CYTOLOGICAL STUDIES IN THE TASMANIAN CONIFERS, THE INDIAN SCILLA AND DIPCADI

Thesis

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by

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PREFACE.

There has been a considerable speculation with regard to the systematic and phylogenetic position of the Tasmanian endemic Conifers in the so-called "Taxares" and "Pinares". Restricted to alpine terrains at an altitude of 3,500-4,500ft., they bear a superficial resemblance, although belonging to different families *per se*. In any scheme of Conifer phylogeny based on external characters alone, allocation of these genera to their proper systematic position would be difficult. Previous cytological studies were wholly confined to the reports of chromosome numbers of two genera. In the first part of this dissertation an attempt has been made not only to provide detailed information about their karyotypes but also to critically evaluate and integrate the karyological data with the available knowledge of their comparative morphology. The ultimate object is to assess the phylogenetic status of the Tasmanian Conifers. Two methods that enabled a critical study of their somatic chromosomes have been outlined in the second part.

The probable trends in evolution of the two Indian Liliaceae namely *Dipcadi* and *Scilla* have so far not received any detailed consideration. The species of *Dipcadi* exhibit considerable convergence in their morphological characters; the area of the genus is wide and at the same time highly disjunct; several of its species are endemic. Providing a cytological basis for all these features has been the aim of the third paper. The mode of origin of the different cytotypes, the prevailing differences between the local populations in their meiotic behaviour, the potentialities that are in store for further evolution and speciation within a collective species like *Scilla indica* have been outlined in the fourth section. The variation in the heterochromatin content of the different individuals of *S. hohenackeri* has also been appended to the same paper.

The writer is indebted to Professor H.N. Barber for suggesting some of the above mentioned problems and for advice and helpful criticism during the course of the work. His thanks are duesto all those in the department who co-operated with him and made the work possible. The award of a Colombo Plan Fellowship by the Commonwealth of Australia, which enabled the writer to undertake the work, is gratefully acknowledged. He is also thankful to Miss Margaret Symons, who spared no pains in neatly typing the manuscript.

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CYTOTAXONOMY AND PHYLOGENY OF TASMANIAN CONIFERS.

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I. INTRODUCTION

The naturally occurring Tasmanian Conifers belong to 3 families, as they are comprehended by Pilger (1926). They are:

Podocarpaceae

> *Pherosphaera hookeriana* Hook.f. Archr.,
> *Microcachrys tetragona* (Hook.) Hook.f.,
> *Dacrydium franklinii* Hook.f.,
> *Podocarpus alpina* Hook.f.,
> *Phyllocladus aspleniifolius* (Labill.) Hook.f.;

Taxodiaceae

> *Athrotaxis cupressoides* Don.,
> *A. laxifolia* Hook.,
> *A. selaginoides* Don.,

Cupressaceae

> *Callitris tasmanica* (Benth.) Baker & Smith.,
> *C. oblonga* Rich.

In addition many others have been introduced into gardens and one *Pinus radiata* Don., the only representative of Pinaceae in Tasmania, is used commercially to a great extent in plantations. It has apparently been naturalized in certain localities, young seedlings appearing in profusion. It was introduced from California in about 1860. It belongs to a typical northern family.

All the foregoing families have had a tortuous taxonomic history either with regard to their position in the phylogenetic scheme of the so-called "Taxares" and "Pinares" or in the segregation of the families into smaller taxa. For instance, Podocarpaceae without *Phyllocladus*, which is now generally included in it, was regarded as the tribe Podocarpeae of Taxaceae by Eichler (1887). *Phyllocladus*, however, was included in the tribe Taxeae, along with such genera as *Ginkgo*, *Cephalotaxus*, *Torreya* and *Taxus*, which are now known to belong to different families and orders. Negar (1907) raised Eichler's Podocarpeae into the family Podocarpaceae but continued to group Phyllocladus with Taxus and Torreya in the family Taxaceae. In his classification of Taxaceae, Pilger (1903) recognised three sub-families namely, Podocarpoideae, Phyllocladoideae and Taxoideae. The same author (1926)later on reconstituted the first two subfamilies into Podocarpaceae as it is now understood and raised the status of Taxoideae into the Taxaceae. Further more, Cephalotaxus was removed from Taxoideae to form a monotypic Cephalotaxaceae. In Vierhapper's system (1910) the present Podocarpaceae formed a part of Taxocupressaceae, which had a large number of small tribes, each composed of one or two genera.

Unlike heterogeneous Podocarpaceae, Pinaceae has remained relatively uniform with little or no change in the taxonomic history. The present day Pinaceae corresponds to Eichler's (1887) tribe Abietinae, which was subsequently called Abietaceae by Negar (1910). Vierhapper (1910) included Araucariaceae in his Abietaceae, which was split into 3 subfamilies and a large number of tribes and subtribes. The inclusion of Araucariaceae in Abietaceae has not received approval from any taxonomist so far. Saxton (1913) was first to name Negar's Abietaceae as Pinaceae, with sub-families Abietoideae and Sciadopitoideae. The latter consists of Sciadopitys, which is now generally grouped in Taxodiaceae. Pilger's classification (1926) of the family Pinaceae with its subfamilies Pinoideae and Abietoideae are now generally used.

Eichler's (1859) tribe Taxodiinae and Negar (1910) Taxodiaceae are same with regard to generic composition. The second sub-family Taxodioideae of Taxocupressaceae in Vierhapper's system (1910) corresponds to Taxodiaceae of Pilger (1926). It is interesting to find that Saxton (1913) included Taxodiaceae in Cupressaceae. Whether it is justifiable or not, it shows that Saxton recognised the close relationship of these two families.

Similar to all the above-mentioned families Cupressaceae has been variously classified (Saxton, 1910, 1913 a, b; Pilger, 1926; Moseley, 1943; Id, 1953).

