

## FRESH LOOK AT THE CYTOLOGY, GENETICS AND EVOLUTIONARY POTENTIAL OF AGAMOSPORY IN HOMOSPOROUS FERNS

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### ABSTRACT

Obligate asexually reproducing ferns provide an important window into the evolution and maintenance of sexual reproduction cycle, and these are also challenging our concept of a species. Relatively speaking, there is high frequency of obligate apomictic/ agamosporous homosporous ferns when compared to the sister clade angiosperms, and more importantly its incidence is disparate amongst the ferns. Reasons for such a high frequency of apomictic taxa together with their unequal distribution in ferns are unclear. Agamospory adds to the enigmatic nature of homosporous ferns. Contemporary studies in ferns are focussing on several questions, and are providing valuable insights into the evolutionary dynamics of agamospory in ferns. A brief overview of the phenomenon, genetics, and evolutionary potential/ significance of agamospory in ferns is presented bringing into focus some recent revelations so as to impress upon the need to revisit the agamosporous mode of reproduction in agamic complexes and hybrids.

Keywords: Fern biology, agamospory, Döpp-Manton pathway in apogamy, sub-sexuality in agamospory, Novel meiosis in apogamous ferns, Autobivalents.

### INTRODUCTION

Pteridophytes comprise an enigmatic group of spore-bearing vascular plants of antiquity, the ancestors dating back to approximately 410 Myrs. The group commands evolutionary importance due to its phylogenetic position and the characteristic alternation of two independent generations in their life cycle, *gametophyte* and *sporophyte*. The surviving descendants (c. 13,600 species) are currently classified into two distinct clades, namely Lycophytes and Monilophytes or the Ferns, the latter being sister to the Angiosperms (Pryer *et al.*, 2004, Ranker & Haufler, 2008). The Ferns include the familiar ferns, Equisetales and Psilotales. The Ferns flourished during the Carboniferous (*Age of Ferns*), and the 'modern' ferns evolved and diversified during the Cretaceous period in the shadow of Angiosperms (Schneider *et al.*, 2004), yet they diverged sharply from the Angiosperms. Vast majority of the Ferns (~93 %) are not only homosporous but are notoriously characterised by high basic chromosome numbers and high frequency

of polyploids (Manton 1950; Manton & Vida 1968; Walker, 1979; Klekowski 1973,1979; Verma 2000, 2008).

As a group, the homosporous pteridophytes attracted the attention of evolutionary biologists soon after Manton's (1950) inspiring treatise- *Problems of Cytology and Evolution in the Pteridophyta*-, to investigate their chromosome numbers and undertake cytogenetic analyses of cytological-complex taxa. As the data on chromosome numbers accumulated, Klekowski & Baker (1966) speculated on the significance of high basic chromosome numbers and rampant polyploidy in the homosporous pteridophytes. They advanced a very provocative hypothesis, elaborated subsequently by Klekowski (1973, 1979), which perceived an evolutionary intimate relationship between the existence of high basic chromosome numbers in the extant diploids (conceived as paleopolyploid!) and the fern life cycle, assuming habitual *gametophytic selfing* within potentially 'hermaphrodite' gametophytes. This form of extreme inbreeding would generate 100%

homozygous progeny in one act of such fertilization. It was further conceived that the high basic numbers in the extant diploids reflected internal polyploidy, their genomes consisting of at least two sets of homoeologous chromosomes, and that heterozygosity would be stored at homoeologous loci (i.e. sexual diploids will be homologous homozygote, but homoeologous heterozygote). Further, the stored heterozygosity would get released via *random-occasional pairing between homoeologous chromosomes*, together with a *genetic restriction* to form only bivalents. Such an inclusive hypothesis generated tremendous and as yet unending inquiry and debate to test all elements of the challenging hypothesis (Klekowski, 1973, 1979; Haufler, 2014). Recently, Haufler (2014) has reviewed critically the post-Klekowski (1973) investigations on elements of the debated hypothesis, raising issues that still need to be resolved.

The sexual life cycle of majority of the extant homosporous diploids follow inter-gametophytic mating (Soltis & Soltis, 1987, 1989, 1990; Haufler, 2014; Haufler *et al.*, 2016; Sessa *et al.*, 2016). The conceptualized Klekowski's (1973) thought-provoking hypothesis of *homoeologous chromosome pairing* together with a *genetic restriction* to form only bivalents as an operative *polyploid genetic system* in the extant diploid homosporous pteridophytes which is based on his conception of being at least tetraploid assuming past *whole genome duplication* (WGD). Homoeologous chromosome pairing was the means to generate variation, it would serve to retain the chromosome form and gene content. Such an operating mechanism would run contrary to the findings of silenced genes, and demonstration of diploid genetic system in the extant 'diploids', including the high base-numbered genus *Equisetum*,  $x = 108$

(Haufler & Soltis, 1986; Soltis, 1986; Gastony, 1991) [see Haufler, 2014 for discussion]. Nevertheless, it must be stated that Klekowski's (1973) theoretical, yet logically appealing, hypotheses to fathom the evolutionary biology of sexually reproducing homosporous pteridophytes served admirably to bring into focus the importance of exploring ferns and fern-allies for better understanding the dynamics of plant genetic systems.

Besides the sexual mode of reproduction and sterile hybrids, the ferns are notorious for propensity of obligate apogamous reproduction, and such taxa have freed themselves of the  $n-2n$  chromosome cycle, both generations being  $2n$ . Approximately 10% of the homosporous ferns for which breeding system is known follow repetitive *apogamous* life cycle, wherein the two alternating generations (gametophyte and sporophyte) possess the same chromosome number ( $2n$ ), by innovating compensatory mechanisms to circumvent meiotic reduction during sporogenesis. This in-itself raises interest in the evolution of the phenomenon of apogamy in ferns. In these ferns the sporophytes produce chromosomally *unreduced* spores or  $2n$  spores (called diplospores), which in turn develop into gametophytes, generally lacking archegonia [where present, the archegonia are non-functional (Mehra, 1938; Laird & Sheffield, 1986)], and the embryo sporophyte develops directly from somatic cells of the gametophyte without gametic fusion (hence apogamy). Since spores are produced in the apogamous ferns, it has been named agamospory (Löve & Löve, 1975; Walker, 1979). The noteworthy part is that *agamosporous life cycle is obligate*, [Facultative apmixis and polyploidy is reported in *Matteuccia orientalis* (Hook.) Trevis. by Lloyd, 1973], although it is

possible to induce apogamy in otherwise sexual species under experimental manipulations, in order to find answers to several questions (Manton, 1950; Manton & Walker, 1954; Mehra, 1975, Palta & Mehra, 1983; Cordle *et al.*, 2012; Bui *et al.*, 2017). The gametophytes of agamosporous ferns usually bear functional antheridia, and the sperms can fertilize eggs of sexual species, the progeny being agamosporous, suggesting dominance of apogamy over sexuality. Thus the functional antheridia harbour the potential to enlarge the agamic complex, raising taxonomic issues. Such agamic complexes are now well-known in ferns and deserve careful and critical study for taxonomic assessment (Evans, 1969; Gastony & Windham, 1989). Lang's (1898, 1929) unusual observation of the bearing of sporangia on gametophyte is intriguing and such out-of-turn expression of genome still needs to be replicated and resolved.

