



MEIOTIC BEHAVIOUR IN TEN SPECIES OF PTERIDOPHYTES FROM BANGLADESH

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Abstract

Meiotic behavior of ten pteridophyte species from Bangladesh was analyzed in the present investigation. In SMCs of *Adiantum capillus-veneris*, thirty distinct bivalents were enumerated. In *A. caudatum* 16 SMCs resulting 64 spores were observed in sporangium which appeared to be diploid sexual. *A. lunulatum* was found to be triploid apogamous and as diploid sexual forms, thus this species of *Adiantum* exhibited wide range of morphological variations. *Pteris biaurita*, *P. graffithi* and *P. vittata* were recorded to be $n = 29$ as diploid. In thelypterids, all the four species were observed to be diploid sexual except *C. dentata* when it was tetraploid ($n = 36$, $4x = 144$). In *C. arida*, meiosis was almost regular with $2n = 72$ chromosomes. *C. cylindrothrix* and *Ampelopteris prolifera* were also found to be diploid sexual, with $2n = 72$ chromosomes.

Key words: Meiosis, Pteridophytes, Spore mother cells

Introduction

Basic chromosome number along with other cytological information could be considered as important feature for diagnostic descriptions of new plant families or sub-families and may provide clues to the affinities existing between families and their classification into higher categories (Walker 1973). Detection of regular or irregular pairing at meiotic cell division is a powerful tool for detecting the fate of a hybrid plant authentically where other evidence is inconclusive or misleading. Pteridophytes are not exceptions of these. Role of cytology in fern taxonomy has been discussed by many authors (Abraham 1958, Panigrahi 1962, Bir 1970). One important example can be referred here; Copeland (1947) placed *Adiantum* under Pteridaceae family. The basic number of *Adiantum* was determined as $n = 30$ whereas $n = 29$ was enumerated in *Pteris*. Due to possessing different basic chromosome number, *Adiantum* was segregated from Pteridaceae and placed in Adiantaceae.

Cytogenetical work helped in the determination of the origin and taxonomic status of several species of *Dryopteris*, *Polysticum*, *Asplenium*, *Polypodium*, *Adiantum*, *Cyclosorus*, etc. The application of cytomorphological knowledge for studying interrelationship at family or group level has been well demonstrated in Thelypteridaceae (Loyal 1963), and in Polypodiaceae (Bir 1973, Bir and Trikha 1979). In case of cytological study no report is available in Bangladeshi fern except Kabir and Mannan (2001) and Azad et al. (2012). So it is evident that a wide gap exists in respect of fern research in Bangladesh. It was observed that three families i.e. Adiantaceae, Pteridaceae and Thelypteridaceae are predominating in Bangladesh. Under these three families only ten species could be procured abundantly from Bangladesh. Besides the ten species, a few doubtful members were observed which could not be included due to scarcity of the samples. From the

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previous reports it was noticed that these families are also occupying the top position in respect of species number and distribution in Bangladesh. This is one of the reasons to consider these ten species under these three families for meiotic study.

Material and Methods

Ten species of pteridophytes were used as experimented material in this study. A brief description of all these plants is presented in Table 1.

Table 1. Habitat, distribution and place of collection of ten species of pteridophytes in Bangladesh

Specimen	Habitat	Distribution	Place of collection
<i>Adiantum capillus-veneris</i> L.	Dilapidated wall, wet creeks, sunshades, terrestrial in few places	Throughout the zone	Panchagarh, Madaripur, Rajshahi, Jhenaidah, and Khulna
<i>A. caudatum</i> L.	Mainly dilapidated brick wall	Rajshahi, Pabna, Jessore, Jhenaidah, Kushtia, Chuadanga	Rajshahi, Jhenaidah and Jessore
<i>A. lunulatum</i> Burm.	Old wall, sunshade of buildings, few places as terrestrial	Throughout the zone	Dinajpur, Bogra, Jessore, Rajshahi, Jhenaidah and Rangpur
<i>Pteris biaurita</i> L.	Terrestrial, shade and sunny places	Panchagarh, Pabna, Dinajpur, Thakurgaon, Rangpur, Bogra	Dinajpur, Panchagarh, Rangpur and Nilphamari
<i>P. griffithii</i> Hook.	Terrestrial, wet and shady dilapidated brick wall	Rajshahi, Nilphamari	Rajshahi
<i>P. vittata</i> L.	Brick wall, terrestrial	Fairly common throughout the zone	Jhenaidah, Rajshahi, Faridpur and Magura
<i>Ampelopteris prolifera</i> (Retz.) Reed.	Marshy place, terrestrial	Most common throughout the zone	Natore and Rajshahi
<i>Christella arida</i> (D. Don) Holtt.	Terrestrial	All over the zone	Panchagarh, Jhenaidah and Rajshahi
<i>C. cylindrothrix</i> (Rosenst.) Holtt.	Terrestrial, moist places	Throughout the zone	Madaripur, Satkhira and Naogaon
<i>C. dentata</i> (Forssk.) Brownsey & Jermy.	Terrestrial, occasionally on dilapidated wall.	Most common throughout the zone	Jhenaidah, Rajshahi, Dinajpur and Barisal

