CYTOLOGY OF SOME HIMALAYAN FERNS

By

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In some previous communications (Mehra *et. al.* 1955, 56, 57*a*, *b*) the cytology of some ferns belonging to the Darjeeling Himalayas was described. In this communication some more ferns belonging to the same area and representing the families Dennstaedtiaceae, Hypolepidaceae, Lindsayaceae, Davalliaceae and Oleandraceae (classification according to Ching 1940) are worked out. We do not agree with the classification of Polypodiaceae by Ching entirely, but we do feel that on the basis of chromosome data gathered so far and other morphological considerations the earlier part of Copeland's Pteridaceae should best be split into the families proposed by Ching, and we have followed him in the present paper. Manton and Sledge (1954) have already pointed out the unnaturalness of Copeland's comprehensive family Pteridaceae.

The technique used was the same as previously described.

All the plants were not uniformly suitable for cytological investigation. The members of Lindsayaceae and Davalliaceae were easy to work because of the large size of their spore mother cells and the readiness with which their chromosomes pick up the stain. About *Dennstaedtia*, the statement that Britton (1953) made should be repeated. "The cytologist is confronted with another difficult subject. The chromosomes and spore mother cells are both small, consequently it is difficult to obtain outstanding plates". This in fact is true also of other members of Dennstaedtiaceae investigated. *Hypolepis* deserves special mention because of its small sized chromosomes and difficulty in staining them; with more mordanting than usual they would go black and stain erratically. Better methods could not be attempted as the material was tried in the field. Hence it has not been possible to give an unequivocal number for this genus.

A summary of the results obtained in the present investigation along with those of other authors on the same genera is presented in the Table I preceding discussion.

Our sincere thanks are due to Mr. A. H. Alston of the British Museum for identifying the species of *Microlepia* and to Mr. R. S. Pathania of this department for taking the microphotographs.

OBSERVATIONS

Dennstaedtia: This is an ancient genus which is known in the fossil state from the upper Cretaceous of Patagonia. There are 70 living species mostly distributed in the tropics of both hemispheres. In Darjeeling it is represented by two well marked species.

D. appendiculata (Wall.) J. Sm. is very common in and around Darjeeling. It grows with a shrubby habit on stony walls along the roadside and also on open ground. It is essentially a plant of higher altitudes and extends from 5,000—10,000 ft. above sea level. At Tanglu (10,000 ft.) very flourishing specimens were seen in abundance. The material for cytological study was, however, taken from Nehru Road, Darjeeling.

The chromosome number was confirmed from a number of permanent aceto-carmine preparations. Meiosis is perfectly regular and 66 bivalents were counted without the slightest ambiguity (Pl. 1 fig. 1, Text-figs. 1, 2).

Text-Fig. 1.
Dennstaedtia appendiculata n=66 ×1800

Text-Fig. 2.
Dennstaedtia appendiculata n=66 ×2640

Text-Fig. 3. *Dennstaedtia scabra* n=66 ×2640

D. scabra (Wall.) Moore is also very common in and around Darjeeling. The most favoured habitat, however, is the edges of the forests. It extends from an altitude of 5,000-9,000 ft. The material for cytological study was taken from Lloyd Botanical Gardens, Darjeeling.

Meiosis is regular and the chromosome number as studied from a single but very clear permanent aceto-carmine preparation is $n=66$ (Text-fig. 3).

Britton (*loc. cit.*) reports $n=34\pm1$ in *D. punctilobula* (Michx.) Moore. This species is undoubtedly diploid. Both the present species are, however, tetraploid on the base number 33. Manton and Sledge (1954) have worked out *D. scabra* from Ceylon and they report n as not less than 64 and not more than 65. The Himalayan plant, however, definitely shows $n=66$. If their report is correct it would mean a slight degree of aneuploidy in the Ceylon form, which would not be unexpected in a tetraploid species.

Microlepia:

The genus is represented by three species in Darjeeling area.

M. speluncae (L.) Moore is one of the largest members of the group and is essentially a plant of the lower altitudes. It has never been seen growing above 3,000 ft., whereas it is quite common at an altitude as low as 500 ft. The plant is exceedingly common near Manjitar Bridge and Tista Bridge where it grows either singly or in formations. The cytological material was taken from Manjitar nearly 10 miles from Darjeeling.

The chromosome number $n=43$ (Text-fig. 4) was confirmed from a number of meiotic plates both temporary and permanent.

Text-Fig. 4. *Microlepia speluncae* n=43 ×2640

This species has been worked out by Manton and Sledge (*loc. cit.*) from Ceylon. There it occurs as a tetraploid with $n=86$.*

M. rhomboidea (Wall.) Presl is found commonly growing on the forest floor of Lebong (approx. 5,000 ft.) in two forms, a diploid and a triploid. The latter is rare and possesses larger fronds and sterile sporangia. The diploid is on the average 80 cms. tall while the triploid is 125 cms. tall.

