BOX 14

HYBRIDIZATION AND SPECIATION

IN THE

FERNS.

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Bot. Hons.

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INTRODUCTION.

Since the time of the early Greeks, philosophers and later botanists have wondered how the present organisation in nature could have arisen. The paradox of all plants being in groups, yet every individual being different from all other individuals was inexplicable, untill Darwin and Mendel provided mechanistic explanations.

One aspect of this problem is the origin of species. During the last three centuries several mechanisms were proposed. With the acceptance of the population concept, and the understanding of a species as a group of populations which are joined by gene flow patterns, speciation has been appreciated as being the result of an interruption of these flow patterns.

For flowering plants, the following isolating mechanisms have been found:

1) Spatial isolation
2) Physiological isolation
   - A. Isolation between parents
     - a) Ecogeograhical isolation
     - b) Temporal or seasonal isolation
     - c) Mechanical isolation
     - d) Prevention of fertilization
   - B. Isolation between hybrids
     - a) Hybrid weakness
     - b) Hybrid sterility

(Adapted from Stebbins, 1950)

Theoretically, in ferns all these isolating mechanisms should be functional. But it has been found that most fern species are isolated by the failure of fertilization; and if fertilization does occur, the hybrids formed are sterile.

In this seminar, I shall discus the consequences of a breakdown in the isola ting mechanisms, with the resultant formation of hybrids in the ferns.

HYBRIDIZATION:

Hybridization occurs frequently among plants, even though it is rare among animals. (Mayr, 1963) Hybrids can be variously defined - technically any crossfertilised seedling is a hybrid. I shall, however, restrict its use to define crosses between taxonomically differentiated populations. A classification of hybrids is given below:

A. Fertile hybrids.

1) Introgression swarms

2) Reproductively isolated populations, giving rise to new species

B. Sterile hybrids.

1) Non - reproducing individuals

2) Asexually reproducing individuals

3) Sexually reproducing individuals, after chromosome doubling

Except for Pteris quadriaurita and Pteris multiaurita (both from Ceylon) there are no known fertile hybrids in the ferns. (Wagner, 1965) Among flowering plants, fertile hybrids are common. The reasons for this pattern can be found in the reproductive strategies of these two groups - in the flowering plants, fertilization is controlled by a complex and highly evolved system, centred around the flower. This system has sufficient variables (temporal spacing, pollinating agents, mechanical isolation) which can be used for reproductive isolation, so that the selective value of chromosomal isolation is reduced. But ferns have to rely heavily upon chromosomal isolation to maintain species as distinct entities.

POLYPLOIDY:

1) THEORETICAL CONSIDERATIONS:

"Polyploidy is now widely recognised as one of the principle methods for the formation of new species among the higher plants" (Stebbins, 1950) This

applies to an even larger extent to the ferns, (Manton, 1950) where the number of sterile hybrids is estimated at 5 to 10% of all known species. (Wagner, 1965)

Diploid hybrids with chromosome sterility can grow well, but during meiosis the haploid sets are so differentiated that no homologous pairs are formed. If not all the chromosomes can pair off into bivalents, the resultant spores will be sterile. With polyploidy, the chromosomes double. As a result each chromosome has an exact homologue, and meiosis can proceed normally.

Polyploidy has been classified into two main types: (a) autopolyploidy (parents are from the same species, so that the autopolyploid genome has four homologous chromosomes, and forms quadrivalents at meiosis), and (b) allopolyploids (parents belong to different species, so that the allopolyploid genome forms bivalents at meiosis). Even though species are real biological units, a continuous grade exists from pure alloploidy to pure autoploidy. Thedegree of differentiation between genomes can vary from nothing to complete homology. Stebbins (1950) recognised these difficulties, and postulated the following scheme:

(a) Autopolyploids - offspring from plants from the same or genetically closely related populations.

