ON THE SEX OF THE PROTHALLIA OF *CERATOPTERIS PTERIDOIDES* (HOOK.) HIERON.[1]

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Abstract: Female and male prothallia were developed from the spores of *Ceratopteris pteridoides* grown in culture. The female prothallia remained female throughout the investigation, but the male prothallia underwent further development to produce either female parts to become hermaphroditic or adventitious female prothallia. Most of the prothallia were insensitive to external GA_3, but some male prothallia were promoted by this growth hormone to produce prothallial extensions which bore archegonia. GA_3 might possibly be an endogenous hormone opposed to antheridiogen, promoting the growth and *feminization* of the prothallium. ABA inhibited antheridial formation and prothallial growth as well.

INTRODUCTION

The *Ceratopteris* belongs to the monotypic family Parkeriaceae (water horn fern family). It is an aquatic to semi-aquatic leptosporangiate fern distributed in tropical and subtropical regions. The genus consists of about four species, and is generally considered to be homosporous. S. H. Chiang (1963) however opened the question as to whether it might possibly be incipiently heterosporous. The voluminous earlier works on *Ceratopteris* were mostly restricted to *C. thalictroides*. Recently, *C. pteridoides* has also been studied. Opinions concerning the interpretation of the sexuality of its prothallia however are still controversial (Y. L. Chiang, 1975). Accordingly, the mechanism operating on sexual determination in the *Ceratopteris* gametophytes is needed for further clarification. In the present investigation, *C. pteridoides* was studied in this aspect, applying gibberellic acid and abscisic acid, one being a growth promotor and the other an inhibitor of higher plants.

MATERIALS AND METHODS

The prothallia of *Ceratopteris pteridoides* (Hook.) Hieron. were raised from spores which were collected on Feb. 5, 1975 in the greenhouse of our Department, where the plants collected from Taipei Zoo in 1960 had been kept growing in water tanks. Without doubt this plant was imported together with tropical fish. The original source of this plant however is unknown. So far, *C. pteridoides* has not been collected from the field in Taiwan.

The spores used were sieved through lens paper. They were sterilized with 70% alcohol for two minutes and added to the medium. The spores were sown by pouring this medium onto a filter paper laid on a layer of cotton (1 gm) in the Petri-dish (10 cm diameter). The medium used was 1/2 strength of Hoagland's inorganic nutrient solution, and the hormones used were gibberellic acid (GA_3) and abscisic acid (ABA). The concentrations of hormones in the media were 0, 0.01, 0.1, 1, and 10 ppm. The amounts of spores and medium per dish were 1 mg and 20 ml respectively. The spores were sown on Apr. 20, 1975 and the prothallia were

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harvested for study on May 15 to 18, 1975. In single spore culture, the spore was picked up by a needle under the stereomicroscope and sown in the center of the Petri-dish on May 24, 1975. The prothallia were examined a month later on June 24. The medium used in the single spore culture was also Hoagland's. Media and associated utensils were autoclaved.

All prothallia were grown under artificial illumination of two 40 W white fluorescent lamps and one 60 W incandescent lamp (6 a. m.–6 p. m.) plus natural diffuse light from the north window of the laboratory (approximately 2,500 luxes). The room temperature was uncontrolled.

RESULTS AND DISCUSSION

Sex of the spore. Female, male and hermaphroditic prothallia were found in the multi-spore culture materials of *C. pteridoides* (Figs. 1–9, control) as in the former study (Chiang, 1975). Since different sexes appeared constantly in the same Petri-dish or the same environment, it was considered that the sex was destined in the spores. However, mutual action between spores in population on sex expression may operate, so the monospore culture test was also used. If each isolated spore gives rise to only a female or male prothallium, the sex of the prothallium should be determined in the spore-self. The experiment showed a positive result, i. e. the separate spore of *C. pteridoides* in the monospore cultures gave rise to either female or male prothallia after an adequate period of growth. Detailed data from mono- and dual-spore cultures regarding the interaction between spores will be presented in a future paper.

