CYTO-TAXONOMIC STUDIES IN *ASPLENIUM AETHIOPICUM* (BURM.) BECHERER COMPLEX

by G. PANIGRAHI,* *Botanical Survey of India, Shillong*

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The genetical relationships of three cytotypes of *Asplenium aethiopicum* (Burm.) Bech. complex have been elucidated on the basis of chromosome pairing in the F_1 hybrids between them. *A. aethiopicum* agg. has been established as an old polyploid complex. A scheme suggesting the phylogenetic origin of the three cytotypes from 7 unknown diploid ancestors has been postulated.

Taxonomic history

Asplenium aethiopicum (Burm.) Bech. represents a species complex of a most natural genus, *Asplenium* L., comprising nearly 700 recognized species. In a genus so large there are of course natural groups of species, which are, however, 'remarkably undefinable' (Copeland 1947). Hooker, Sp. Fil. III, p. 93, observes, 'I have endeavoured in vain to find tangible characters for the larger, or even any group, into which the genus *Asplenium* (section Euasplenium) can be conveniently divided. Others have met with the same difficulty. Presl, who was the first to give a list of a really large number of species, has only two divisions, (1) 'frons coriacea', (2) 'frons herbacea', and nothing can be more unsatisfactory. Copeland (1947) agrees with Hooker and maintains, '... the largest natural group I have been able to recognize is characterized by texture. A great number of species have distinctly fleshy fronds ...'

The difficulties experienced by taxonomists in dividing the genus *Asplenium* into distinct groups are also met with in the treatment of the species complex *A. aethiopicum* (Burm.) Bech. Burmanii (1768) first named the species as *Trichomanes aethiopicum* Burm. from South Africa, and described it as having 'frondibus bipinnatis, pinnis alternis, pinnulis pinnatifidiincisis glabris...'. Subsequently Swartz (1788) described Jamaican plants with 'frondi 3-pinnatifida, pinnis fubcuneiformibus, pinnulis apice erofodentatis' under *A. praemorsum* Sw. and Thunberg (1800) described plants from Cape Colony under *A. furcatum* Thunb. (We could not yet trace out the

* *Present address:* Botanical Survey of India, Allahabad.

original description given by Thunberg.) In addition to these, nearly a dozen more epithets, either of specific or varietal status, have been suggested prior to 1868 and are cited by Kuhn (1868) as synonyms for *A. praemorsum* Sw., apparently unaware of the relationship of these plants with *Trichomanes aethiopicum* Burm. It is of interest to note that although Kuhn (*loc. cit.*) quotes *A. furcatum* Thunb. as one of the synonyms for *A. praemorsum* Sw., Sim (1892) described plants from South Africa under *A. furcatum* Thunb. and quotes *A. praemorsum* Sw. as a synonym. Jenman (1909) describes *A. furcatum* Thunb. from the West Indies and the Guianas and observes 'spread through nearly all the tropical countries of the globe and beyond to Australia and Cape Colony', whereas Ewart (1930, p. 54) describes plants from Australia as *A. praemorsum* Sw. and cites *A. furcatum* Thunb. as synonym. Again, *A. filare* (Forsk.) Alston refers to plants from Sudan which are morphologically very similar to plants of *A. praemorsum* Sw., except for the presence of very thick-walled cells in the scales, with smooth or much less dentate margin. Alston (1934) quotes *A. praemorsum* Sw. as a synonym for *A. filare* (Forsk.) Alston. All these taxonomic anomalies appear to be due to the fact that the differences in the expression of morphological characters of the different individuals of the species from the same or different localities show a continuous grade of variation. This accounts for the failure to detect constant sharp discontinuities which would justify the splitting of the complex into more than one species. Becherer (1935), however, recognized *T. aethiopicum* Burm. f. as having morphological resemblance to *A. praemorsum* Sw., etc., and on the authority of the rules of priority constituted *A. aethiopicum* (Burm.) Becherer and cited *T. aethiopicum* Burm. as basinym and *A. praemorsum* Sw. and *A. furcatum* Thunb. as its synonyms. Alston, in Excell (1944), recognizes Becherer's new combination and describes the relevant specimens from S. Tomé and nearby islands under *A. aethiopicum* (Burm.) Bech., which, therefore, includes not only *praemorsum*, *furcatum* and *filare*, but also all the other 14 different synonyms cited by Kuhn (1868).

