POLYMORPHICITY AND CYTOGENETICS OF *ADIANTUM LUNULATUM* COMPLEX

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INTRODUCTION

THE Malabar maidenhair fern, *Adiantum lunulatum* Burmann f. (*Fl. Ind.* 235, **1768**) is one of the prettiest and most delicate species of Indian Adiantums. The species derives its name from lunate (half moon-shaped) subdimidiate, glabrous pinnae. It is better known in literature as *A. philippense* Linn. (Sp. Pl. 2, 1094, **1753**). However, recently Verma (1961) has advocated valid reasons for the preference of specific epithet *lunulatum* over *philippense* from a cytotaxonomic view-point.

Mehra (1938, 1944) was the first to report apogamy in the species from Sikkim and Dehra Dun. Since then it has been investigated from various parts of its range and so far reported, the species occurs largely as a triploid apomict, from S. Africa, Ceylon to Himalayas (*cf.* Manton, 1959; Manton and Sledge, 1954; Mehra and Verma, 1960; Verma and Loyal, 1960; Roy and Sinha, 1961, 1962). Extensive collections made in the Himalayas during four consecutive years (1955–58) have revealed a high degree of polymorphicity in the species. A cytological analysis of the different populations has shown that besides the triploid apomict three additional cytotypes, diploid sexual, diploid apogamous, and tetraploid sexual also exist in the Eastern Himalayas. A brief reference to this effect was made earlier in a paper listing the chromosome numbers of Himalayan Ferns (*cf.* Mehra, 1961).* In the present contribution a detailed study of this complex as it occurs in the Himalayas is presented.

MATERIAL AND METHODS

The species occurs plentifully on forest margins and moist rock crevices throughout the Himalayas at lower elevations, usually up to 1,050 m. It prefers gravelly and acidic soil or moist limestone. The present investigations are largely based upon plants growing all along the pony path from Badamtam (600 m.) to Teesta (150 m.) near Darjeeling in the E. Himalayas.

The material was collected during the months of July–September, the peak season of growth for the ferns in the Himalayas. For cytological preparations, fixations of unripe sporangia was done both in 1 : 3 acetic-alcohol and modified Carnoy's (1 part acetic acid + 3 parts

* More recently diploid and tetraploid sexuals have also been reported from South India (Abraham *et al.*, 1962).

absolute alcohol + 2 parts chloroform). Acetocarmine squashes of S.M.C. were made in the usual fashion.

MORPHOLOGICAL VARIATION

The characters which best exhibit variation in the different populations are frond size, pinna shape, size and extent of lobing, soral length and the basal angle formed by the acroscopic and basiscopic edges of pinnae at the junction of the stalk. In general, the entire range of variation agrees broadly to *A. lunulatum* plan in the presence of a short ascending rhizome, clothed with brown narrowly lanceolate scales, and bearing naked polished deep brown-black stipes supporting nearly as long or rarely far larger simply pinnate lamina, either terminating in an apical pinna or the rachis apex is prolonged bearing gradually reduced pinnae and terminating in a vegetative bud. Pinnae are more or less typically lunate, a character specific of *A. lunulatum*, and absolutely glabrous. The spores are similar in structure, being trilete with smooth-slightly rough exine. Some of the important variable characters which need be mentioned are given below.

Frond size.—The size variation is by no means continuous in this complex. The data on the size of the individuals and their relative frequencies (*cf.* Table I) representing a random sample of 775 indi-

TABLE I

Frequency of individuals in various size classes (in cm.)

Cl.	Fr.	Cl.	Fr.	Cl.	Fr.	Cl.	Fr.	Cl.	Fr.
4	2	17	4	30	23	43	41	56	6
5	5	18	3	31	25	44	29	57	5
6	7	19	2	32	24	45	37	58	..
7	15	20	5	33	..	46	27	59	9
8	23	21	5	34	27	47	..	60	..
9	24	22	7	35	..	48	21	61	3
10	30	23	6	36	..	49	20	62	3
11	37	24	15	37	26	50	15	63	..
12	36	25	10	38	32	51	9	64	2
13	21	26	..	39	..	52	..	65	..
14	13	27	10	40	38	53	7	66	1
15	8	28	..	41	36	54	7	67	2
16	5	29	6	42	..	55	..	68	1

Cl. = Class, Fr. = Frequency.