Female prothallium. The female prothallium of *C. pteridoides* is a well-developed, rather large (in comparison with the male) cordate plant (Fig. 1). It has one meristematic notch and only bears archegonia. No sign of it turning into a hermaphrodite by producing antheridium was ever noted in the female prothallium throughout the experiment. It seemed that the femaleness of the female prothallium was even intensified as the prothallium grew, i. e. the larger prothallium produced more archegonia. Moreover, no female prothallium was found producing male prothallium adventitiously. The female prothallium was thus purely female.

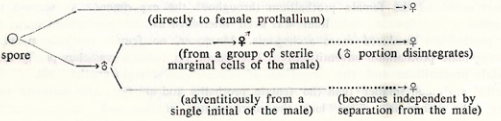
Male prothallium and the formation of adventitious female prothallium. The male prothallia are much smaller than the female prothallia and are typically spatulate in shape, bearing only antheridia. They lack the meristematic notches and thus their growth is restricted (Fig. 9). The sterile part of the male prothallium however is totipotent, occasionally undergoing further development after the cessation of antheridium-formation (i. e. after the antheridial phase) to produce one to two adventitious prothallia. The new prothallium was cordate in shape and without exception are female, bearing only archegonia. It was confirmed by the light microscope that the adventitious prothallium was derived from a single initial cell on its "mother" plant (actually the male). The male prothallium never produce new male individuals adventitiously.

Male prothallium and the development of hermaphroditic prothallium. The new part formed by expansion of the sterile margin (not one cell but a group of cells) of the male prothallium was also cordate and always female. The formation of a female part from the male prothallium inevitably results in a hermaphroditic prothallium. All hermaphrodites found in this experiment were formed in this way. Accordingly, the male part of the hermaphrodite, which is always formed prior to the female part, was without exception located at the base of the prothallium. Although many hermaphrodites are difficult to identify at first glance, they are formed by means of the vegetative expansion of the males, they are actually the male prothallia together with their associated post-female extensions. Generally, the fewer antheridia a male prothallium has, the more frequently it develops into a hermaphrodite. GA_3 appears to be effective in stimulating the male to form female extensions, and to turn it into archegonial phase. (Figs. 15, 16, 25 and 26).

The synchronizing development of both sex organs on the same prothallium (usually cordate in shape as in the female) however is not unusual in *C. thalictroides* (Chiang and Chiang, 1967; Yabe and Yasui, 1913) and *C. pteridoides* as well (Momose, 1964). But such hermaphrodites were not seen in the present material.

Evidence of antheridiogen. In ferns, antheridiogens or antheridium-inducing hormones have been widely demonstrated (for review see Näf, Nakanishi and Endo, 1975). In *C. thalictroides* it has been called antheridiogen C by Schedlbauer (1974). So, is there any evidence of antheridiogen in *C. pteridoides*? In the present experiment, the number of antheridia formed per prothallium was found varying greatly from none to 93. All antheridia were formed during a relatively short period of time, or the antheridial phase, which was soon after the spore had germinated. No antheridia were produced in the later stage of the prothallial development. Besides, the adventitious prothallium which were derived from the male prothallium never produce antheridium. All these observations indicate that the antheridium-forming factor lurked in the spore and/or juvenile prothallium is of a *quantitative* and *consumable* nature. Accordingly, the occurrence of a hormone or antheridiogen in this fern is predictable.