The species complex is, therefore, a taxonomical tangle. Considering its very wide geographical distribution almost all over the tropical and subtropical belts of both the hemispheres, it is no wonder that it has undergone considerable genetical differentiation.

Cytotypes of Asplenium aethiopicum *complex and their morphology*

Three cytotypes were available for the present investigation, viz. a tetraploid with $n = 72$ together with an octoploid with $n = 144$ (Fig. 3*a*), came from Kenya. The octoploid form with fronds more scaly and fibrillose and spores slightly smaller than the Kenya plants was also collected from Ceylon (Manton and Sledge 1954). A few plants from Madeira proved exceedingly interesting in revealing one of the highest levels

of polyploidy recorded among the ferns, though not with the highest number of chromosomes. The stock plants from Madeira, presumed to be an octoploid with $n = 144$ (cf. Manton 1950, p. 283) turned out to be a dodecaploid with $n = 216$ (Fig. 3*b*) (Panigrahi 1962). All these plants showed regular pairing of chromosomes and formed no multivalents. They produced good spores, were sexual and bred true without any noticeable sign of segregation.

Whether or to what extent *natural hybridization* occurs within the members of the species complex is not known.

(i) *Tetraploid from Kenya*

Fronds tripinnatifid, larger and more cut throughout than the others, less coriaceous and less paleaceous (Fig. 1*a*). Fronds 12–22 cm broad, 45 cm to 75 cm long with a stipe 15 to 22 cm long. Pinnae shortly stalked, deltoid, 4 cm broad, 7–10 cm long, with woolly scales on the lower side only. Pinnules distinct, deltoid, shortly stalked, pinnately cut to the midrib into 5–7 cuneate or flabellate, 3-lobed, toothed pinnules. The stipe is fibrillose; the rachises of the pinnae and underside of the pinnae are more or less fibrillose, but the upper surface generally almost naked and shining. The lower pinnae rather shorter than those at the middle of the frond.

Scales on the base of the stipe are few and small, broad at base and slowly tapering to a point with a toothed 'midrib', orange-brown in colour (Fig. 2*a*). Not enough scales, however, have been seen due to their rarity on the only frond that was available for study; the largest of them seen is illustrated. Small fibrils (Fig. 2*d*) with a broad base and abruptly tapering apex are scattered about on rachis and costae. Annulus of sporangia are with 19–30 indurated cells; spores small, reniform, and measure 40·1μ long and 21·1μ broad (Fig. 2*g* and *j*).

The first paragraph of this description differs very little from that of *A. furcatum* var. *tripinnatum* Baker given by Sim (1892) which, therefore, has a morphological similarity with the tetraploid from Kenya. Sim, in discussing the localities for this variety, says, 'Localities have not formerly been recorded for this as separate from *A. furcatum*, but it is the more common form, and possibly some quoted for the species belong to this variety'. Then he cites the following localities:

> East—Grahamstown (Dr. Atherstone); Boschberg (MacOwan).
> Kaff—Komgha (Flanagan); Main (Mrs. Young, and through all the Amatolla forests).
> Natal—Noodsberg (Wood); Drakensberg (Dr. Rehman, 7217), Transvaal and Rhodesia, etc.

It may be seen from the foregoing that the Kenya tetraploid plant has

in all probability a considerable distribution, at least in the South African flora. In addition, a few odd plants resembling the tetraploid from Kenya

FIG. 1. Fronds from: (*a*) *Asplenium tripinnatum* (Baker) Panigr. (4*n* from Kenya × ½); (*b*) *A. aethiopicum* (Burm.) Becherer (8*n* from Kenya × 1/5); (*c* and *d*) *A. aethiopicum* (Burm.) Becherer (12*n* from Madeira × ½); (*c*) showing ontogeny of frond development; (*e*) F_1 (6*n* hybrid between 4*n* and 8*n* Kenya plants × ½); (*f*) F_1 (10*n* hybrid between 8*n* Kenya and 12*n* Madeira plants × ½).

in morphology have been collected from the Canary Islands, S. India, and Ceylon, and are at the British Museum (Natural History), London.

