Shigeo MASUYAMA*: **The sequence of sex expression in the prothallia of *Adiantum pedatum* L. and *A. capillus-veneris* L.**

益山樹生*: クジャクシダとホウライシダの前葉体における性表現の推移

There are numerous reports on the morphology of fern prothallia (cf. Atkinson and Stokey, 1964; Nayar and Kaur, 1971). Most of these reports have been aimed to find taxonomical characters in the gametophyte, and so the subjects of investigations have generally centered around the mode of spore germination, development of prothallia, and various features of well-developed prothallia with male and female sex organs. Recently, Klekowski and his co-workers (1968, 1969) have paid attention to the sequence of sex expression during development of the prothallium for the study of the mating system in ferns. However, the relationship between the sequence of sex expression and that of gametangium formation of the prothallium still remains to be investigated.

In his comprehensive studies on fern prothallia, Momose (1967) pointed out the difference in the spatial arrangement of gametangia between two species of *Adiantum*, *A. pedatum* L. and *A. capillus-veneris* L.; that is, though archegonia are usually situated in the meristematic region or on the cushion in well-developed prothallia, antheridia are located in the posterior region far from archegonia in the former species, while in the latter species antheridia are often located in the neighbourhood of archegonia.

In the present study, the sequence of sex expression in these two species of *Adiantum* was investigated from the view points of the duration of gametangium formation and the spatial arrangement of gametangia during development of the prothallium.

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Materials and methods The spores of *A. pedatum* were collected from a plant growing at Mt. Kiyosumi in Chiba Pref. and those of *A. capillus-veneris* at Koishikawa Botanical Garden of the University of Tokyo. Within two months after collection, spores were sowed on autoclaved soil in small pots, which were placed in a growth chamber illuminated by white fluorescent tubes at 800 to 1000 lux in light intensity and kept at 23°±1°C. Bottoms of those pots were dipped in tap water throughout cultivation.

Two pots for each species, in which prothollia were growing in almost same density, were chosen to provide samples of prothallia. The average numbers of prothallia per square centimeter in these pots were 13 for *A. pedatum* and 10 for *A. capillus-veneris* at 1.5 months afte sowing. Twenty to thirty prothallia of various size from each pot were checked at the intervals of one week from 1.5 months to 4 months after sowing. They were rinsed in tap water for a few seconds, and then stained with 45% acetocarmine solution for about 6 hours.

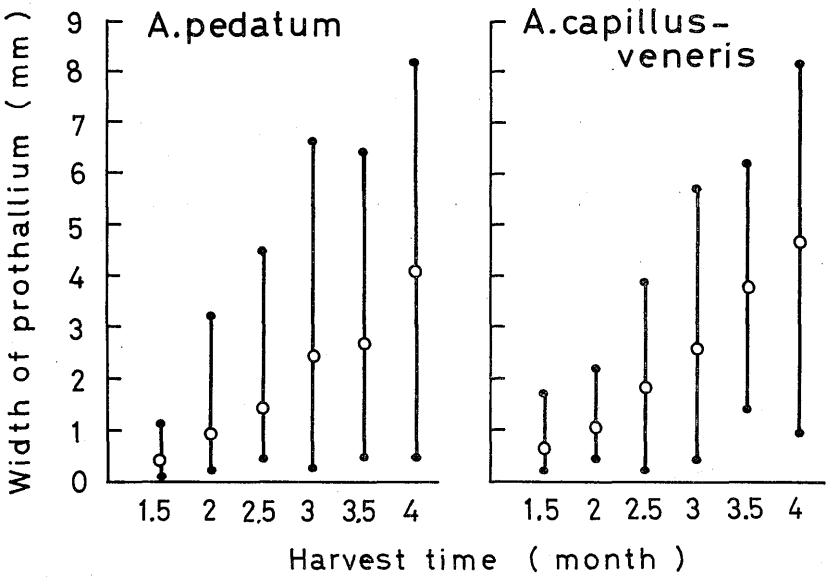

Fig. 1. The relation between the width of the prothallium and the harvest time. Open circle indicates the mean width of prothallia. The maximum or the minimum width of prothallia is represented by solid circle.

Stained prothallia were mounted in water on slide glass and observed under a microscope. Only cordate prothallia were used for the present investigation. The width of a prothallium was measured at its widest part. Occasionally semicircular outgrowths were observed on the ventral surface of a prothallium, and if such an outgrowth had one reddish spherical cell stained with acetocarmine surrounded by a clearing zone, it was regarded as an antheridial initial (Fig. 4). When a red-stained cell or a cell group of two or four red-stained cells were observed in the meristematic region, they were regarded as archegonial initials (Fig. 5). According to the gametangia observed on each, prothallia were classified into four types as follows:

Male: the prothallium with antheridia or antheridial initials.

