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The Cytology of Athrotaxis

By

HEATHER F. GULLINE

Botany Department, University of Tasmania

WITH 1 TEXT FIGURE

SUMMARY

On the grounds of morphology and distribution, it has been suggested that *Athrotaxis laxifolia* is a hybrid between *A. cupressoides* and *A. selaginoides*. To test this hypothesis the cytology of the genus was investigated. All three species of *Athrotaxis* have 2n = 22 chromosomes in somatic cells and n = 11 in pollen grains. Meiosis is normal in all three species. No conclusive evidence that *A. laxifolia* is a hybrid was found. A final decision on the taxonomic status of *A. laxifolia* must await the results of breeding experiments.

1. INTRODUCTION

The genus *Athrotaxis* Don (1839) is an endemic Tasmanian genus of the family Taxodiaceae (Coniferae). Three species have been described. Two of these, *A. cupressoides* Don ('Pencil Pine') and *A. selaginoides* Don ('King Billy Pine') are markedly different from one another and plentiful in their native habitats, often forming pure stands. The third, *A. laxifolia* Hooker, is intermediate in size of leaves and cones between the first two species, and is comparatively rare. It is usually found as isolated trees near stands of one or both of the other two species.

These facts have given rise to the opinion that *A. laxifolia* is a hybrid between *A. cupressoides* and *A. selaginoides* (R. E. Smith, unpub.). In order to test this theory and also to provide further evidence on the relationships between the genera of the Taxodiaceae (Stebbins, 1948), I have investigated the cytology of all three species.

The life-histories of *A. selaginoides* and *A. cupressoides* have been studied by Saxton and Doyle (1929) and Elliott (1951) respectively, but no account of *A. laxifolia* or any observations on the chromosomes of the genus have been published.