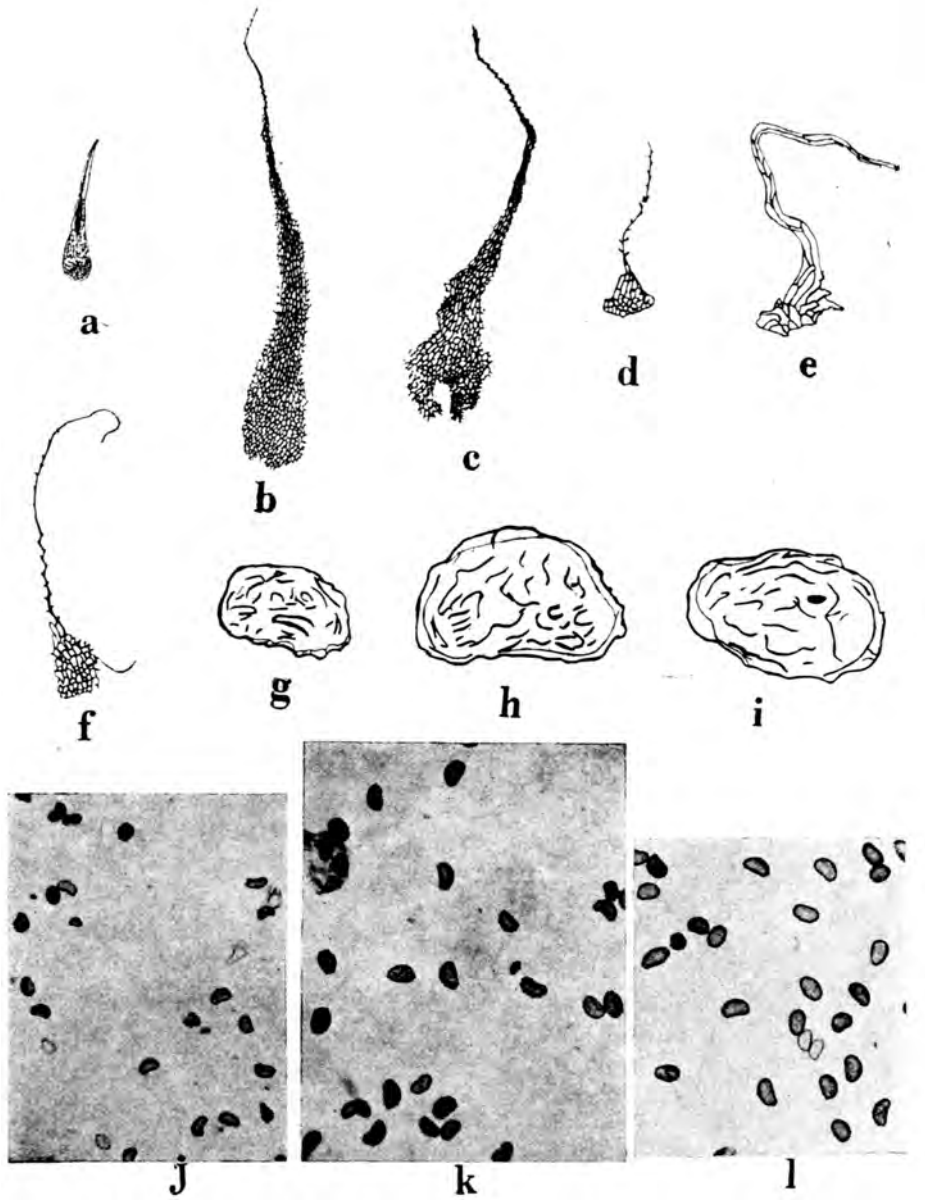

FIG. 2. (*a–c*) Scales (× 5) from: (*a*) 4*n* plant; (*b*) 8*n* plants; (*c*) 12*n* plants; (*d–f*) fibrils (× 20): (*d*) 4*n* plant; (*e*) 8*n* plants; (*f*) 12*n* plants; (*g–i*) spores (× 500): (*g*) 4*n* plants; (*h*) 12*n* plants; (*i*) 8*n* plants; (*j–l*) spores (× 66): (*j*) 4*n* plants; (*k*) 8*n* plants; (*l*) 12*n* plants.