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* This species is stated to occur in 'countless slightly different forms' in its pantropic distribution (Copeland 1947).

THE DIPLOID: The chromosome number counted from permanent aceto-carmine preparations was $n = 43$ without doubt (Text-fig. 5). From the study of various phases of meiosis, the plant is found to be perfectly normal.

Text-Fig. 5. *Microlepia rhomboidea* (diploid) n=43 ×2640

THE TRIPLOID: The plant is abnormal as is evidenced from a close study of meiosis. During diakinesis we observed chromosomal chains, trivalents, bivalents and many univalents. At first metaphase many of the univalents are outside the equatorial plate whereas others are irregularly arranged on it. (Pl. 1 fig. 2). The univalents are not able to reach the poles in time; they lag behind. The second meiotic division is also irregular with numerous laggards (Pl. 1 fig. 3). As a result the chromosomes are not all included in the four nuclei of the mother cell at the end of meiosis but accessory micronuclei are formed. These latter become included within the tetrad members (Text-fig. 6.). The spores are irregular in shape and size and are abortive.

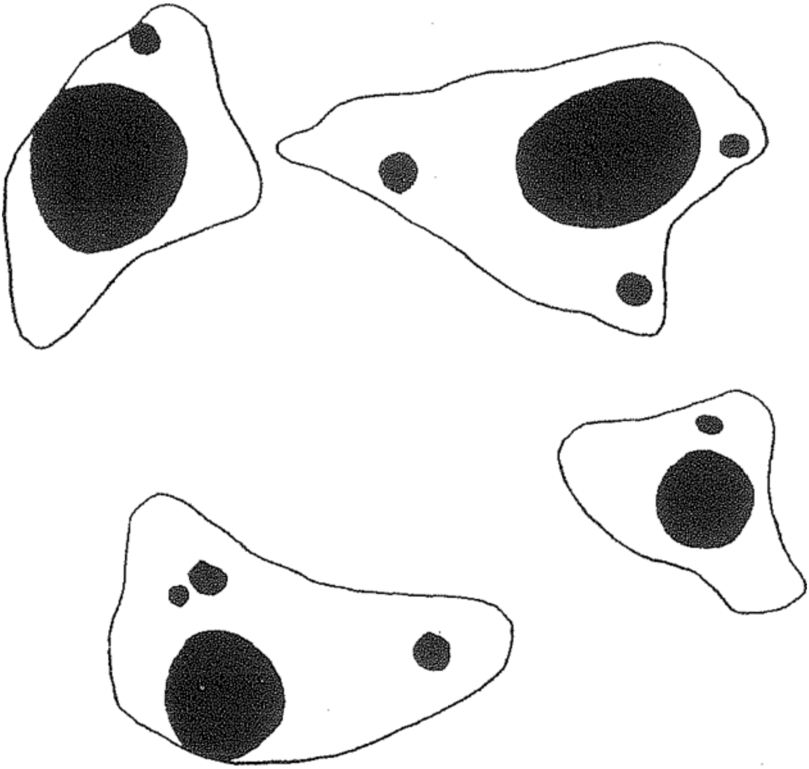
Counting of the exact chromosome number was rather difficult due to irregular meiosis, but a total of up to 120 chromosomes have been observed. From a study of morphological characteristics, chromosomal configurations, and the frequent occurrence of trivalents, it is suspected that the plant is an autotriploid in which perhaps some segmental translocations have also occurred.

M. pilosula (Wall.) Prantl: This species is also met with commonly in Lebong Forest along with the previous one. In its meiotic behaviour it shows perfect regularity and forms uniform tetrahedral spores. 86 bivalents were clearly observed (Text-fig. 7).

The above observations confirm 43 as the base number for the genus arrived at previously by Manton and Sledge (*loc. cit.*).

Hypolepis:

Only one species, *H. punctata* (Thunb.) Mett grows in the area. The plant resembles



Text-Fig. 6. *Microlepia rhomboidea* (triploid) spores with micronuclei ×1760

Text-Fig. 7. *Microlepia pilosula* n=86 ×2460

Pteridium aquilinum very much in habit. It grows like a weed along the roadside, on open ground, and the edges of forest floors at an altitude of nearly 6,000 ft. Material from various localities was studied including the plants cultivated in the Lloyd Botanical Gardens.

The chromosome size is very small as reported previously by Manton and Sledge, and this feature may perhaps be characteristic of the genus. Due to this and the difficulty with which the chromosomes stain, the present study has not progressed beyond the conclusion that the number of bivalents is approximately 102 (Pl. 1 fig. 4, Text-figs. 8 & 9). The base number for the genus is shown to be 52 by Brownlie (1957) on which, therefore, the present species is a tetraploid.