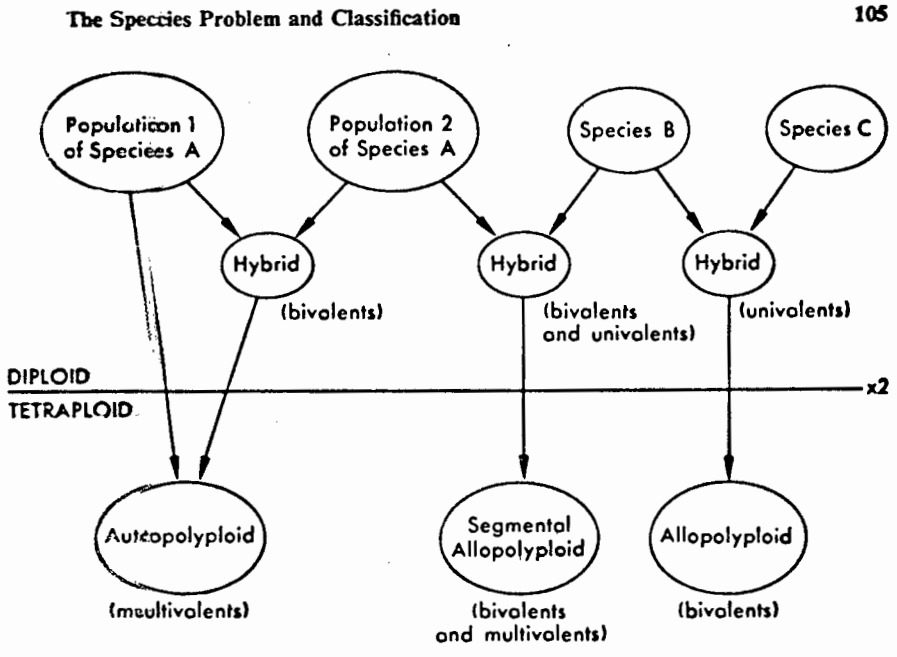
(b) Segmental allopolyploids - these contain two genomes that have a considerable number of chromosome segments or even chromosomes in common, but they are sterile at the diploid level.

(c) True allopolyploids - in this type the two genomes are completely different, and the allopolyploid is sterile to its parents.

(d) Autoallopolyploid - this can only occur above the tetraploid level, and describes the chromosome doubling of an allopolyploid.

Clausen, Keck amd Hiesey proposed the term amphidiploid to cover all types of polyploids which have arisen after hybridization between two or more diploid species separated by hybrid sterility barriers.

FIGURE 1: Diagram showing types of polyploidy. (From Solbrig, 1970)



2) METHODS OF STUDYING HYBRIDS:

Determining whether a taxon is of hybrid origin, and determing its parentage, is usually inductive and speculative. Methods for determing hybrids fall into three wide categories: (Wagner& Chen, 1965; Stebbins, 1971)

(a) Morphological studies, dterminig the intermediacy of the characters.

(b) The viability and efficiency of the reproductive system.

(c) Artificial hybridization.

Morphological studies usually indicate the presence of hybrids. Hybrids tend to be exactly intermediate between the parents, thus reducing the two extremes. This rule has high predictive value, examples exist where one of the parents has been predicted on the morphological characters of one parent and the hybrid (Wagner, 1968). Crosses between tertraploids and diploids result in the hybrids being closer to the tetraploids. In backcrosses, the backcross is more similar to the hybrid, than to the parent (stebbins, 1971). This becomes clear when one regards the genome situation: the degree of morphological resemblance dpends on the amount of genetic material inherited. American pteridologists rely heavily on the morphological characters in elucidating

hybrid complexes. (Wagner, 1965)

Studies in the reproductive system can be conducted at various levels:

(a) Phenetically: the absence of sporangia, or the deformation of the pollen often indicate sterility in terms of aborted spores.

(b) Cytogenetically, the behaviour of the genome at meiosis shows sterility or the level of ploidy, and the degree of chromosome homology.

Phenetic changes in the reproductive organs are indicative of hybrid sterility. These changes are ultimately due to genetic aberrations. The spores do not develope normally, and often abort early. The size variation of the spores increases drastically. If sufficient spores abort, the sporangia and even the sori can abort.

Cytogenetic studies (made popular by Manton, 1950) are much more laborious, but they can provide a large amount of information. If the chromosome pairs are not homologous, bivalent formation during meiosis will not proceed normally. As a result, the chromosomes are not normally distributed to the spores, resulting in sterile and aborted spores. In fertile polyploids, patterns revealed during pairing at meiosis is a rich source of information on the relationships between the constituent genomes of the hybrid. These studies are usually combined with hybridization programmes.

Hybridization programmes can serve two purposes: they can either be aimed at remaking the hybrid, or they can be means of studying the degree of chromosome homology between the species involved in the hybrid complex. It is technically difficult to synthesize a hybrid, as the gametophytes are nearly all hermaphrodite. (Klekowski et al. 1968) It is, however, the best way of analysing hybrid complexes.

Manton et al (1967, 1970a, 1970b) used these methods to analyse the Adiantum caudatum complex in India and Africa. The interrelationships which they found are shown in figure 2.