Apart from the general interests of the families mentioned above, the Tasmanian Conifers are unique in themselves and present problems both to the taxonomist as well as the plant geographer. Restricted to alpine and subalpine terrains at an altitude of 3,500 - 4,500 ft., they bear a superficial resemblance, although belonging to different families per se and have therefore lead to a great taxonomic confusion. In any sequence of Conifer phylogeny based on external characters alone, allocation of these genera to their proper systematic position would be difficult. Pherosphaera hookeriana is an admirable instance to illustrate the point in question. All Conifers native to Tasmania have suffered greatest synonymy and the whole taxonomic confusion is well summarised in the words of Hooker (1860) "We have come to the conclusion, that it will create the least perplexity to retain the name Microcachrys tetragona for the plant figured originally as Athrotaxis tetragona, and whose male flowers I originally described as Microcachrys; its small, regularly formed cone renders the name applicable. The name Pherosphera we transfer to the plant whose female flowers I confounded with Microcachrys, and whose male flowers being collected into almost globose amenta, will justify the appellation; and for the plant which Mr. Archer supposed to be my female Microcachrys, we propose the name Diselma, in allusion to the two ovuliferous scales". Athrotaxis belongs to Taxodiaceae, which finds a wide geographic distribution in the northern hemisphere. Its occurrence as a Tasmanian endemic is a great geographic anamaly. It would be a matter of considerable theoritical significance if a solution to all these problems is arrived at on the basis of exomorphic and endomorphic characters, of which cytology is the most important. There has been no cytological survey of Tasmanian Conifers except that of Culline (1952) and the three species of Athrotaxis and one species of Callitris (cf. Darlington and Wylie, 1955).

II. CHROMOSOME NUMBERS.

1. Podocarpaceae

Name of the species	n	2n	Median & Submedian	Sub-terminal	Terminal	SAT-chromosomes	Secondarily constricted chromosomes	Author
Phyllocladus aspleniifolius (Labill.) Hook.f.	-	18	16	2	-	-	-	This paper
Pherosphaera hookeriana Hook. f. non Archer	-	26	4	-	22	-	-	This paper
Microcachrys tetragona Hook.f.	-	30	10	-	20	2	-	This paper
Dacrydium franklinii Hook.f.	-	30	10	-	20	2(?)	-	This paper
Podocarpus								
Sect. Stachycarpus:								
Podocarpus falcatus R.Br.	-	24	4	20	-	-	-	Flory, 1936.
" "	-	24	8	16	-	-	-	Mehra and Khoshoo,1956b
P. gracilior Pilger.	12	24	2	14	8	-	-	Mehra and Khoshoo,1956b
P. andinus Pilger.	-	c.40	-	-	-	-	-	Flory, 1936
Sect. Eupodocarpus:								
P. acutifolius	19	-	-	-	-	-	-	Stiff, 1952.

Name of the species	n	2n	Median & Submedian	Sub-Terminal	Terminal	SAT-chromosomes	Secondarily constricted chromosomes	Author
P. nivalis Hook.f.	19	-	-	-	-	-	-	Stiff, 1952
" " Hook.f.	-	38	-	-	-	-	-	Snoad, 1952
P. latifolius R.Br.	11	22	10	8	4	-	-	Mehra and Khoshoo,1956b
P. neriifolius D. Don.	-	38	4	-	34	-	-	Flory, 1936
P. macrophyllus D. Don.	-	38-40	6	14	18	-	-	Flory, 1936
" "	-	38	-	-	-	-	-	Hirayoshi, 1942
" "	19	38	-	-	-	-	-	Tahara, 1941
" "	19	38	-	-	-	-	-	Mehra and Khoshoo,1956b
" as chinensis	-	40	-	-	-	-	-	Flory, 1936
P. alpina	19	-	-	-	-	-	-	Stiff, 1952
" " Hook.f.	-	38	-	-	38	2	-	This paper

2. Taxodiaceae.

Name of species	n	2n	Median & Submedian	Sub-Terminal	Terminal	SAT-chromosomes	Secondarily constricted chromosomes	Author
Sciadopitys verticillata Sieb. & Zucc.	-	20	-	-	-	-	-	Tahara, 1937
Athrotaxis selaginoides Don.	11	22	-	-	-	-	-	Gullino, 1952
" "	-	22	22	-	-	-	2	This paper
A. laxifolia Hook.	11	22	-	-	-	-	-	Gulline, 1952
" "	-	22	22	-	-	-	2	This paper
A. cupressoides Don.	11	22	-	-	-	-	-	Gullino, 1952
" "	-	22	22	-	-	-	2	This paper
Cryptomeria japonica (Linn.) Don.	11	-	11	-	-	-	-	Sax and Sax, 1933
" "	-	(44)	-	-	-	-	-	Zinnai & Chiba, 1951
" "	-	22	22	-	-	-	4 "inconspicuous"	Mehra and Khoshoo, 1956a
Cunninghamia lanceolata (Lamb) Hook.	11	-	-	-	-	-	"	Sugihara, 1941
" "	-	22	22	-	-	2	-	Mehra and Khoshoo, 1956a
Taiwania cryptomerioides Hayata	-	22	-	-	-	-	-	Sax and Sax, 1933

Name of the species	n	2n	Median & Submedian	Sub-Terminal	Terminal	SAT-chromosomes	Secondarily constricted chromosomes	Author
Taxodium distichum Rich. (?)	-	22	-	-	-	-	-	Sax and Sax, 1933
" "	-	22	-	-	-	-	-	Stebbins, 1948
T. mucronatum Tenore	-	22	22	-	-	-	2	Mehra and Khoshoo, 1956a
Metasequoia glyptostroboides	-	22	-	-	-	-	-	Stebbins, 1948
Sequoiadendron giganteum Buchholz.	-	22(44)	20	2	-	-	-	Jenson and Levan, 1941
Sequoia sempervirens Endl.	-	66	-	-	-	-	-	Hirayoshi and Nakamura, 1943.
" "	-	66	-	-	-	-	-	Stebbins, 1948

3. Pinaceae

sub family 2. Pinoideae.