(ii) *Octoploid from Kenya*

This is a large plant with fronds which are more or less of the same dimensions as those of the tetraploid from Kenya and are more or less tripinnatifid but with much less toothed pinnules than the tetraploid (Fig. 1*b*);

the stipe and rachis are more scaly; the pinnae are almost sessile, or slightly stalked, lanceolate and narrower than those of the tetraploid plants; the upper surface of costae of pinnae are also fibrillose; the pinnae not or hardly reduced than the one above and the subsequent ones are gradually diminished from base towards tip.

Scales on the stipe and rachis are many cells broad at the base and gradually taper to an end (Fig. 2*b*). The cells are isodiametric and dentate at the margin of the scales. The fibrils are of two types: one is similar to the fibrils of the tetraploid form, but the other type of fibril (Fig. 2*e*) is with 5-6 rows of cells at the base and gradually tapers towards the end. Cells are elongated rather than isodiametric; deep brown in colour; a transparent covering to the fibril conspicuous. Annulus of sporangia with 22–24 indurated cells; spores nearly oval, slightly wavy and measures 42·6 μ long and 29·8 μ broad (Fig. 2*i* and *k*).

(iii) *Dodecaploid from Madeira*

This has much the fleshiest fronds (Fig. 1*c* and *d*). Stipe and rachis are nearly black, abundantly set with rusty, brown and fibrillose scales, which increase in size downwards. Pinnae are set more or less thickly on both sides with scattered, brown woolly scales. The entire frond is 30–45 cm long and 7–10 cm broad; bipinnate; pinnae slightly stalked, cut nearly to the rachis into 4–6 cunneate pinnules; the basal anterior pinnule has no opposite posterior pinnule next to it; the apex of the pinnae slightly toothed and tapering to a point; never cut into sharp pointed teeth, the upper surface of the rachis grooved but the lower surface round.

Scales are broader at base, 11-12 cells wide (Fig. 2*c*); transparent covering conspicuous; cells almost isodiametric. Fibrils (Fig. 2*f*) like those of the tetraploid from Kenya. Annulus of sporangium with 22-23 indurated cells; spores kidney-shaped or oval and measure 53·5 μ long and 37·7 μ broad (Fig. 2*h* and *i*).

It appears from a rough survey of the specimens both at Kew Herbarium and the British Museum (Natural History), London, that plants resembling the dodecaploid plants in morphology are by far the commonest and are reported from Madeira, Kenya, Uganda, S. India, S. Celebes, Ceylon, Peru, Venezuela, Panama, Mexico, Guatemala and Jamaica. It cannot, however, be assumed that all these are identical with the Madeira cytotype until some have been tested cytologically.

Materials and methods

Spores of tetraploid and octoploid cytotypes from Kenya and octoploid from Ceylon and dodecaploid from Madeira were sown separately with a

view to crossing the octoploids with the tetraploid and dodecaploid respectively and also to attempt crossing the latter two with each other, if possible. From the nature of the chromosome-pairing in the hybrids produced, if any, it was intended to determine the nature of polyploidy in this species complex and also to establish the genetical relationships between the cytotypes derived from such widely distant geographical areas as Ceylon, Kenya and Madeira.

The methods followed for cytological analysis and hybridization remained essentially the same as for *Cyclosorus* (*see* Panigrahi and Manton 1958).