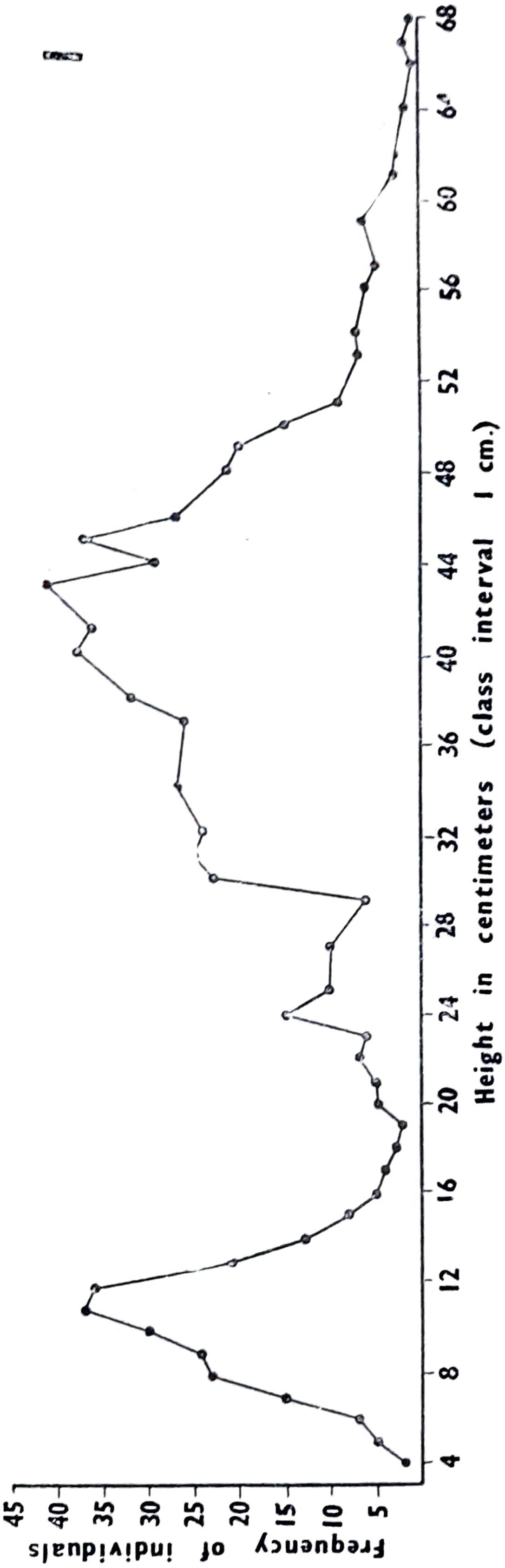
viduals from the populations growing by the side of Badamtam-Teesta pony path is very significant. Fractional measurements have purposely been avoided and these have been grouped in one class up or below depending upon the fraction. The entire *lunulatum* element seems to contain at least two widely different modes. If this data, without selecting an appropriate class interval, be plotted as such in a frequency polygon (Text-Fig. 1), the above conclusion becomes quite apparent, *i.e.*, the size factor sharply delimits the entire element into two major categories. One of them ranges between 4 and 18 cm. (8–13 cm. in general, $m = 10 \cdot 71$ cm), while the other category ranges between 20 and 58 cm. (largest number falling between 30 and 49 cm., $m = 40 \cdot 34$ cm). This data is highly significant since the former class represents in large majority the diploid sexuals while the second comprises mainly of triploid apogamous types.

On the whole the fronds in the small-sized category are delicate and occupy moist, shady and well-protected rock crevices (limestone in general), whereas the large-fronded individuals inhabit shaded aspects of open forest margins or roadsides and the fronds are comparatively much tougher. It should be pointed out that so far the lower size limits given in various authentic works for *A. lunulatum* does not decrease beyond 20 cm and the general size range given is 30–45 cm (*cf.* Ching, 1957). In fact, the sharp discontinuity in the size-range was the first indication that led us to suspect some intrinsic cause rather than sheer environmental modification to be responsible for this variability.

Pinnae.—Frond size in general is directly proportional to the pinna size and what is more important some of the size-overlap individuals (extremes of the two categories) from the two size-groups do not generally have the overlapping of the pinna size. The large size category possesses thickly herbaceous larger pinnae borne on longer stalks (total range of pinna size $2\text{–}5 \cdot 0 \times 0 \cdot 8\text{–}2$ cm.), which at least in the lower few pairs are fully lunate or more often with the basal angle (where acroscopic and basiscopic edges of pinnae meet at stalk) more than 180° (*cf.* Text-Fig. 2). The basal angle for the pinnae placed higher up gradually decreases to roundabout 120° and in the distal region it becomes nearly 90°.