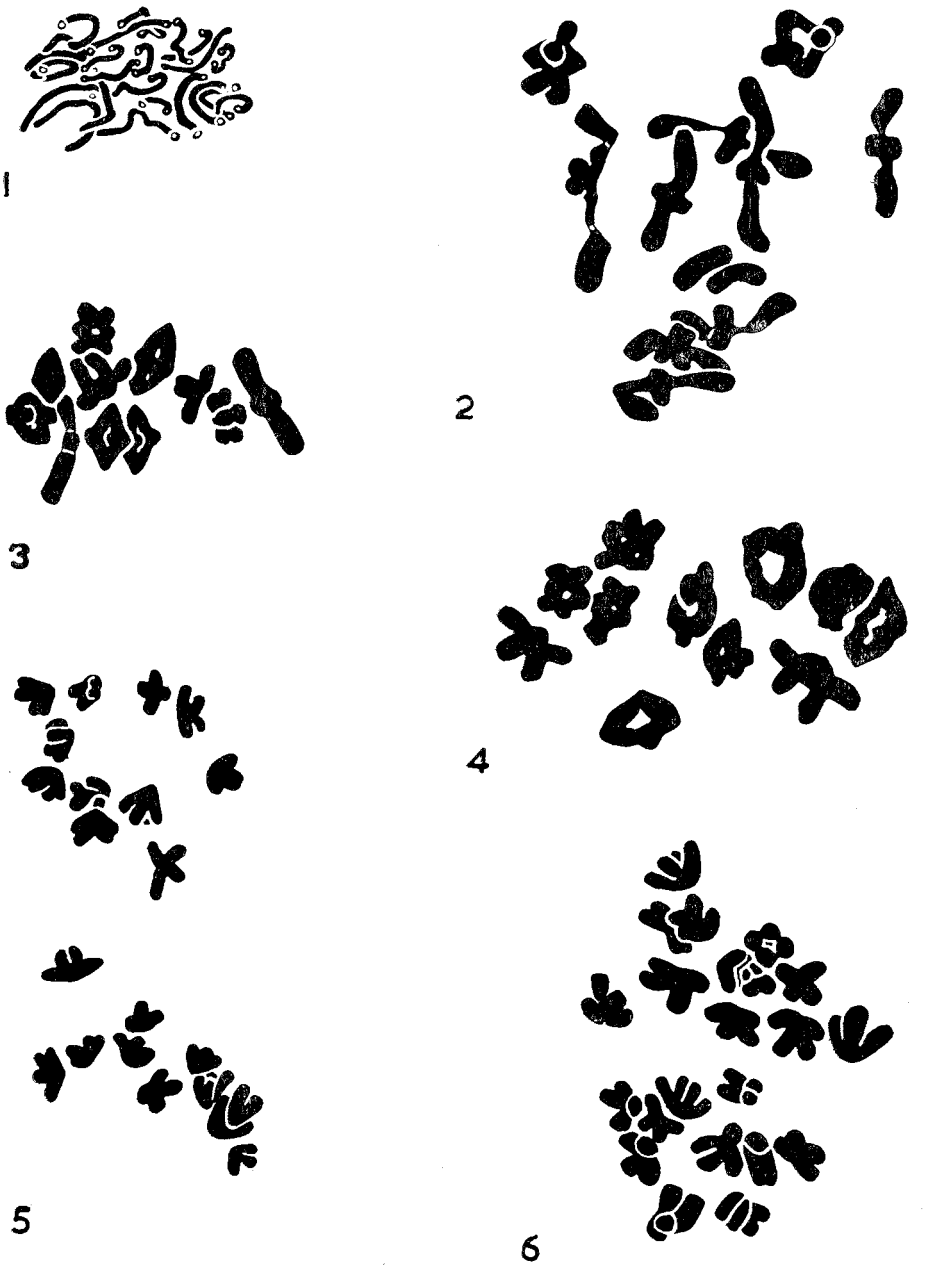

FIG. 1.—Mitotic metaphase in root tip of *Athrotaxis selaginoides*.
FIG. 2.—First meiotic metaphase in p.m.c. of *A. cupressoides*.
FIG. 3.—First meiotic metaphase in p.m.c. of *A. laxifolia*.
FIG. 4.—First meiotic metaphase in p.m.c. of *A. selaginoides*.
FIG. 5.—First meiotic anaphase in p.m.c. of *A. laxifolia*.
FIG. 6.—First meiotic anaphase in p.m.c. of *A. cupressoides*.
p.m.c., pollen mother cell.

Reports on chromosome studies of the other members of the family Taxodiaceae have been published by a number of authors. (See Table 1). It would appear that the remaining two genera, *Cunninghamia* and *Glyptostrobus*, have not been counted.

TABLE 1

Chromosome Numbers in the Taxodiaceae

Species	Chr. No.	Author
Athrotaxis cupressoides	2n = 22	Gulline (this paper)
Athrotaxis laxifolia	2n = 22	Gulline (this paper)
Athrotaxis selaginoides	2n = 22	Gulline (this paper)
Cryptomeria japonica	2n = 22	Sax and Sax (1933)
Metasequoia glyptostroboides	2n = 22	Stebbins (1948)
Sciadopitys verticillata	2n = 20	Tahara (1937) (cited by Stebbins, 1948)
Sequoia sempervirens	2n = 66	Stebbins (1948)
Sequoiadendron giganteum	2n = 22	Buchholz (1939, *a*, *b*) ; Jensen and Levan (1941)
Taiwania cryptomerioides	2n = c. 22	Sax and Sax (1933)
Taxodium distichum	2n = 22	Stebbins (1948)

2. Materials and Methods

A summary of the localities from which material of each species was obtained and the stages observed in that material is given in Table 2.

Cuttings of *A. cupressoides* and *A. laxifolia* rooted after being kept in moist sand, with bottom heat, for three months. Root tips were squashed and stained in aceto-orcein or fixed in 2BE, sectioned at 20μ and stained in crystal violet.

Branchlets of all species bearing young male cones were collected from as many localities as possible from May 13th 1951 onwards. It was found possible to keep these alive in jars of water in the laboratory where the development of the young cones progressed at a more rapid rate than in trees growing in their natural habitat. Thus meiosis was first observed in the laboratory some six weeks before it occurred in the field. The microsporangia were squashed and stained in aceto-orcein.

Details of the methods used are given by Darlington and La Cour (1947).

Drawings were made with a camera lucida at an initial magnification of 3150X and reduced to 1575X for publication.

TABLE 2

Materials and Localities

Species	Locality	Material	Stages Observed
A. cupressoides	National Park	Seedlings	Mitosis in root tips
	National Park	Branchlets	P.m.c. meiosis to mature pollen
	Pine Valley	Branchlets	P.m.c. meiosis to mature pollen
	Hartz Mountains	Branchlets	Mature pollen
A. laxifolia	National Park	Cuttings	Mitosis in root tips
	National Park	Branchlets	P.m.c. meiosis to mature pollen
	Cradle Mountain	Branchlets	P.m.c. meiosis to mature pollen
A. selaginoides	National Park	Branchlets	P.m.c. meiosis to mature pollen
	Wyld's Craig	Seedlings	Mitosis in root tips
	Cradle Mountain	Branchlets	P.m.c. meiosis to mature pollen
	Hartz Mountains	Branchlets	Mature pollen
	Snowy Mountains	Branchlets	Mature pollen

Abbreviation:—P.m.c., pollen mother cell.

3. THE CYTOLOGY OF *Athrotaxis*

The resting nuclei of *Athrotaxis* regularly have two nucleoli. At mitotic metaphase, 22 chromosomes can be counted in the vegetative cells of all three species. The chromosomes are all long and rod-shaped with median or sub-median centromeres (fig. 1). The chromosomes cannot be accurately measured in sectioned material as they rarely orientate themselves completely in the plane of the equatorial plate. The arms are usually lying parallel to the long axis of the cell. Squashes proved unsatisfactory because of the large amounts of resinous materials present.