It may not be out of place here to record the extreme difficulties experienced in carrying out the scheme of experimental hybridization in *Asplenium aethiopicum* complex. The prothalli of all the cytotypes are slow-growing and are morphologically indistinguishable from each other, unlike those of *Aleuritopteris* (Panigrahi 1962). In addition, it is difficult to get the spermatozoids to swim even after free water has been supplied for a quarter of an hour, unlike *Cyclosorus* (cf. Panigrahi and Manton 1958). Unless the spermatozoids actively swim about, the breeder is at his wit's end to effect successful interbreeding between individuals carrying different genomes.

Experimental hybridization

Table I lists the replicates of hybridization attempted, both with positive and negative results:—

TABLE I

	No. of attempts made	No. of hybrid plants produced	Remarks
1. ♀ 4*n* Kenya × ♂ 12*n* Madeira	12	1	Sporeling grew to 2·5 cm height in 6 months. Hybridity not confirmed cytologically.
Reciprocal cross	20	×	
2. ♀ 8*n* Kenya × ♂ 12*n* Madeira	48	2	Decaploid hybrids with hybrid vigour.
Reciprocal cross	48	×	
3. ♀ 8*n* Ceylon × ♂ 12*n* Madeira		1	Sporeling shows hybrid vigour. Hybridity not confirmed cytologically.
Reciprocal cross	12	×	
4. ♀ 8*n* Kenya × ♂ 4*n* Kenya	30	1	Sporeling was a hexaploid hybrid.
Reciprocal cross	30	×	

(An octoploid plant from Ceylon was introduced into the hybridization scheme. In its morphology it is similar to the octoploid form from Kenya, although slightly more scaly and fibrillose and spores smaller.)

Hexaploid (8n Kenya × 4n Kenya) hybrid

This revealed 216 somatic chromosomes in root tip analysis and while displaying great hybrid vigour was intermediate between parental plants in

