CYTOLOGY OF SOME BLECHNOID FERNS TOGETHER WITH A NOTE ON THE AFFINITIES OF *STENOCHLAENA*

by P. N. MEHRA, F.N.I. *and* S. S. BIR, *Botany Department,*
Panjab University, Amritsar

ABSTRACT

Chromosome numbers from meiosis have been studied in the type species of three genera of the Blechnaceae. In *Blechnum orientale* and *Woodwardia radicans* the *n* number is 34, while in *Stenochlaena palustris* it is $n = 73$.

The phylogenetic affinity of the much debated genus *Stenochlaena* is discussed in the light of cumulative evidence of characters of its sporophytic and gametophytic generation. A new character of value is brought out which consists in the incurving of the margin of the fertile pinnae and its significance is discussed.

In this paper cytology of three members of the family Blechnaceae (sensu Copeland, 1947) met with in the Darjeeling Himalayas, namely *Blechnum orientale* L., *Woodwardia radicans* (L.) Smith and *Stenochlaena palustris* (Burm.) Bedd. has been investigated. There is no dispute regarding the systematic position of the first two while the last one is a problematic genus.

Bower (1928) and Copeland (*loc. cit.*) consider *Stenochlaena* as a Blechnoid member. It has been treated under the Aspidiaceae by Ching (1940) and Dickason (1946). Christensen (1938) placed it with *Acrostichum* in the group of 'Acrostichoid genera derivated from the Pteridoideae'. In this he was followed by Holttum (1949, 1954) who placed it in the sub-family Pteridoideae of his large family Dennstaedtiaceae. Recently Alston (1956) has included it in the family Polypodiaceae (sensu stricto).

The cytological data on the Blechnoid ferns is very meagre. The three genera *Blechnum, Woodwardia,* and *Stenochlaena* have collectively about 225 species but to date only a dozen out of these have been worked out. Furthermore, some measure of uncertainty exists about the exact basic chromosome numbers of these genera. The present investigation deals with the meiotic chromosome numbers of the type species of these genera.

Blechnum orientale and *Stenochlaena palustris* are essentially low-land ferns growing very abundantly near Teesta, District Darjeeling, between 500–700 ft. altitude. The former is an occupant of rather exposed places along Teesta—Gangtok Road, while the latter is a luxuriant climber in the forest alongside the same road covering tree trunks and reaching tree tops. On the other hand *Woodwardia radicans* has an extensive range, from Kashmir to Bhootan in the Himalayas (Beddome, 1892), occurring on calcareous soil in moist ravines at an elevation of 3,000–8,000 ft.

For meiotic chromosome counts the usual aceto-carmine squash technique was employed. The fixations were made in Carnoy's Fluid for 24–48 hrs. Counts were made from a large number of mother cells to ascertain the correct number. In the case of *Woodwardia radicans* the chromosome number was investigated from plants growing in two areas, at Mussoorie in the Western Himalayas, and Darjeeling in the Eastern Himalayas, in both cases with identical results.

