

THE CHROMOSOMES OF *OPHIOGLOSSUM RETICULATUM* L.

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MANTON² in her pioneering work on the "Problems of Cytology and Evolution in the Pteridophyta" has shown that by a combination of modern cytological technique and the necessary manipulative skill and patience, the chromosomes of this group of plants can be studied and may well yield information of great value to the student of evolution. Taking advantage of the wealth of uninvestigated material of this group available in South India a cytogenetical study of this was started recently.

The purpose of this paper is to draw attention to certain striking observations on the cytology of *Ophioglossum reticulatum* L. The plants used in the study were all collected in the wild condition from four localities in South India and grown in pots in a fern house. The cytological and photographic technique followed were essentially similar to what was adopted by Manton.

collection showed 572 bivalents. The difference probably indicates the margin of error to be expected in determinations of such high numbers, though counts were made from very clear preparations and every care was taken to avoid errors.

Second material (from Parambikulam, 200 miles north of Trivandrum, in dense forest at an elevation of about 2,000'). One plant from this collection showed 566 bivalents at first meiotic metaphase, and this count agrees very closely with the first count of 564 bivalents from the Trivandrum material. But count from another plant from the same collection, and from a preparation which was exceptionally clear showed 631 bivalents and what appeared to be 10 fragments (or very small univalents?) (Figs 1 and 2). This shows that the somatic cells of the sporophyte of this plant have over 1,260 chromosomes, the highest chromosome number yet discovered in any species. Attempts were made to get a somatic chromosome count from archesporial mitosis, and preparations

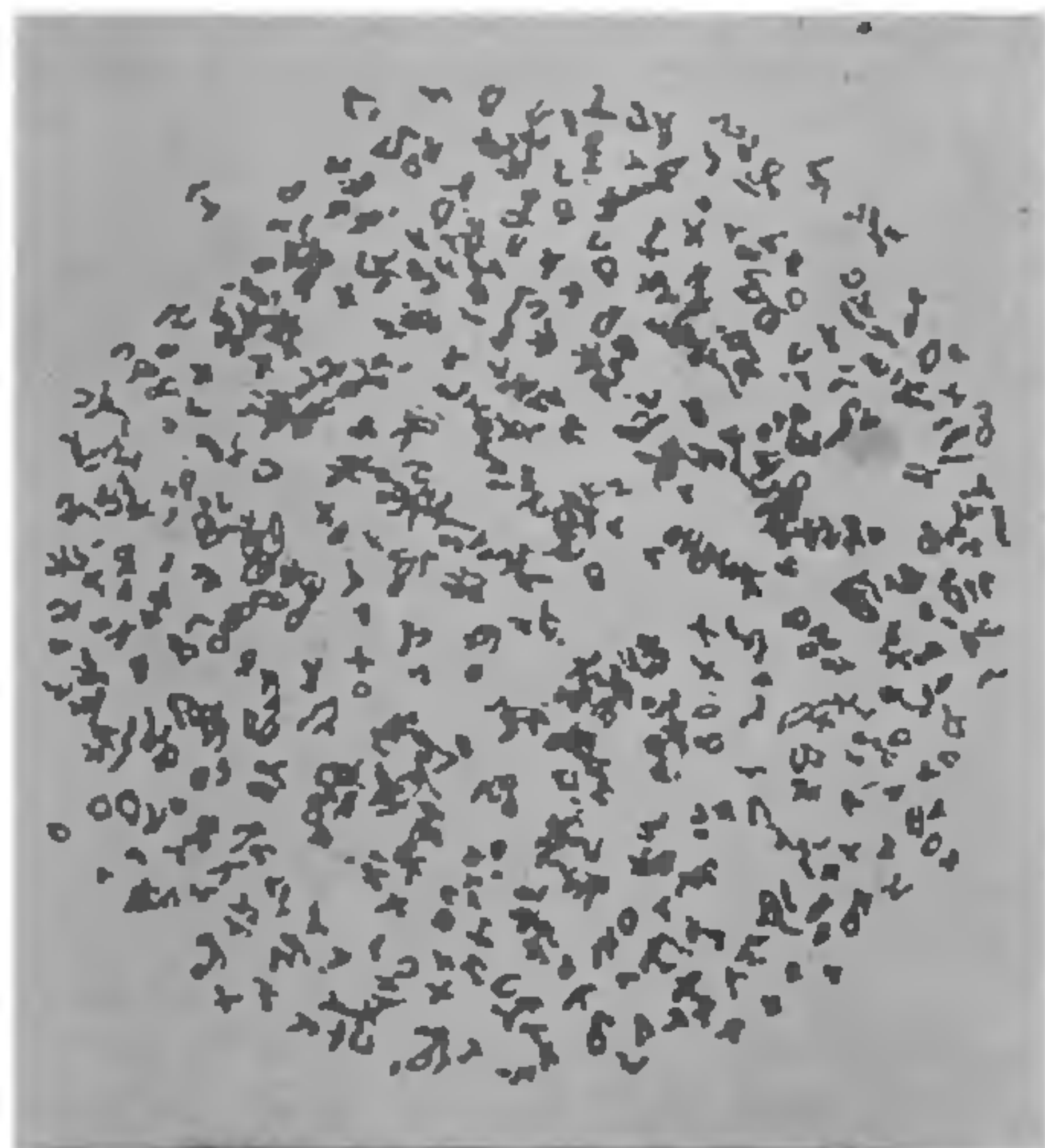


FIG. 1. Photomicrograph showing the first meiotic metaphase chromosomes in *Ophioglossum reticulatum*. (Parambikulam material). There are 631 bivalents and 10 fragments, $\times 500$.

