Cytological Studies in a Natural Hybrid of *Egenolfia* Schott

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Egenolfia Schott, a terrestrial leptosporangiate fern of the family Aspidiaceae, is distributed throughout the Indo-Malayan region (Copeland 1947). Eleven species are reported in this genus of which 7 occur in India (Holttum 1949). The cytology of only two species viz. *E. appendiculata* and *E. asplenifolia* has been reported previously from India (Abraham *et al.* 1962, Bhavanandan 1977). During the course of cytological investigation in the fern flora of Kerala, a natural hybrid of *Egenolfia* has been detected and the results of the study are presented in this paper.

Materials and methods

For cytological studies, young sporophylls of the materials collected from different localities in Kerala and grown in the green house, were fixed in 3:1 alcohol acetic acid. Acetocarmine stain technique was followed for cytological studies.

Stomatal studies were carried out from the epidermal peelings from fresh pinnae.

Observations

The spore mother cells of the hybrid material showed irregular chromosome behaviour during meiosis with the maximum of 34 bivalents and 14 univalents at metaphase I (Fig. 1). The univalents were found scattered in the cytoplasm and later organized to form varying numbers of micronuclei at telophase I (Figs. 2, 3). Micronuclei were formed at telophase II (Fig. 4) and also in the tetrads (Fig. 5). As a result of irregular meiotic behaviour 60% of the spores formed were found to be sterile (Fig. 6).

The meiotic behaviour of the spore mother cells of all the accessions of *E. appendiculata* and *E. asplenifolia* was perfectly normal showing 41 bivalents each at metaphase I (Figs. 7, 8). Spore sterility was found to be 20% and 40% in *E. appendiculata* and *E. asplenifolia* respectively (Figs. 9, 10).

The stomata are restricted to lower epidermis and are of Polocytic type in all three cases. The margin of subsidiary cell is less sinus in the hybrid, deeply sinus like the other epidermal cells in *E. asplenifolia* and almost even in *E. appendiculata* respectively (Figs. 11, 12, 13).

Discussion

The spore mother cells of the hybrid showed irregular meiosis with bivalents and univalents at metaphase I. The univalents failed to move to the poles at anaphase I, were found scattered in the cytoplasm and later organized to form micronuclei at telophase I. In the second division also the micronuclei were formed at telophase II and in the tetrad cells. This results in 60% spore sterility. The meiotic behaviour in *E. appendiculata* and *E. asplenifolia* was perfectly normal with 41 bivalents each at metaphase I, and the subsequent stages were normal.

There are reports on the occurrence of natural as well as synthetic hybrids among the species of angiosperms and ferns. In order to individuate cytogenetically homologous chromosomes between *Chrysanthemum makinoi* ($2x=2n=18$) and *C. vulgare* ($2x=2n=18$) of the family Compositae, Tanaka and Shimotomai (1968) synthesised a hybrid ($2x=2n=18$) of these two species. They found only one bivalent in the hybrid that was the longest chromosomes of both parental complement, which are the only homologous chromosomes. The hybrid ($2x=$

Figs. 1-13. 1-6. *Egenolfia* hybrid. Figs. 1-5, 7, 8. $\times 550$. 1, spore mother cell showing bivalents and univalents at metaphase I. 2, late anaphase I showing laggards. 3, telophase I nuclei with micronuclei. 4, telophase II nuclei with micronuclei. 5, tetrad with micronuclei. 6, spores. $\times 85$. 7, *Egenolfia appendiculata* $n=41$. 8, *E. asplenifolia* $n=41$. 9, *E. appendiculata*. Spores. $\times 85$. 10, *E. asplenifolia*. Spores. $\times 85$. 11, *Egenolfia* hybrid. Lower epidermis. $\times 215$. 12, *E. asplenifolia*. Lower epidermis. $\times 215$. 13, *E. appendiculata*. Lower epidermis. $\times 215$.

$2n=22$) synthesised from the cross between *Phaseolus aureus* ($2x=2n=22$) and *P. trilobus* ($2x=2n=22$) of the family Papilionaceae showed sufficient bivalent pairing (39.9%) in PMCs at metaphase I, even though later resulted in underdeveloped hybrid seeds and hybrid mortality in F_1 (Dana 1966). In spite of high percentage of sterility in the hybrid synthesised from *Emilia sonchifolia* and *E. coccinea* (Compositae) the presence of bivalents and multivalents besides univalents, bridges, and fragments in the hybrid is clear indication of structural homology between the constituent genomes (Omotoye 1973).