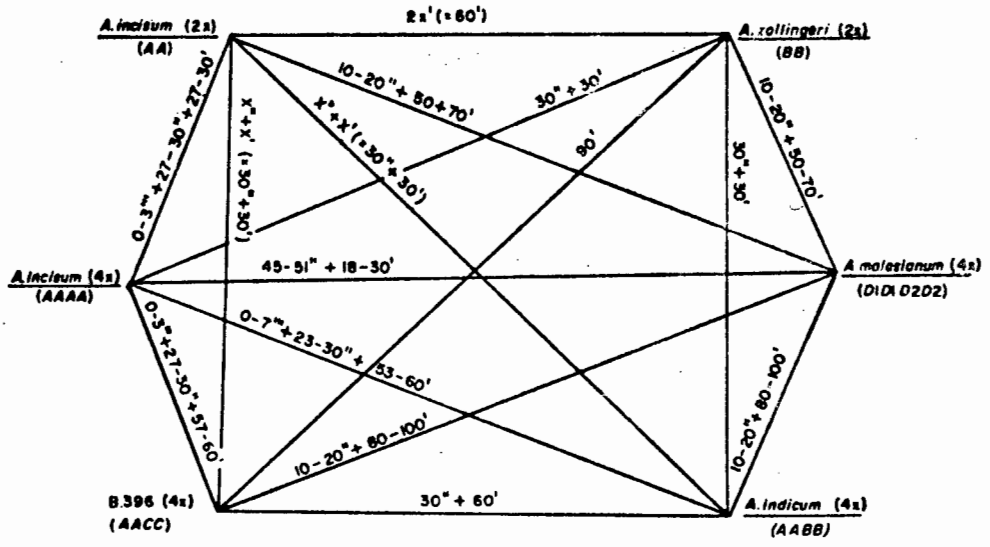


FIGURE 2 Hybridization polygon summarizing the cytology and genome analyses resulting from the completed hybridization programme discussed in this and the two preceding papers.

Evidence for the postulated relationships is as follows:

Crosses between A. incisum (4x) and A. zollingeri (2x) show 30 bivalents and some trivalents. These trivalents are significant, as they reveal autosyndetic pairing. Morphologically, the African tetrevalent A. incisum and the Indian divalent A. incisum are very alike. These two facts indicate that the African A. incisum is probably of autopolyploid origin. The absence of more trivalents can be attributed to a genetic factor which suppresses multivalence.

The allopolyploid origin of A. indicum Ghatak is indicated by the crosses listed in Table 1. The chromosome patterns at meiosis show the A. incisum Forsk and A. zollingeri Mett. ex Kuhn are the likely parents. The triploid hybrids (crosses 4, 5 and 6 in Table 1), which are with the putative parents, all have 30 bivlents and 30 univalents. This shows that of the three genomes involved two are homologous. As other crosses have shown that A. indicum does not have an extensive capasity for autosyndesis, the only explanation is that one homologous genome must be supplied by a parent. The parents, when crossed, give 60 univalents (crosses 1 and 2) and very few hybrids (Table 2). A. indicum must

Table 1. *Summary of cytological observations*

	Hybrids investigated	Cells analysed	Nature of pairing	Investigated by
Diploid hybrids				
(1) *A. incisum* 2x ♀ × *A. zollingeri* 2x ♂ (Patna)	2	39	60 univalents	Sinha
		11	1 bivalent + 58 univalents	Sinha
(2) *A. zollingeri* 2x ♀ × *A. incisum* 2x ♂ (W. Bengal)	1	25	60 univalents	Sinha
(3) *A. incisum* 2x ♀ × *A. incisum* 2x ♂ (Patna) (W. Bengal)	2	25	30 bivalents	Sinha
Reciprocal	1	10	30 bivalents	Sinha
Triploid hybrids				
(4) *A. zollingeri* 2x ♀ × *A. indicum* 4x ♂	4	10	30 bivalents + 30 univalents	Ghatak
Reciprocal	6	15	30 bivalents + 30 univalents	Ghatak
(5) *A. incisum* 2x ♀ × *A. indicum* 4x ♂ (Patna)	3	25	30 bivalents + 30 univalents	Sinha
Reciprocal	5	30	30 bivalents + 30 univalents	Sinha
(6) *A. incisum* 2x ♀ × *A. indicum* 4x ♂ (W. Bengal)	1	20	30 bivalents + 30 univalents	Sinha
Reciprocal	1	10	30 bivalents + 30 univalents	Sinha
Tetraploid hybrids				
(7) *A. malesianum* 4x ♀ × *A. indicum* 4x ♂ (Malaya)	1	3	Mainly univalents with a few bivalents (not exceeding 15)	Manton
(8) *A. malesianum* 4x ♀ × *A. indicum* 4x ♂ (S. China)	3	19	mainly univalents + a few pairs (not exceeding 15)	Sinha
Reciprocal	2	10	Same pairing as above	Sinha