Of utmost concern in our understanding of the genetics of agamospory in ferns is Klekowski's (1973) conceived perception that the commonness of the Döpp-Manton pathway of sporogenesis in obligate agamospory is due to the presence in it of an opportunity for *homoeologous chromosome pairing*, foreseeing in it an element of sub-sexuality. One would *then* expect genetically variable spores and progeny. Concealed in Klekowski's perception of sub-sexual system in the Döpp-Manton pathway, via '*homoeologous chromosome pairing and recombination and restriction to form only bivalents*' is the tacit presumption of hybrid origin of apogamy. Klekowski's hypothesis demands a reassessment and to discover the variety of apogamous systems in ferns. Neither Haufler (2014), nor Grusz (2016) and Grusz *et al.*, (2017), have touched upon in detail the debatable issue of sub-sexuality in the common Döpp-Manton or other systems of

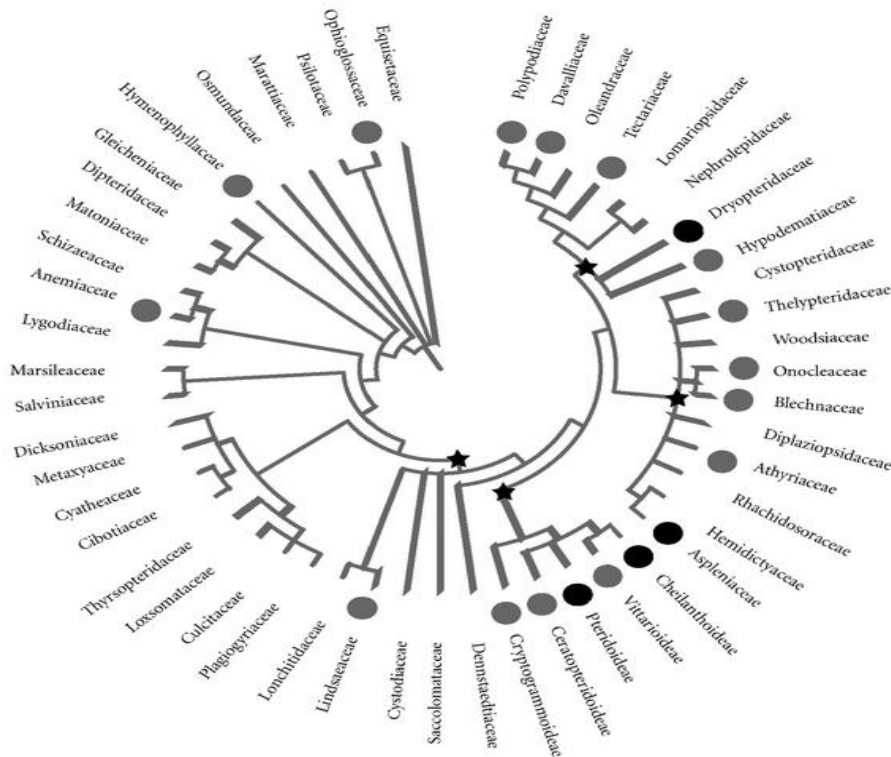
agamospory, although referring to literature reporting genetically variable progeny in agamosporous ferns. In this brief overview, it is intended to discuss issues pertaining to cytology and genetics of sporogenesis pathways in agamosporous ferns, producing 'unreduced' (2n) spores. It is also intended to impress upon the need to undertake incisive cytological research in apogamous and hybrid taxa in ferns, keeping in view the recent revelations of far reaching consequence.

### **AGAMOSPORY IN FERNS AND ITS DISPARATE DISTRIBUTION**

The phenomenon and evolutionary significance of '*agamospory*' has engaged a large number of botanists ever since the first report of asexual growth from the prothallus of *Pteris cretica* by Farlow (1874). Walker (1966, 1979, 1985) has dealt with several aspects of obligate agamospory in ferns, while Raghavan (1989) devoted a chapter (No. 13) on '*Apogamy- an alternate developmental program of gametophytes*'. More recently Grusz (2016) has in-depth reviewed it toward a clearer understanding of the current perspectives on apogamous reproduction in ferns. The evolutionary significance of the phenomenon of obligate agamospory in ferns may be judged from its propensity. Whereas nearly 10% of the living ferns are estimated to follow one of the pathways to produce unreduced spores in their agamosporous life cycle, surprisingly less than 1% of the angiosperms, a sister clade to ferns, are apomictic (Bicknell & Koltunow, 2004; Becks & Alavi, 2015). Why so!, and how much has agamospory contributed to fern diversity is yet to be estimated (Liu *et al.*, 2012). It necessitates recording of the mode of reproduction in fern floras. It is probable that agamosporous reproduction plays a more dynamic role in fern diversity, mirrored in their distinctive

pre-meiotic and meiotic innovations to produce chromosomally unreduced (2n) spores, developmentally linked to the asexual mode of sporophyte production, ensuring regular 2n - 2n alternation of generations, and also in the faithful perpetuation of the adapted genotype over generations, barring mutations. Because the life cycle of agamosporous ferns is

uncoupled from the haploid-diploid chromosome cycle of sexual ferns, this feature of apogamy has contributed immensely to our understanding of the alternation of generations in the sexual fern life cycle (Mehra, 1975; Bell, 1979, 1988, 1989, 1992; Sheffield, 1994, 2008; Sheffield & Bell, 1987; Verma, 2005).



**Fig. 1. of Liu et al., 2012\*: Global phylogeny of fern families as recovered in recent phylogenetic analyses with some poorly resolved nodes collapsed. Circles indicate occurrence of apomixis in this family. Grey circle: < 5% of known apomictic fern taxa belong to this family; black circles: > 5% of known fern taxa belong to this family. Pteridaceae are replaced by five subfamilies to illustrate the uneven distribution of apomixis in the highly diverse family. Fern families with more than 400 extant species are printed in bold. Stars indicate lineages of importance: 1 = Polypodiales, 2 = Pteridaceae, 3 = Eupolypods 1, and 4 = Eupolypods 2.]**

[Source: Open Access Journal, Journal of Botany, Liu et al., 2012; gratefully acknowledged.

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Although the incidence of apogamy in nature has been estimated to be 10% of the extant ferns, it is indeed curious, if not surprising, to observe disparate distribution of agamospory amongst the families of ferns, displayed very impressively by Liu *et al.*, (2012) on a phylogenetic frame-work (Fig. 1). Grusz (2016) has also focused on it through an equally impressive portrayal on the recent classification by PPG 1 (see Schuettpelz *et al.*, 2016). Amongst the 24 families of leptosporangiate ferns displayed by Grusz (2016), 50% of the families (i.e. 12) contain one or more genera with apogamy. And, this mode of reproduction is prevalent in fewer families/genera, being relatively common in members of family Pteridaceae, Dryopteridaceae, Polypodiaceae, Aspleniaceae and Thelypteridaceae. According to Walker (1985) agamospory is 'confined to only 18 out of the 350-400 genera commonly recognized' (p.59). This observation requires inquiry into the genetic make-up of such genera that pre-adapt/favour the evolution of agamosporous mode of reproduction so as to free themselves of the probable homozygous progeny from gametophytic selfing in sexual reproduction, and to perpetuate faithfully the favoured (hybrid!/ heterozygous) genotypes, capable of invading relatively xeric habitats.