In both *Blechnum orientale* and *Woodwardia radicans* the *n* number is 34 without doubt (Pl. IV, figs. 1,2 ; Text-fig. 1). The meiotic chromosomes in the latter are somewhat larger than in the former and possess a greater affinity for staining. In *Stenochlaena palustris*, on the other hand, 73 bivalents were counted with certainty but these like *Blechnum orientale* take light stain (Pl. IV, fig. 3 ; Text-fig. 2). The meiosis in all the three species is normal and 64 well filled spores are formed
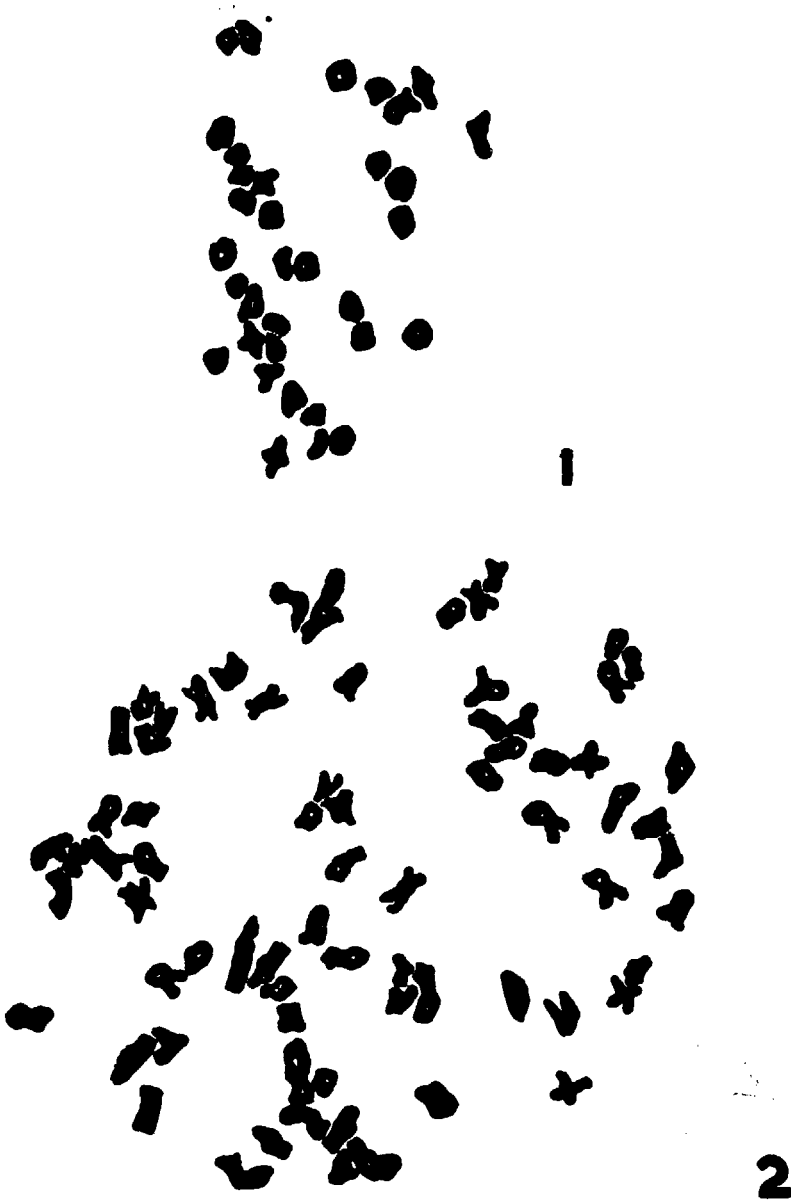
TEXT-FIG. 1.   *Blechnum orientale.*   Explanatory diagram to plate **IV.**
Fig. 1. $n = 34.$ $\times$ 2100.