The course of meiosis is identical in all three species (figs. 2, 3, 4). At first metaphase, the 22 chromosomes almost always form 11 pairs with 1-3, usually two, chiasmata. Failure to pair was observed in one plant only, of *A. cupressoides* (see fig. 2). The appearance of the bivalents is exactly like that of other Conifers illustrated by Sax and Sax (1933). The chiasmata are usually interstitial or sub-terminal. The frequency of chiasmata per pollen mother cell is given in Table 3. This chiasma frequency is lower than that for many other Conifers (Sax and Sax, 1933). In each species the figures were calculated from 10 cells, i.e., 110 bivalents.

At first anaphase, the chromosomes segregate regularly, and the median or submedian positions of the centromeres become obvious (figs. 5, 6). There is a short resting stage between the first and second divisions, during which it can be seen that the cytoplasm has divided into two parts but no cell wall is formed between the two daughter nuclei. The second division results in the formation of four haploid nuclei arranged tetrahedrally within the pollen mother cell wall. The young microspores then develop cell walls and before shedding undergo a division so that at maturity each pollen grain is bicellular, as described by Elliott (1951). During 1951, in *A. cupressoides* meiosis occurred in the field in mid-July, and pollen was shed in early September, about six weeks later. The corresponding stages occurred in *A. laxifolia* approximately a week later, and in *A. selaginoides* about four weeks later, than in *A. cupressoides*. The mature pollen grains are spherical and show no obvious differences between the three species. The fertility of the pollen is given in Table 3. The estimates are based on counts of 200-300 pollen grains from mature cones of one tree of each species.

TABLE 3

Species	P.M.C. Meiosis		% Pollen Fertility
	Chiasmata per cell	Mean Chiasmata per bivalent	
A. cupressoides	21·00 ± 0·39	1·91	95·0
A. laxifolia	20·90 ± 0·40	1·90	87·73
A. selaginoides	21·20 ± 0·32	1·93	96·7

4. Relationships Within the Taxodiaceae

The basic number of chromosomes found almost uniformly throughout the Taxodiaceae is x = 11. Comparison of illustrations of the chromosomes of *Athrotaxis* (this paper), *Cryptomeria japonica* (Dark, 1932), *Metasequoia glyptostroboides* (Stebbins, 1948), and *Sequoiadendron giganteum* (Jensen and Levan, 1941) shows that the group is a very uniform one in size and morphology, as well as in number, of chromosomes. It appears that in the course of evolution, the differentiation of the genera has progressed chiefly through gene mutations unaccompanied by changes in the number or gross morphology of the chromosomes.

A notable exception to this generalisation is *Sequoia sempervirens* which is a polyploid with 6x = 66 chromosomes. On the basis of vegetative characters, Stebbins (1948) put forward the theory that it is descended from a cross between an ancestor of the diploid *Metasequoia* and another species closely related to either *Sequoiadendron, Taiwania,* or *Athrotaxis.* The chromosome numbers of these genera make this relationship theoretically possible. The present view of paleontologists, however, (Florin, 1940) is that *Athrotaxis* has always been restricted to the Southern hemisphere, and all the other Taxodiaceae to the Northern hemisphere. It is therefore improbable that *Athrotaxis* has taken any part in the evolution of *Sequoia sempervirens.*

5. The Status of *Athrotaxis laxifolia*

It is difficult to reach any conclusion on the origin of *A. laxifolia* from cytological studies alone. As can be seen from the text-figures the chromosomes of *A. cupressoides* are indistinguishable from those of *A. selaginoides.* Thus *A. laxifolia* would be expected to have 22 long, metacentric chromosomes whether it is a hybrid between the two other species or a " good " species of independent origin.

The course of meiosis in *A. laxifolia* is similar to that of any normal diploid plant. The frequency of chiasma formation is not significantly different from that of the other species and the chromosomes segregate normally at anaphase. The pollen resulting from the pollen mother cell divisions also appears normal in all respects. This is evidence, although not conclusive, that *A. laxifolia* is not a hybrid. It is possible for a hybrid between two closely related species to undergo meiosis in a normal manner and to produce fertile pollen (Darlington, 1937). Sax and Sax (1933) record that many Conifer species hybrids show regular chromosome pairing, normal chiasma frequency at meiosis and a high degree of fertility. It is however possible that many of these " species " should properly be regarded as geographical subspecies.

In the field, *A. cupressoides* and *A. selaginoides* are found in different ecological habitats, although not necessarily growing further apart than their wind-borne pollen could be carried to effect a cross between them. However, in the season these observations were made, the flowering seasons of these species did not overlap. It is possible that in other years, altitudinal variations in flowering time might make natural hybridisation possible.

It is known that *A. laxifolia* forms female cones which develop to maturity and dehisce, but no records of the viability of the seed or of any progeny are known to the author. Final proof whether *A. laxifolia* is a hybrid or not can be made only by re-creating the species artificially or observing the occurrence of segregation of specific characters of *A. cupressoides* and *A. selaginoides* in the progeny of *A. laxifolia*.

6. Acknowledgments

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