The evolution of apogamy in ferns is thought to be of recent geological origin, as far back as <20 mya! The data referred to by Grusz (2016, p. 660) is reproduced here for information: 'that apomictic lineages themselves are apparently young, with initial estimates placing the ages of extant apomictic ferns in relatively recent evolutionary time (e.g., ~0.4 my in *Astrolepis*, Beck *et al.*, 2011; <8 my for the polystichoid ferns, Liu *et al.*, 2012; <15 my for the crown groups of apogamous ferns in Japan, Tanaka *et al.*, 2014)'.

It would be of interest to fathom the environmental conditions prevailing around 20 mya that favoured the evolution of agamosporous life cycle in some lineages of ferns, as compared to its sister clade angiosperms with which ferns co-shared the habitat! And, how do such genera differ in their genetic-makeup from those lacking agamospory!

#### **TYPES OF AGAMOSPORY: INNOVATIONS OF SPOROGENESIS**

At present, it seems to be customary to refer to only two types of obligate agamospory in ferns. Klekowski (1973), Walker (1979, 1985), and Grusz (2016) recognize only two compensating sporogenesis-pathways for the production of chromosomally unreduced (2n) spores, namely, (1) the *Döpp-Manton pathway* wherein the compensation to meiotic reduction occurs at *premeiotic mitosis*, which is 'incomplete' resulting in a restitution nucleus with twice the sporophytic nuclear content (i.e. 4n), and the following meiosis is perfectly bivalent forming that generates 2n spores in tetrads (Döpp, 1932; Mehra, 1944, Manton, 1950). This pathway is referred to by Grusz (2016) as *premeiotic endomitosis* (PE); in leptosporangiate ferns PE occurs at the 8-celled stage of sequential mitoses of the archesporial cell in sporangium development; resulting in sporangia containing 8 spore-mother-cells, instead of the usual 16, with twice the nuclear content (4n), followed by regular bivalent-forming meiosis that yields 32 2n-spores in tetrads; and (2) the *Braithwaite pathway* characterized by usual four successive mitoses of the archesporial cell to yield 16-spore-mother-celled sporangia, followed by *meiotic first division restitution* (MFDR), and the second meiotic division finally producing 32 unreduced spores in diads. According to Walker (1985) the 16 spore-mother-cells

undergo 'a modified first meiotic division in which all the chromosomes are unpaired at metaphase. No chromosomal division occurs and restitution nuclei are formed without anaphase separation. The second meiotic division is normal with the chromosomes and cytoplasm dividing, resulting in 32 diplospores arranged in diads and having an unreduced chromosome number' (p.59-60). These two systems have been described and illustrated 'fully' by Raghavan (1989), Haufler (2014) and Grusz (2016). Walker (1985) enlarged the scope of the Braithwaite system to include within it the Mehra & Singh's (1957) observation of some homologous pairing during meiosis-I in *Trichomanes insigne* (v.d.B.) Bedd. var  $\beta$ , and the Evans (1964) report of 'ameiotic alternation of generations' in *Polypodium dispersum* A.M. Evans. More importantly, the spore output in the Braithwaite scheme frequently falls short of the expected 32 diplospores, whereas full complement of 32 diplospores characterizes the Döpp-Manton scheme of agamospory (Walker, 1985). The complex of three types within the *Braithwaite system* [i. Mehra & Singh, 1957; ii. Braithwaite 1964; iii. Evans, 1964] offers the challenge to analyse the details of sporogenesis, variety of spores produced, and their consequence. In addition to the two principal systems, Morzenti (1967) reported the formation of 16 sporocytic spores in *Asplenium plenum* [*Asplenium x plenum* E. St. John ex Small (pro sp.)], i.e. spore-mother-cells transform themselves into unreduced (2n) spores, by which hybrid ferns may reproduce, and it suggested an unusual mode of species formation. It is another area of challenge to investigate the production of such viable spores in hybrids and how the progeny reproduces.

It is well-known that the Döpp-Manton pathway [PE] is the most common and indeed successful pathway adopted by ferns

to 'faithfully' perpetuate the adapted genotypes. In contrast, Walker (1985) listed some 8 taxa in the Braithwaite type of agamospory. It is possible to reasonably suspect the occurrence of Döpp-Manton agamospory in fern species, if the spore output per sporangium is commonly half of the usual number in sexual relatives (i.e. 32 instead of 64 in the leptosporangiate ferns) and spores are produced in tetrads. It is therefore important to record spore output per sporangium in all investigations on the cytology of ferns, and whether the spores are produced in tetrads or diads, to distinguish between the two principal types of agamospory. Thus, the output of spores per sporangium and whether produced in tetrads or diads can provide an initial information on the mode of reproduction.

Evans (1969) introduced the possibility of 'failure of at least some, if not all, of the spore mother cells of certain sporangia to divide at all, thereby producing a few to as many as 16 unreduced spores per sporangium (Morzenti, 1967)' (p.710). It is not unlikely that well-established Döpp-Manton agamosporous ferns may experience variable pathways in disturbed habitats and the spore-output and form of spores depart from expectation! Such situations may be expected in hybrids in process of evolving regular agamospory. It is a challenging area indeed. Only 'future in-depth surveys of reproductive mode in ferns will help to shed light on the impact of either meiotic pathway on fern evolution' (Grusz, 2016).

#### **WHY IS DÖPP-MANTON PATHWAY COMMON IN FERNS!**

The Döpp-Manton pathway during sporogenesis of agamosporous ferns contains a situation of *transient polyploidy* at the 8-celled stage, arising from 'incomplete'

4th mitosis of the archesporial cell being a restitution mitosis (termed *endomitosis* by Grusz, 2016), and the following meiosis in the 4n spore-mother-cells may permit, as per Klekowski's (1973) hypothesis, pairing between homoeologues with 'genetic' restriction to form only bivalents, and release thereby genetically different spores/progeny. This possibility must be viewed in the back-drop of Klekowski's all inclusive concept which considers the extant diploids as containing at least two pairs of homoeologous sets (internal polyploidy at 4n level, presumably the consequence of past whole genome duplication). Thus, if homoeologous chromosome pairing occurs at first meiosis of the Döpp-Manton spore-mother-cells, restricting chromosomal associations to only bivalents, it would demonstrate sub-sexuality. Klekowski (1973) reasoned that this possibility of homoeologous chromosome pairing was the determining factor favouring natural selection of the Döpp-Manton scheme of agamospory, and hence it led to the commonness of the system. The perceived notion of 'genetic' restriction to form only bivalents runs throughout the *homoeologous chromosome pairing hypothesis* of Klekowski (1973, 1979). Such genetic controls are yet to be discovered however.