Text-Fig. 8. *Hypolepis punctata* n = ca. 102 × 1650

Lindsaya:

The genus is comprised by about 200 species mostly pantropic in distribution. It is represented in the area by a single species *L. cultrata* (Willd.) Sw. This small sized plant is quite common on rocks in shady and moist places and often grows gregariously to form dense patches. It differs from the other species of the genus in possessing a typical solenostele instead of a 'Lindsaya stage'. Its altitudinal range is between 5,000-7,000 ft. The material for the cytological study was taken from Katapahar.

150 bivalents were counted with certainty (Pl. 2 fig. 5, Text-fig. 10) in a number of mother cells. No meiotic irregularity of any sort was observed. This confirms the findings of Manton and Sledge who reported $n = ca. 150$ for the same species growing in Ceylon.

A perusal of Table I shows that five species of the genus have so far been worked out, but, unlike the other genera of ferns, there is no consistency in the base number. The genus requires thorough investigation cyto-taxonomically.

Text-Fig. 9. *Hypolepis punctata* n = ca. 102 ×2640

Sphenomeris:

There are 18 species known of this genus. *S. chusana* (L.) Copel. is the only species commonly growing along the roadsides and pathsides at an altitude a little lower than Darjeeling proper. It was seen growing abundantly on the hilly side of the road to Chungthung (Sikkim). The material for cytological investigation was taken from a tea estate on the way to Sedrapong Hydro-electric Station.

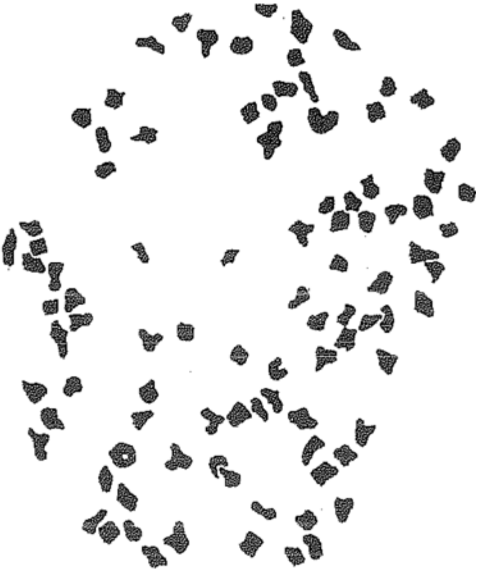
The meiotic chromosome number was confirmed from a number of permanent preparations to be precisely $n = 94$ (Pl. 3 fig. 6, Text-fig. 11). No meiotic irregularity of any sort was observed.

The present findings seem somewhat interesting in the light of Manton and Sledge's investigations. They report a tetraploid form of this species with $n = ca.$ 100 from Ceylon and a hexaploid form $n = 145$-147 from Malaya. It is evident from above that the present material, though undoubtedly tetraploid as is Manton and Sledge's material from Ceylon, is so on a slightly different base number 47. A comparison of the

Himalayan tetraploid with the silhouettes of the tetraploid of Ceylon and hexaploid of Malaya presented by Manton and Sledge (1954, p. 148) reveals that the Himalayan plant is more like the hexaploid from Malaya than the Ceylon form in external morphology.

A glance at Table I shows that this is the only species of the genus that has been investigated. The plants from different areas show different chromosome numbers. It is thus not possible to come to a final conclusion with regard to the base number of the genus, nor the way in which the cytological evolution has proceeded within the species, until further investigations are carried out.

Text-Fig. 10. *Lindsaya cultrata* n = 150 × 1350



Text-Fig. 11. *Sphenomeris chusana* n = 94 × 1750

Araiostegia:

This genus is represented by two species in the Darjeeling area, both of which are epiphytes or lithophytes.

A. pulchra (J. Sm.) Copel. is a graceful fern with much dissected fronds, and is abundant round about Darjeeling. The study was carried on cultivated material from Lloyd Botanical Gardens and wild material from Lebong forest. Very clear chromosome plates at diakinesis have made the accurate counting possible showing $n=40$ (Pl. 2 figs. 7, 8 and Text-figs. 12, 13).