Name of the species	n	2n	Median & Submedian	Sub-terminal	Terminal	SAT-chromosomes	Secondarily constricted chromosomes	Author
Pinus canariensis C.Sm.	-	24	24	-	-	-	-	Bowden, 1945
" "	-	24	24	-	-	-	3	Mehra and Khoshoo, 1956a
P. caribaea More.	-	24	24	-	-	-	2	Mehra and Khoshoo, 1956a
P. contorta Dougl.	-	24	24	-	-	-	-	Langlet, 1934
P. densiflora Sieb. & Zucc.	-	24	24	-	-	-	-	Hirayoshi, 1942
P. gerardiana Wall.	-	24	24	-	-	-	8	Mehra and Khoshoo, 1956a
P. halepensis Mill.	-	24	24	-	-	-	-	Mehra and Khoshoo, 1956a
P. lambertiana Dougl.	-	24	24	-	-	-	6	Mehra and Khoshoo, 1956a
P. nigra (laricio) Arnold.	12	-	12	-	-	-	-	Sax and Sax, 1933
" "	-	24	24	-	-	-	-	Mehra and Khoshoo, 1956a
P. sylvestris Linn.	12	-	12	-	-	-	-	Sax and Sax, 1933
P. palustris Mill.	-	24	-	-	-	-	-	Matheus, 1932
P. patula Schl., & Cham.	-	24	-	-	-	-	-	Bowden, 1945

Name of the species	n	2n	Median & Submedian	Sub-Terminal	Terminal	SAT-chromosomes	Secondarily constricted chromosomes	Author
P. pinaster Aiton	-	24	-	-	-	-	-	Saxton, 1909
" "	-	24	24	-	-	-	-	Mehra and Khoshoo, 1956a
P. pinea Linn.	-	24	-	-	-	-	-	Lane (unpublished)
P. radiata D. Don.	-	24	-	-	-	-	4	Mehra and Khoshoo, 1956a
" "	-	24	-	-	-	-	10	This paper
P. roxburghii Sarg.	12	-	-	-	-	-	-	Sethi, 1928
" "	-	24	24	-	-	-	6	Mehra and Khoshoo, 1956a
P. wallichiana A.B. Jacks.	-	24	24	-	-	-	6	Mehra and Khoshoo, 1956a
P. khasya Royle	-	24	-	-	-	-	4	Mehra and Khoshoo, 1956a
P. ponderosa Doug.	-	24	24	-	-	-	-	Mehra and Khoshoo, 1956a
P. merkusii Jun. & de Vric.	12	-	-	-	-	-	-	Mehra and Khoshoo, 1956a
11 other species	12	-	12	-	-	-	-	Sax and Sax, 1983

4. Cupressaceae.

Name of the species	n	2n	Median & Submedian	Sub-terminal	Terminal	SAT-chromosomes	Secondarily constricted chromosomes	Author
Callitris rhomboidalis R. Brown	-	22	-	-	-	-	-	Culline (from Darlington and Wylie 1955)
C. calcarata R. Brown	-	22	22	-	-	-	2	Mehra and Khoshoo, 1956a
C. cupressiformis Vent	-	22	22	-	-	-	2 "inconspicuous"	" "
C. glauca R. Brown	-	22	22	-	-	-	2 "inconspicuous"	" "
C. morrisoni R.T. Baker	-	22	22	-	-	-	2	" "
C. propinqua R. Brown.	-	22	22	-	-	-	2 "inconspicuous"	" "
C. robusta R. Brown	-	22	22	-	-	-	2	" "
C. verrucosa R. Brown	-	22	22	-	-	-	2	" "
C. tasmanica (Benth.) Baker & Smith. (= *C. rhomboidea* R. Brown)	-	22	22	-	-	-	2	This paper
C. oblonga Rich.	-	22	22	-	-	-	2	This paper
Chamaecyparis lawsoniana Parl.	11	-	11	-	-	-	-	Sax and Sax, 1933

Name of the species	n	2n	Median & Submedian	Sub-Terminal	Terminal	SAT-chromosomes	Secondarily constricted chromosomes	Author
C. obtusa Endl.	-	22	-	-	-	-	-	Hirayoshi, 1942
" "		22(44)	-	-	-	-	-	Kanezawa, 1951
Cupressus funibris Don.	-	22	-	-	-	-	-	Mehra and Khoshoo, 1948b
" "	11	-	10	1	-	1	-	Mehra and Khoshoo, 1956a
C. lusitanica Mill.	-	22	22	-	-	-	2	Camara and Jesus, 1946
C. lusitanica Miller var. benthami carr.	11	-	-	-	-	-	-	Mehra and Khoshoo, 1956a
C. torulosa Endlicher	-	22	-	-	-	-	-	Mehra and Khoshoo, 1948, 1956a.
" "	11	-	10	1	-	1	-	Mehra and Khoshoo, 1956a
C. sempervirens Linn.	-	22	20	2	-	-	2	Mehra and Khoshoo, 1956a
C. cashmeriana Royle	11	-	-	-	-	-	-	" " "
Libocedrus plumosa Druce.	-	22	-	-	-	-	-	Lane (from Darlington & Wylie, 1955).
Thuja occidentalis Linn.	11	-	22	-	-	-	-	Sax and Sax, 1933
T. occidentalis Linn. var. compacta carr.	-	22	22	-	-	-	2	Mehra and Khoshoo, 1956a

Name of the species	n	2n	Median & submedian	Sub-Terminal	Terminal	SAT-chromosomes	Secondarily constricted chromosomes	Author
T. orientalis Linn.	11	-	11	-	-	-	1(?)	Sax and Sax, 1933
" "	11	-	10	1	-	1	1	Mehra and Khoshoo,1956a
T. plicata D. Don.	11	-	11	-	-	-	-	Sax and Sax, 1933
T. standishii Carr.	11	-	-	-	-	-	-	Sax and Sax, 1933
Tetraclinus articulata Masters.	-	22	22	-	-	-	-	Mehra and Khoshoo,1956a
Actinostrobus pyramidalis Miquel.	-	22	22	-	-	-	2	Mehra and Khoshoo,1956a
Widdringtonia cupressoides End.	-	22	22	-	-	-	2	Mehra and Khoshoo,1956a
Juniperus communis Linn.	11	-	-	-	-	-	-	Sax and Sax, 1933
J. procera Hochst.	-	22	20	2	-	-	-	Mehra and Khoshoo,1956a
J. virginiana Linn.	11	-	11	-	-	-	-	Sax and Sax, 1933
" "	-	22	-	-	-	-	-	Ross and Duncan, 1949
" " "	-	22,33	-	-	-	-	-	Stiff, 1951
J. virginiana Linn. var. *scopulorum* Jones.	11	-	-	-	-	-	-	Mehra and Khoshoo,1956a
J. rigida Sieb.& Zucc.	11	-	11	-	-	1(?)	-	Sax and Sax, 1933