According to Gastony & Windham (1989) the higher incidence of Döpp-Manton pathway is probably attributable to genome stability imparted by high fidelity in bivalent chromosome pairing following premeiotic genome duplication.

Grusz (2016) essentially concurs: 'The retention of functional meiosis in PE apomicts has been previously hypothesized to confer an evolutionary advantage, possibly as a means of generating genotypic diversity (DeBenedictis, 1969; Klekowski, 1973). Evidence of genetic variation

obtained by Lin *et al.*, (1995), Ishikawa *et al.*, (2003), Ootsuki *et al.*, (2011, 2012) and Grusz (2014) tend to support this postulation, that 'PE could bestow long-term benefits in apomictic ferns, whose evolutionary potential may be more closely resemble a selfing mating system than the purely clonal apomixis with which they are often associated' (Grusz, l.c., p. 6). The most important observation by Ishikawa *et al.*, (2003) of genetic variation in the progeny derived from spores of a single parental sporophyte of Döpp-Manton triploid apogamous *Dryopteris nipponensis* Koidz., was accommodated within the *homoeologous chromosome pairing hypothesis*. It would be necessary to revisit the published data on genetic variation obtained from a sporophyte of Döpp-Manton agamosporous ferns, like the one obtained by Ishikawa *et al.*, (2003) in triploid apomict *Dryopteris nipponensis*, for critical analyses considering other alternative possibilities, because the Klekowski's (1973) homoeologous pairing hypothesis has complex and still debated, provisions (Hauffer, 2014). The chromosome pairing behaviour in the 8-spore-mother-celled sporangia (regular bivalents) and in the usual 16-celled sporangia ought to be matched, as pairing is homology dependent. There is stark mismatch in chromosome associations in the 8-celled SMC:s (regular bivalents) when compared with meiosis in 16-smc:s sporangia of diploid apomicts showing several bivalents and few univalents, and in triploid apomicts revealing x-pairs + x univalents (see Table 1). The causal genetic system of this mismatch is unknown. It is an open field of inquiry pregnant with unexpected revelations.

Agamosporous species are not entirely clonal throughout its range. In this context, it may be said that four possibilities have been envisaged in literature that may cause

variation in agamosporous fern species: (1) somatic mutation (Manton, 1950), (2) recurrent origin (Walkler, 1962; Watano & Iwatsuki, 1988), (3) homoeologous chromosome pairing (Klekowski, 1973; Klekowski & Hickok, 1974; Klekowski, 1976; Hickok, 1978; Chapman *et al.*, 1979; Ishikawa *et al.*, 2003) and (4) unequal meiosis (Lin *et al.*, 1992).

Verma (1979, 2000) while discussing the apogamous systems in ferns referred to Klekowski's perceived element of sub-sexual system in the Döpp-Manton pathway. It was held that 'it is the retention of the entire sequence of events of the meiotic cycle which ensures that evolutionarily coordinated sequence of cytokinesis to follow is not disturbed. As a result spores are produced in tetrads following the cytokinetic events characteristic of their related sexual species. The adaptive relations of spore form and structure remain thereby unaltered, and hence their spores are not expected to be functionally inferior to their sexual relatives. Spore is the most important constituent of the fern life-cycle, and the production of the usual kind of spores characteristic of related sexual species, in tetrads, that permitted natural selection and prevalence of the Döpp-Manton system of agamospory in ferns' (Verma, 2000, p.65).

#### **Cytology of Döpp-Manton Sporogenesis Pathway in Apogamous Ferns: Suggestive Evidence against Sub-sexuality!**

Amongst the variety of mechanisms adopted by obligate agamosporous ferns, the Döpp-Manton pathway is the most successful in producing chromosomally 'unreduced' spores following seemingly regular meiosis, and it is the most widespread. The all inclusive hypothesis of

Klekowski (1973) confers an evolutionary advantage upon Döpp-Manton agamospory perceiving the possibility in it of generating genetic-diversity among spores, or sub-sexuality, through homoeologous chromosome pairing. According to Klekowski (1973) the incomplete premeiotic mitosis at the 8-celled stage results in 4n spore-mother-cells, and in the following meiosis the bivalents may form randomly rather than strictly between sister chromosomes, and thereby release genetic variability. It is reflective of a logical assumption of hybrid origin of apogamy. As an alternative, mutations would accumulate over a period of time to augment heterozygosity, which gets transmitted faithfully over several generations. (see Grusz, 2016).

The issue of sub-sexuality in Döpp-Manton agamosporous ferns has already been discussed by Verma (1979, 2000) arguing against Klekowski's postulate of homoeologous chromosome pairing during meiosis. Obviously, advances in chromosome painting and other chromosome techniques may reveal the secrets, but one can make some headway from available observations on record regarding some cytological details relevant to the issue.

The basic tenet of Klekowski's postulate of the likelihood of homoelogous chromosome pairing to generate variable spores, that gained subsequent support (see Ishikawa *et al.*, 2003; Haufler, 2014; Grusz, 2016), revolves around the events between the end stages of premeiotic mitosis and the onset of bivalent forming meiosis. Detailed cytological observations in support of Klekowski's (1973) hypothesis have not been made in recent years. The available data is revealing, however. Mehra (1938) who had discovered apogamy in *Adiantum*



*lunulatum* Burm. f. (= *A. philippense* L., Verma & Fraser-Jenkins, 2008), subsequently detailed the cytological events in this species (Mehra, 1944), which are very similar to what has been described with clarity by Manton (1950). In the higher ferns four successive mitoses of the archesporial cell result in 16 spore-mother-cells, which undergo meiosis to form 64 spores, in tetrads. In the ontogeny of sporangia of Döpp-Manton pathway, the premeiotic mitosis is incomplete, terminating in a restitution nucleus with twice the nuclear contents. Cytokinesis is omitted. A regular bivalent-forming meiosis-I follows in the 8 4n-spore-mother cells, to form 32 2n-spores in tetrads. Manton (1950) emphasized that the observed chromosome pairing is confined to sister chromosomes formed during premeiotic mitosis. According to Manton (1950) meiosis begins while the halves of split chromosomes are still in close contact. In that case the observed bivalents at meiosis-I would be of the nature of *autobivalents*, as reported in the case of female meiosis in the liliaceous species *Allium odorum* L. (Håkansson & Levan, 1957). There is seemingly some kind of physiological control as a part of the total switch mechanism which brings about restitution mitosis and suppression of cytoplasmic cleavage at definite developmental stage. At pre-meiotic mitosis the split chromosomes do not undergo the formal separation, and presumably they had no chance of moving out randomly, so that the sister chromosomes paired at the following meiosis. Support is derived from the occasional cleavage-type sporangia in Döpp-Manton agamosporous *Asplenium cheilosorum* Kunze ex Mett. discovered by Mehra & Bir (1960) [triploid apomict  $2n = 'n' = 108$ ; 16-celled sporangia generally show  $36 \text{ II} + 36 \text{ I}$ ,  $x = 36$ ; but there are many other associations as well, e.g.  $3 \text{ III} + 40 \text{ II} + 19 \text{ I}$ ; see Bir & Verma, 2010, p.186], wherein the

cytoplasmic cleavage at pre-meiotic mitosis is not completely suppressed, thereby often ending up in unequal-sized spore-mother-cells entering meiosis. All such unequal-sized spore-mother-cells display surprisingly complete regularity of chromosome pairing [e.g. ' $n$ ' = 36,45,63,72,117 IIs] as all bivalents, confirming thereby the contention of the observed bivalents in Döpp-Manton sporogenesis being of the nature of '*autobivalents*'.