Apart from general shape and size, the extent of lobing varies considerably. The largest forms usually possess lobed pinnae, lobes extending to $\frac{1}{2}$–$\frac{1}{3}$ the breadth (Text-Fig. 2 A). In another set of individuals the pinnae have almost a continuous outer edge being only interrupted by a few narrow notches (Text-Fig. 2 B). Still others have a completely continuous outer edge with a continuous sorus (Text-Fig. 2 C).

Distinctly small-sized populations (Pl. I, Fig. 1; Pl. II, Figs. 7, 8), on the other hand, bear correspondingly small-sized pinnae (average dimensions $0 \cdot 4\text{–}1 \cdot 0 \times 0 \cdot 2\text{–}0 \cdot 6$ cm) borne on relatively much smaller stalks. There is lesser variability in the extent of lobing and shape of pinnae. The lower pair seldom becomes fully lunate and the outer



TEXT-FIG. 1. Frequency polygon on size of individuals from a sample population of 775 individuals from Badamtam-Teesta pony path (Darjeeling). Note two widely different modes.

M 8

TEXT FIG. 2

TEXT-FIG. 2. Line drawings of triploid apogamous *Adiantum lunulatum*, showing architecture of pinnae, lobing, soral construction and basal angle, $\times \frac{1}{2}$ nat. size. (A) Pinnae lobed, soral lines straight (from P.U. Coll. No. 212). (B) Pinnae simply notched (P.U. Herb. No. 4531). (C) Pinnae entire, soral line continuous (P.U. Herb. No. 1540). (D) Pinnae lobed or notched, sori concave (P.U. Herb. No. 4530).

edge is shallowly lobed or simply notched. The soral line never becomes continuous. The basal angle in the large majority falls between 90° and 150° in the lower $\frac{1}{3}$ of the frond, while in the upper $\frac{2}{3}$ region it approaches 90°.

Sori.—The range of variability in soral construction among the large-sized forms is comparatively far more than in the second category, where the situation is more or less constant. In the deeply-lobed or shallow-lobed forms, one lobe possesses in general a single elongate soral line, although more than one sorus may occur occasionally. In extremely shallow-lobed forms, the lobing is often obscured by the overlapping of adjacent indusia (Pl. II, Figs. 3, 4). Forms with entire pinnae have a continuous line of sorus (Pl. II, Fig. 5; Text-Fig. 2 C), and in some cases with lobed pinnae each sorus becomes protruded outwardly at the edges and curves centripetally, so that the concavity faces outwards (Pl. II, Fig. 6; Text-Fig. 2 D).

The category of small-sized fronds has nearly uniform type of sori, each lobe possessing a smooth-elongate sorus or a slightly concave sorus (Pl. II, Fig. 7).

CYTOLOGY OF MORPHOVARIANTS

As stated above cytological studies of these variants have revealed the existence in the E. Himalayas of four cytotypes, namely, triploid apogamous, diploid sexual, tetraploid sexual, and diploid apogamous, all based on $x = 30$.

Triploid apogamous.—This is by far the commonest cytotype in which 8-celled sporangia far outnumber the 16-celled ones and show 'n'$= 90$ at diakinesis (Text-Fig. 3). The frond size ranges between 20 and 68 cm. Morphologically three categories of individuals are recognizable here on the basis of pinnae and soral construction:

1. Pinnae variously lobed with discrete smooth sori (Text-Figs. 2 A, B).
2. Pinnae simple with continuous soral line (Text-Fig. 2 C).
3. Pinnae shallow lobed, sori prominently concave (Text-Fig. 2 D).

All the three categories broadly adhere to the description of *Adiantum lunulatum* (*see* Holttum, 1954). The illustrations by Beddome (F.S.I., t.1., **1863**), Ching (*Icones Fl. Sinicarum*, Fas. 5, pl. 215, **1958**), Pichi-Sermolli (Fig. 4, p. 667, **1957**) and Nayar (1962) only conform to the category 1, although most authors do mention about the variability in the size and lobing of pinnae. The forms in this category are the