Name of the species	n	2n	Median & Submedian	Sub-Terminal	Terminal	SAT-chromosomes	Secondarily constricted chromosomes	Author
J. horizontalis Moench.	-	22	-	-	-	-	-	Ross and Duncan, 1949
J. sabina Linn.	-	22-24	-	-	-	-	-	Reese, 1952
J. phoenicea Linn.	11	-	-	-	-	-	-	Mehra and Khoshoo, 1956a
J. bermudiana Linn.	11	-	-	-	-	-	-	Mehra and Khoshoo, 1956a
J. chinensis Linn.	11	-	11	-	-	-	1	Sax and Sax, 1933
J. chinensis Linn. var. *pfitzeriana*	22	-	-	-	-	-	-	Sax and Sax, 1933
J. squamata Duc. Ham. *meyeri*	-	44	-	-	-	-	-	Jensen and Levan, 1941

III. OBSERVATIONS.

As revealed by the observations presented below, the chromosomes of all species of Tasmanian Conifers are long except the medium-sized chromosomes of *Pherosphaera hookeriana, Dacrydium franklinii, Podocarpus alpina* and the small chromosomes of *Microcachrys tetragona*. While the chromosomes in different families remained constant, it is indeed remarkable that Podocarpaceae exhibits considerable variation in number, morphology and size. Not withstanding this, they have a pattern of organisation of their own and morphologically similar chromosomes could be detected in their complements. The karyotypes of some of the genera are unique to themselves in several ways and probably speak of their phylogenetic history. Satellited chromosomes are rare in the complements. Their function however is fulfilled by the chromosomes with secondary constrictions. The latter form a characteristic feature of the Tasmanian species, as is the case with their allies, occurring in other geographical areas.

Considerable evidence for structural changes, mostly of the nature of segmental interchange involving homologous or nonhomologous chromosomes was encountered and described below. Chromosome number remains unaltered despite the segmental rearrangements. These Conifers appear to be more flexible to structural alterations and rigid to the numerical balance. Sometimes the characteristic "symmetry" of the karyotype which is known to be so essential for the species to maintain its fertility is disturbed in Tasmanian Conifers due to the sporadic appearance of morphologically dissimilar homologous chromosomes in their complements. Still species like Phyllocladus asplenifolius and Athrotaxis cupressoides survive in nature. Some of these critical observations on the chromosomes proved difficult due to their length and the consequent foreshortening. Nevertheless well flattened and well spaced metaphases were chosen for the chromosome analysis. Technical difficulties attending on the presence of tannin in their cells added to the difficulties. This explains why some of the details concerning the karyotypes and the structural alterations in different individuals reflecting their evolutionary history were missed by the previous investigators in Tasmanian Conifers and in members related to them.

A word need be said about the terminology used in the following descriptions of chromosomes. Median and submedian chromosomes are those that have equal or unequal arms respectively. Chromosomes are designated subterminal when the second arm is short having the same diameter as the distal arm. They are "terminal" when the proximal arm is minute and scarcely visible as in Podocarpus alpina or when the chromosome is rod-like with no visible second arm as in Dacrydium franklinii.

Podocarpaceae.

1. Phyllocladus asplenifolius (Labill.) Hook.f. $2n = 18$.

The genus Phyllocladus was first established by L.C. Richard (Con. 130,t.) in 1826 and comprises 6 species which are distributed in Tasmania, New Zealand, the Philippine Islands and Borneo. The most distinctive features of the whole genus are the flattened, entire or lobed, leaf-like branchlets, the true leaves being reduced to small appressed scales and the hard small seeds borne in short fleshy receptacles at the margin of phylloclades.

P. asplenifolius commonly called "Celery top Pine" is native to Tasmania. The diploid chromosome number is 18 (Fig. 1.). The homologous chromosome could not be sorted out for want of distinctive morphological features. However the following 9 pairs of chromosomes could be distinguished (Fig. 2). There is a sudden drop in length from B to C and another from H to I. This justifies to some extent their designation as long, medium and short chromosomes.

Long chromosomes

(1) 1 pair of chromosomes (A) with median or submedian constrictions; the two homologous chromosomes appear to be a little unequal in length;

(2) 1 pair of chromosomes with distinctly submedian constrictions (B);

Medium-sized chromosomes

(3) 6 pairs of chromosomes, 3 with median (C, E, G) and 3 with submedian constrictions (D, F, H); while the submedian chromosomes are distinct, there still remains a doubt whether some of the chromosomes described as median could be slightly submedian; one pair appears to be heteromorphic for their unequal primary constrictions (F' and F'' in Fig. 1);

Short chromosomes

(4) 1 pair with subterminal constrictions (I); in some metaphases, the short proximal arms were found to be unequal in length.

The chromosomes in general are fairly long and are most unlike any other Tasmanian Conifer both in number and morphology. A close scrutiny has revealed that the karyotype is asymmetrical in having a greater proportion of submedian chromosomes and a pair of medium chromosomes (F' and F'') with unequal primary constrictions. Such long primary constrictions were reported by Mehra and Khoshoo (1956) in *Widdringtonia cupressoides*, which is characterised by a pair of chromosomes with exceptionally long centromeric constrictions. Another important feature which has perhaps a bearing on the general evolution of the karyotypes in Conifers is the unequal proximal arms of the short chromosomes (i) sometimes observed in metaphases, presumably due to segmental interchange between two homologous chromosomes. That short arms only are affected in P. aspleniifolius is an interesting fact to note.

2. Pherosphaera hookeriana Hook.f. non Archer ($2n = 26$).

The genus Pherosphaera, first established by Archer in Hooker's Journal of Botany in 1850, is much confounded in taxonomic literature and is regarded as closely allied to Dacrydium. It is restricted to two species, namely P. Hookeriana and P. fitzgeraldi. The former is an endemic to Tasmania, occurring in alpine regions near Lake St. Clair and Mt. Field East (3,500 - 4,500 ft.) and the latter on the Blue Mountains, West of Sydney. The two species differ but slightly in their habit.