TEXT-FIG. 2.   *Stenochlaena palustris.*   Explanatory diagram to plate **IV.**
Fig. 3. $n = 73.$ $\times$ 1600.
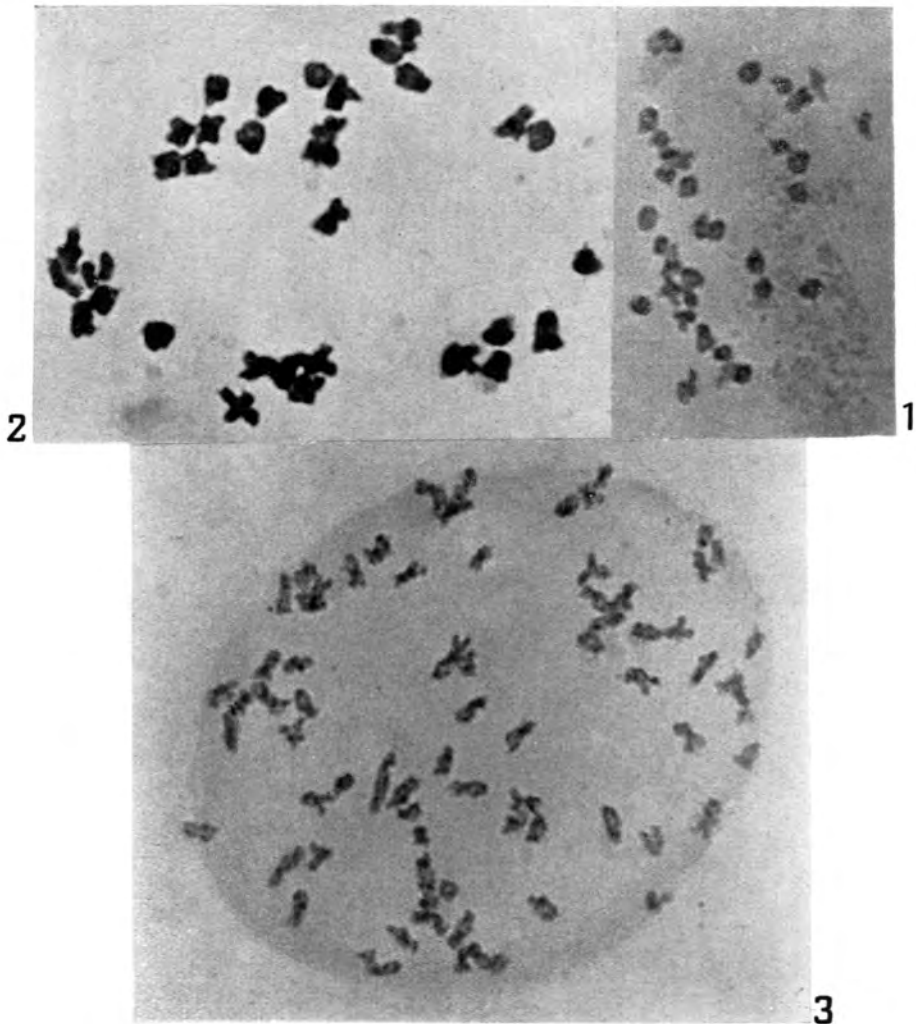
Fig. 1. A spore mother cell of *Blechnum orientale*, $n = 34$. ×1700.
Fig. 2. A spore mother cell of *Woodwardia radicans* showing $n = 34$. ×1300.
Fig. 3. 73 bivalents at meiosis in a spore mother cell of *Stenochlaena palustris*. ×1300.

within a sporangium in each case. The first two plants are diploid while the degree of ploidy in the last one cannot be ascertained until more species belonging to the genus are worked out. But in all probability this is also a diploid with compounded number.

A perusal of the literature shows that different chromosome numbers have been reported for *Blechnum* ; *B. spicant* possesses $n = 34$ and $2n = 68$ (Manton, 1950) while *B. orientale, B. cartilagineum, B. procerum* and *B. nudum* are reported to possess $n = $ ca 33, $n = 32$, $n = 56$ and $n = 28$ respectively (Manton and Sledge, 1954). Copeland (*loc. cit.*) considers the genus to be highly diversified and this is fully supported by the different base numbers $n = 28, 32, 34$ present within the genus.

Britton (1953) reported $n = 36 \pm 1$ for *Woodwardia* (*Anchistea*, Presl.) *virginica* while Wagner (1955) found $n = 35$ for the same species. In *Woodwardia* (*Lorinseria*, Presl.) *areolata* Wagner (*loc. cit.*) too reported $n = 35$. But in *Woodwardia radicans* and *W. chamissoi* the number is $n = 34$ (Manton and Sledge, 1954). Copeland (*loc. cit.*) considers *Lorinseria areolata* as a type which he separates from *Woodwardia*. The cytological evidence supports this segregation. Similarly *Anchistea* Presl. typified by *A. virginica* ($n = 35$) which has been merged in *Woodwardia* by Copeland, on the basis of cytology may deserve a generic rank if supported by other characters.

The same chromosome number $n = 34$ in *Blechnum* and *Woodwardia* (in part at least) confirms a close relationship between the two genera.

Two species of *Stenochlaena* have been investigated by Manton (1954) and Manton and Sledge (1954), in both cases with uncertain chromosome numbers. In *S. palustris* n is stated to be 70–80 with a probability of 74 and $2n = $ ca 148. The number has now been shown to be $n = 73$ for this species. In *S. tenuifolia* the report is $n = 72$—74. It is obvious that $n = 73$ for *Stenochlaena* bears no relationship with the numbers reported for the other two afore-mentioned genera.