Female: the prothallium with archegonia or archegonial initials.

Hermaphrodite: the prothallium with both antheridia and archegonia or their initials.

Asexual: the prothallium without any sexual organ.

Results The frequency of four sexual types of prothallia in each population varied with the culture time (Table 1). In both species, males occurred earlier than females and hermaphrodites, being more abundant than those two types in the early period of cultivation. As the culture time proceeded, however, both of the female and hermaphrodite increased and the male decreased in number in *A. pedatum*, while in *A. capillus-veneris* only the hermaphrodite increased. As shown in Fig. 1, values of the maximum and the mean widths of prothallia increased successively with the culture time in both populations.

Näf (1958) has observed the female prothallium of *Pteridium aquilinum* (L) Kuhn, which grew rapidly without prior phase of antheridium formation in the early period of cultivation. In the present culture, such a female was not found.

Fig. 2 shows the relationship between the sexual type and the width of the prothallium regardless of culture time. These data are based on total prothallia that were harvested from 2 months on after sowing, because about a half of prothallia harvested from that culture time were found to bear gametangia or gametangial initials as shown in Table 1. As the width increased, the frequency of each sexual type varied successively and the

Tab. 1. The frequency of four types of prothallia in population.

Months from spore sowing	Number of samples	Asexual %	Male %	Female %	Hermaphrodite %
A. pedatum					
1.5	73	86	14	0	0
2	56	11	68	7	14
2.5	58	0	74	12	14
3	60	0	47	20	33
3.5	55	0	49	16	35
4	43	0	34	33	33
A. capillus-veneris					
1.5	28	100	0	0	0
2	24	54	42	0	4
2.5	42	23	27	0	50
3	44	10	25	0	65
3.5	31	0	13	3	84
4	38	0	8	0	92

sexual types that marked the highest frequency at each width occurred in the following order: male at first, then hermaphrodite, and finally female in *A. pedatum*; asexual at first, then female, and finally hermaphrodite in *A. capillus-veneris*.

Fig. 3 shows the formation of gametangial initials in each sexual type of the prothallia represented in Fig. 2. In *A. pedatum*, the rate of occurrence of the male prothallia with antheridial initials decreased successively with the increase of the width. Most hermaphrodites had archegonial initials but had not any antheridial initial. Nearly all the females had the archegonial initials. In *A. capillus-veneris*, the most males had antheridial initials at any width. The majority of hermaphrodites had both antheridial and archegonial initials, though some of them in the range between 1.6 to 4.0 mm in width had archegonial initials alone. Every female had archegonial initials. Besides, the antheridia without any sperm (Fig. 6) were frequently observed especially on the hermaphrodite prothallia of *A. pedatum*.

Spatial arrangement of gametangia varied with development of the prothallium. In *A. pedatum*, when prothallia grew about 1 mm wide, antheridia were observed over a central area and antheridial initials were