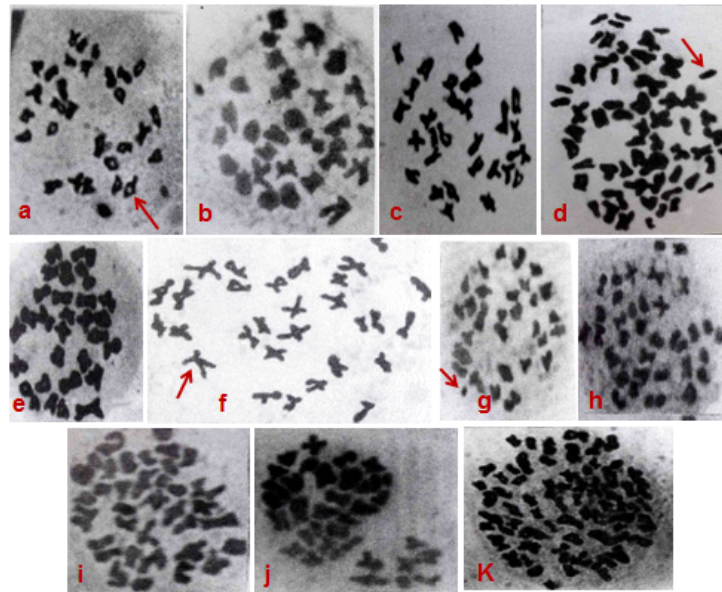
To study the meiotic behaviour including chromosome association and chiasma frequency young sporophylls were collected between 8.00 am and 5.00 pm directly from the original habitat (north western part of Bangladesh) or from the plants grown in pots or field. The frequency of diakinesis was found abundant between 9.00 am and 3.00 pm. It was observed that first half of the day time was best for collection regarding the maximum number of dividing cells. However, grossly there was no hard and fast rule for meiotic timing like mitosis. The young sporophylls were fixed in 1:3 aceto-alcohol immediately after collection. For *Adiantum*

36 h fixation time was enough whereas 48 h fixation time rendered good results for *Pteris*, *Ampelopteris* and *Christella*. After 36-48 h of fixation they were transferred to 80% alcohol and kept in a refrigerator till use.

Suitable sori were placed on clean slide and a drop of 1% aceto-carmine was added. The sporangia were then ruptured with a flat-ended needle and the indusia and sporangial wall were removed from the slide. Thereafter, the spore mother cells (SMC) were covered with a cover glass, warmed gently over an alcohol flame and a slight pressure was exerted by fingertip to spread out the SMCs as well as the chromosomes. Extra heating was applied with 45% acetic acid when needed to make the cytoplasm clear. Temporary slides were observed under compound microscope and photomicrographs were taken from the desired preparations. Cytological screening was carried out at all stages of meiosis. A total of thirty stages of diakinesis or metaphase I of each species were studied to analyze the chromosome association. Data on chromosome association and chiasma frequency were recorded from the desired preparations. Chromosome configurations at diakinesis and metaphase I were used to determine the pairing and number of univalent, bivalent or multivalents. The ring or rod bivalents with position or number of chiasmata were also recorded. Other notable observations during meiosis were also recorded.