TEXT-FIGS. 3–6. Fig. 3. Diakinesis in a spore mother cell of triploid apogamous *A. lunaatum*, ' ' = 90, $\times$2,000. Fig. 4. Silhoutte of tetraploid sexual cytotype. (P.U. Herb. No. 3812), $\times \frac{1}{2}$ nat. size. Fig. 5. A S.M.C. of tetraploid, showing *n* = 0, 6H $\times$ 2,000. Fig. 6. Silhoutte of diploid apogamous cytotype (P.U. Herb. No. 3811), $\times \frac{1}{2}$ nat. size.

commonest occurring most often in open and shaded localities preferably on soil. These forms were collected under P.U. Herb. Coll. No. 212, a specimen out of which was sent to Dr. R. E. Holttum (Kew) for comparison with the Burmann's type. He expressed (letter dated March 24, 1959) "the specimen (Burmann's type) comes nearest your No. 212 but is rather less deeply incised". To be very exact, it should therefore be proper to relate strictly the shallow lobed forms as nearer to the type of *A. lunulatum*. From the spore dimensions and the occurrence of abortive spores, it has been inferred that Burmann's type represents a triploid apomict (*cf.* Verma, 1961).

Diploid sexual.—It is characterized by its much smaller delicately herbaceous fronds, 4–18 cm, the basal pinnae seldom assume a lunar shape (Pl. I, Fig. 1). Generally, the pinnae are small, 0·4–1·0×0·2–0·6 cm, and the basal angle lies between 90° and 150° but never more than 180°. In the upper $\frac{2}{3}$ region it is often 90°. Sixty-four normal and viable tetrahedral spores result in a sporangium and at diakinesis the spore mother cell shows 30 bivalents (Pl. I, Fig. 2).

As compared to triploids, this cytotype is plentiful only nearabout Teesta. As expected, the spore and stomatal dimensions are smaller when compared to the triploids.

Tetraploid sexual.—In addition to the above stated common cytotypes met with in Darjeeling (Teesta), tetraploid sexuals (Text-Fig. 4) have also been discovered based on the presence of 60 bivalents at M_I (Text-Fig. 5). Meiosis in these is perfectly regular resulting in a normal output of 64 spores per sporangium. This form is midway between the triploid apogamous and diploid sexuals in frond size, but the texture and the basal angle resembles more or less the diploids in a wider sense.

Diploid apogamous.—Very few individuals (Text-Fig. 6) which were at first taken to be the larger forms of the diploid taxon proved to be cytologically different. These were diploid apogamous showing 'n' = 60 and released 32 spores per sporangium. From morphological appearance it is hard to isolate these from the tetraploid sexual cytotype.

Discussion

The Himalayan *A. lunulatum* Burm. is in fact an "aggregate species" and so far known is comprised by four cytotypes, namely, triploid apogamous, diploid sexual, tetraploid sexual, and diploid apogamous. Out of these, triploids are widely distributed having been reported from S. Africa, W. Trop. Africa, Ceylon, Bombay, Mt. Abu, and Western and E. Himalayas. Diploid and tetraploid sexual cytotypes have been recently reported also in S. India (Abraham *et al.*, 1962). There is no report yet of the occurrence of diploid apogamous elsewhere.

Cytogenetic Considerations

It has been indicated all along that all the cytotypes broadly agree to the *lunulatum* plan and there is probably no other species which may be considered to be involved in this complex. The obvious assumption, thus, which one is likely to make on gross morphology is that the entire range represents an "autoploid series". This assumption, however, is not supported by the pairing behaviour of chromosomes in the spore mother cells.

At M_I the tetraploids show complete bivalent formation, a condition prevalent in genomic alloploids. The remaining two cytotypes are apogamous and in apogamous ferns the chromosomal association analysis in the 16-celled sporangia are taken to be of paramount importance, since they supposedly reflect the genetic constitution of the species. This information is not known for the diploid apomict. Ghatak (1960), however, has reported approximately $30_{II} + 30_I$ in the

16-celled sporangia of triploids from W. Bengal. Same is corroborated by the investigations of Roy and Sinha (1961). This would again suggest that two diploid species are involved in it, one represented twice. In other words the tetraploid sexual or the diploid apomict which could serve as the other parent in the triploid are of hybrid origin or genomic alloploids. The situation requires experimental evidence but on the face of it and in cognizance with morphological data, it appears that there are to be suspected at least two distinct genetical lines in the diploids which although morphologically similar would create on hybridization a cytological situation akin to alloploids.