P. hookeriana is an erect, much branched shrub attaining a height of 1 - 2.5 m., and having leaves closely imbricate, thick, very obtuse, keeled, arranged in 4 or 5 rows. The male cones are small, erect, terminal with subsessile microsporophylls, which are spirally arranged. The female cones are decurved, small, with 4 - 8 imbricate scales, thickened at the base, acuminate at the apex, ovule single anatropous to start with but ultimately orthotropous.

The root-tip cells of P. hookeriana showed 26 chromosomes (Fig. 3). The idiogram consists of (Fig. 4):

(1) 2 pairs of long chromosomes, one with terminal (A) and the other with submedian constrictions (B);

(2) 11 pairs of chromosomes, of which one with submedian (F) and the rest with terminal constrictions; all these show a gradation in length; the last pair (M) is $\frac{2}{3}$ the length of the third pair (C).

Such a chromosome complement bears a close resemblance to that of Dacrydium frankliniі, both in size and general morphology of chromosomes. P. hookeriana with 26 and Dacrydium franklinii with 30 somatic chromosomes are however totally divergent. From the point of view of number alone, Pherosphaera is unique among Podocarpaceae, none of which , as far as our knowledge goes, are characterised by 26 somatic chromosomes. The significance of this chromosome complement will be discussed later in this paper.

3. Microcachrys tetragona (Hook.) Hook.f. (2n = 30).

Microcachrys established by Hooker in 1845 (Jour. Bot. vol. IV, p. 150) is a monotypic genus, which occurs in alpine situations on the summit of Western and South Western range and Central Plateau of Tasmania (3,500 - 4,500 ft.) in exposed ridges and wet moors. It has much in common with the cognate genera, like Diselma, Pherosphaera and Dacrydium and is much confused with them.

Microcachrys tetragona is a prostrate shrub with 4-angled, whip-like branchlets; the leaves are small, imbricate, keeled and arranged in 4 rows; male cones are terminal, oval with 20 or more stamens; female cones terminal, egg-shaped with 20 - 28 fertile scales, spirally imbricate, bright red and translucent; the inverted ovules are enveloped by an integument and partially by an epimatium; seeds have scarlet arils.

The chromosome number as could be determined in the root-tip cells is 30 (Fig. 5). The idiogram consists of (Fig. 6):

(1) 5 pairs of chromosomes of approximately the same size, 4 with submedian (A, B, C, D) and 1 with either median or submedian constrictions (E);

(2) 10 pairs with terminal constrictions (F - O); in all these chromosomes the short proximal arms are minute; out of those, the smallest pair (O) is satellited; the satellites are prominent and are attached to the short arms (?) which are not visible at all; the satellites could be distinguished from the short proximal arms by their relatively large size and are usually separated from the chromosomes by fairly long SAT-threads; on the contrary the short proximal arms are very close to the long distal arms.

Compared with other Tasmanian Conifers, the chromosomes of _Microcachrys tetragona_ are very small, perhaps smallest in all Conifers known cytologically. The shortest pair is approximately half of the longest chromosome and in between these two extremities there is an imperceptable gradation in the lengths of the different chromosomes. To a great extent, this is true of _Dacrydium franklinii_ ($2n = 30$) too. Karyologically _Microcachrys tetragona_ has much in common with _Dacrydium franklinii_.

When the root-tips are treated with 0.5% colchicine solution for 2 hours followed by another treatment with 0.003M 8-hydroxyquinoline for the same duration, _Microcachrys tetragona_ shows peculiar "reductional groupings" (_cf._ Huskins, 1948). In Fig.7, ten chromosomes are at one pole and 20 at the other. The two groups are bridged by a chromosome.

Similar cases have been reported by other workers during C-mitosis. The "disturbed mitosis" of Nybom and Knutsson (1947), the "psedo-anaphase" of Witkus and Berger (1944), the "exploded metaphase" of Barber and Callan (1943) and the induced "reduction groupings" reported by Huskins (1948) and his co-workers after a treatment with sodium nucleate (1 - 4%) simulate the condition described for *Microcachrys tetragona*. It is interesting that in the present case, treatment with two different chemicals is needed to induce a condition which resembles the so-called "reduction groupings" reported by Huskins (1948). When the roots are treated with either of the chemicals, no such phenomenon was noted.

The most important cause for such a phenomenon in *Microcachrys* appears to be a change in the viscosity of the cytoplasm both by colchicine and oxyquinoline. This would facilitate passive movement of the chromosomes. Even if the centromeres of the C-pairs were to be active, it is improbable to imagine the existence of an "active imperfect spindle" (Witkens and Berger, 1944) or a centrosome spindle (Barber and Callan, 1943) in *Microcachrys* after a treatment with colchicine of high concentration. Hence any explanation which involves the activity of the centromere and the spindle mechanism must be discarded. The only possible explanation would be a passive movement of the C-pairs to the 2 ends due to the transverse forces in the spindle tactoid, the particles of which push the chromosomes to opposite regions. This leads to the formation of 2 groups with variable number of chromosomes. The existence of transverse forces has been propounded by Östergren (1945) and it is believed that they are not destroyed after colchicine treatment in *Microcachrys*.

4. *Dacrydium franklinii* Hook.f. ($2n = 30$).

The genus *Dacrydium* with about 20 species finds a distribution in Chile, New Zealand, Tasmania and Malaysia. *D.franklinii* popularly called Huon Pine is endemic to Tasmania. The tree attains a height of about 100 ft., and yields one of Tasmania's finest timbers. The branchlets are pendulous, with very small, imbricate and closely appressed leaves arranged in 4 or 5 rows. Male cones are cylindrical and terminal. The female cones are small, terminal and decurved, with 8 - 10 fertile scales. As is the case with *Microcachrys tetragona*, the ovule is surrounded by an inner integument and partially by an epimatium. The ovule is nearly erect at maturity (Sahni, 1921 p. 290) like *D. cupressinum*, *D. intermedium* and *D. colensoi*. It resembles *Pherosphaera* in having a nearly erect ovule.