Table 2 *Numbers of prothalli etc. involved in the hybridization programme*

	Prothalli used	Selfs	Hybrids	Hybridizer
Diploid hybrids				
(1) *A. incisum* 2x ♀ × *A. zollingeri* 2x ♂ (Patna)	132	3	5	Sinha
Reciprocal	12	0	0	Sinha
(2) *A. zollingeri* 2x ♀ × *A. incisum* 2x ♂ (W. Bengal)	–	–	1	S. K. Roy
(3) *A. incisum* 2x ♀ × *A. incisum* 2x ♂ (Patna) (W. Bengal)	13	0	3	Sinha
Reciprocal	16	0	4	Sinha
Triploid hybrids				
(4) *A. zollingeri* 2x ♀ × *A. indicum* 4x ♂	88	–	4	Ghatak
Reciprocal	111	–	12	Ghatak
(5) *A. incisum* 2x ♀ × *A. indicum* 4x ♂ (Patna)	65	0	5	Sinha
Reciprocal	54	0	6	Sinha
(6) *A. incisum* 2x ♀ × *A. indicum* 4x ♂ (W. Bengal)	8	0	2	Sinha
Reciprocal	–	–	2	S. K. Roy
Tetraploid hybrids				
(7) *A. malesianum* 4x ♀ × *A. indicum* 4x ♂ (Malaya)	–	–	1	Ghatak
(8) *A. malesianum* 4x ♀ × *A. indicum* 4x ♂ (S. China)	175	24	3	Sinha
Reciprocal	42	0	11	Sinha

then be an allopolyploid.

A. malesianum Ghatak illustrates the concept of segmental polyploidy. In crosses with all other species and collections, the hybrid shows 10 to 20 bivalents at meiosis. The hybrids between A. malesianum and A. incisum 4x show 40 to 51 bivalents. As A. incisum is an auto allopolyploid, 30 of those pairs will be autosyndetic A.incisum chromosomes. The remaining 10 to 21 bivalents must then be autosyndetic A. malesianum chromosomes. A. malesianum then has no genetic homology to the A. caudatum complex, but is itself of segmental allopolyploid origin. Parts of its two genomes are homologous, and can form bivalents but other parts cannot. This is confirmed by a cross between two strains of A. malesianum. Each strain behaves like a diploid, but the hybrid showed a few quadrivalents. So there must be multiple homologies within A. malesianum. Local populations probably developed genetic factors to prevent homeoelogical pairing, thus only allowing pairing between true homologues. Such factors have been found in wheat. (Barber, 1970)

Table 3. Range of bivalents in tetraploid hybrids involving *A. meisenum* from two sources (Malaya and South China) crossed with *A. incisum* Forsk. sens. str. from five African localities

Parents of the cross	Number of cells with														
	Less than 40″	40″	41″	42″	43″	44″	45″	46″	47″	48″	49″	50″	51″	More than 51″	Total
(1) Ghana (Shai Hills) × Malaya	—	2	2	—	—	—	—	—	—	—	1	1	—	—	6
(2) Ghana (Shai Hills) × S. China	—	1	—	—	1	—	—	—	—	—	—	1	1	—	4
(3) Ghana (Krobo Hill) × S. China	—	3	1		—	1	1	—	1	—	—	1	—	—	13
(4) Sudan × Malaya	—	5	1	—	—	2	3	—	1	—	—	—	—	—	12
(5) Sudan × S. China	—	—	—	—	—	—	—	1	2	2	1	2	1	—	9
(6) Transvaal × S. China	—	—	1	—	1	1	—	—	—	—	—	1	—	—	4
(7) S.W. Africa × S. China	—	2	1	—	—	—	1	—	—	—	2	—	1	—	7
Total	—	13	6		2	4	5	1	4	2	4	6	3	—	55

Table 4. Distribution of bivalents in triploid hybrids involving *A. malesianum*

	Number of cells with bivalents:													
	Below 10	10	11	12	13	14	15	16	17	18	19	20	Above 20	Total
A. malesianum × *A. incisum* 2x	1	1	2	3	3	4	13	5	6	11	1	3	—	53
A. malesianum × *A. zollingeri*	1	1	1	4	2	3	10	3	6	5	—	2	—	37
Total	2	2	3	7	5	7	23	8	12	17	1	5	—	90

Braithwaite (1972) used the same methods to analyse the Asplenium splendens complex in South Africa. His results are given in Table 5. From this he postulated the follwing relationships:

A. splendens ssp. splendens (n=72)

→ A. multiforme (n=144)

A. splendens ssp. drakensbergensis (n=72)

A. multiforme is designated as an segmental allopolyploid. Yet the parental genomes are homologous. It is probably an intermediate case, being between a pure autopolyploid and a segmental allopolyploid.

TABLE 5

Details of hybridisation experiments involving *A. splendens*, *A. multiforme* and tetraploid *A. aethiopicum*.