Affinities of Stenochlaena :

Sufficient information has now accumulated on the genus *Stenochlaena*, based chiefly on the much investigated species *S. palustris*, to justify assessment of characters for deducing phylogenetic relationship of this controversial genus.

The members of the genus are all tropical climbers which possess usually pinnate dimorphic fronds with stiff texture and parallel venation. The scales borne on the rhizome are ovate-orbicular and unequally peltately attached.

The anatomy of the stem is highly complicated (Mehra and Chopra, 1951). The central vascular system is a dictyostele due to the presence of narrow and much elongated foliar gaps and perforations, both of which open in the same fashion by the departure of a basal root-trace. This main stelar system is enveloped on the outside by a peripheral double system of smaller strands which form reticulate net-works. The peripheral system owes its origin to the basal root-trace strands which on their way out leave a residuum of traces in the cortex before passing out into the roots. The leaf trace, which arises from the central system, is binary (one strand arising from each side of the gap) but is supplemented by several subsidiary strands from the peripheral system before it enters the petiole.

The anatomy of fertile pinna is studied by Holttum (1932). In the rather thick midrib are present three vascular bundles, two upper and one lower. In addition there are present two lateral ones which supply the veinlets to the lamina. Besides these a continuous vein designated as 'soral vein' runs on each side of the midrib at its junction with the lamina and depressed below the general level of veins supplying the lamina. Branches from the latter which supply the sporangia similarly lie at a lower level giving a so-called 'diplodesmic' condition.

The lamina is soriferous all over excepting for some distance from the margins all along its length. A feature of great phyletic interest which has been overlooked by Holttum is that the naked margins of the fertile pinnae are strongly reflexed downwards and inwards not only in the young state but also in the mature fronds (Text-figs. 3,4) giving the appearance of a 'false indusium'. This is very similar to what has been figured by Bower (1928) in the primitive species of *Blechnum* belonging to the section *Lomaria* which possess a poor development of the 'flange'. The 'incurved indusium' is 4–6 cells in thickness. The junction of the 'indusium' with the lamina is marked on the under-surface by the presence of 2–4 rows of somewhat thick walled, wavy, epidermal cells with brown contents
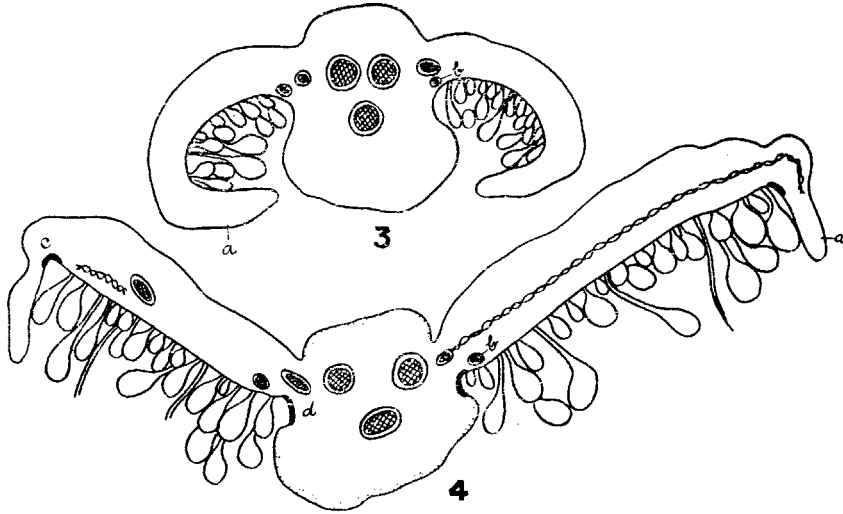
TEXT-FIGS. 3,4. T. S. of a young and mature fertile pinna, (a) incurved margin (b) soral vein (c) dark cells at the junction of the margin and the lamina (d) similar cells at the junction of the mid-rib with lamina. × 30

(Text-fig. 5). This region contrasts markedly with the rest of the epidermal surface. Similarly differentiated rows of cells are present at the junction of the midrib with the lamina. Holttum (1932) figured the margins of the fully ripe fertile pinnae reflexed outwards which may probably occur under strong conditions of dessication.