In the somatic metaphase, there are 30 chromosomes (Fig. 8a). In root-tips treated with 0.5% colchicine, considerable variation in the chromosome number was encountered (2n = 22, 26, 31 and 32). One such abnormal metaphase with 32 chromosomes is represented in 8b. The most frequent number is 30. The idiogram consists of (Fig. 9);

(1) 1 pair of chromosomes with submedian or subterminal constrictions (A);

(2) 13 pairs of chromosomes, 4 pairs with submedian constrictions (E, F, H, J) and 9 pairs with terminal constrictions (B, C, D, G, I, K, L, M, N); the dot-like very short proximal arms are visible only in one pair (D) and in the rest they are not visible; it is possible that they may be satellited chromosomes;

(3) 1 pair of short chromosomes also with terminal constrictions (O).

It is pertinent to mention here that while there is no distinction between long and medium-sized chromosomes, one short pair could however be picked out. In other words, while all the chromosomes from A to N merge imperceptably into one another, there is a sudden drop from the chromosome N to the chromosome O. There are neither satellited chromosomes (?) nor chromosomes with secondary constrictions. But for the size of the chromosomes, the complement of *Dacrydium franklinii* is almost similar to that of *Microcachrys tetragona*.

Evidence for segmental interchange without any change in the chromosome number has been found in Dacrydium franklinii . One chromosome pair (marked A' and A" in Fig. 8a) is clearly unequal. It is difficult to predict whether they are homologous or non-homologous chromosomes. The rest of the chromosomes in the complement appear to have remained unchanged and could be sorted out into respective homologous pairs. Hence A' and A" in question are probably homologous. The unequality of their length is explicable on the assumption that A" translocated a piece to A'.

5. Podocarpus alpina Hook.f. ($2n = 38$).

The genus Podocarpus is by far the largest and most widely distributed of the Podocarpaceae. It consists of about 60 species which show much diversity of structure in its various species and are distributed throughout most of the Southern Hemisphere and in India, China and the West Indes. It is divided into 4 main subgenera on the character of the female strobitus (Pilger, 1926; Wilde 1944). They are: Eupodocarpus, Stachycarpus, Nageia and Dacrycarpus. Buchholz and Gray (1948) however have given a different scheme of classification.

Podocarpus alpina is a much branched low straggling shrub, growing in alpine and subalpine elevations in Victoria and Tasmania (3,000 - 4,500 ft.). Plants growing in higher altitudes become stunted and heath-like. The leaves are crowded, linear, straight, blunt or acute $\frac{1}{4}-\frac{1}{2}$" long; male conés are solitary in the axil of the upper leaves; or 3 or 4 on the short axillary branches; the female cone is solitary on the axillary branches; the ripe seed is covered with a greenish-black ovuliferous scale.

Figs. 10 and 11 show the somatic metaphases with 38 chromosomes. The idiogram (Fig. 12) shows that the chromosomes are not differentiated into long medium and short chromosomes. All the chromosomes are characterized by terminal constrictions. One pair is satellited. The satellites are probably attached to the short proximal arms, which are not visible at all. In fact, the short proximal arms in other chromosomes too are not visible in all the metaphases (compare figs. 10, 8, 11). Very few somatically doubled cells were observed despite the fact that the roots are treated with a fairly high concentration of colchicine (0.5%).

CUPRESSACEAE

Cupressaceae is represented by Callitris and Diselma in Tasmania. The genus Callitris with about 20 species is wholly confined to Australia and New Caledonia. C. tasmanica and C. oblonga are the only two species that occur in Tasmania. The former is abundant on the east coast from Prosser River to Elephant Pass in Tasmania and extends its range up to S. Australia, Victoria and N.S.W. C. oblonga is a local endemic on the banks of South Esk River near Launceston and Avoca.

Dallimore and Jackson (1948) distinguished C. rhomboidea R. Brown with very small bright green leaves from C. tasmanica with large and coarser leaves. However, C. rhomboidea is generally treated as synonymous with C. tasmanica.

Callitris has received very little cytological attention. Gulline (from Darlington and Wylie, 1955) reported the somatic chromosome number of C. tasmanica (= C. rhomboidalis) as 22. Mehra and Khoshoo (1956) described the karyotypes of seven species Callitris namely, C. calcarata, C. cupressiformis (= C. rhomboidea), C. glauca, C. morrisoni, C. propinqua, C. robusta and C. verrucosa. All have essentially the same karyotype with 22 somatic chromosomes. The chromosomes are neither median or submedian and one pair has secondary constrictions. The length of the secondary constrictions is variable and so the distal segments cut off by these secondary constrictions. The secondary constrictions in <u>C. cupressiformis</u>, <u>C. glauca</u> and <u>C. propinqua</u> are described as "inconspicuous".

6. <u>Callitris tasmanica</u> (Benth.) Baker & Smith. ($2n = 22$).

<u>Callitris tasmanica</u> is a tall pryramidal tree which usually attains a height of 7 meters, rarely reaching 14 m. The leaves are in whorls of 3, less than 1 mm. long, closely pressed to the branchlets making them angled. The male cones are small, terminal, cylindrical, each scale with 3 pollen sacs. The female cones are clustered on short branchlets, spherical, 1.2 - 1.8 cm. in diameter, 6 rhomboidal thick scales, in whorls of 3 each, with central columella.

The diploid chromosome number is 22 (Fig. 20). The homologous chromosomes could be sorted out with considerable difficulty due to the absence of distinct morphological features. The idiogram (Fig. 21) shows the following chromosomes in descending order of their lengths:

(1) Three pairs of chromosomes with distinctly submedian constrictions (A, B, C);

(2) one pair of chromosomes with median or slightly submedian constrictions (D);

(3) one pair of chromosomes with submedian constrictions (E);

(4) one pair of chromosomes with median constrictions (F);

(5) one pair of chromosomes with distinctly submedian constrictions (G);

(6) next in order comes the pair of chromosomes with a median primary constriction and a subterminal secondary constriction (H);

(7) one pair of chromosomes with a median or slightly submedian constrictions (I);

(8) two pairs of chromosomes with submedian constrictions (J, K).