Results and Discussion

Chromosome association and chiasma frequency along with the pairing habits of the chromosomes were observed at diakinesis of prophase-I or metaphase-I. Photomicrographs showing diakinesis in SMCs of ten fern species are given in Figs. 1(a-k). The data on chromosome association and chiasma frequency were tabulated and these are given in Table 2.



Figs. 1(a-k): Photomicrographs showing diakinesis in SMCs of *Adiantum*, *Pteris* and thelypteroid species. a) *A. capillus-veneris*, b) *A. caudatum*, c) *A. lunulatum* (2x), d) *A. lunulatum* (3x), e) *P. biaurita*, f) *P. griffithii*, g) *P. vittata*, h) *C. arida*, i) *A. prolifera*, j) *C. cylindrothrix*, k) *C. dentata*. Arrow headed univalent (1d, 1g), rod bivalent (1f), ring bivalent (1a).

Table 2. Chromosome number and association determined at diakinesis/ metaphase-1 of ten fern species

Species	Chromosome number (2n)	Univalent  ±SE	Bivalent		X-ma per SMC  ±SE	X-ma per bivalent  ±SE	% of Univalent	% of Rod bivalent	% of Ring bivalent
			Rod  ±SE	Ring  ±SE					
<i>A. capillus-veneris</i>	60	-	5.50±0.27	24.50±0.27	55.30±0.37	1.84±0.01	-	15.98	84.02
<i>A. caudatum</i>	60	0.80±0.33	6.50±0.34	23.10±0.35	53.90±0.50	1.79±0.02	2.63	21.38	75.98
<i>A. lunulatum</i>	60	-	5.90±0.23	24.10±0.23	54.70±0.30	1.82±0.01	-	19.66	80.34
<i>P. biaurita</i>	58	0.80±0.44	8.90±0.31	19.70±0.26	49.10±0.43	1.71±0.01	2.72	30.28	67.00
<i>P. griffithii</i>	58	0.40±0.43	15.00±0.61	13.80±0.46	42.80±0.48	1.48±0.01	1.37	51.37	47.26
<i>P. vittata</i>	58	-	2.40±0.31	26.60±0.30	57.8±0.70	1.99±0.02	-	8.27	91.73
<i>A. prolifera</i>	72	-	5.10±0.38	30.90±0.38	67.60±0.50	1.88±0.01	-	14.16	85.84
<i>C. arida</i>	72	-	7.60±0.45	28.40±0.45	65.60±0.50	1.82±0.01	-	21.11	78.89
<i>C. cylindrothrix</i>	72	-	5.50±0.34	30.50±0.34	67.40±0.91	1.87±0.03	-	15.28	84.72
<i>C. dentata</i>	144	-	22.40±0.42	49.60±0.42	121.80±0.93	1.69±0.02	-	31.11	68.89

SMC = spore mother cells, SE = standard error.

In general, the nucleus of SMC was eccentric in *Adiantum* and *Pteris* species whereas it was central in thelypterids. In *A. caudatum* central nucleus and in *C. dentata* few eccentric nuclei were also observed. *Adiantum capillus-veneris*, a diploid sexual ($n = 30$) exhibits extremely uniform cytological status throughout the Indian regions (Roy and Sinha 1956, Mehera and Verma 1960, Manickam 1984, Manton 1950) though exceptions are there. Munshi (1994) observed $n = 30$ and $n = 31$ having 64 spores in *A. capillus-veneris*. Singh and Roy (1988) observed two types of *A. capillus-veneris*, one tetraploid $n = 60$ and the other apogamous triploid with $n = 2n = 90$. The cytotypes differed morphologically vary slightly. In the present investigation thirty distinct bivalents were enumerated (Fig. 1a&c).

In *A. caudatum*, different cytotypes are available. Manton et al. (1967) and Ghatak (1962) reported wild diploid and sexual forms of *A. caudatum* from West Bengal, Assam, Bihar state, etc. In the present study sixteen spore mother cells resulting 64 spores were observed in sporangium which appeared to be diploid sexual. Triploid apogamous, $n = 2n = 90$ is the commonest form of *A. lunulatum* (Mehera and Verma 1960, 1963, Verma and Loyal 1960, Singh and Roy 1969, Verma 1961, Ghatak 1963a, Roy and Sinha 1961). In addition to this diploid sexual, $n = 30$ (Bir and Verma 1989, Mahable and Kamble 1981), diploid apogamous, $n = 2n = 60$ (Bir and Verma 1989); and tetraploid sexual, $n = 60$ are also available (Bir and Verma 1989, Verma 1961, Abraham et al. 1962).