Mixed sporangia of all ages are present in the fertile region. The sporangial stalk is much elongated and three rowed. The spores are wedge-shaped and without a perisporium.

The gametophyte has been elaborately studied by Stokey and Atkinson (1952a). A filament is formed on the germination of spore. Unicellular hairs appear rather early in the history of the gametophyte and are abundantly present at its maturity. The brick-shaped meristem is apical and located within the central notch of the cordate prothallus. The sex organs are similar to those met with in the higher leptosporangiate ferns. The basic chromosome number is $n = 73$ (present report).

Ching's inclusion of the genus in Aspidiaceae is unjustified on several grounds. The spores in Aspidiaceae (senu Ching) invariably possess a perisporium which is lacking in *Stenochlaena*. We have investigated some members of Aspidiaceae anatomically and find fundamental differences in the basic plan of the stelar system. There is never a basal root-trace given off in the Aspidiaceae before the

opening of a leaf-gap. The root-traces are numerous and these arise at all levels from the meristeles enclosing a leaf-gap. Splitting of the stele through perforations such as we find in *Stenochlaena* is not manifested in Aspidiaceae. The leaf-trace is mostly formed of more than two strands. The venation in Aspidiaceae is also widely different from that in *Stenochlaena*. Furthermore, the characteristic incurving of the margin of the fertile pinnae cannot be accounted for if we were to consider the relationship of *Stenochlaena* with the Aspidiaceae. It is true that in the gametophytic generation the two resemble in the matter of profuse development of unicellular hairs but now these have been shown to be present on gametophytes of ferns of diverse origins (Stokey, 1951). Finally there is no correlation between the chromosome number $n = 41$ present in most members of the Aspidiaceae and $n = 73$ observed in *Stenochlaena*.
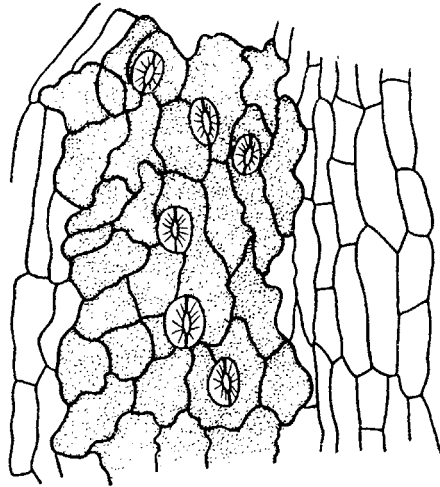
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TEXT-FIG. 5. A portion of epidermis from the under face at the region of incurving of margin showing 4 rows of thick walled cells with brown contents. × 210

Christensen (1938) and Holttum (1954) relate the genus to Pteroids placing it near *Acrostichum*. This also seems unjustified. In *Acrostichum* (as in most other Pteroids) the spores are tetrahedral in strong contrast to the wedge-shaped ones in *Stenochlaena*. Then there is a fundamental difference in the vascular system. In *Acrostichum* the stele is a dictyostele formed in a typical fashion and possesses a few *medullary strands* in the centre which do not contribute to the leaf-trace (Thomas, 1905). The position is entirely different in *Stenochlaena* where instead of medullary strands, cortical ones are present. The opening of a leaf-gap in *Acrostichum* is never accompanied by the formation of a basal root-trace. Further, the leaftrace in *Acrostichum* is built on a C-shaped type characteristic of the Pteroids but splits early into numerous ones arranged in the same pattern during its departure from the cauline stele, while it is binary in *Stenochlaena*. In *Acrostichum* characteristic peltate and lobed paraphyses are interspersed among the sporangia but in *Stenochlaena* paraphyses are absent. The gametophytic generation is also widely different in the two cases (Stokey and Atkinson, 1952a,c). As in all the other Pteroids so far investigated, the gametophyte in *Acrostichum* lacks emergences of any kind but in *Stenochlaena* unicellular hairs are present. The meristem in the gametophyte of *Acrostichum* is lateral in strong contrast to its apical position in

Stenochlaena. Finally in the strictly Pteroid members starting from the genus *Pteris* in Copeland's Pteridaceae including *Acrostichum*, the *n* number is consistently 29 or 30 (Manton and Sledge, 1954), while $n = 73$ in *Stenochlaena* bears no relationship whatsoever with this number.