As pointed out already, the chromosomes show a gradual gradation in length and the 11th pair is $\frac{2}{3}$ the length of the first pair. In spite of the fact that a large number of metaphases were observed, no sign of structural changes could be detected in the species. Only in some metaphases, the secondary constrictions were of unequal lengths in the homologous chromosomes, the significance of which is not clearly understood. This was certainly not due to the foreshortening of the chromosomes.

7. C. oblonga Rich ($2n = 22$)

C. oblonga is similar to C. tasmanica in all essential respects, except for the erect branches and elliptical seed scales, which are narrow and blunt at the apex, where a small terminal projection is located.

It is characterized by 22 chromosomes in the root-tip cells. (Fig. 13), as is the case with C. tasmanica. The idiogram represented in Fig. 19 is almost similar to that of C. tasmanica.

Two plants apart from others received a detailed consideration during the present study and they are remarkable for illustrating the structural changes possible in the species. Attention was mainly focussed on a pair of chromosomes with secondary constrictions in relation to other chromosomes in the complement. Strikingly enough, the chromosomes with secondary constrictions are heteromorphic for their lengths (Figs. 16, 17 and 18). Some times only the secondarily constricted chromosomes are effected when other chromosomes remained constant as far as the present observations go (Fig. 18). At other times a variation in the length of any one of the SAT-chromosomes is associated with an alteration in the morphology of the other chromosomes in the complement. Two such cases are illustrated in Figs. 14 and 15 and the effected chromosomes are blackened. In Fig. 14, one non-nuclear chromosome has lost a piece and it is translocated to one of the SAT-chromosomes. Such a conclusion is supported by the fact that no other chromosome in the complement seems to be visibly altered including the second SAT-chromosome. In Fig. 15 in addition to such a change, there is a fragment and the total number of bodies including the fragment is 22. In other words, 2 non-nucleolar chromosomes have suffered a structural change in this metaphase.

All these are of the nature of segmental interchange, in homologous and nonhomologous chromosomes. Navashin (1934) reported spontaneous structural changes in Crepis, some of which were translocations. Morgan (1939) discussed somatic exchanges in Drosophila. Jones (1937, 1938) adduced genetic evidence to show that nonhomologous interchanges are possible in the endosperm of maize. Translocations in the somatic tissues of the type described above in Callitris are mainly due to the following reasons. Accidental entanglement of chromosomes during prophases provide opportunities for interchanges. (Darlington, 1931). Sometimes breakages are followed by fusions of broken ends resulting in segmental interchanges. What is of interest in Callitris oblonga is that in a majority of cases, translocation is confined to chromosomes with secondary constrictions, indicating that homologous breaks and fusions are more frequent than the nonhomologous ones. Whether it is homologous or nonhomologous, the translocation is confined to the long distal arms of the SAT- chromosomes, showing thereby that this species is an example of localised breakages and fusions for which the SAT-chromosomes are particularly susceptable. Giles (1940) reported localisation of breakage in regions proximal to the centromere in Tradescantia. Mehra and Khoshoo (1956) not only reported variation in the length of secondary constrictions (ranging from "inconspicuous" to very much exaggerated) but also a variation in the length of the distal segments cut off by these secondary constrictions in different species of Callitris. This situation appears to be similar to the variation in the length of distal arms of the same type of chromosomes in C. oblonga. There appears to be every reason to believe that the variations reported by Mehra and Khoshoo (1956) are brought about by segmental interchanges.

Some of these structural changes give an insight into the possible mechanism of isolation and interspecific sterility and hence the mode of origin of new species of Callitris in particular and Cupressaceae in general. In the light of evidence presented above, it seems clear that most of the species of Callitris have originated on account of extensive repatterning of chromosomes. Perhaps in Callitris it is a method by means of which new species could originate without any numerical unbalance. It only requires a careful study to elucidate these changes, That such changes both structural and numerical are possible at a population level is admirably illustrated by Holocarpus in which 14 populations belonging to 4 species exhibit extensive changes in chromosome number and morphology, which were attributed to segmental interchanges (Clausen, 1951).

8. Diselma archeri Hook.f. ($2n = 22$).

Diselma is a monotypic genus endemic to Tasmania, growing at alpine and subalpine elevations of Central Plateau, West Coast Ranges and Lake St. Clair (3,500 - 4,500 ft.). It is an erect, much branched, dioecious shrub (5 - 20 ft.), compact in certain situations. The leaves are minute, scale-like, closely appressed, imbricate, opposite decussate making the branches 4-angled. The male cones are small, terminal with stamens in 3 or 4 pairs. The female cones are solitary, terminal with 2 pairs of scales, the upper pair with 2 three-winged seeds.

Fig. 22 represents a somatic metaphase showing 22 chromosomes. The chromosome number is fairly constant in different cells of the roots. A few exceptions were however noted and one suchcase is represented in Fig. 24, which shows 19 chromosomes. Chromosomes in the idiogram (Fig. 23) are described below according to the descending order of their lengths:

(1) Two pairs of chromosomes, one with median (A) and the other with submedian constrictions (B);

(2) one pair of chromosomes with a median primary constriction and a subterminal secondary constriction (C); a small granular body is located in the "secondary constriction";

(3) one pair of chromosomes with median constrictions (D);

(4) one pair of chromosomes with distinctly submedian constrictions (E);

(5) 3 pairs of chromosomes with median constrictions (F, G, H);

(6) two pairs of chromosomes with submedian constrictions (I, J);

(7) one pair of chromosomes with a median constriction (K).

It need not be overemphasised that the chromosome complement of Diselma archeri is typical of Cupressaceae with little or no variation in the size of the different chromosomes. In fact each chromosome type is similar to those of the two species of Callitris described in this paper. It however differs from any Cupressaceae known so far cytologically in the structure of the chromosome with "secondary constriction", which is unusual in having a granule in it. The mode of origin and its significance in the systematics of *Diselma* will be discussed later in this paper. Closely associated with this fact is the shift in its position in the idiogram when compared with *C. tasmanica* and *C. oblonga*. Relatively the chromosomes with secondary constrictions are much longer in *Diselma* than what they are in the karyotype of *Callitris* species.