	No. of prothalli	*No. of hybrids*	*%age success*	*Chrom. pairing at meiosis*
Tetraploid hybrids				
1) ♀ A. splendens subsp. splendens X ♂ A. aethiopicum 4x. Reciprocal hybrid	50, 20 } 70	1, 7 } 8	11%	Virtually no pairing. 144 univalents
2) ♀ A. aethiopicum 4x X ♂ A. splendens subsp. drakensbergense.	48	0	0%	- - - -
3) ♀ A. splendens subsp. drakensbergense. X ♂ A. splendens subsp. splendens. Reciprocal hybrid	16, 32 } 40	13, 17 } 30	75%	Complete pairing. 72 bivalents.
Hexaploid hybrids				
4) ♀ A. multiforme X ♂ A. splendens subsp. splendens.	8	5	63%	71-72 paired groups (trivalent & bivalent) + 48-54 univalents.
5) ♀ A. multiforme X ♂ A. splendens subsp. drakensbergense.	22	12	55%	70-72 paired groups (trivalent & bivalent) + 45-51 univalents.

A chromatographical method for analysing hybrid complexes was developed by Smith and Levin. (1963) They applied it to the Appalachian Asplenium complex. The complex relationships between all the taxa were analysed between 1935 and 1955. This is given in Table 6. Two dimensional chromatograms were run with extracts prepared by powdering a dried frond and soaking the material in 2ml of absolute methanol. The results for three of the taxa are shown in Figure 3.

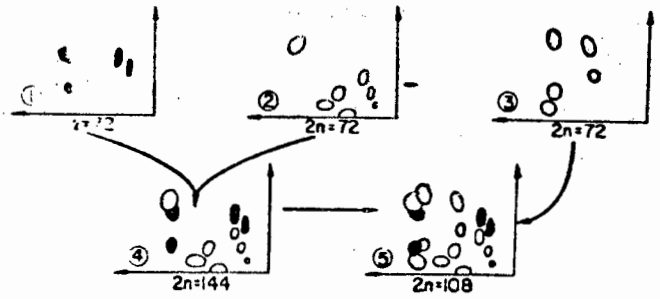


Fig. 3 Diagrammatic representations of two-dimensional chromatograms of two species of *Asplenium* and their hybrids, showing the complementation of flavonoids referred to in the text. (a) *A. rhizophyllum*; (b) *A. montanum*; (c) *A. platyneuron*; (d) bigenomic allopolyploid (*A. rhizophyllum* × *A. montanum*); (e) trigenomic allopolyploid, *A.* × *kentuckiense* (*A. rhizophyllum* × *A. montanum* × *A. platyneuron*). (After SMITH and LEVIN, 1963 *Amer. J. Bot.*, 50, 952–958.)

Table 6: Relationships between the Appalachian Aspleniaceae.

Taxon	Relationship	n
A. montanum Willd.	ancestral diploid	72
A. platyneuron Oakes	ancestral diploid	72
A. rhizophyllum L.	ancestral diploid	72
A. ebenoides Scott	A. platyneuron x rhizophyllum	72
A. ebenoides Scott	A. platyneuron x rhizophyllum	144
A. bradleyii Eaton	A. montanum x platyneuron	144
A. pinnatifidum Nutt.	A. montanum x rhizophyllum	144
A. x kentuckiense McCoy	A. platyneuron x pinnatifidum	108
A. xgravesii Maxon	A. bradleyii x pimnatifidum	144
A. x trudelii Wherry	A.montanum x pinnatifidum	108
A. x wherryi Smith	A. bradleyi x montanum	108

These results clearly show the hybrid patterns. This provides a much quicker way than cytological analyses of unravelling hybrid patterns. But it does not always work, as parental forms sometimes have the same chemical patterns Herbarium material can also be used in these studies. This technique should

be studied more critically, and be used more.

3. PATTERNS IN POLYPLOID COMPLEXES:

There are some definite patterns discernable in polyploid complexes. These can be roughly separated into two categories: (a) the irreversibility of polyploidy and (b) climatic-geographic correlations in the distribution of polyploidy.

Although De Wet (1972) observed some cases pf polyhaploidy, polyploidy is in general irreversible. (Stebbins, 1971; 1975) This irreversibility has two main aspects - the extinction of the lower ploidy levels, and the gradual `diploidization` of the amphidiploids.

The extinction of the lower ploidy levels was already noticed by Manton in 1950. It is very obvious among ferns, where the basic chromosome number (n) is around 30. The original chromosome number of all plants was probably less than ten. (Stebbins, 1971) Grant postulated that the main groups of recent pteridophytes are in fact the remnants of geologically ancient polyploid complexes.