In conclusion, it shall suffice to state that *lunulatum* complex promises an excellent material for further experimental investigation. Morphologically it appears as a range of "autoploid" series but available cytological evidence disproves it. The situation seems to be rather parallel to N. American *Pityrogramma triangularis* (*cf.* Alt and Grant, 1960) where also three cytological races occur, all morphologically similar and two genetical types are suspected to occur at the diploid level.

Incidentally, a reference should be made here to the karyotypic studies in the genus *Adiantum* by Roy and Sinha (1960, 1962), which includes triploid *A. philippense* (= *A. lunulatum*). The authors state "In *A. philippense* one pair again has both primary and secondary constrictions. Nineteen pairs of chromosomes have median centromeres and 25 pairs have sub-median to sub-terminal centromeres". It is evident that Roy and Sinha (*loc. cit.*) detected 45 pairs in the triploid ($2n = 3x = 90$) and they did not take into account $x = 30$ in species of *Adiantum* of its affinity. The position thus cannot be taken as correct and requires reinvestigation. Presently looking from any angle it neither corroborates Ghatak's (1960) analysis nor approves of its being an "autoploid".

Taxonomic Evaluation of Cytotypes

It is rather curious to note that all the sheets of *A. lunulatum* present in the Central National Herbarium, Sibpur (Howrah, India) belong to the group of triploids. The small-sized individuals (diploids) are not at all represented and this fact is reflected in the range of size mentioned in earlier literature. The triploids in general and P.U. Coll. No. 212 (Group I here) in particular conform to Burmann's type. Three morphological forms were recognized in the triploids and cytotaxonomically speaking each form, since it can breed true effectively apogamously, could possibly be segregated as a variety, especially the one with a continuous line of sorus. This form seems to be widespread as evident from Dr. Holttum's letter (dated April 12, 1957), "*A. philippense* occurs in West Tropical Africa and in West Indies and in both areas is very variable with some forms having continuous sori". However, at present the varietal names already existent in *A. philippense* or *A. lunulatum* are not known to us. Hence the matter of nomenclature is postponed. It may be remarked that the morphovariants in the triploid apomict could have evolved through gene mutations or chromosomal rearrangement during a long history of its apogamous nature evident in its present-day wide distribution from S. Africa to Himalayas. Apogamous reproduction would preserve all such changes, if otherwise not detrimental to the proper functioning of growth processes and able to withstand the test of natural selection.

The diploid sexual cytotype is morphologically distinct from the triploids and on this basis it demands a separate specific status. It has been named *Adiantum teestae* sp. nov. (Pl. I, Fig. 1), based on its place of collection, Teesta.

The tetraploid sexual has more similarities in general aspect to the diploid sexual than the triploid apomicts but for the size of pinnae and frond. The diploid apomict similarly comes nearer to the diploid sexual cytotype (*A. teestae*) than the common triploids. Both the cytotypes can, for the present, be retained as additional elements in *A. teestae* (diploid). The specific name *Adiantum lunulatum* (s.s.) is thus limited only to the triploid apomicts.

Summary

Adiantum lunulatum Burm. (= *A. philippense* L.) is widely distributed in subtropical and tropical regions of Asia, Australia and Africa. The extremely variable elements of the species from the E. Himalayas (about Teesta) have been analysed.

In all, four cytotypes have been found, namely, triploid apogamous (commonest), diploid sexual (quite common in the area), tetraploid sexual (occasional) and diploid apogamous (rare). Burmann's type specimen of *A. lunulatum* resembles the triploid cytotype as regards the size and general configuration of the fronds, as well as the spore size. *A. lunulatum* (s.s.) may, therefore, be regarded to represent triploid apogamous taxon. Because of the overall morphological distinctness of the diploid sexual cytotype (small-sized individuals), it has been discussed to segregate it into a new species, *A. teestae*. The two other cytotypes, namely, diploid apogamous and tetraploid sexual because of their general resemblance with *A. teestae* are also retained in it for taxonomic convenience although each one of these has a distinct biological status from evolutionary point of view.

It has been pointed out that all the cytotypes and morphovariants broadly agree to *lunulatum* plan and no other species is involved in the *lunulatum* complex. The cytogenetic considerations have been discussed on the available cytological and morphological data. Gross morphology suggests a case of autoploid series whereas cytology disproves it and indicates "alloploid" nature of cytotypes. In conclusion, it has been indicated that in the diploid sexual cytotype there probably exist at least two distinct genetical lines which on hybridization yield a situation like the genomic alloploids.