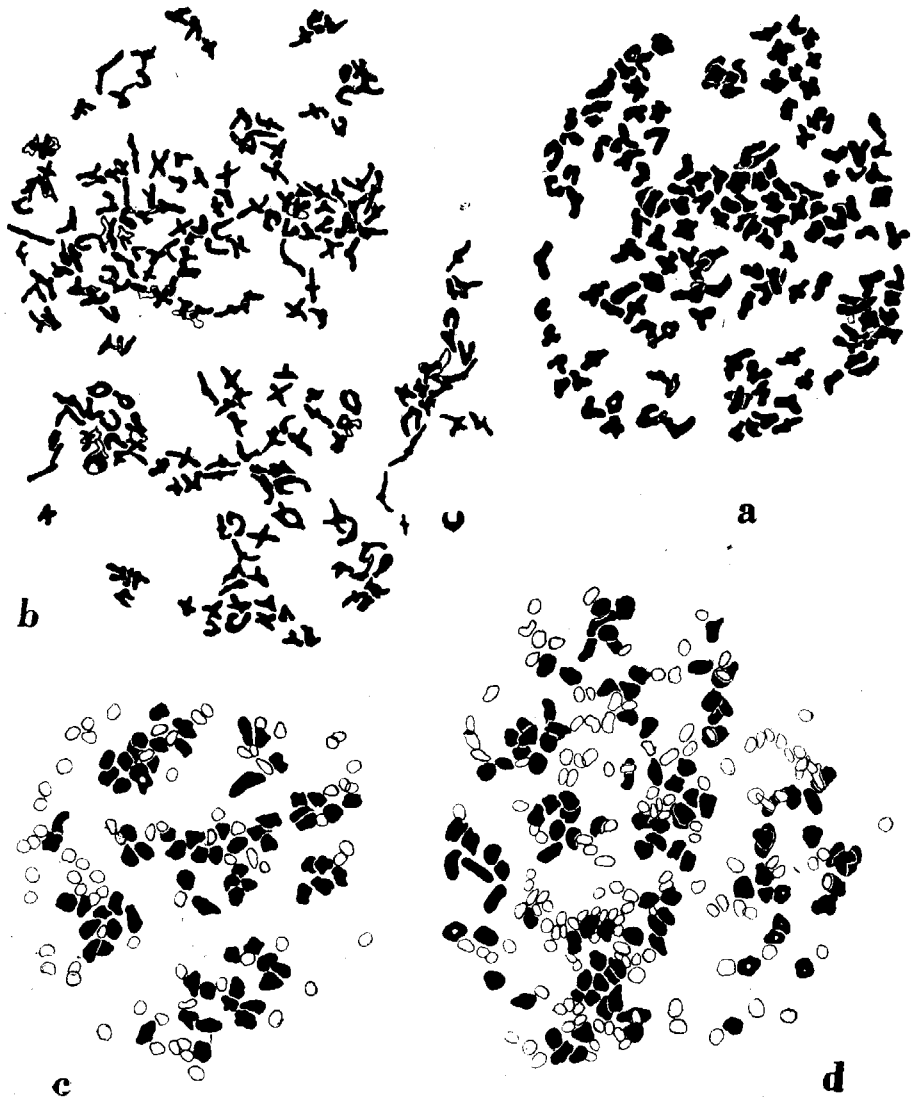

FIG. 3. Bromide prints (× 1,300) from photo-micrographs (× 1,000) of cells: (*a*) 8*n* plant from Kenya with $n = 144$; (*b*) 12*n* plant from Madeira with $n = 216$; (*c*) 6*n* F_1 hybrid between 4*n* Kenya and 8*n* Kenya showing $72^{II}+72^{I}$; (*d*) 10*n* F_1 hybrid between 8*n* Kenya and 12*n* Madeira plants showing $108^{II}+144^{I}$ (bivalents in black, univalents in outline).

its morphology. The frond is tripinnatifid (Fig. 1*e*) and the distribution of the scales and fibrils was very much like the tetraploid male parent. The

plant produced only one sporangiferous frond in 11 months from the date of crossing, was sterile and formed bad spores.

Limited cytological analysis showed 3-4 cells, each with approximately 72 bivalents and 72 univalents; no multivalents were seen (Fig. 3*c*). Absence of multivalents coupled with the presence of 72 bivalents and 72 univalents, which are exact multiples of the basic no. $x=36$ for *Asplenium*, may rule out suggestions of autopolyploid origin of the octoploid form from the tetraploid, unless the former is so old that the capacity for formation of multivalents in course of its long history has been lost. On the other hand, the meiotic pairing in the hybrid might suggest that the octoploid cytotype from Kenya is an allopolyploid to which a tetraploid form with similar morphology to that of the tetraploid cytotype from Kenya, viz. *A. furcatum* Thbg. var. *tripinnatum* Baker, is part-parental.

Decaploid (8n Kenya × 12n Madeira) hybrid

Of the two synthesized decaploid hybrids, one was transferred to Kew Gardens for successful cultivation. The hybrids were intermediate between the two parents in their morphology. They share the bipinnate frond, fleshier and thicker texture and abundant scaly and fibrillose characters of the dodecaploid male parent, but possess more deeply-cut pinnules characteristic of the octoploid female parent (Fig. 1*f*). One of the plants became sporangiferous in 12 months from the date of crossing. It is sterile and forms bad spores.

The study of meiosis in about half a dozen cells shows approximately 108 bivalents and 144 univalents; no multivalents are formed (Fig. 3*d*). Thus, a total of approximately 252 univalents represents 360 chromosomes derived from both the parents. In the absence of multivalents and also by reason of formation of a number of bivalents which is an exact multiple of the basic haploid set (viz. $x=36$), there is no evidence to suggest the autopolyploid origin for the dodecaploid cytotype from Madeira, which might represent a more complex alloploid than the types met with in *Cyclosorus* (Panigrahi and Manton 1958). (These results may be summarized in Table II.)

An analysis of the pairing in the hexaploid and decaploid hybrids and a theoretical reasoning as to how the tetraploid, octoploid and dodecaploid forms could have been produced in nature indicate that the $12n$ Madeira plant might have originated by the intercrossing of the allopolyploid derivatives of six different diploid species, as postulated in the phylogenetic scheme below.