The `diploidization' of old polyploids is, as we shall see below, related to the extinction of lower ploidy levels. This process, by which polyploids gradually become like diploids in appearance and behaviour, was noticed by Manton (1967) and Stebbins (1971).

The other basic pattern - a correlation between the level of ploidy and the past climatic-geographic history of the area, is basic to the explanation of the first pattern. This correlation was very coherently argued by Wagner (1968). The occurence of high levels of polyploidy are correlated to the degree of disturbance, and the date of that disturbance. It supercedes the old correlation of ploidy with severe climate regimes, which was proposed before the tropical ferns had been investigated cytologically. Manton (1950) and Bobrov (1973) presented evidence that the glaciations of Europe in the Pleistocene have triggered the still active processes of polyploidization (ferns) and introgression (Pinaceae), as compared with the more stable situation in the tropics. Stebbins

(1971) showed that areas recently disturbed by man were colonated by higher ploidy level plants. If this theory is correct, it follows that the tropics must have been disturbed some time before the Pleistocene.

To understand these patterns, we have to look at the effects of chromosome doubling.

Higher levels of ploidy have a slower rate of change or mutation of the genome. (Manton, 1950; Stebbins, 1971) This is expected, as a single mutation in the vast number of gene loci in a hexaploid will have much less chance of being expressed, than one in a diploid. This then reduces the actual range of variation, and selection can only work directly on the dominant genes which are expressed. As a result, the rate of evolution of polyploids is slower than that of diploids. On this basis, Stebbins (1971) claimed that polyploids in general were conservative. He ignored the effects of polyploidy on the heterozygosity of the genome. Barber (1970) showed that tetraploids retain their heterozygosity 3,8 times as long as diploids when they are repeatedly selved. This is due to tetrasomic gene loci. Amphidiploids being permanent hybrids for related genes on two combined genomes, an increase genetic heterozygosity is obvious. A third factor, also postulated by Barber (1970), complicates the issue even more. He formed the concept of 'biochemical diversity'. He found that hybrids did not only have all the enzymes found in the parents, but that some new ones also appeared. These he postulated to be due to combinations of parental enzymes. He used haemoglobin as an example: it could be the result of a hybrid, one parent contributing the α chain, and the other the β chain.

From this data, it is clear that Stebbins claim that polyploids are conservative, is a bit oversimplified. Phenetically it might well be the case. But where diploids can only adapt to fairly extreme situations by mutations, polyploids have a store of unexpressed genes, some of which might well be more suited for the new situation. On this basis one could s w that they can adapt more rapidly to new niches and habitats. This is then what is observed in the

correlation btween level of ploidy and degree of past disturbance.

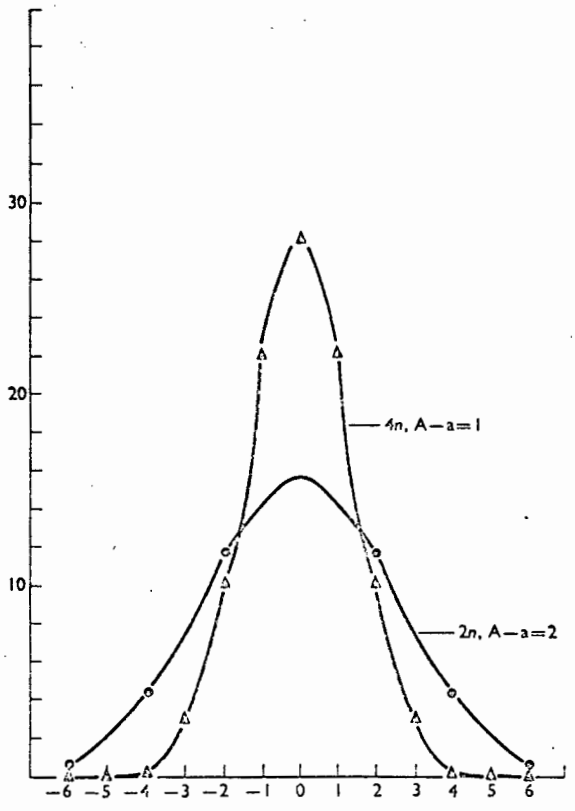


Fig. 4 Diagram showing the reduction in variation which would be expected in an F_2 progeny segregating for a quantitative character at the tetraploid level as compared to the diploid level. Further explanation in the text. (From Stebbins.[210])

Raw polyploids have often been claimed to have a low fertility (Stebbins, 1971). Experimental doubling of the chromosomes resutts in sterility after three to four doublings. Yet polyploids appear to be as fertile as the diploids, and some ferns have somatic chromosome numbers of over 500.