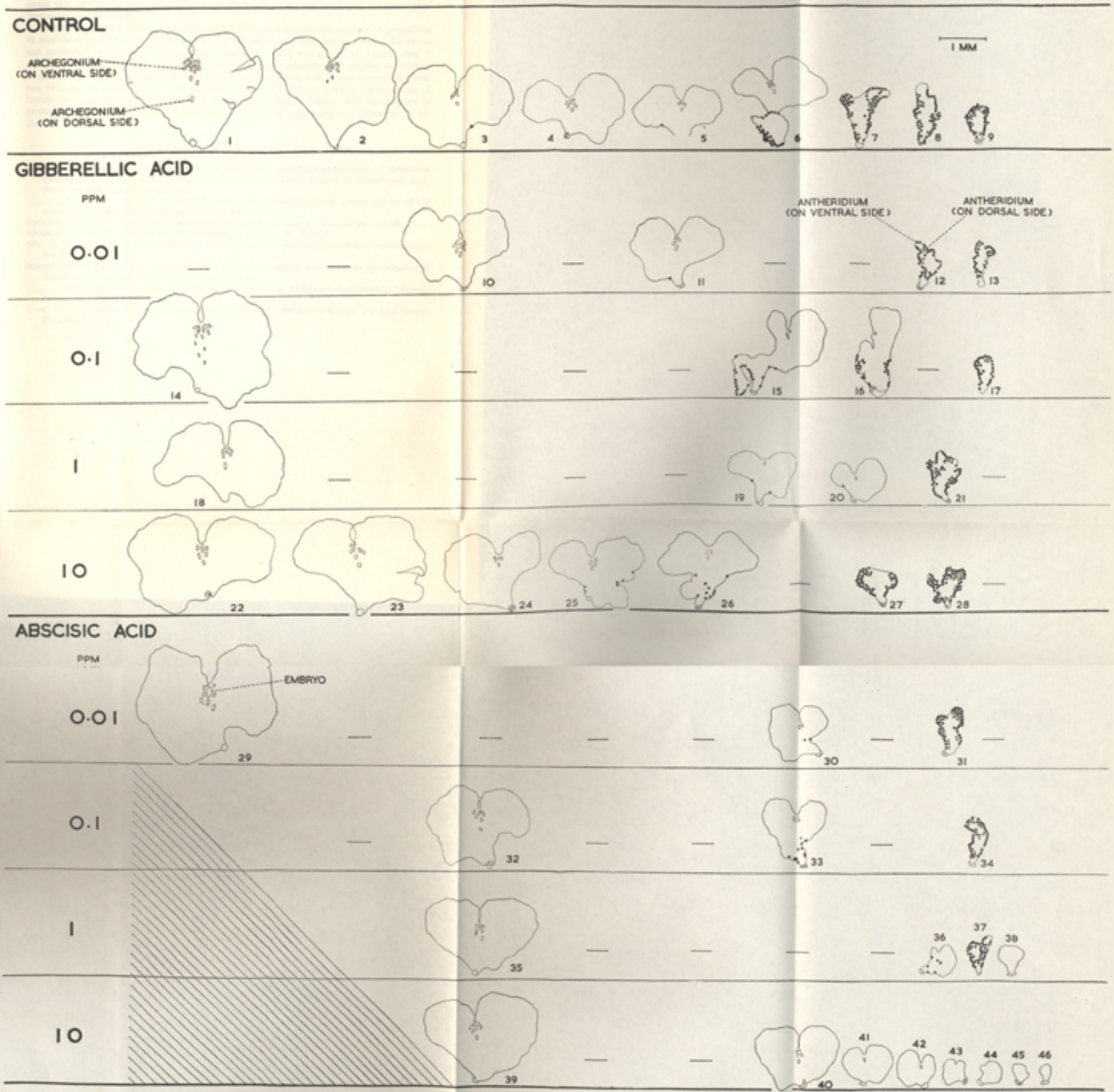
Sex of the prothallium. As mentioned earlier, the female prothallium remained as a female throughout, but the male prothallium frequently underwent a further development to modify its sex after its antheridial phase. Two cases of the post development of the male prothallium were observed. In one case: the production of adventitious female prothallium (-ia), in the other case: the formation of a female part to become hermaphrodite. In both cases, the original "mother" prothallia (actually the males) disintegrated or lost their function as males at a later stage and thus eventually the prothallia all became females:



Schedlbauer and Klekowski (1972) considered that in *C. thalictroides* as much as 100% of gametophytic population would finally become hermaphroditic.