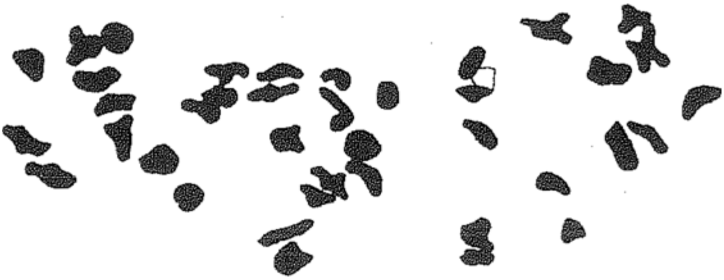
A. pseudocystopteris (Bedd.) Copel. is also common at Darjeeling, and extends westwards to Mussoorie and Simla hills where it is met with as an epiphyte thickly clothing tree trunks and branches (Mehra 1939). Meiotic studies carried out from Mussoorie material gave a positive count of $n=40$ chromosomes (Pl. 3. fig 9, Text-figs. 14, 15).

Leucostegia:

This genus is closely allied to *Araiostegia* as is evidenced from the fact that the species now assigned to it were formerly included in *Leucostegia.* The anatomy of the rhizome in the two cases, however, is different. It is represented by only a single species *L. immersa* Presl. in the area under investigation and occurs both as an epiphyte and lithophyte. The characteristic yellowish green colour with large impressed sori differentiates it from *Araiostegia.* The species extends westwards to Mussoorie hills from where it was investigated. The chromosome number is $n=41$ (Pl. 3 fig. 10, Text-fig. 16).

Text-Fig. 12. *Araiostegia pulchra* n=40 ×1600

Text-Fig. 13. *Araiostegia pulchra* n=40 ×1600



Text-Fig. 14. *Araiostegia pseudocystopteris* n=40 ×1820

Text-Fig. 15. *Araiostegia pseudocystopteris* n=40 ×1760

Text-Fig. 16. *Leucostegia immersa* n=41 ×1460

Oleandra:

The genus is represented in the area by two species *O. neriiformis* Cav. and *O. wallichii* (Hook.) Presl. Both the species possess a sprawling habit, growing abundantly on huge rocks or cliffs along the roadside forming dense thickets at an altitude of 6,000 ft. The latter species also extends westwards to Mussoorie and Simla hills but decreases considerably in frequency (Mehra 1939).

The chromosome number for *O. neriiformis* was determined from a wild population growing in the vicinity of Lebong road, Darjeeling. *n* is 41 beyond any doubt (Pl. 3 fig. 11, Text-figs. 17, 18).

Text-Fig. 17. *Oleandra neriiformis* n=41 ×2000

Text-Fig. 18. *Oleandra neriiformis* n=41 ×2640

Text-Fig. 19.
Oleandra wallichii n=41 ×2640

Text-Fig. 20.
Nephrolepis cordifolia n=41 ×2640

Cytological investigation of the other species was done from plants growing in Mussoorie hills. The number *n* for this species is also 41 (Text-fig. 19).

Manton and Sledge worked out two species, *O. musifolia* and *O. distenta* from Ceylon and Kew respectively. The former is reported to have *n*=*ca.* 40 and the latter a tetraploid with *n*=*ca.* 80. As precise counting was not possible in either of these two species, the base number for the genus remained uncertain. The present findings show the base number to be definitely 41. *Oleandra* thus conforms more with *Nephrolepis* which similarly has a base number 41, rather than *Davallia* with $n=40$ and thus supports Holttum's (1947) suggestion of the relationship between the two genera.

Nephrolepis:

This genus is represented in Darjeeling by a single species *Nephrolepis cordifolia* (L.) Presl. which occurs rather sporadically. The species, however, is exceedingly common at low altitudes along the roadside from Darjeeling to Gangtok in Sikkim. The cytological investigation was undertaken from specimens under cultivation in the Company Garden, Amritsar. Squashes revealed clearly $n=41$ (Text-fig. 20) thus confirming the result of Manton and Sledge for plants of the same species from Ceylon.

Table I

*Resume of chromosome number of all the species so far investigated in the genera dealt with here.**