The initial low fertility is attributed to physiological disruptions due to multisomic gene loci. This problem is especially acute if some of the chromosomes are slightly homologous. As a consequence, autopolyploids often haveaberrent meiosis, which results is aborted spores. To correct this, the chromosome pairs appear to diverge - that is, if two bivalents are very similar, so that accasionally a quadrivalent is formed, they will tend to diverge. There is thus a change from tetrasomic gene loci to disomic gene loci. The genome starts behaving like a diploid genome, and acquires its characteristics. This restores its fertility, and allowes chromosome doubling to occur again.

APOSPORY:

Some 80 species of genera such as Adiantum, Asplenium, Cheilanthes, Dryopteris and Pteris have a nonsexual or apogamous type of life cycle. (Wagner, 1965) Apospory occurs when the egg in the gametophyte germinates and forms a normal sporophyte, without having been fertilized. The sporophyte can also be formed from buds on the gametophyte. This means that the gametophyte, sporophyte and the spores have the same chromosome number. This can be achieved by one of two mechanisms:

(a) There is an automatic doublingof the chromosomes in the spore mother cells just before meiosis. Meiosis then produces a diploid set of chromosomes. This is the normal process of apospory. (Wagner et al, 1965)

(b) Diploid gametophytes can be formed from unreduced spore mother cells. This process was postulated in 1967 by Morzenti to explain the existence of the fern Asplenium plenum E.P. St. John.

A. plenum is probably a hybrid between A. abscissum Willd. (2x, sexual species) and A. curtissii Underw. (sterile 3x hybrid). A. curtissii is the hybrid between A.verecundum Chapm. (4x) and A. abscissum.

Meiotic behaviour of A. plenum shows 10 to 20 univalents out of the 144 chromosomes. This indicates a genome of AAB_1B_2, where AA can all pair, but only some of B_1B_2 can pair. A. curtissii will be AB_1B_2 (A from A. abscissum, B_1B_2 from A. verecundum). A cross between A. abscissum and a diploid gamete from A. curtissii would produce an AAB_1B_2 genome.

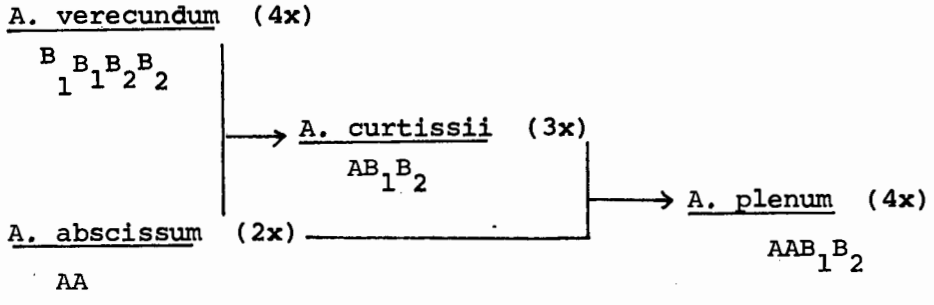


FIGURE 5: Relationships of Asplenium plenum E.P. St John

Morzenti did find unreduced spores in A. curtissii. These germinated to form fertile gametophytes, which can give rise to new A. curtissii plants by apospory. They also produced viable sperm, which could affect the back-cross with A. abscissum.

This is thus a method by which sterile hybrids can perpetuate themselves as well as establish other hybrids. But it is probably very rare, and only a headache to taxonomists, and not to evolutionists.

HYBRIDIZATION, ECOLOGY, SPECIATION AND EVOLUTION:

The concepts of evolution and speciation are, although related, not identical.

Evolution can be understood on two levels: (a) On the teleological level it implies a directional developement from a primitive to an advanced form. (b) On the empirical level , it means the formation of something out of something else, ou of an existing thing. We usually think of evolution in the teleological sense, and that is how I shall apply the term here.

Speciation is the formation of a species. By a species I mean an evolutionary unit, a collection of plants or populations which at this stage in time normally influence one another's genome changes.

From this it is already clear that hybridization will have an influence upon the process of speciation. But in the ferns, because of the absence of introgression, the original parental species are not at all affected by hybridization. The sole effect of hybridization is the establishment of new species. We have seen how this is possible, and that it actually happens. The extent to which new species are formed by hybridization, and made capable of sexual reproduction by polyploidy, has been debated. As we have seen, polyploidy is virtually the norm among the ferns. But to what extent is this autopolyploidy? Manton (1950) claimed that autopolyploidy is insignificant. Wagner (1964) would have it that it is probably responsible for most of the higher basic numbers. This is a controversy which we cannot resolve with the infor-

mation at our disposal.

What is the role of hybridization in the formation of present day biotas? This is best revealed by the role which present day hybrids play in an ecosystem.

Hybridization between two specialised species will produce a plant which is intermediate between the two extremes. It will have lost most of the specialisations of the two parents, and be more similar to the ancestral type of that group.