A tendency of the male prothallia towards feminity in the later stage of development implies that the final role of the gametophyte has been the production of embryo. If there was a "fundamental" sex existing in this fern, it should be the female, not the male. The female prothallium is usually large and has sufficient ability to fulfil its mission to nourish the embryo.

Effect of gibberellic acid. Since the larger prothallia found were always females whereas the smaller ones were males, it was reasonable to suggest that these might be a connection between the sex and the growth of the prothallium. A typical growth promotor of higher plants, gibberellic acid (GA_3), was therefore tested to find out whether it might be a sex hormone towards *C. pteridoides* prothallium or not. GA_3 has been assumed as a functional antheridiogen in some ferns, but not in *C. thalictroides* (Schedlbauer, 1974). As shown in Figs. 10-28, *most* of the prothallia of *C. pteridoides* were insensitive to external GA_3, i. e. GA_3 neither inhibited nor promoted the sex organ formation. The growth of prothallia was also not obviously affected. The only notable effect of GA_3 was that it stimulated the sterile margin of *some* male prothallia to form female parts or extensions (Figs. 15, 16, 25, 26). This last result interested the author. It indicates that external GA_3 is effective in growth and *feminization* of some male



Figs. 1–46. Camera lucida drawings of *C. powderi* prothallia showing their responses to gibberellic acid and abscisic acid. Forty-six prothallia from total of more than 60 materials studied were included in this figure. Dashes indicate the analogous prothallia actually occurred but they were not studied. Shaded area indicates that no prothallium occurred in that area.

prothallia which are *critically* sensitive to it. Spores collected in a mass are heterogenous in their growth ability. They obviously contain the growth factor, presumably GA_3, in different amounts. In higher plants the shoot where the vitamins are synthesized is insensitive to external vitamins. Similarly, the insensitivity of most prothallia, especially of the females, to external GA_3 can be explained on the basis of the possible occurrence of GA_3 (or some other homologue) on the inside.

Effect of abscisic acid. Another attempt was made to discover the response of prothallium to abscisic acid (ABA). As shown in Figs. 32–46, ABA inhibited both antheridial formation and prothallial growth. The effective concentration of ABA was as low as 1 ppm. And it almost inhibited antheridial formation completely when the concentration reached as much as 10 ppm; only one out of 20 prothallia examined was found that had formed a single antheridium on it at this concentration. On the other hand, ABA did not prevent the formation of female sex organ although the number of archegonia decreased slightly with the decrease of prothallial size. External ABA feminized the prothallia by suppressing the formation of antheridia.

LITERATURE CITED

CHIANG, S. H., 1963. A study on growth and development of sex of the gametophyte of *Ceratopteris thalictroides* (L.) Brongn. with a special reference to the systematic position of Parkeriaceae. M. S. thesis. National Taiwan Univ., Taipei.

_____, & Y. L. CHIANG, 1967. Influence of shading and gibberellic acid on growth and sex of the prothallia of *Ceratopteris thalictroides*. Taiwania, **13**: 179–193.

CHIANG, Y. L., 1975. Prothalli of *Ceratopteris pteridoides* (Hook.) Hieron. Taiwania, **20**: 172–178.

MOMOSE, S., 1964. The prothallium of *Ceratopteris*. J. Jap. Bot., **39**: 225–235.

NĀF, U., K. NAKANISHI & M. ENDO, 1975. On the physiology and chemistry of fern antheridogens. Bot. Rev., **41**: 316–355.

SCHEDLBAUER, M. D., & E. J. KLEKOWSKI, JR., 1972. Antheridogen activity in the fern *Ceratopteris thalictroides* (L.) Brongn. Bot. J. Linn. Soc., **65**: 399–413.

_____, 1974. Biological specificity of the antheridiogen from *Ceratopteris thalictroides* (L.) Brongn. Planta, **116**: 39–43.

YABE, Y., & K. YASUI, 1913. On the life-history of *Ceratopteris thalictroides* Brongn. Bot. Mag. Tokyo, **27**: 233–245.