(iv) *Discussion*

It has been shown that the tetraploid from Kenya (i.e. *Asplenium furcatum* var. *tripinnatum* Baker) forms a component in the genomic constitution of the octoploid from Kenya, though it forms sterile hybrids with it.

TABLE II

Name of species complex	Parental plants			Experimental hybrids			Parental plants		
	Ploidy	Somatic chromosome number	Meiotic behaviour of chromosomes	Ploidy	Somatic number of chromosomes	Meiotic pairing of chromosomes	Nature of polyploidy	Biological status	Taxonomic status proposed
Asplenium aethiopicum (Burm.) Bech. complex	4*n* Kenya	144	72^{II}	6*n*	216	72^{II}+72^{I} i.e. 2*n*^{II}+2*n*^{I}	Alloploid	A distinct species	A new species *A. tripinnnatum* (Baker) Panigr. sp. nov.
	8*n* Kenya	288	144^{II}				Alloploid	Do.	
	12*n* Madeira	432	216^{II}	10*n*	360	108^{II}+144^{I} i.e. 3*n*^{II}+4*n*^{I}	A complex alloploid	Do.	Both are best treated as *A. aethiopicum* (Burm.) Becherer for the time being

The taxonomic importance of this finding, together with the cytological evidences of alloploidy of both the octoploid and dodecaploid cytotypes may