Bhavanandan (1977, 1985) has reported two natural hybrids of allopolyploid origin with little intergenomic pairing in two leptosporangiate ferns, *Tectaria paleocnemoids* and *Bolbitis quoyana*. The constituent genomes in *T. paleocnemoids* ($5x=2n=205$) were reported to be highly non-homologous in the sense that its spore mother cells showed 170–180 univalents and 10–15 bivalents at metaphase I and degenerated without completing the subsequent stages of meiosis. *Bolbitis quoyana* was shown to be an allotriploid hybrid ($3x=2n=123$) with the genome constitution AAB in which he (Bhavanandan 1985) has suggested that this hybrid has originated by the fusion of a $2n$ gamete of a diploid species (AA) and an 'n' gamete from another species (BB). The AA genomes in this hybrid complement formed 41 bivalents while the chromosomes in the B genome remained as 41 univalents.

Instances of complete bivalent formation in the synthesised hybrids ($2n=24$) of spinous Solanums have been reported by Kirti and Rao (1978, 1980). Appearance of 12 bivalents in 22.8 and 76.36% of the total PMCs analysed at diakinesis and metaphase I in the hybrids of *Solanum integrifolium* ($2n=24$) and *S. surattense* ($2n=24$) (Kirti and Rao 1978) and *S. indicum* ($2n=24$) and *S. integrifolium* ($2n=24$) (Kirti and Rao 1980) respectively is the clear indication that the parental species have retained sufficient ancestral homologies to permit intergenomic pairing in the F_1 heterozygotes.

The present hybrid showed sufficiently large percentage (82.8%) of bivalent pairing, 30–34 bivalents, as against 41 bivalents in *Egenolfia* species. The chromosome pairing suggests the persistence of homologous chromosomes in the hybrid complements. The spore sterility (60%) in the hybrid may be due to irregular behaviour of unpaired chromosomes at anaphase I and II and micronuclei formation.

It is suggested that the present hybrid has originated spontaneously from *E. appendiculata* and *E. asplenifolia*. The hybrid has more resemblance to *E. asplenifolia* in the size and shape of the pinnae, subsidiary cells etc. It is suggested that *E. asplenifolia* is likely to be the female parent and *E. appendiculata* the male parent. It is possible that these two parents have had a common origin, and cryptic structural changes and other mutations have led them into distinct species, but still retaining enough chromosomal homologies which facilitate chromosome pairing in their hybrid derivative.

Summary

Cytological and morphological studies in a natural diploid hybrid of *Egenolfia* have been carried out. The hybrid showed irregular meiosis with the formation of bivalents and univalents at metaphase I and micronuclei at telophase I and II and in the tetrads. The hybrid showed close resemblance with two diploid species of *Egenolfia*, *E. appendiculata* and *E. asplenifolia* which showed normal bivalent formation during meiosis. It is suggested that *E. appendiculata* and *E. asplenifolia* would be the putative parents of the present hybrid taxon. The high percentage of bivalent formation (82.8%) in the hybrid is suggestive of the persistence of sufficient chromosomal homologies in the parents which facilitate appreciable chromosomal pairing in the hybrid derivative.

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References

- Abraham, A., Ninan, C. A. and Mathew, P. M. 1962. Studies on the cytology and phylogeny of the Pteridophytes VII. Observations on one hundred species of South Indian ferns. *J. Ind. Bot. Soc.* **41**: 339–421.
- Bhavanandan, K. V. 1977. Studies on the cytology and gametophytes of South Indian Aspidiaceae. Ph. D. Thesis, University of Kerala.
- 1985. Cytological studies on *Bolbitis quana* (Gaud.) Ching. *Cytologia* **50**: 453–459.
- Copeland, E. B. 1947. *Genera Filicum*. Waltham, Mass., Chronica Botanica Co.
- Dana, S. 1966. Species cross between *Phaseolus aureus* Roxb. and *P. trilobus* Ait. *Cytologia* **31**: 176–187.
- Holtum, R. E. 1949. The classification of ferns. *Biol. Rev.* **24**: 267–296.
- Kirti, P. B. and Rao, B. G. S. 1978. Meiosis in the inter specific hybrid of two spinous *Solanums* and its bearing on their affinities. *Curr. Sci.* **47**: 696–697.
- and — 1980. Chromosome pairing in reciprocal hybrids of *Solanum integrifolium* and *S. indicum* var. *multiflora*. *Caryologia* **33**: 289–294.
- Omotoye, O. 1973. Meiotic studies on diploid hybrids between *Emilia sonchifolia* and *E. coccinea* (Compositae). *Cytologia* **38**: 725–729.
- Tanaka, R. and Shimotomai, N. 1968. A cytogenetic study on the F₁ hybrid of *Chrysanthemum makinoi* × *C. vulgare*. *Cytologia* **33**: 241–245.
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