Name of the plant	Source	Investigator	Chromosome number	Degree of ploidy and remarks
Dennstaedtia punctilobula (Michex.) Moore	South Ontario (Canada)	Britton (1953)	$n=33\pm1$	Diploid
D. appendiculata (Wall.) J. Sm.	Darjeeling	Authors	$n=66$	Tetraploid
D. scabra (Wall.) Moore	Ceylon	M & S (1954)	$n=64$-65	Tetraploid
	Darjeeling	Authors	$n=66$	Tetraploid
D. cicutaria (Sw.) Moore	Kew	M & S (1954)	$n=94$	Hexaploid
Microlepia platyphylla (Don.) J. Sm.	Kew	M & S (1954)	$n=43$	Diploid
M. speluncae (L.) Moore	Darjeeling	Authors	$n=43$	Diploid
	Ceylon	M & S (1954)	$n=86$	Tetraploid
M. rhomboidea (Wall.) Presl	Darjeeling	Authors	$n=43$	Diploid
M. rhomboidea (Wall.) Presl Triploid	Darjeeling	Authors	$2n=$ca. 120	Triploid, irregular
M. pilosula (Wall.) Prantl	Darjeeling	Authors	$n=86$	Tetraploid
Hypolepis punctata (Thunb.) Mett.	Ceylon	M & S (1954)	$n=51$-53	Diploid
	Malaya	M & S (1954)	$n=$ca. 100	Tetraploid
	Australia	M & S (1954)	$2n=$ca. 150	Triploid, sterile
	Darjeeling	Authors	$n=$ca. 102	Tetraploid
H. rugulosa (Labill.) J. Smith	New Zealand	Brownlie (1957)	$n=52$	Diploid
H. tenuifolia (Forst. f.) Bernh.	New Zealand	Brownlie (1957)	$n=104$	Tetraploid
Lindsaya cuneata (Forst. f.) C. Christ	New Zealand	Brownlie (1957)	$n=42, 43$	Diploid

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*Since sending this paper to press Brownlie (Trans. Roy. Soc. New Zealand 1958) has reported chromosome numbers in two species relevant to the present paper, *Hypolepis millefolium* Hook. with $n=52$ and *Lindsaya linearis* Swartz with $n=34$.

Lindsaya caudata Hk.	Ceylon	M & S (1954)	$n=82$	Tetraploid
L. pectinata Bl.	Malaya	M & S (1954)	$2n=ca.\ 100$	Diploid
L. decomposita Willd.	Malaya	M & S (1954)	$n=ca.\ 50$	Diploid
	Malaya	M & S (1954)	$n=ca.\ 100$	Tetraploid
L. cultrata (Willd.) Sw.	Ceylon	M & S (1954)	$n=ca.\ 150$	Hexaploid
	Darjeeling	Authors	$n=150$	Hexaploid
Sphenomeris chusana (L.) Copel.	Darjeeling	Authors	$n=94$	Tetraploid
	Ceylon	M & S (1954)	$n=ca.\ 100$	Tetraploid
	Malaya	M & S (1954)	$n=145\text{-}147$	Hexaploid
Araiostegia pulchra (J. Sm.) Copel.	Darjeeling	Authors	$n=40$	Diploid
A. pseudocystopteris (Bedd.) Copel.	Mussoorie	Authors	$n=40$	Diploid
Leucostegia immersa Presl	Mussoorie	Authors	$n=41$	Diploid
Oleandra musifolia (Bl.) Pr.	Ceylon	M & S (1954)	$n=ca.\ 40$	Diploid
O. distenta Kunze	Kew	M & S (1954)	$n=ca.\ 80$	Tetraploid
O. neriiformis Cav.	Darjeeling	Authors	$n=41$	Diploid
O. wallichii (Hk.) Pres.	Mussoorie	Authors	$n=41$	Diploid
Nephrolepis cordifolia (L.) Presl.	Ceylon	M & S (1954)	$n=41$	Diploid
	Company Garden Amritsar	Authors	$n=41$	Diploid
N. hirsutula (Forst.) Pr.	Ceylon	M & S (1954)	$n=41$	Diploid

DISCUSSION AND CONCLUSIONS

The most interesting question that arises is the origin of diversified base numbers in such closely related genera as *Dennstaedtia*, *Microlepia* and *Hypolepis*. There is an undoubted relationship between them and particularly between the first two in regard to the general habit, and morphological and anatomical features, the chief difference being the slightly greater deflection of the sorus in *Microlepia* compared to *Dennstaedtia;* yet there appears to be a wide gap between their base numbers 33 and 43 respectively. We have at present very little data with regard to the cytology of the related family Dicksoniaceae to consider this question, but going backwards to the 'gradate marginales' we find in the Hymenophyllaceae one member *Trichomanes obscurum* with $n=33$ (Manton and Sledge 1954). This ancient group has already shown itself to be most interesting in possessing a series of monoploid numbers like 7, 9, and 13 (Manton 1950, Mehra and Singh 1957*a*). May it not be that we are dealing with another prime base number 11 and that this has been carried forward in *Dennstaedtia*, and also in *Microlepia* with a slight degree of aneuploidy ?* At the present stage of our knowledge this suggestion must remain purely tentative. It would, however, be exceedingly desirable to work out

*Since sending the paper to press Brownlie (Trans. Roy. Soc. New Zealand, 1958), has reported $n=11$ in *Hymenophyllum peltatum* Desv., and $n=22$ in *H. revolutum* Col. and *Meringium bivalve* (Forst.) Copeland.

more of the Hymenophyllaceae and also in particular the members belonging to the Dicksoniaceae to get clues that might be helpful in throwing further light on this problem.