Further support to the '*autobivalent*' nature of the bivalents in Döpp-Manton sporogenesis comes from cases where the normal 16-celled sporangia occur along with restituted 8-celled sporangia, and chromosome pairing in the former is based on chromosome homologies, and such data are useful in interpreting the genomic makeup of the Döpp-Manton agamosporous taxon (see Mehra, 1961b, Mehra & Verma, 1963; Bir & Verma, 2010 : and also Table 1 and the data on *Asplenium cheilosorum* given above). The frequency of 16-celled sporangia can be increased by transplanting individuals from nature to fern-house (Manton & Sledge, 1954). The case of *Pteris cretica* L. may be cited, a species wherein apogamy was first reported by Farlow (1874). The data on various cytotypes is summarized in Table 1. Several such examples can be noticed in the chromosome atlas of Indian pteridophytes by Bir & Verma (2010).

Thus, whereas the meiosis in 16-celled sporangia reveals chromosomal associations from univalents to multivalents, reflecting homologies, meiosis in the 8-celled sporangia, subsequent to restituted pre-meiotic mitosis, invariably shows regular bivalents. In the agamosporous *Adiantum lunulatum* (= *A. philippense*) [Mehra, 1944; Mehra & Verma, 1960, 1963; Ghatak, 1977], with ' $n$ ' = 90 (triploid apomict,  $x = 30$ ) in

**Table 1. Chromosomal associations in the two main types of sporangia in three cytotypes of *Pteris cretica* that follows the döpp-manton scheme**

Taxon	2n-Chr. No.* (x = 29)	Chromosome associations in smc of sporangia		Reference
		16-celled	8-celled**	
<i>P. cretica</i> L.	58	Some I, Many II, Some III, Few IV	58 II	Manton, 1950
	58	2-6 I + 26-28 II	58 II	Verma & Khullar, 1965
	87	19-25 I + 28 II + 2-4 III	87 II	Verma & Khullar, 1965
-- var. <i>albolineata</i> Hk.	87	Few unpaired More multivalents	87 II	Manton, 1950
	87	27.8 I + 21.8 II + 5.2 III	87 II	Verma, 1972
-- var. Hort. (from Uganda)	116	Few unpaired + more multivalents	116 II	Manton, 1950
--var. <i>crinata</i> Hort.	116	35.5 I + 36.2 II + 2.7 III	116 II	Verma, 1968

\* 2n-chromosome number = 'n' -number of autobivalents, at meiosis of 8-celled sporangia\*\* of Döpp-Manton agamospory.

\*\* Meiosis in 8-celled sporangia show 'n' -number of 'autobivalents'.

8-celled sporangia, meiosis in the 16-celled sporangia shows 30 II + 30 I (Ghatak, 1959). It is postulated that the kind of elusive genetic control which produces the physiological block at pre-meiotic mitosis of 8-celled sporangia, thereby allowing the formation of 'autobivalents' also suppresses as a consequence both homologous and homoeologous chromosome pairing. Additional support to the postulate of 'autobivalents' comes from sporangia where two pre-meiotic mitoses are of the nature of restitution mitoses resulting in four spore-mother-cells with four times the nuclear contents (i.e. 8n), and meiosis in these spore-mother-cells is exceedingly regular with only bivalents, despite each chromosome represented four times (Manton, 1950). These 4n spores may give rise to 4n apomicts. Thus, the bivalents at meiosis of 8-celled and the 4-celled sporangia are 'autobivalents'. Hence, there would be no possibility of the perceived homoeologous chromosome pairing, and no sub-sexuality in the most common Döpp-Manton system of agamospory in ferns, a conclusion contrary to that of Klekowski (1973).

It may be said that there is likelihood of sub-sexuality in the Mehra-Singh (1957) pathway if recombination occurs during meiosis-I to generate genetically variable spores. There is scope to discover such type of agamospory occurring along with, in variable frequencies, in otherwise Döpp-Manton agamosporous ferns. Nature does not restrict herself to standard patterns conceived on available data/ hypothesis. Possibility of mixed type of development during sporogenesis needs to be investigated. There are reports in literature concerning variable progeny in ferns otherwise known to be following the common agamospory, e.g. *Pteris cretica* (Suzuki & Iwatsuki, 1990), *Dryopteris varia* group (Lin *et al.*, 1995), *Dryopteris nipponensis* (Ishikawa *et al.*, 2003), and these and other such cases must attract our attention.

#### SOME CONCERNS ABOUT AGAMOSPORY

Agamospory is common in a few families, like Aspleniaceae, Athyriaceae, Dryopteridaceae, and Pteridaceae. One

must ask why so? Also, "why is apomixis so common in ferns"?, and is it that there is genetic 'preadaptations to apomixis in ferns!' (Liu *et al.*, 2012). There is another question: Do asexual polyploid lineages have short evolutionary lives! (Beck *et al.*, 2011). Several questions still require resolution (Liu *et al.*, 2012, Vicent *et al.*, 2014).

Regalado *et al.*, (2010) and Dyer *et al.*, (2012) have addressed to some issues in Aspleniums, and Liu *et al.*, (2012) in Polystichoid ferns. The 'apogamous embryo' lacks foot and root (Regalado *et al.*, 2010). The absorbing review, titled 'Insight into fern evolution: a mechanistic approach to main concepts and study techniques', by Vicent *et al.*, (2014) includes an important aspect of 'Apogamy with some more thoughts around the species concept in ferns' (p.16 ), because in many cases it is related to the processes of hybridization and polyploidy. Hori *et al.*, (2016) utilized two nuclear markers (PgiC and GapCp ) to establish the origin of the triploid apogamous fern *Dryopteris hondoensis* Koidz., as a result of wide hybridization between two sub-genera of *Dryopteris* (subg. *Erythrovariae* sect. *Erythrovariae* and sect. *Aemulae* of subg. *Dryopteris*). Tanaka *et al.*, (2014b) impressed upon the ecological and phylogenetic approaches for diversification of apogamous ferns in Japan. All such issues need to be addressed to in the understanding of agamosporous ferns occurring in India.

