the present study it was observed that a mature or old male prothallus of *C. pteridoides* in an isolated culture is endowed with a potential to continue its life, by producing vegetatively one or more new individual prothalli before its death. As the sporophyte produces adventitious buds which give rise to new plants, so the sterile cells of the male prothallus of this fern give rise vegetatively to adventitious (aposporous) prothalli. In this observation, two typical female prothalli were found developing vegetatively from a single mature male prothallus. Whether the adventitious prothallus arises from a single initial or from a group of initials is unknown. However, since the adventitious prothallus is always just as typical as a prothallus produced from a spore, it is presumable that it arose from a single initial. Williams (1938) mentions that the production of adventitious prothalli appears to be correlated with a removal of the influences exercised by the apex. He states that experimental removal of the apex of the fern prothallus leads to the development of adventitious prothalli from any region of the prothallus, while in undamaged prothalli they are formed only on the

older basal regions which presumably are no longer influenced by the growing point.

DISCUSSION

The spore size varies widely in *Ceratopteris*. The fossil spores studied by Sohma (1973) were somewhat smaller than those of extant species, ranging from 77 to 100 μ in diameter. The variation of spores in size in *C. thalictroides* was investigated by Chiang (1963). More than 1,000 spores were carefully measured and then treated biometrically. The curve of frequency distribution of spore size shows that there is a tendency toward bimodism. A large heterogeneity chi-square resulted, indicating that there were two different sizes of spores being sampled, not one. The spores of this fern are thus 'heterosporous' or 'incipient heterosporous', producing 'microspores' (100 μ in diameter) and 'megaspores' (110 μ in diameter) although they are indistinguishable with the naked eye. This was further confirmed with *C. pteridoides* by the same author, in which the diameters of 'microspores', and 'megaspores' are respectively 90 and 100 μ . Recently, Schedlbauer and Klekowski (1972) studied the spores of *C. thalictroides* and concluded that this fern is homosporous. The number of spores they studied were only 30. This number is less than the spore number of one sporangium, in which typically 32 spores are produced. No biometrical analysis was given by them. The significant variability of the spore size of this fern is, however, above suspicion. More recently, Hickok and Klekowski (1973) have observed larger diad and triad spores in addition to the tetrad spores of normal size in *Ceratopteris*. They think that those large spores were produced by abnormal meiosis. The nature or developmental mechanism of the 'heterospory' and its relationship to the sex of the prothalli in *Ceratopteris*, however, is not known.

Since *C. thalictroides* and *C. pteridoides* have a tendency to be 'incipient heterosporous', both male and female prothalli can naturally be expected to be found. The results of the work of Chiang and Chiang (1967) showed that this was true, since both male and female prothalli were really obtained in the cultures in addition to the hermaphroditic prothalli in *C. thalictroides*. In *C. pteridoides*, both male and female prothalli were also obtained in addition to the bisexual prothalli.

The correlation between spore size and the sex of the prothalli has been studied in *Ceratopteris* by Schedlbauer and Klekowski (1972). They reported that incipient heterospory does not exist in *C. thalictroides* and that there is no correlation between spore size and the sex of the resulting gametophyte. They reported that all 30 spores they studied gave rise to hermaphroditic prothalli. In *Platyzoma* the spore size is correlated with gametophyte morphology, i. e. large spores give rise to female gametophytes and small spores give rise to male gametophytes (Tryon, 1964). *Equisetum* is believed to be homosporous, but the male and female prothalli arise respectively from separate spores.

As was mentioned in the introduction, there is a difference of opinion concerning the sexuality of the prothalli of *C. thalictroides*. According to Kny (1875) and Leitgeb (1878), the prothalli are dioecious. Yabe and Yasui (1913), however, did not agree this view. Although Yabe and Yasui did observe the spatulate, smaller male prothalli bearing only antheridia, like those described by Kny, they considered that those prothalli would continue to grow and finally produce archegonia on them if circumstances were suitable. Purely female prothalli were not observed by them. Thus, Yabe and Yasui described the prothalli of *C. thalictroides* as monoecious.

Mahabará (1948) studied the prothalli of this fern from its natural habitat in India and reported that all the prothalli harvested by him were bisexual. The prothalli of *C. thalictroides* were again studied in India by Javalgekar in 1960. He concluded that the prothalli were hermaphroditic in culture as well as in nature. As he only found an extremely small proportion of male prothalli, like those observed by Kny, he neglected this small exception. Prothalli bearing only archegonia were not found by him. Later Pal and Pal (1963) reported that the gametophytes of *C. thalictroides* are bisexual although they did observe some purely antheridial and some purely archegonial prothalli in their cultures. Recently Nayar and Kaur (1969) and Schedlbauer and Klekowski (1972) reported that the spores of *C. thalictroides* produced male and hermaphroditic gametophytes. Female prothalli were not found by them.

The pure archegonial prothalli of *C. thalictroides* have so far been observed only by Pal and Pal (1963), Chiang (1963) and Chiang and Chiang (1967). As all types of prothalli were found in the same culture dish, the Pals have mentioned that their occurrence probably cannot be attributed to the effect of light or any other external factor. Chiang and Chiang (1967), however, found that 1/8 of full sun light was the best light condition for producing a higher percentage of female prothalli, while 1/2 sun light was favorable for the production of male and hermaphroditic prothalli. No archegonial prothalli were found in the cultures under 1/2 or full sun light. They also found many female prothalli in the cultures grown under continuous white fluorescent light. These results indicate that light is a factor which determines the sex expression of the prothalli in *C. thalictroides* although the sex of the prothalli is primarily predetermined by the spores. In the present study, it was found that the sex of the prothalli was related to the meristematic activity of the prothalli. It is thus reasonable to conclude that light primarily affects the vegetative growth of the prothallus, and secondarily the sex of the prothallus.

If the male prothalli are present, the possible occurrence of female prothalli is a matter of course. The development of the female prothalli is the step next to that of the hermaphroditic prothalli, i.e. male→hermaphroditic→female, since the sex of prothalli changes quantitatively and progressively. In more detail, the progressive change of the sex is as follows: male→hermaphroditic with male tendency→hermaphroditic with female tendency→female. Now the prothalli of all of these types have been observed both in *C. thalictroides* and *C. pteridoides*.

Tryon (1964) reported that a polypodiaceous fern, *Platyzoma* has some affinities with *Ceratopteris* with regard to their sporangial characters. Now its incipient heterospory is an another characteristic which resembles *Ceratopteris*. Tryon also mentioned that *Platyzoma* grows in deep sand under conditions ranging from inundation to drought and that it occurs mainly along the coast. It seems to the present author that *Platyzoma* is terrestrial in all outward appearance, but it really hides an aquatic nature. If this suggestion is true, all aquatic ferns in the Filicales are, with no exception, heterosporous or incipiently heterosporous, whereas the terrestrial members are all homosporous.

So far little has been made on the study of prothalli of *C. pteridoides* except the Momose's (1964). Momose (1964) described the diagnostic character of the prothalli grown from the spores which were obtained from the Botanic Gardens of the University of Copenhagen. He observed that the elongate monoecious prothallus had its long axis parallel with the 'notch-spore axis'. He mentioned the

monoecious prothalli only, but did not refer to the differentiation of the sex. In the present observation, the monoecious and female prothalli are rather short and broad with two large laterally expanding wings.

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