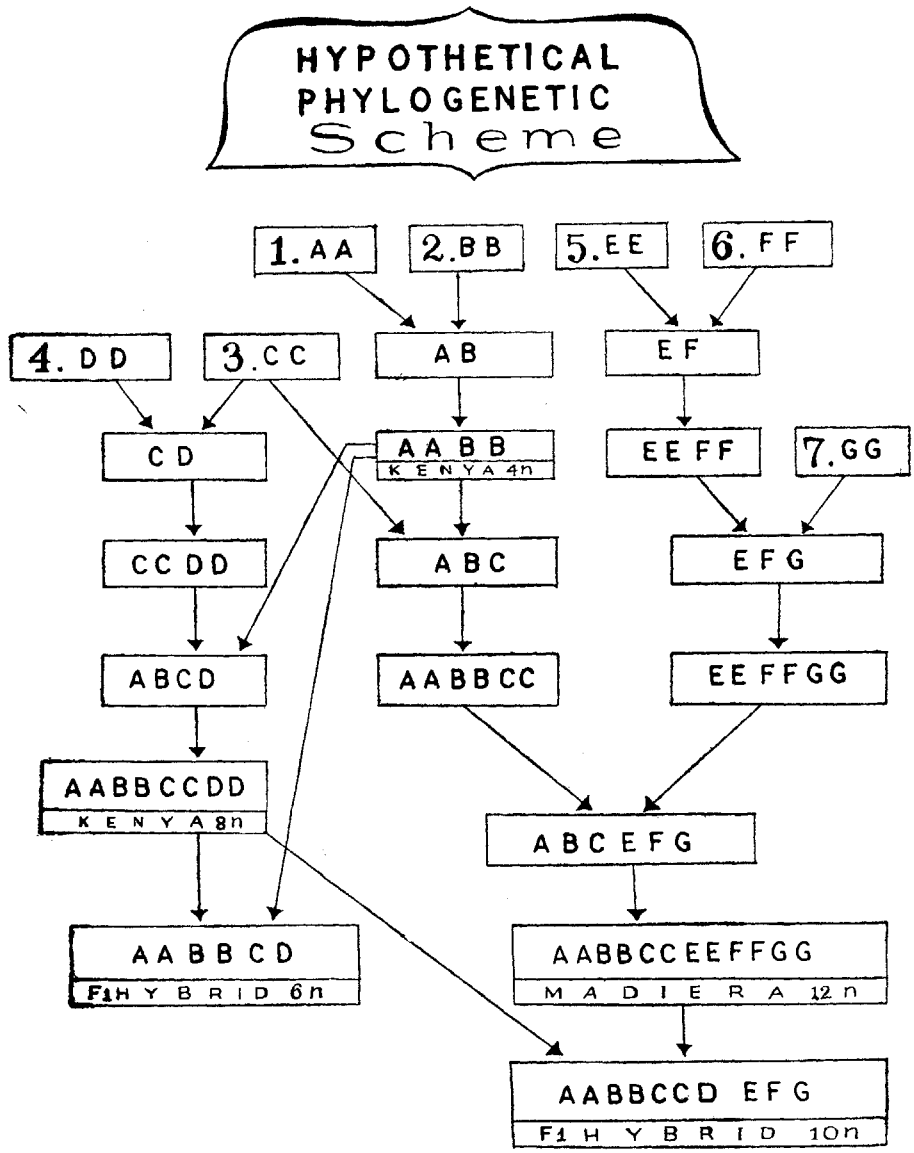

FIG. 4. Phylogenetic scheme hypothesizing cytogenetics of evolution of 4*n* Kenya, 8*n* Kenya and 12*n* Madeira cytotypes respectively.

justify the segregation of the tetraploid form resembling *A. formosum* var. *tripinnatum* Baker as a distinct taxonomic species. It is, therefore, proposed

to raise Baker's variety to a specific rank as *A. tripinnatum* (Baker) Panigr. sp. nov.*

The cytogenetic and taxonomic treatment of the octoploid from Kenya and the dodecaploid from Madeira is more complicated and the taxonomic tangle cannot be resolved satisfactorily in the present state of our knowledge. Both cytotypes are alloploids and form sterile hybrids when bred together, have distinctive morphological features and, therefore, warrant their segregation as two distinct taxonomic species. But it may be seen from the phylogenetic scheme above, that both of them are related to each other through a number of hypothetical diploids, tetraploids, hexaploids, etc., resulting in a network of inter-related forms, which makes classification extremely difficult, according to the usual concepts of the species.

This accounts for the difficulties experienced by earlier workers in maintaining all the different specific epithets proposed and explains their tendency to merge all of them as synonyms under *A. aethiopicum* (Burm.) Bech., as discussed earlier.

Even if one were tempted to split the dodecaploid Madeira plants from the octoploid Kenya plants on the basis of evidences already cited, this would necessitate a thorough examination of all the type specimens together with the original descriptions of the 16 different species and varieties quoted as synonyms for *A. praemorsum* Sw. by Kuhn (1868), together with the type specimen of *Trichomanes aethiopicum* Burm. This is a long and arduous task, which may be usefully attempted by the herbarium taxonomists.

Therefore, *A. aethiopicum* (Burm.) Bech. has not only turned out to be species complex of 'a very old genus', *Asplenium* (Copeland 1947, p. 167), but also is established as an instance of an old polyploid complex, much more advanced cytologically than *Cyclosorus*. The proposed scheme illustrates not only how this complex cytotype may have been built up in 10 successive steps of hybridization and chromosome-doubling, but might perhaps suggest the presence of similar mechanisms in other species and genera with very high grades of polyploidy in the tropics (cf. Tables 11, 12, 13, Manton 1953).

These findings, based on a few plants collected from only two areas, viz. Kenya and Madeira, although significant, are only a part of the story. A more extensive search in the wilds may bring to light the hypothetical diploids, tetraploids and hexaploids (unless these are already extinct) about the occurrence and morphological nature of which we know next to nothing. The hybrids between the octoploid from Ceylon and the dodecaploid from

* The citation is as follows:—
ASPLENIUM TRIPINNATUM (Baker) Panigr. Basinym: *A. furcatum* Thunb. var. *tripinnatum* Baker in Hooker, W. J. and Baker, J. G. (1865–68), *Syn. Fil.*, p. 487, Hardwicke, London and in T. R. Sim (1892). The Ferns of South Africa (item 83). Cape Town; Aberdeen (printed).

Madeira and between the latter and the tetraploid from Kenya and also between the two octoploids from Ceylon and Kenya, if and when produced, might throw a good deal of light on their inter-relationships, and would also show how octoploid from Ceylon and octoploid from Kenya are genetically related. These studies, to which one may look forward, may in the long run help taxonomists by supplying suitable criteria with which to view the species complex in its proper perspective.

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