## ORIGINS OF AGAMOSPORY IN FERNS

Historically, the evolutionary origins of agamospory have been ascribed to hybridisation, polyploidy, and female sterility (Manton, 1950; Lovis, 1977; Walker, 1979, 1985; Mogie, 1992). This is reflected in the meiotic chromosome associations and behaviour observed in 16-celled sporangia

of apomictic taxa, the high proportion of triploid apomicts, and because agamosporous taxa are usually found in complex reticulation networks with other apomictic and sexually reproducing lineages (Walker, 1966; Liu *et al.*, 2012).

However, the inquiry is still unresolved, although a hybrid origin is favoured. Park *et al.*, (2003) reported incipient apomixis in the inter-specific triploid hybrid *Cornopteris christenseniana* (Koidz.) Tagawa. Hori *et al.*, (2016) reported hybrid origin of the triploid apogamous fern *Dryopteris hondoensis* Koidz.

It must be realized that for faithfully regulated operation of obligate agamospory necessitates a highly coordinated system of 'linked' genes/ or a 'gene complex' that assures sequential switching-on of at least three features across the two generations, gametophyte and sporophyte: (i) restitution of pre-meiotic mitosis (in the common Döpp-Manton Scheme) forming chromosomally unreduced (2n) spores via regular bivalent-forming meiosis, (ii) spores develop into gametophytes either completely lacking archegonia, or when present (few cases) these are non-functional (Mehra, 1938, Laird & Sheffield, 1986), and (iii) the development of embryo from vegetative cells, commonly behind the notch meristem, to complete the 2n - 2n alternation of the two morphological generations in the fern life cycle. The elusive 'gene complex' conferring agamospory, which is dominant to sexuality, as hybrids between apomicts -providing spermatozoids- and sexual species (archegonia) are invariably apomicts. That 'agamospory' can develop in otherwise sterile hybrids is shown by Hori *et al.*, (2016) in *Dryopteris* (case referred above). How do such 'gene-complexes', functioning across the two generations in the life cycle, get triggered into action!, and at a specific stage

during sporogenesis! Probably, the hybrid-combination itself may provide the 'signal' assuming that such 'genes' are otherwise repressed components of the fern genomes, within families where apogamy is observed to be prevalent! Such hypothesized predisposition to apogamy, as an alternative to sexuality, requires genomic studies. It is worth exploring. In support, it is known that apogamous sporophytes may develop in sexual species by growing their gametophytes under water stress, in soil deficient in mineral salts, or in light regimens of varying fluencies. 'Several investigators have successfully induced growth of apogamous sporophytes of numerous ferns which reproduce sexually in a perfectly normal way' (Raghavan, 1989, p. 268). The technique of inducing apogamy in sugar-supplemented media is beginning to reveal some secrets (Mehra, 1975, Cordle *et al.*, 2012).

#### **ARE THE OFFSPRING OF AGAMOSPORY 'CLONAL'!**

It has been commonly held that obligate agamosporous species reproduce faithfully, barring mutation, through 'unreduced' spores, produced following either the Döpp-Manton or the Braithwaite pathways (Walker, 1979, 1985). Implicitly, it is conceived that all offspring from an agamosporous parent are expected to be 'clonal'. This needs to be experimentally analysed, utilizing modern molecular techniques. The case of *Dryopteris nipponensis* analysed by Ishikawa *et al.*, (2003) is revealing. Besides the two recognized pathways of agamospory, there is another pathway discovered by Mehra & Singh (1957) in *Trichomanes insigne* var.  $\beta$ , where the chromosomes do experience some chiasmate pairing during early meiosis-I, mimicking thereafter the Braithwaite scheme (see Verma, 1979).

Thus, species following Mehra-Singh pathway are expected to generate variation to the extent of recombination during meiosis-I.

Even species following the very common Döpp-Manton pathway, like *Pteris cretica* L. (Manton, 1950; Verma & Khullar, 1965), have been reported to exhibit detectable genetic variation. Suzuki & Iwatsuki (1990) utilized enzyme electrophoresis technique to distinguish five diploid and six triploid clones within *Pteris cretica* L. from Japan. The enzyme electrophoresis helped also in finding the diploid sexual species, *P. kidoi* Sa. Kurata, involved in the generation of the triploid apomict clones, through recurrent hybridizations (Suzuki & Iwatsuki, 1990). More recently, Jaruwattanaphan *et al.*, (2013) have attempted to reconstruct the hybrid speciation events in the *Pteris cretica* group in Japan and adjacent regions. Diploid and triploid apomicts in *P. cretica* from the western Himalaya have been worked out chromosomally by Verma & Khullar (1965), and the triploid apomict from the eastern Himalaya (Verma in Mehra, 1961a) is morphologically distinct. The triploid apomict from the Himalaya is suspected to be different from that in Japan, and its parentage needs to be investigated. Huang *et al.*, (2006) reported that cryptic characteristics distinguish diploid and triploid varieties of *Pteris fauriei* Hieron.. Thus, all fern species in India characterized by agamospory need not only to be listed and within-species morphological variation classified, but also to be reinvestigated for chromosome status, chromosome behaviour, and analyses of genetic variation in progeny from raising gametophytes.

Watano & Iwatsuki (1988) reported genetic variation in the "Japanese apogamous form" of the fern *Asplenium*

*unilaterale* Lam. Ishikawa *et al.*, (2003) produced 'electrophoretic evidence for homoeologous chromosome pairing in the apogamous fern species *Dryopteris nipponensis* (Dryopteridaceae)'. Ootsuki *et al.*, (2011, 2012) also produced evidence of genetic segregation in the apogamous fern species *Cyrtomium fortunei* (Dryopteridaceae). In case the regularly formed bivalents at meiosis-I in the 8-celled sporangia of Dopp-Manton pathway are taken to be 'autobivalents', as discussed earlier, the causal explanation in reports of genetic segregation needs to be revisited. Ishikawa *et al.*, (2003) gave elaborate genetic analysis in the Dopp-Manton type triploid apomict, *Dryopteris nipponensis* ('n' = 123, Kurita, 1966, 1967). They named the three genomes as **a**, **b**, **c**, in an attempt to find a plausible explanation for genetic variation in progeny of a single sporophyte based on Klekowski's (1973) hypothesis of homoeologous chromosome pairing. Their reasoned discussion, despite being appealing, does not fit into the view of bivalents being 'autobivalents'. Besides, any conceived pairing between the genomes **a-b**, **b-c**, or **a-c**, if it occurs, ought to be referred to as non-homologous chromosome pairing! Genetic explanation of the important data of Ishikawa *et al.*, (2003) still needs to be searched. There is the possibility of mix-type sporangia or 16-celled sporangia where pairing depends on homologies; and even the ongoing mutations may be invoked for the genetically variable spores in the progeny! Obviously there are lot of issues that remain to be addressed to and resolved.