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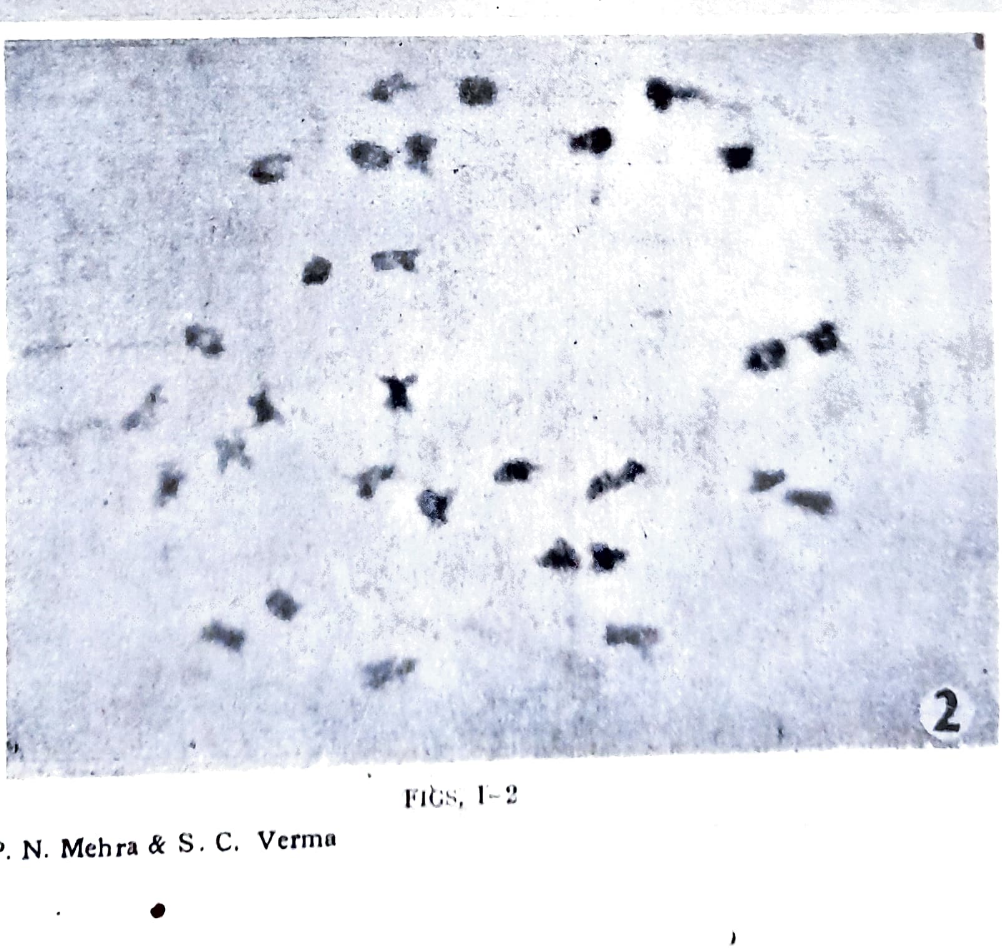

FIGS. 1–2

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FIGS. 3-8

P. N. Mehra & S. C. Verma

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EXPLANATION OF PLATES I & II

PLATE I

FIG. 1. Individuals of diploid sexual *A. teestae* Verma. This sheet represents the Typus deposited in the Panjab University Herbarium (No. 4534), × approx. ½.

FIG. 2. Diakinesis in a S.M.C. of *A. teestae*, showing $n = 30$, × 1,200.

PLATE II

FIGS. 3–8. Close-up photographs of some of the major forms in *A. lunulatum*, both large and small-sized category, showing details of soral construction. A two-centimetre scale is attached along with each. Fig. 3. Pinnae lobed, soral lines straight, triploid apogamous (P.U. Herb. No. 3813). Fig. 4. Pinnae simply notched, sori restricted one to each lobe, triploid apogamous (P.U. Herb. No. 4531). Fig. 5. Pinnae entire, soral line continuous, triploid apogamous (P.U. Herb. No. 4532). Fig. 6. Pinnae lobed or notched, sori sharply concave, triploid apogamous (P.U. Herb. No. 4530). Fig. 7. Pinnae shallow lobed, sori straight-concave, diploid sexual (*A. teestae*), note the small size and basal angle (P.U. Herb. No. 4534). Fig. 8. A small individual of diploid sexual *A. teestae* to show the range of size (P.U. Herb. No. 4533).