With the establishment of base number 52 for *Hypolepis* (Brownlie 1957) and *Pteridium* (Manton 1950, Britton 1953, Brownlie l.c.) and 26 for the closely allied genus *Paesia* (Brownlie l.c.), a case seems to have been made out for treating these genera together in one evolutionary series. They are otherwise also known to have a good deal in common (Ching 1940, Copeland 1947).

The cytological evolution within the family Lindsayaceae seems to be fairly complex. Many more members must be investigated before the position is clarified.

The inclusion of *Araiostegia* in the Davalliaceae is supported on cytological grounds and the base number 40 is in general agreement with the other members of the family so far worked out, as pointed by Manton and Sledge.

Oleandra, which has a disputed phylogenetic position, resembles *Nephrolepis* cytologically rather than the Davalliaceae. Considering its other diverse features it should not be put under Davalliaceae, but perhaps its best place will be along with *Nephrolepis* in a separate family Oleandraceae. Holttum (1947) has placed both the genera together in his sub-family Oleandroideae of the comprehensive family Dennstaedtiaceae.

SUMMARY

Meiotic studies have been carried out on 14 species representing 9 genera of ferns from the Darjeeling Himalayas belonging to families Dennstaedtiaceae, Hypolepidaceae, Lindsayaceae, Davalliaceae and Oleandraceae according to Ching's classification.

The two species of *Dennstaedtia*, *D. scabra* and *D. appendiculata* each with $n=66$ chromosomes, are tetraploid.

A diploid form of *M. speluncae* with $n=43$ is present in the Darjeeling Himalayas, its tetraploid form being reported by Manton from Ceylon. *M. pilosula* is also tetraploid with $n=86$. Diploid and triploid forms of *M. rhomboidea* with $n=43$ and $2n=ca.$ 120 respectively have been found growing side by side in Lebong forest. A morphological comparison of these forms shows a general grossness of the latter accompanied by greater dissection of the fronds.

Hypolepis hostilis shows $n=ca.$ 102, and the base number seems to be of the same order as in *Pteridium*, which it otherwise resembles considerably.

Lindsaya cultrata possesses $n=150$ chromosomes exactly.

Sphenomeris chusana has been found to possess $n=94$.

Araiostegia pulchra and *A. pseudocystopteris* with $n=40$ confirm the correctness of the allocation of this genus to Davalliaceae. The other members of the family studied show a similar base number.

Oleandra neriiformis and *O. wallichii* with $n=41$ resemble *Nephrolepis* with a similar base number rather than *Davallia*. The placing together of these genera (*Oleandra* and *Nephrolepis*) in a separate family Oleandraceae is justified on cytological grounds.

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PLATE 1

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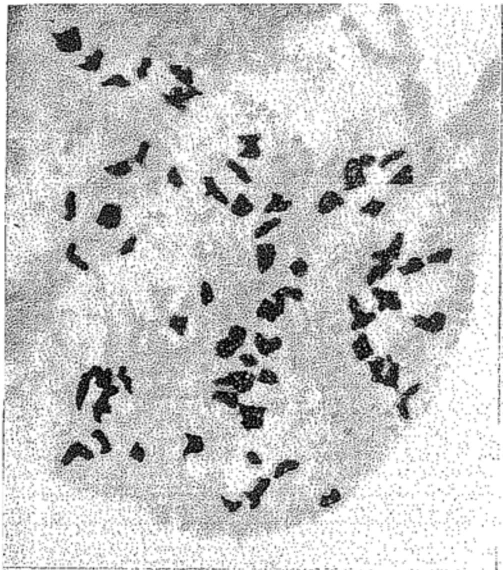

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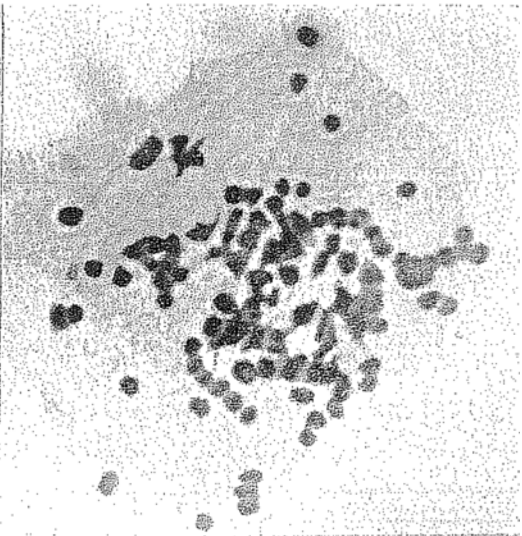