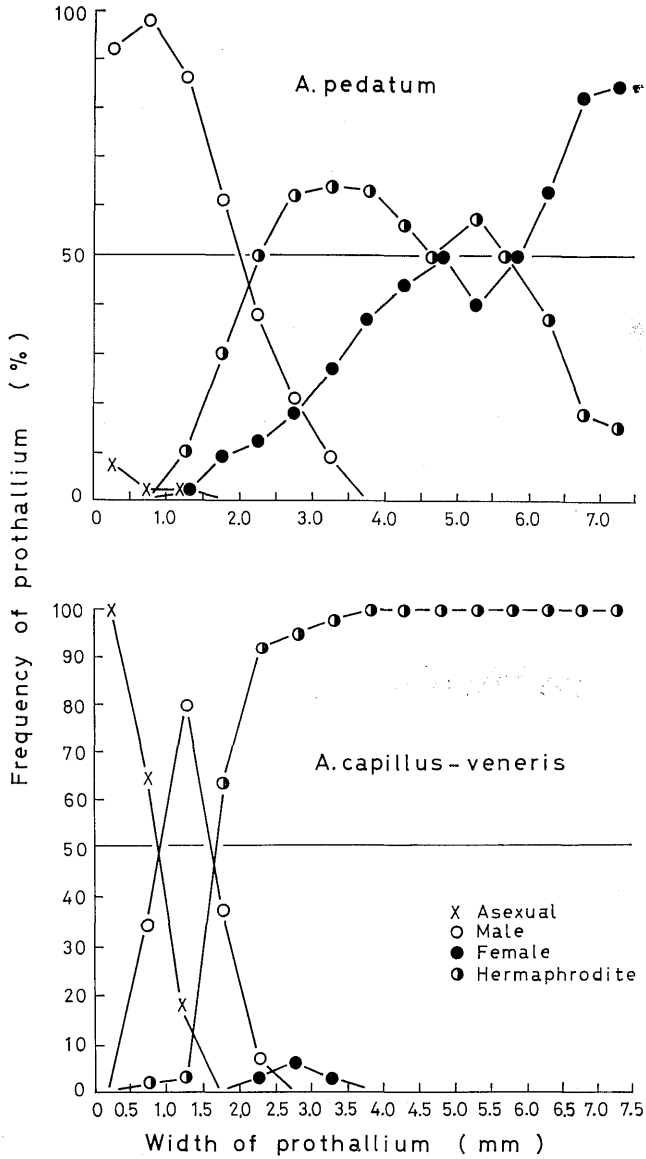

Fig. 2. The relation between sex expression and the width of the prothallium. Total number of samples is 446 in *A. pedatum* and 379 in *A. capillus-veneris*.

located on the anterior part of that area (Fig. 7). In prothallia about 2 mm wide, one or more archegonial initials appeared in the meristematic region, while mature antheridia were located in a posterior half of a prothallium. Any antheridial initial was not obseved in this stage (Fig. 8). In well-developed prothallia, archegonial initials were still observed in the meristematic region and older archegonia were found on the cushion. Mature antheridia, if present, were located in a posterior region of a prothallium far from archegonia (Fig. 10). In the prothallia of *A. capillus-veneirs* at the stage of 1 mm wide, antheridia occupied the central area as in those of *A. pedatum*. In prothallia 2 to 3 mm wide, archegonial initials appeared in the meristematic region, and antheridial initials were still observed on an anterior part of the central area that was occupied by mature antheridia (Fig. 9). In well-developed prothallia, archegonial initials were still observed in the meristematic region and older archegonia were on the cushion. Antheridial initials were often observed in the neighborhood of

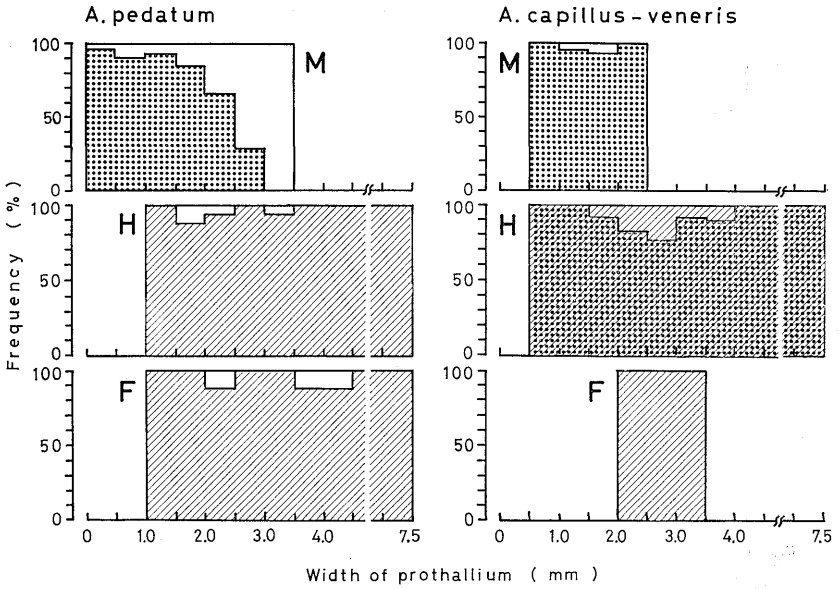

Fig. 3. Occurrence of the prothallium with gametangial initials in each sexual type. Dotted part: with antheridial initials, hatched part: with archegonial initials, unmarked part: without any gametangial initials. M; male, H; hermaphrodite, F; female.

young archegonia on the meristematic region (Fig. 11, 12).

Discussion The morphological study of the prothallia of *Adiantum pedatum* and *A. capillus-veneris* has already been carried out by Kawasaki (1956) and Momose (1967). In these works, however, the attention was put mainly on the morphology of the well-developed prothallium, so that the sequence of sex expression during development of the prothallium has remained unexplained.

