Cytology of the Genus *Selaginella*

P. I. Kuriachan

Department of Botany, University of Kerala,
Trivandrum, India

Received May 18, 1963

Introduction

The ancient heterosporous genus *Selaginella* includes over 800 species (Manton 1950), distributed mostly in the tropics. *Selaginella* has been found to be a very difficult cytological material owing to the extreme smallness of the spore mother cells (Graustein 1930, Manton 1950). Compared to the other groups of Pteridophytes, only very little work has been done on the cytology of this genus and so far the chromosome numbers of only eight species are known. Cytological investigations on a number of species of *Selaginella* indigenous to South India have been made during the present study and this report embodies the results on eight species.

Previous work

Heinsen (1894) studied the megaspore and female gametophyte of *Selaginella*, but failed to observe any case of mitotic division either in spores or in vegetative tissues and as he saw elongated nuclei he concluded that amitotic division was possible. Denke (1902) reported the haploid chromosome numbers in *S. emmeliana* ($n=8$) and *S. serpens* ($n=8$). Heitz (1926) observed $n=8$ in *S. vogelii*, $n=10-11$ in *S. ouvrardii* and $n=26-28$ in *S. martensii*. Manton (1950) studied the cytology of three European species viz. *S. spinulosa* ($2n=18$ and $n=9$), *S. helvetica* ($2n=18$) and *S. denticulata* ($2n=18$ and $n=9$).

Material and methods

Species of *Selaginella* were found to thrive in plains and high altitudes in South India. Most of the species examined during this study occur at high elevations in the Western Ghats like Kodaikanal (6,000 ft.), Munnar (4,500 ft.), Ponmudi (3,000 ft.) and Muthukuzhi (2,500 ft.) and fixation of materials for cytological studies was made from wild collections in such cases. However, in the case of a few, only garden materials of unknown origin were available. Verification of the species determinations was done at the Botany Department, British Museum of Natural History.

Sporangia for meiotic studies were fixed in Carnoy's fluid (3:1:1; absolute alcohol, acetic acid and chloroform). Somatic chromosome counts were

made from root tip cells or rhizophores fixed in Carnoy's fluid after a pre-fixation treatment in melting ice for 2-3 hours. A fixation period of 10-15 days was found to yield best results both in meiotic and mitotic studies. A trace of iron acetate added to the fixative increased the stainability of the chromosomes.

Observations

1. *S. gracilis* Moore

Materials of this species were collected from Munnar. Ten bivalents were clearly observed at diakinesis in the spore mother cells (Fig. 1). This was further confirmed by somatic counts from root tip cells which showed 20 chromosomes (Fig. 2).

2. *S. wallichii* (Hook. and Grev.) Spring

This was collected from Muthukuzhi. This species also showed 20 chromosomes in root tip cells (Fig. 3) and 10 bivalents in spore mother cells. Meiosis was normal except for the delayed separation of a bivalent at anaphase I in most of the spore mother cells.

3. *S. braunii* Bak.

This was collected from the Trivandrum Public Gardens. The spore mother cells showed 10 chromosomes each at metaphase II of meiosis (Fig. 4).

4. *S. plana* (Desv.) Hieron

Materials of sporangia and root tips were obtained from plants grown in the Trivandrum Public Gardens. The haploid and diploid numbers were observed to be 10 and 20 respectively.

5. *S. crassipes* Spring

This species is very common in the plains. It grows vigorously in the rainy season and dries up in the summer. Materials used for this study were collected from Trivandrum. Nine bivalents could be seen clearly in the spore mother cells at metaphase I (Fig. 5).

6. *S. involvens* (Sw.) Spring emend Hieron

This species was collected from Kodaikanal. Nine bivalents were observed at metaphase I of spore mother cells (Fig. 6).

7. *S. willdenovii* (Desv.) Bak.

Sporangia and root tips for cytological study were obtained from materials collected from Ponmudi. Root tip preparations showed 18 chromosomes at metaphase (Fig. 7) and 9 bivalents were observed in spore mother cells.

8. *Selaginella* sp. (unidentified)

This species was collected from Kodaikanal. Mitotic study showed 36 chromosomes in the root tip cells (Fig. 8) and meiotic counts at diakinesis of spore mother cells showed 18 bivalents (Fig. 9).

The somatic chromosomes of all the above species of *Selaginella* were found to be very small and their length varies from 2.6 μ (largest ; observed

Figs. 1-9. $\times 1,500$. 1, diakinesis in a spore mother cell of *Selaginella gracilis* Moore showing 10 bivalents. 2, somatic metaphase in root tip cell of *Selaginella gracilis* Moore showing 20 chromosomes. 3, somatic metaphase in a root tip cell of *Selaginella wallichii* (Hook and Grev.) Spring showing 20 chromosomes. 4, second meiotic metaphase in a spore mother cell of *Selaginella braunii* Bak. showing 10 chromosomes in each group. 5, first meiotic metaphase in a spore mother cell of *Selaginella crassipes* Spring showing 9 bivalents. 6, first meiotic metaphase in a spore mother cell of *Selaginella involvens* (Sw.) Spring emend Hieron showing 9 bivalents. 7, somatic metaphase in a root tip cell of *Selaginella willdenovii* (Desv.) Bak. showing 18 chromosomes. 8, somatic metaphase in a root tip cell of *Selaginella* sp. showing 36 chromosomes. 9, diakinesis in a spore mother cell of the above species showing 18 bivalents.

in *S. gracilis*) to 0.8μ (shortest; observed in the unidentified species with $2n=36$). Morphological details of the somatic chromosomes could not be studied as the positions of centromeres were not clearly visible.