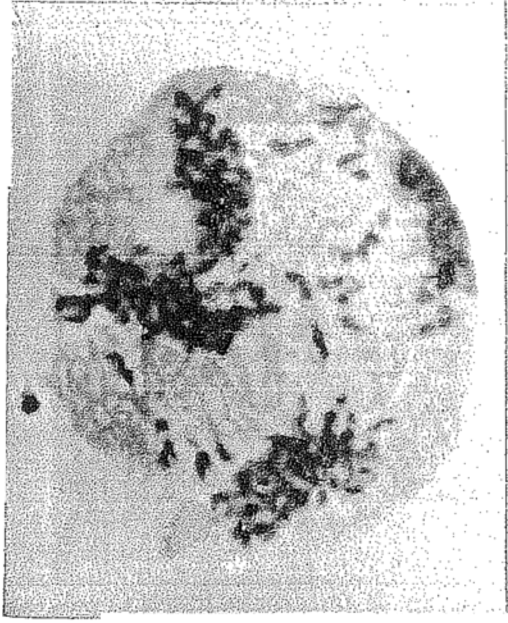

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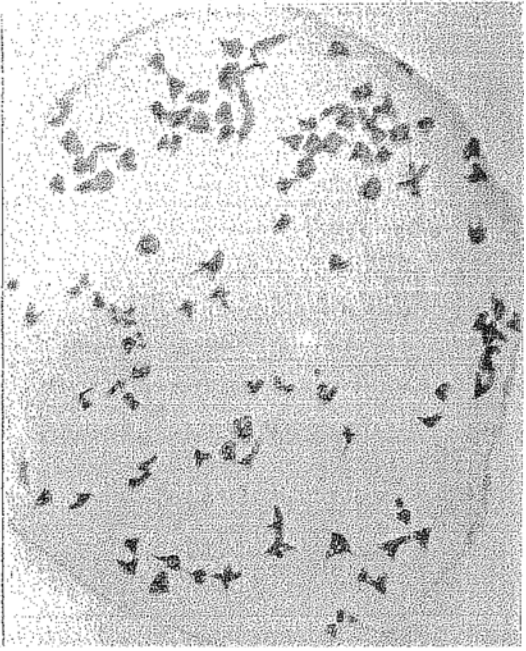

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Fig. 1. *Dennstaedtia appendiculata*, n=66 ×1800 same as Text-fig. 1.

Fig. 2. *Microlepia rhomboidea* (triploid) 2n=ca. 120 ×1350.

Fig. 3. Same as above at second meiotic division ×1350.

Fig. 4. *Hypolepis hostilis* n=ca. 102 ×1650 same as Text-fig. 8.

PLATE 2

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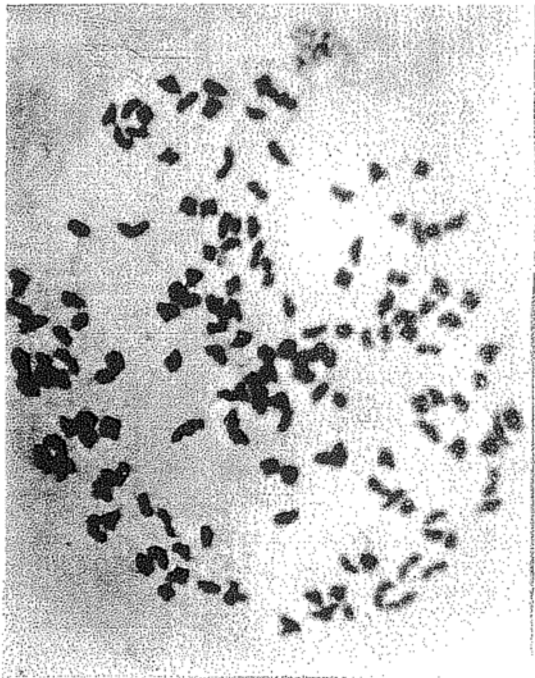

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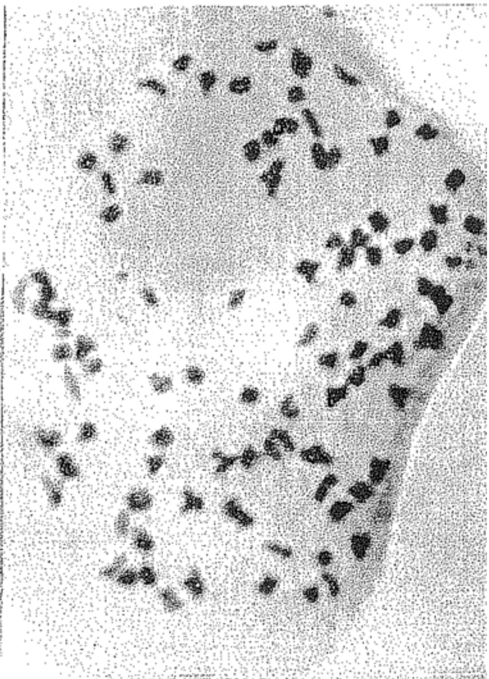