However, *A. lunulatum* was found with wide range of morphological variations. Tetraploid sexual and triploid apogamous did not yield any significant morphological differences (Bir and Irudayaraj 2001). In the present

study triploid apogamous and diploid sexual forms were investigated and its wide morphological variation was followed. However, Mehera (1944), Mehera and Verma (1960 & 1963) reported four cytotypes of *A. lunulatum* from Darjeeling area, e.g. diploid apogamous rare, triploid apogamous commonest, diploid sexual quite common and tetraploid sexual occasional. Mehera and Khullar (1977) from eastern Himalaya observed 4 types of this species and the smaller form of this area was renamed as *A. teestae*.

Pteris biaurita, *P. griffithii* and *P. vittata* were recorded to be $n = 29$ as diploid in the present study. However, different cytotypes were discovered by various cytologists. *Pteris biaurita* was reported as $n = 29$, $2n = 58$ diploid sexual, $n = 2n = 58$, diploid apogamous (Abraham et al. 1962), $n = 87$, triploid apogamous (Verma and Khullar 1965), $2n = 87$, triploid, irregular meiosis (Ghatak 1963b) whereas *P. vittata* was found with $n = 29$, diploid (Ammal and Bhavanandan 1991), $n = 58$, tetraploid (Roy and Pandey 1962, Kuriachan 1973, Kuriachan and Ninan 1976, Verma 1973, Srivastava and Pandey 1985), $2n = 174$ hexaploid (Abraham et al. 1962).

In thelypterids, all the species were observed to be diploid sexual except *C. dentata* when it was tetraploid (Fig. 1 h, i, j & k). *C. arida* was reported earlier as $2n = 72$, diploid sexual (Loyal 1961b & 1991), *C. cylindrothrix* as $2n = 72$, diploid sexual (Loyal 1961b & 1991, Vasudeva and Bir 1982) and *A. prolifera* as diploid sexual, $n = 36$ (Loyal 1961b & 1991, Manton and Sledge 1954, Singh and Roy 1988, Mehera and Loyal 1956, Abraham et al. 1962, Vasudeva and Bir 1982). Mahable and Kamble (1981) reported different basic number as $n = 41$ whereas *C. dentata* was reported as $n = 36$, 4x tetraploid (Loyal 1961a,b & 1991, Paingrahi 1992, Manickam 1984). Manickam (1984) also reported diploid *dentata* with $n = 36$ referring Loyal (1961a) and Ghatak (1961). However, this may be erroneously cited because in his later publication Loyal (1991) mentioned it as tetraploid sexual or amphidiploid. Meiosis was almost regular in the species observed in the present study. In *A. caudatum*, bridges were observed in a few of anaphase-1 and presence of univalent in triploid apomictic *A. lunulatum* was common feature. An intensive cytological sampling in various populations of *A. caudatum* and *A. lunulatum* was, however, shown that the pairing behaviour was not very much consistent in the SMCs of 16-celled sporangia. Sometimes there may be all pairs or pairs and singles, or multivalents, pairs and singles, and sometimes all univalents (Sinha 1987).

In *P. biaurita* lagging chromosomes and univalents were observed in some cases. Among the species of *Adiantum*, highest ring bivalent was observed in *A. capillus-veneris* followed by *A. lunulatum* and *A. caudatum*. Highest number of univalent was found in *A. lunulatum* triploid apogamous form. However, during the study of meiosis, in *A. caudatum* (Fig. 1b), both dyads and triads were observed. Irregular distribution of chromatin material in *P. biaurita* (Fig. 1e) was observed. In one case of *C. dentata*, triad was observed. In the present investigation the haploid number of *Pteris* ($n = 29$) and *Adiantum* ($n = 30$) was in conformity with the earlier reports.

Conclusion

The present findings are primarily in conformity with many of the researcher's observation as because the conclusive comment can be drawn after working with most of the ferns available in Bangladesh. Due to

differences of chromosome number many authors suggested in separating *Adiantum* from Copeland Pteridaceae.

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