This is revealed by the ecological requirements of the parents and the hybrids. Because each species is highly adapted to its ecological niche, a hybrid will not have a niche, and will consequently not survive, (Anderson, 1949) But if an area had been disturbed, the hybrid will not have much competition, and as it is likely to have more general characteristics, it is more likely to survive there than the specialised parental species. If chromosome doubling takes place, that hybrid might become part of the biota of that once disturbed area. It will then act as a normal species.

Information about distribution changes can also be abstracted from a knowledge of relationships and present distributions. For hybridisation to occur, the parents have to be sympatric. If they are now allopatric, a knowledge of the ecological requirements of the parents could reveal past changes in the climate.

The Adiantum caudatum complex shows how these theories can be applied. The distribution of the group is given in fiure 6. The fact that only the tetraploid version of A. incisum is found in Africa, can lead to two postulations.

(a) A. incisum is originally from India, and the tetraploid happened to be preadapted for conditions in Africa.

(b) A. incisum originated in Africa, Because of its long stay in Africa, polyploidy could occur. It has not been in India long enough for polyploidization to have taken place.

This argument cannot as yet be definitely be settled, but the weedy nature

of raw polyploids supports the first hypothesis.

FIGURE 6 Map of the known distribution of genomes involved in the investigation recorded here and in the two preceding papers. For reasons of space only the gametic analysis is provided, a single letter denoting a diploid and two letters a tetraploid. The approximate northern limit of *A. malesianum* in China is indicated by the thick broken line (for further details see Fig. 1). *A*, The genome of *A. incisum*, both 2x and 4x; *B*, the genome of *A. zollingeri*; *AB*, the genome of *A. indicum*; *AC*, the genome of "B396"; *D1D2* = the genome of *A. malesianum*.

From the above it becomes clear that hybridization (with polyploidy) probably acts as a speciation mechanism for the formation of new species for open niches. Once these species have been established, they are gradually diploidised, and so are turned into respectable diploids that can repeat the performance.

HYBRIDIZATION AND TAXONOMY:

Hybridization between taxa raises questions as to the validity of those taxa. And reproductive isolation barriers within species would indicate that the taxon includes several species.

These criteria, if applied dogmatically, would cause chaos. Degree of fertility cannot be taken as an absolute measure of the affinity between groups. Affinity is a function of all the characters derived from the genome.

Nor can isolation barriers within species be regarded as proof of a too wide species delimitation - many perfectly valid species have sections which have been isolated for millions of years by oceans of mountains.

In the Aspleniaceae, hybridization occurs between several genera. But among the rest of the ferns, this is a rare phenomenon. This does not invalidate the genera: intergeneric hybridization is also known from the Poaceae and the Orchidaceae. It is probably an indication that we are dealing with a rapidly evolving group.

On the specific level, the problem has two aspects:

(a) autopolyploidy

(b) allopolyploidy

Autopolyploidy creates reproductive barriers within a species, without any morphological differentiation (e.g. Adiantum incisum) Many cytologists would regard each level of ploidy as a separate species. These 'species' can conveniently be called cytospecies. I should think that a cytospecies only differs from the parent with regard to its number of chromosomes. Some species have strings of increasing ploidy, without changing anything else.. It could be regarded as sibling species. But until it has differntiated sufficiently from the original diploid, it cannot be separated of as a distinct species.

Allopolyploidy provides a completely different case. Here a group is formed which does not relate to any other group. Genetically, it contains nothing new, but the whole genome represents a unique combination of genes. It cannot be regarded as a sibling species, as it is first formed as a fully distnct species. It conforms with all the major criteria of a species:

(a) morphologically unique

(b) genetically isolated

(c) a narrow and consistent set of ecological requirements.

Allopolyploids can thus be regarded as fully fledged species.

Sterile hybrids will also have to be descrided and named. They are often common, and one cannot but describe all plants.

CONCLUSIONS:

Hybridization, accompanied by chromosome doubling, is widespread among the ferns. It has been carrying on for millions of years. As polyploids are better adapted for colonising new habitats than diploids, the consequence of disturbances is the eradication of the diploids.

There are several ways of studying hybrids: morphological comparisons, cytogenetic studies and hybridization programmes. The latter two yield the most conclusive and repeatable evidence. This also indicates what type of polyploidy took place.

Hybridization is an important method of speciation, but it does not have much importance for evolution, until the hybrids have been diploidised. The real importance of hybrids lies in their ability to colonise newly disturbed areas. This they are as important part of the pioneer vegetation. Among ferns hybridization is an important method of forming new evolutionary lines.

Thus hybridization is of great importance, both to speciation and to the formation of present day biotas.

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