From the facts that values of the maximum and the mean width of prothallia in a population increased successively as the culture time proceeded (Fig. 1), it seems that the width can be used as a convenient measure to estimate the growth rate of a prothallium. In this point of view, Fig. 2 may indicate that, when cultured in a population, most of the prothallia of present two species will change sex expression during development as follows; a juvenile asexual prothallium of *A. pedatum* will turn into the male phase at first, and then into the hermaphroditic phase followed finally by the female phase, while that of *A. capillus-veneris* will turn into the male phase initially, and then into the prolonged hermaphroditic phase.

Although the prothallia of present two species are both protandrous, there is a clear difference in the duration of antheridium formation between these two species as shown in Fig. 3. The prothallia of *A. pedatum* cease to produce a new antheridium before they begin to produce archegonia, while most prothallia of *A. capillus-veneris* continue to produce new antheridia even after they begin to produce archegonia.

Considering these facts, occurrence of the female phase in *A. pedatum* may be due to the cessation of antheridium formation and collaps of old antheridia in the hermaphroditic phase. This probability is supported by the fact that all or most antheridia in some hermaphroditic prothallia of this species were empty probably through the release of their sperms. As for *A. capillus-veneris*, the prolonged hermaphroditic phase may result from the simultaneous formation of both antheridia and archegonia in that phase. Some prothallia, however, failed to produce new antheridia only in the early period of the hermaphroditic phase (Fig. 3). A few of these hermaphroditic prothallia may proceed to the female phase in the same way as *A. pedatum*, and they may return to produce new antheridia and become