#### **UNEXPECTED PROGENY OF AGAMOSPOROUS SPECIES- A NEW CHALLENGE**

An important report by Lin *et al.*, (1992) on the diploid and triploid offspring of the

triploid agamosporous fern, *Dryopteris pacifica* (Nakai) Tagawa, remained seemingly unnoticed. In fact it concerns all such apogamous triploids which reveal at meiosis within SMCs of their 16-celled sporangia, a chromosome situation of  $x \text{ II} + x \text{ I}$ . In fact chromosome pairing behaviour within 16-celled sporangia (which occur at varying frequencies) is an important key to cytogenetic interpretations. In the case of triploid apogamous *D. pacifica*, meiosis-I in SMCs of 16-celled sporangia shows  $41\text{III} + 41\text{I}$ . ( $x = 41$ ); the 8-celled ones show  $123\text{II}$ . Surprisingly, Lin *et al.*, (1992) observed that 'spores from one of the triploid plants developed into either diploid or triploid gametophytes, which further apogamously produced diploid or triploid sporophytes, respectively' (p.443). They attributed it to a novel mechanism of ploidy reduction for the origin of diploid apogamous forms from the triploid apomicts. It was conceived 'that spores with 82 chromosomes probably developed from the 16 spore mother cells with  $n = 41\text{III} + 41\text{I}$  by means of extraordinary meiotic division accompanied with unequal segregation'. Accordingly, 'the earlier hypotheses that agamosporous diploids are derived from hybrids of diploid sexual species by acquiring agamospory (Manton, 1950) or directly from such species by genic changes (Manton 1950, Bir 1973), should be critically tested' (p.449). It is a field of inquiry waiting to be taken up.

In India, several agamosporous species-complexes are reported which display  $x \text{ II} + x \text{ I}$  chromosome-associations in spore-mother cells of 16-celled sporangia of triploid apomicts (see Bir & Verma, 2010). *Adiantum philippense* L. is one such species complex (Verma & Fraser-Jenkins, 2008; Gautam & Rajkumar, 2017), where the commonest form is triploid apogamous and it shows  $30 \text{ II} + 30 \text{ I}$  in SMCs of 16-celled

sporangia (reported as *A. lunulatum* Burm.f., Ghatak 1959, Mehra & Verma 1963). Khullar & Mehra (1972) reported 1 III + 30 II + 27 I instead. And, there is a much less-common diploid apogamous form in the western Himalaya,  $2n = 'n' = 60$  whose origin could be conceived from the reported pairing data in its 16-celled sporangia (29 II + 2 I, Khullar & Mehra, 1972; Mehra & Khullar 1977). Diploid apomict *A. lunulatum* is reported as well from Kerala (Western Ghats) by Mathew (1965). It is probable that the W. Himalayan (Kalka) diploid apomict in *A. lunulatum* complex originated from the common triploid apomict in a manner similar to the one reported in *D. pacifica* by Lin *et al.*, (1992)! All of such cases, including that of *Adiantum philippense*, ought to be investigated critically. In fact, the entire *A. philippense* species-complex (Verma & Fraser-Jenkins, 2008; Gautam & Rajkumar, 2017) is required to be analysed utilizing modern molecular methods.

*Pteris cretica* species-complex with diploid and triploid apomicts from the western Himalaya (Verma & Khullar, 1965) is waiting to be reinvestigated in the backdrop of Lin *et al.*, (1992), together with the similar complexity in the Darjeeling Himalaya (Verma in Mehra, 1961a) and the report of sexual diploid by Walker (1962) under *P. cretica* aff. Recently, Kumar *et al.*, (2015) have provided evidence of novel microsatellite markers for identification and diversity characterization in *Pteris cretica*. Besides several ferns with obligate apogamy, there are more than 100 sterile hybrids discovered (Fraser-Jenkins *et al.*, 2017) whose mode of reproduction requires investigation. One may ask the question: Does hybridization drive the transition to apogamous reproduction in ferns?

## AN EXCITING RECENT DISCOVERY INVITING SERIOUS ATTENTION

Ekrat & Koutecky (2016) under an informative title "Between sexual and apomictic: unexpectedly variable sporogenesis and production of viable polyploids in the pentaploid fern of the *Dryopteris affinis* agg. (Dryopteridaceae)", impressed upon the fact that 'reproduction of ferns with potentially mixed systems and inheritance of apomixis remains largely unknown'. They investigated reproduction of the pentaploid *Dryopteris x critica*, a hybrid of triploid apomict *D. borneri* and tetraploid sexual *D. filix-mas*. The results as summarized by the authors are: 'The hybrid is partly fertile (89–94% of aborted spores) and shows unstable sporogenesis with sexual and apomictic reproduction combined. The number of spores per sporangium varied from approx. 31 to 64. Within a single sporangium it was possible to detect formation of either only aborted spores or various mixtures of aborted and well-developed reduced spores and unreduced diplospores. The spores germinated into viable gametophytes with two ploidy levels: pentaploid (5x, from unreduced spores) and half of that (approx. 2.5x, from reduced spores). Moreover, 2–15% of gametophytes (both 2.5x and 5x) formed a viable sporophyte of the same ploidy level due to apogamy' (p. 97). Ekrt & Koutecky (2016) utilized genome size, spore output, spore-size variation and abortion, sporogenesis, and spore-germination and sporophyte formation in formulating the conclusion: 'This study documents the mixed reproductive mode of a hybrid between apomictic and sexual ferns. Both sexual reduced and apomictic unreduced spores can be produced by a single individual, and even within a single sporangium. Both types of spores give rise to viable F2 generation gametophytes and

sporophytes' (p. 97). This study is indeed inspiring. It demands to have a fresh look at apomicts which produce both types of spore-mother-cells within a sporangium, or sporangia following the normal course of 16-celled sporangia and others following the 8-celled scheme (Döpp-Manton pathway). Fresh studies on apomicts are full of promise.

In India, there is an interesting case of a pentaploid *Pteris vittata* L. Meiosis in 16-celled sporangia of the pentaploid revealed 29II + 87I, whereas in a triploid *Pteris vittata* the chromosome associations in 16-celled sporangia were 29II + 29I (Khare & Kaur, 1983a; Khare, 1995). Some observations were made on germination of spores of the pentaploid (Khare & Kaur, 1983b). Thus, in the background of Ekrt & Koutucky (2016) it will be rewarding to reinvestigate the entire *Pteris vittata* complex (Verma, 2013a), and also to determine the mode of reproduction of the triploid and the pentaploid cytotypes, apart from addressing taxonomic issues. It may be noted that spontaneous apogamy was observed in gametophyte cultures of the tetraploid *P. vittata* subsp. *emodi* Fraser-Jenk. by Verma (2013b).