Comments

Manton (1950) reported the chromosome numbers in three of the European species, *S. spinulosa* ($2n=18$ and $n=9$), *S. helvetica* ($2n=18$) and *S. denticulata* ($2n=18$ and $n=9$); all of them being diploids on the basic chromosome number 9. Denke (1902) and Heitz (1926) reported haploid numbers like 8, 10-11 and 26-28 in the genus *Selaginella*. However, the present study clearly indicates the presence of two basic chromosome numbers 9 and 10, in the genus. Abraham *et al.* (1962) have given conclusive evidence to prove that in the South Indian leptosporangiate ferns, the most primitive species have the highest basic numbers and that the trend has been towards a reduction in basic chromosome numbers. Among the primitive Pteridophytes the heterosporous genus *Isoetes* also exhibits an aneuploid change towards a reduction in basic chromosome number from 11 to 10 (Abraham and Ninan 1958, Ninan 1958). In the case of *Selaginella* also the trend seems to be towards a reduction in chromosome number and if so the basic number 10 is to be considered as primitive and 9 as an aneuploid derivative. However, confirmatory evidence for this from karyotype analysis of the different species could not be furnished owing to the extreme smallness of the chromosomes which did not show morphological details clearly.

The unidentified species of *Selaginella* with $n=18$ and $2n=36$ is a tetraploid on the basic number 9. Meiosis in this species was regular with the formation of 18 well spread out bivalents at metaphase I and normal anaphase I separation, which is suggestive of allopolyploidy. Graustein (1930) reports a high rate of hybridity in the genus. It might be that the above tetraploid species arose by hybridization followed by chromosome doubling.

The evolution within the genus *Selaginella* has been attributed to genic changes (Manton 1950) and genic recombination by interspecific hybridization (Graustein 1930). The present study has clearly demonstrated that aneuploidy and polyploidy (allopolyploidy) were also active mechanisms of speciation in the genus. However, the presence of such a large number of species forming a remarkably compact taxonomic unit suggests that various intrachromosomal changes which might have occurred in the long history of the genus were also responsible for speciation in the genus.

Summary

1. Cytological investigations on 8 species of *Selaginella* (including an unidentified species) indigenous to South India were conducted. The chromosome numbers in the species studied are as follows: *S. gracilis* ($n=10$ and

2n=20), *S. wallichii* (n=10 and 2n=20), *S. braunii* (n=10), *S. plana* (n=10 and 2n=20), *S. crassipes* (n=9), *S. involvens* (n=9), *S. willdenovii* (n=9 and 2n=18), *Selaginella* sp. (unidentified) (n=18 and 2n=36).

2. All the species studied except the unidentified species are diploids on basic number 9 or 10. The unidentified species is a tetraploid based on the number 9.

3. Aneuploid change of chromosome number (from 10 to 9 as observed in this study), hybridization followed by chromosome doubling, and probably accumulation of intrachromosomal changes have been suggested to be operative in speciation of the genus *Selaginella*.

Acknowledgment

The author is indebted to Prof. A. Abraham, Head of the Department of Botany, University of Kerala, Trivandrum for valuable guidance and encouragement. His thanks are also due to the Ministry of Education, Government of India, for the award of a Senior Research Scholarship.

References

- Abraham, A. and Ninan, C. A. 1958: Cytology of *Isoetes*. Curr. Sci. 27: 60-61.
- , —, 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. Indian Bot. Soc. 41: 339-421.
- Denke, P. 1902. Sporenentwicklung bei *Selaginella*. Beih. Bot. Centralbl. 12: 182-199.
- Graustein, Jeannet E. 1930. Evidence of hybridism in *Selaginella*. Bot. Gazette 90: 40-74.
- Heinsen, E. 1894. Die Makrosporen und weibliche Prothallium von *Selaginella*. Flora 78: 466-496.
- Heitz, E. 1926. Cited from Manual of Pteridology. The Hague.
- Manton, I. 1950. Problems of cytology and evolution in the Pteridophyta. Cambridge University Press.
- Ninan, C. A. 1958. Studies on the cytology and phylogeny of the Pteridophytes V. Observations on the Isoetaceae. J. Indian Bot. Soc. 37: 93-103.
- Verdoorn, F. 1938. Manual of Pteridology. The Hague.
-