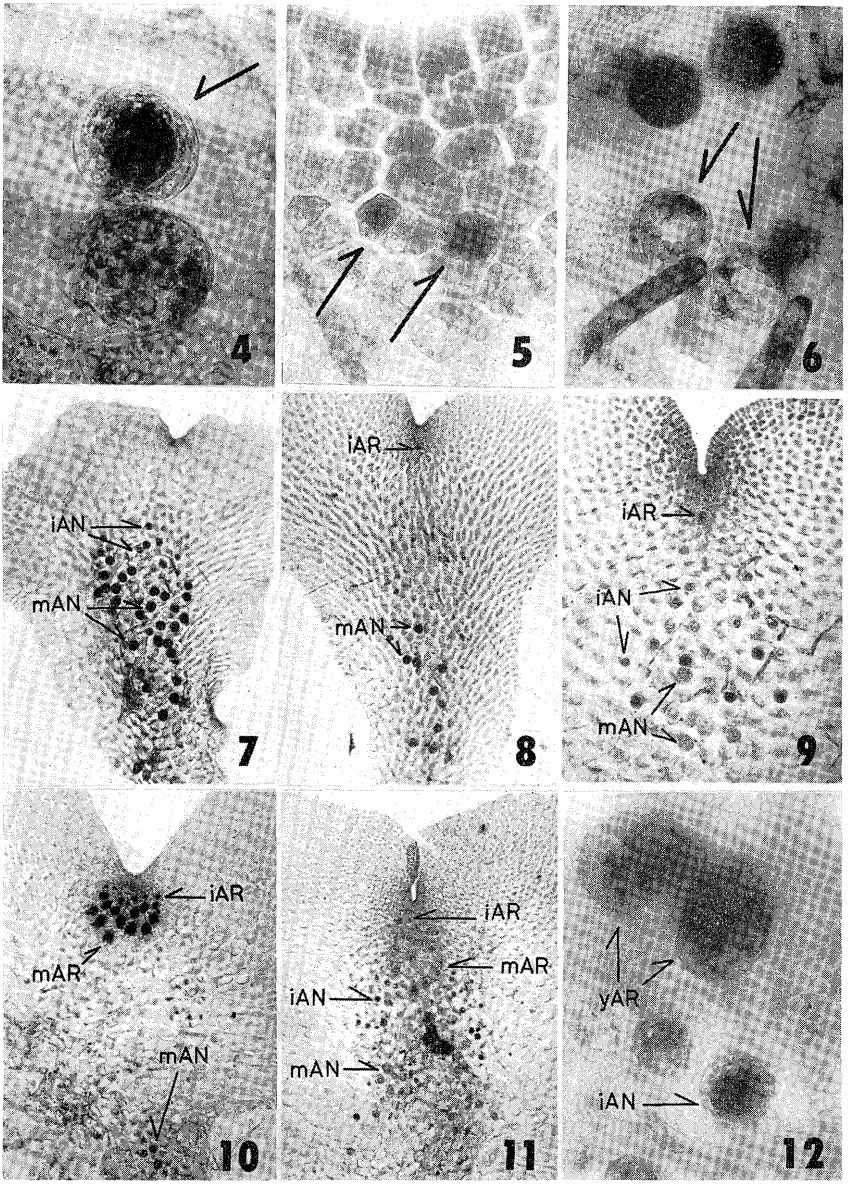

Fig. 4. Antheridial initial of *A. capillus-veneris* (indicated by arrow). ×85. Fig. 5. Archegonial initials of *A. capillus-veneris* (indicated by arrows). ×60. Fig. 6. Empty antheridia of *A. pedatum* (indicated by arrows). ×60. Figs. 7-11. Ventral surfaces of prothallia with gametangia. 7, 8 and 10; *A. pedatum* ×15, ×6, ×5, respectively. 9 and 11; *A. capillus-veneris* ×12, ×5, respectively. (iAN, antheridial initial; mAN, mature antheridium; iAR, archegonial initial; mAR, mature archegonium). Fig. 12. Antheridial initial in the neighborhood of young archegonia (yAR). ×60.

again hermaphroditic.

In the morphological study on the prothallia of seven species in *Adiantum*, Kachroo and Nayar (1953) have observed that archegonia appeared much later than antheridia and marked the end of the formation of fresh antheridia. The sequence of gametangium formation found in *A. pedatum* is identical with this. On the other hand, Klekowski (1969) reported that only cordate male prothallia and cordate hermaphroditic ones occurred in the cultures of *Adiantum pulverulentum* L. and of *A. tetraphyllum* Willd. Occurrence of these prothallia will probably result from the similar sequence of gametangium formation to that of *A. capillus-veneris*, namely, the initial formation of antheridia followed by the prolonged simultaneous formation of antheridia and archegonia.

Various features on the sequence of gametangium formation in fern prothallia have been documented in a number of previous reports on the morphology of the gametophyte. To summerize, there are probably three types of gametangium formation in the ferns with the protandrous prothallia; complete cessation of antheridium formation before archegonium formation, partial cessation of antheridium formation, and indefinite formation of both types of gametangia (Nayar and Kaur, 1971). The prothallium of *A. pedatum* and that of *A. capillus-veneris* may represent the first type and the third type respectively.

It is interesting that the prothallium of *A. capillus-veneris* has a resemblance to that of *Lygodium flexuosum* (L.) SW. (Rashid, 1970), not only in the simultaneous formation of antheridia and archegonia following the initial formation of antheridia but even in the spatial arrangement of gametangia; that is, as the prothallium grows, antheridia come to occupy a more anterior position and are produced around developing archegonia on the meristematic region in the latter period of development. The prothallium of *Adiantum tetraphyllum* also seems to bear similar features (Plate 2A in Klekowski, 1969). These facts suggest that the spatial arrangement of gametangia, particularly antheridia, may be related, to some extent, to the sequence of gametangium formation. Various patterns of the spatial arrangement of gametangia have been reported by numerous investigators (cf. Momose, 1958; Atkinson and Stokey, 1964; Nayar and Kaur, 1971). The relationship between these various patterns and the sequence of gametangium

formation remains to be investigated.

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成長にともなって，前葉体がどのように性表現を変えていくかを，主として生殖器官の新生能の点から調べてみた。材料はクジャクシダとホウライシダを用いた。その結果，両種とも造精器が造卵器に先行して形成されるが，いったん造卵器が形成されるようになると，クジャクシダでは造精器はもはや形成されないのに対し，ホウライシダでは引き続き形成されていくことがわかった。このちがいは両種の性表現のうつり変わりにはっきりしたちがいをもたらしているが，また，前葉体における生殖器官の形成域のちがいともある程度関連しているようである。

○高等植物分布資料 (76) Materials for the distribution of vascular plants in Japan (76)

○アメリカスズメノヒエ この植物は，1969年9月，徳島県で採集されたものにより，アメリカスズメノヒエとして報ぜられ（植研 46 (11), 348），また1971年7月，茨城県でも帰化していることが記録された（植物採集ニュース 52, 2)。これらを見て，どこか見たことがある植物だと記憶をたどって，熊本大学薬学部の標本庫を探してみたところ，1967年7月28日，熊本県鹿本郡植木町平島の路傍で採ったものがあった。その時は私も見なれぬ植物として，その年の秋だったが，当時科学博物館におられた大井次三郎氏の所に標本を持参したことがある。それがこんどのアメリカスズメノヒエであった。標本は現在手元に1点しかないが，記録されたとおりの特徴をもっている。従って日本への帰化は徳島より2年早かったことになる。その後の追跡調査をしていないので，今も生えているかどうかわからないが，当時は道路の両側に相当沢山繁殖していた。この時の標本の一つは，科学博物館にあるはずである。

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