Recently Alston (1956) in his paper dealing with the sub-division of the family 'Polypodiaceae' included *Stenochlaena* in the Polypodiaceae (sensu stricto) which calls for some comments. His chief arguments for this suggestion are the wedge-shaped non-perisporiate spores and peltate scales, characters which are in common with the Polypodiaceae. He, however, realises the absence of articulation of the frond with the rhizome in *Stenochlaena* which character is present in the Polypodiaceae. Some members of the Polypodiaceae in which the gametophytes have been investigated, like *Drynaria, Pseudodrynaria* (Nayar and Kachroo, 1953 Nayar, 1954) and *Pleopeltis* (unpublished observations of the authors), unicellular hairs are present on the prothalli to a varying degree of the type that are met with in *Stenochlaena* and this may further be assumed to lend support to this relationship. The chief objections to this relationship appear to us to be based on completely different general habit of *Stenochlaena*, stiff texture and parallel venation of its fronds and the marked incurving of the margin of the fertile pinnae. Again, the stelar system in advanced Polypodiaceae is a highly disintegrated schizostele in which the leaf-trace arises in a fashion entirely different from *Stenochlaena*. Several strands of the axis on the side of a leaf depart to form a leaf trace and the opening thus caused is repaired by the adjacent strands moving in. This is fundamentally different from the binary trace of *Stenochlaena* arising from the margins of the leaf-gap. Also the characteristic sclerenchyma strands so commonly present in the Polypodiaceae are absent in *Stenochlaena*.

Bower (1928) considered *Stenochlaena* to be related to the *Blechnoid* ferns. This suggestion was originally put forward by Smith and has recently been upheld by Copeland (*loc. cit.*) who places it along with *Blechnum, Woodwardia*, and a few other genera in the Blechnaceae. This relationship is well supported on the basis of cumulative evidence of characters other than the basic chromosome number which itself is shown to be variable not only within the family but even within members of the same genus. The general habit of the plant, the stiff texture of fronds and parallel venation match very well with species of *Blechnum* excepting that *Stenochlaena* is a climber. But Copeland reports that some species of *Blechnum* have scandent rhizomes. The basic pattern of the main stelar system in *Stenochlaena* is in conformity with what is described by Kachroo (1955a) for *Blechnum* and with the other members of Blechnaceae so far worked out although in no species it is complicated by accessory cortical system of strands. The incurved margin of the fertile pinnae in *Stenochlaena* is reminiscent of a similar situation in primitive member of *Blechnum* (Lomaria section) in which the development of 'flange' is rudimentary. The sporangia possess a three-rowed stalk and the spores are wedge-shaped and without a perisporium in both. The earlier stages of germination of the spore in *Blechnum* and *Stenochlaena* are alike and even the mature gametophytes have a good deal in common, particularly in the profuse development of unicellular hairs (Stokey and Atkinson, 1952a,b). These authors, however, remark that in the two species of *Blechnum, B. spicant* and *B. buchtienii* studied by them the antheridia have an elongated basal cell giving a clavate appearance to the antheridium unlike the position in *Stenochlaena palustris* where the basal cell is funnel-shaped and the antheridium globular. This situation described for the above two species of *Blechnum* may not be true for the genus as a whole for in *B. orientale* Kachroo (1955b) subsequently reported the basal cell of the antheridium to be funnel-shaped and the general form of the antheridia globular. The sum total of all these characters leads one to conclude that a real phylogenetic relation-

ship exists between *Stenochlaena* and the members of the family Blechnaceao particularly the genus *Blechnum*.

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