First material (from Trivandrum City, sea coast). The spore mother cells clearly showed 564 bivalents at metaphase of first meiotic division. Count from another plant of the same



FIG. 2. Explanatory diagram of the same metaphase plate shown in Fig. 1, made on enlarged photographic print, and reduced to same size in reproduction.

showing over 1,000 chromosomes in a single cell at metaphase were obtained.

Third material (from Ponmudi hill top, 40 miles north-east of Trivandrum at 4,000' elevation, growing in exposed grassland). The

plants from this locality showed smaller rhizomes, and the sterile part of the sporophyll was a little broader and the apex more rounded compared with specimens from the first two localities. A clear count of 451 bivalents was made in this plant. Also plants from this collection showed the largest chromosomes so far seen in this species.

Fourth material (from Ettapadappu, about 12 miles north-east of Ponmudi, in dense forest at 1,000' elevation). Plants from this area showed 436 bivalents at first metaphase of meiosis, the lowest chromosome number so far encountered in *O. reticulatum*.

In the course of this study we have examined several metaphase plates in preparations made from plants of each of the four localities, and have not so far seen any evidence of multivalent formation. The size of the chromosomes is appreciably large for a cell having such an extraordinarily large number. The spiral structure of the chromosomes is clearly visible in some preparations, and sometimes one component of a bivalent appears longer than its homologue on account of unequal relaxation of the spirals. From comparison of several counts in each material we are inclined to believe that the margin of error in determination of numbers will be under 2 per cent. The observed numbers in *O. reticulatum* from four different localities vary from 436 to 631 bivalents, indicating a range between 872 and 1262 chromosomes in the sporophytic tissue.

Two points deserve consideration in this connection.—(1) the mode of origin of such a large number, and (2) the significance, if any, of the difference in numbers observed in materials of the same species and of closely related species from different geographical regions. A survey of the known chromosome numbers in seed plants¹ shows that higher numbers have evolved from smaller numbers by polyploidy. That more or less the same process has been operative also in the evolution of the lower vascular plants is seen from Manton's observations.² That a polyploid series exists in the genus *Ophioglossum* is evident from Manton's record of $n = 128$ in *O. lusitanicum* and $n = 256$ in *O. vulgatum*. Both species showed only bivalents at meiosis, and in the absence of record of any lower numbers in the genus it was difficult to decide the basic number. The present observations show that while *O. vulgatum* from Manchester has a diploid chromosome number of over 500, the Parambikulam material of *O. reticulatum* has more than 1,260 chromosomes. It seems that allo- or auto-polyploidy or a combination of the two may have

been operative in this genus at some stage in its long history. But the fact that only bivalents are formed at meiosis, whether the somatic number is 256, 512 or 1,262, makes it difficult to decide this question. There is the possibility that these are very ancient polyploids, which in the course of thousands or millions of years might have through the accumulation of genic changes become functionally diploids. And any disadvantage arising from such a multiplication of chromosomes may have been counterbalanced by the parallel attainment of a vegetative mode of reproduction through root buds. Again, it is not improbable that our currently accepted ideas regarding multivalent formation and secondary associations, based largely on observations on favourable angiosperms with small number of medium- or large-sized chromosomes do not hold good in every respect as far as plants with such large numbers and consequently small-sized chromosomes are concerned. A re-examination of these concepts may be necessary in the light of observations on these ancient plants.

It is of interest to enquire whether there is any relationship between chromosome numbers and geographical distribution. If we accept the idea that generally higher numbers are derived from smaller numbers in the course of evolution, it follows that within the same species material from the older strata of the earth's crust, which have longest escaped great geological upheavals would show higher chromosome numbers compared with materials of the same from relatively more recent formations. This relationship may possibly hold good for closely related species as well. The peninsular part of India, comprising the whole of South India, constitutes one of the oldest of such land masses, and it may be expected that in species indigenous to this region higher levels of polyploidy may be seen within any group of closely related species as compared with materials of the same from later formations. An extensive and careful investigation of the tropical Pteridophytes may provide some answer to this and related problems of evolution.

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1. Darlington, C. D. and Janaki Ammal, E. K., *Chromosome Atlas of Cultivated Plants*, George Allen and Unwin, London, 1945.
2. Manton, I., *Problems of Cytology and Evolution in the Pteridophyta*, Cambridge, 1950.