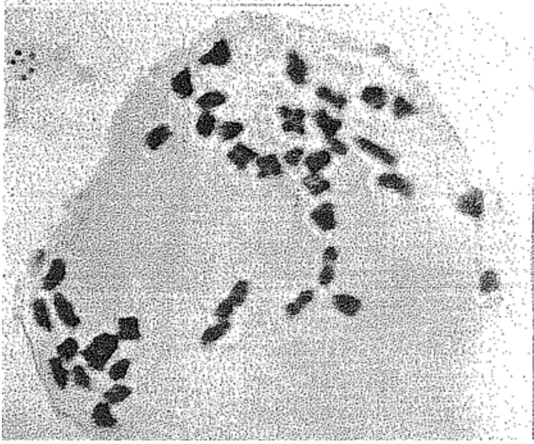

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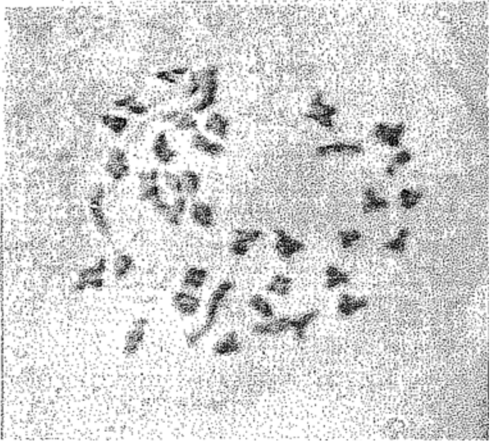

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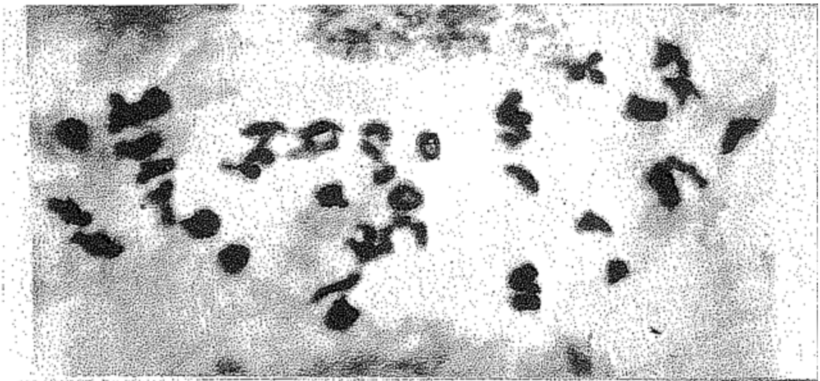
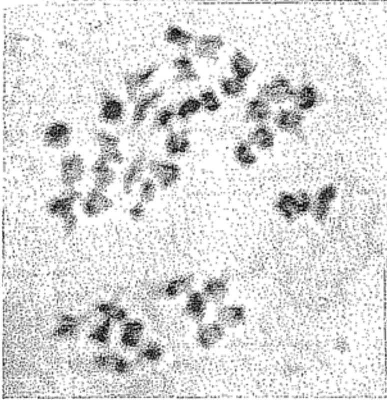
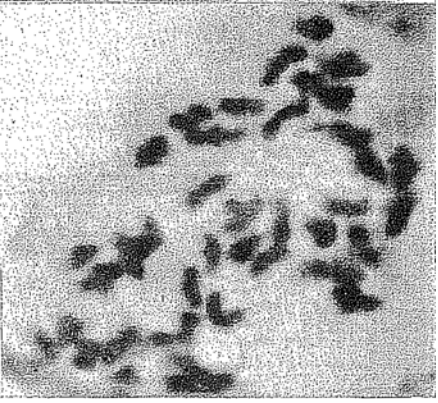
Fig. 5. *Lindsaya cultrata*, n = 150 × 1350. Same as Text-fig. 10.
Fig. 6. *Sphenomeris chusana*, n = 94 × 1750. Same as Text-fig. 11.
Figs. 7, 8. *Araiostegia pulchra*, n = 40 × 1600. Same as Text-figs. 12, 13.

PLATE 3

9







11 10

Fig. 9. *Araiostegia pseudocystopteris*, n=40 ×1820. Same as Text-fig. 14.
Fig. 10. *Leucostegia immersa*, n=ca 41 ×2460. Same as Text-fig. 16.
Fig. 11. *Oleandra neriiformis*, n=41 ×2000. Same as Text-fig. 17.