#### **EVOLUTIONARY POTENTIAL OF AGAMOSPORY**

It is indeed intriguing to notice disproportionately high frequency of agamospory in homosporous ferns (c. 10%) when compared to its sister clade, angiosperms (< 1%). The situation in agamosporous ferns becomes rather paradoxical, when considering the costs involved in such reproduction. On purely theoretical considerations, agamospory confers a 50% cost on reproduction. The first cost lies in spore output: both the Döpp-Manton and the Braithwaite types of sporogenesis end up in 50% less spores

(e.g. 32 instead of 64), and hence 50% less gametophytes. Secondly, male gametes in the common Döpp-Manton pathway are not reduced, these are 2n. When such male gametes are involved in cross-fertilisation with sexual relatives, there is an increase in ploidy level and the 'hybrid' is agamosporous. Subsequently, male gametes of the polyploid progeny may cross-fertilize with other sexual lineages thereby increasing the ploidy levels, and building up a huge agamic complex, causing taxonomic identity issues. However, such incremental increase in ploidy would become 'deleterious' beyond a certain nucleus-cytoplasm ratio, thereby limiting male gamete involvement beyond a certain level. Recent investigations by Liu *et al.*, (2012) on the '*evolutionary dynamics of apomixis in ferns*' focussing on Polystichoid ferns as a case study, have concluded that 'the apomictic lineages showed no increase in speciation rate, instead all apomictic lineages appeared to be short lived despite some evidence for post origin diversification. In general, the results support the hypothesis that apomictic ferns are evolutionary dead ends in the long term but maintain the short-term potential to be highly successful in particular ecological conditions such as climates with strong seasonality' (p.9). Such studies are required in species-rich genera of families with high incidence of agamospory.

#### **IN CONCLUSION: DISCOVER AND ANALYSE DIFFERENT TYPES OF SPORANGIA IN AGAMOSPOROUS FERNS AND HYBRIDS**

When a taxon in ferns is said to be agamosporous, it does not convey that all of its sporangia produce functional diplospores (32 in leptosporangiate ferns), as a result of either the Döpp-Manton pathway (PE) or the

Braithwaite pathway (MFDR). Manton (1950) herself pointed out that in the Döpp-Manton agamospory, a proportion of the sporangia may follow the normal course of development ending up in 16 spore-mother-cells which proceed into meiosis based on chromosome homologies. And such pairing behaviour reflects the genomic make-up of the taxon in question. For example, in triploid apomicts one may observe at meiosis within 16-celled sporangia either of three principal situations: (1) combination of bivalents, univalents and multivalents [AAA/AA'A'/AA'A"], (2) bivalents + univalents in equal numbers ( $x \text{ II} + x \text{ I}$ ,  $x$  being the basic number) [ABB or ABB'], and (3) all univalents ( $3x \text{ I}$ )[ABC]. Thus meiotic analyses within 16-celled sporangia are indeed the most important in interpreting the genome structure of the taxon (see Mehra, 1961b, Verma, 2000). The proportion of 16-celled sporangia in Döpp-Manton agamospory may vary from almost negligible to fairly common, and it is possible to increase their incidence by transplanting the apogamous taxon from natural habitat to the fern house, a suggestion made by Manton & Sledge (1954). Disturbance in habitat seems to alter the balance. Apart from the 8-celled and the 16-celled sporangia, Manton (1950) referred to a third type where two mitoses preceding meiosis are of the restitution type, resulting in 4-celled 8n-sporangia which through regular meiosis end up in 16 giant 4n spores, and these spores have the potential to form higher level ploidy individuals. Meiosis in the 4-celled sporangia display only bivalents despite the 8n nuclear contents. Significance of this observation has been referred to earlier in calling such bivalents as 'autobivalents'. The hybrids may resort to an unusual mode of reproduction, wherein all the 16 spore-mother-cells transform themselves into spores, called sporocytic

spores, as in *Asplenium plenum* (Morzenti, 1967).

Evans (1969) enlarged the dimensions of investigations in apogamous ferns and in so-called sterile hybrids. Evans (l.c.) contemplated: "We now find that the classical concept of apogamy as being clearly indicated by the presence of total spore abortion in some sporangia and 32 good spores in others is no longer satisfactory. We must broaden this to include several different categories all the way from apogamous ferns in which all sporangia contain 32 viable spores to some taxa in which only a few of the spores in a given sporangium are viable and capable of producing apogamous gametophytes, and from the clear-cut situation of apogamous taxa being clearly distinct and incapable of cross-fertilization with normal plants to those in which the apogamous gametophyte may participate as the male parent in crosses with sexually normal gametophytes (Walker, 1958). Therefore, I think that the possibilities are present in any hybrid fern for arriving at a means of self-perpetuation through apogamy. If one considers the mechanisms involved, as I now see it, for producing an unreduced spore in a plant with an unbalanced genome, they are, again, variations on a theme, they involve at least any of the following three possibilities: 1) Development of unreduced spores through the gimmick of division in the pre-spore-mother cells accomplishing mitotic division but withholding cytokinesis. This, essentially, produces half the expected number of spore mother cells, each with double the expected number of chromosomes. This accomplishes perfect pairing due to replication and the reconstitution of these chromosomes without cytokinesis into a single cell. Dr. Manton (1950) has referred to this as the "restitution nucleus," It then follows that these spore mother cells



undergo meiosis in a regular fashion to produce only 32 viable spores with the same chromosome number as the archesporial cell of the parent plant. 2) Failure of meiosis, either producing a simple mitotic division of the 16 spore mother cells to produce 32 diplospores, a situation for which I have coined the phrase "ameiotic alteration of generations"(1964); or possibly producing only a partial meiotic division which essentially accomplishes the same result of 32 diplospores from 16 spore mother cells, as suggested by Braithwaite (1964) in his studies in an African *Asplenium*. 3) Failure of at least some, if not all, of the spore mother cells of certain sporangia to divide at all, thereby producing a few to as many as 16 unreduced spores per sporangium (Morzenti, 1967)' (p. 710). One needs to add to it a variant of the so-called Braithwaite pathway, discovered by Mehra & Singh (1957) in *Trichomanes insigne* forma  $\beta$ , where they observed some chiasmatic bivalents during early meiosis-I, which could permit limited recombination, thereby suggesting release of genetic variation in Mehr-Singh pathway.

The unusual reports in *Pteris cretica* (Suzuki & Iwatsuki, 1990), *Dryopteris pacifica* (Lin *et al.*, (1992), and *Dryopteris affinis* agg. (Ekrt & Koutecky, 2016) referred to earlier ought to motivate for undertaking in-depth studies on apogamous and hybrid ferns utilizing the modern techniques, keeping an eye on the possibility of complex, hitherto unrecorded, situation in sporogenesis. It is known that unreduced spores are sometimes produced by sexual species and these give rise to polyploids. The hybrids have the option, these may take the sexual route or the apogamous route to perpetuate themselves. Thus, production of unreduced spores in-itself is not a distinguishing characteristic of agamospory, it is the entire constellation of the elusive 'gene complex'

sequentially controlling sporangial development, meiosis in spore-mother-cells, diplospores generating gametophytes lacking archegonia or non-functional archegonia, gametophytes bearing functional antheridia in Dopp-Manton pathway whose spermatozoids can fertilize archegonia of sexual species to build up agamic complex, the progeny being agamosporous. So far known, in Braithwaite pathway the antheridia are either absent or release sluggish spermatozoids of no consequence. Studies on the gametophyte generation of agamosporous ferns still require to be addressed to. Considering the possibility of the occurrence of a mixture of different pathways within an apogamous or a hybrid taxon it is essential to record critical observations not only on the spore-output per sporangium but also on the kind of spores produced. There may be some functional spores amongst majority deformed spores. The reproductive method called agamospory continues to offer challenges, which must be accepted.

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