TAXODIACEAE

The genus *Athrotaxis* is an endemic Tasmanian genus of the family Taxodiaceae. It is represented by three species namely, *A. selaginoides*, *A. cupressoides* and *A. laxifolia*, which grow on the central Plateau along the north-west and south-east margins and Mt. Field ranges at an altitude of 3,000 - 4,200 ft. *A. selaginoides* is frequent in localities where there is high rainfall. *A. cupressoides* is common round lakes and in wet soil. *A. laxifolia* is rare and is usually found as isolated trees near one or both the other species.

To add to this fact of distribution *A. laxifolia* is intermediate in the size of leaves and cones between the other two species. This has led the taxonomists to postulate that *A. laxifolia* is a hybrid between *A. selaginoides* and *A. cupressoides*. Culline (1952) has shown that all the 3 species have the same chromosome number ($2n = 22$), that meiosis is normal and that the chiasma frequency is not significantly different in them. The pollen grains appear normal in all the species. This author in other words could not produce any positive evidence to show that *A. laxifolia* is a hybrid. The following is a detailed analysis of the karyotypes of the three species with a view to understanding if possible the hybrid origin of *A. laxifolia*.

9. *A. selaginoides* Don. ($2n = 22$).

Commonly known as "King William Pine", *A. selaginoides* is a tall tree (about 100 ft.) with large, broad, lance-shaped , leathery, sharp pointed, incurved loosely arranged, and imbricate leaves. The female cones are spherical, largest in the genus, $\frac{1}{2} - \frac{3}{4}''$ across, the cone scales thick, woody, ending in a spine-like process.

The somatic chromosome number is 22 and is in conformity with the report of Gullino (1952). This species as well as others are characterised by the largest chromosomes so far encountered in Tasmanian Conifers. They show considerable foreshortening and in well spaced metaphases the following chromosome types are clear (Fig. 26).

Long chromosomes:

(1) one pair of chromosomes with median or slightly submedian constrictions (A);

Medium-sized chromosomes:

(2) seven pairs of chromosomes, out of which 4 pairs are submedian (B, D, F, H) and three with median constrictions (C, E, G);

(3) one pair of chromosomes with almost median or submedian primary constrictions and a subterminal secondary constrictions (I);

Short chromosomes:

(4) one pair of chromosomes with median constrictions (J);

(5) one pair of chromosomes with distinctly submedian constrictions (k).

The overall size variations in the chromosomes is not well marked as to justify their classification into long, medium and short chromosomes. But it has been resorted to not only for the sake of descriptive convenience but also for comparison with other species. The short chromosomes are $\frac{2}{3}$ the size of the long chromosomes.

(10) A. cupressoides Don. ($2n = 22$).

The Pencil Pine (A. cupressoides) is a moderately tall tree, attaining a height of 20 - 40 ft. The leaves are very small, 1/8" long, closely appressed, imbricate, rhomboid-ovate, thick, keeled and blunt at the apex. The seed cones are spherical, scale woody with a wedge-shaped base and rounded apex. It has a spine-like process on the outer side.

The somatic chromosome number is 22 (Fig. 29). The idiogram consists of (Fig. 30).

Long chromosomes:

(1) one pair of chromosomes with a median constrictions (A);

Medium-sized chromosomes:

(2) two pairs of chromosomes with median constrictions (B, I);

(3) four pairs of chromosomes with distinct submedian constrictions (C, D, E, J);

(4) two pairs of chromosomes with either median or submedian constrictions (F, G);

(5) one pair of chromosomes with one submedian primary and a subterminal secondary constrictions (H); of the three segments, the middle segment appears to be the longest in some metaphases.

Short chromosomes:

(6) one pair of chromosomes with a distinctly submedian constriction (K).

In the idiogram, there is a sudden drop from A to B and again another drop from

J to K. This species has only one short pair of chromosomes unlike *A. selaginoides* which it resembles in all other respects.

The karyotype of *A. cupressoides* is interesting in several other respects. The long chromosome pair (A) and a medium-sized chromosome (G) did not find a morphologically similar partner in the metaphase represented in Fig. 29. The supposed homologue of A' is a little smaller in size (A"). This has been found consistently in all the well flattened metaphases observed during the present investigation leading one to think that perhaps it has a characteristic feature of the species or the three plants under investigation. In the case of the chromosomes G' and G" (Fig. 29), which are supposed to be homologous, a similar situation prevails; but it could not be verified consistently in all the metaphases. On the whole it must be admitted that it proved very difficult to sort out the homologous chromosomes in this species. What has been said with regard to these two pairs may also be true of other pairs but they escaped notice during the present study.

(11) *A. laxifolia* Hook. (2n = 22).

A. laxifolia is a tree of 25 - 30 ft., The leaves are larger and less closely pressed, slightly spreading with the apex incurved. It resembles closely the other two species and is regarded as a hybrid between them.

Fig. 27 represents the somatic chromosome complement with 22 chromosomes. The idiogram consists of (Fig. 28):

(1) nine pairs of chromosomes with median or submedian constrictions (A, B, C, D, E, F, G, H, J);

(2) one pair of chromosomes with a median primary constriction and a subterminal secondary constriction (I);

(3) one pair of chromosomes with distinctly submedian constrictions (k).

A close study of the idiogram would at once suggest that the distinction between the long and medium-sized chromosomes so distinct in the other two species has disappeared in *A. laxifolia*. All the chromosomes show a gradation among themselves except for the last pair which corresponds to the short pair of the other two species. In having only one short pair of chromosomes, it resembles more *A. cupressoides* than *A. selaginoides*. Although chromosome morphology and size do not throw any light on the hybrid origin of this species, it is however possible that it might have originated as a hybrid and undergone further structural changes, as evidenced by the lack of distinction between the long and medium-sized chromosomes, which is characteristic of the other two species.

PINACEAE.

(12) *Pinus radiata* Don. (Monterey Pine). $2n = 24$.

Pinus radiata is one of the 80 species of *Pinus*, which are widely distributed in the northern hemisphere. In California (Swanton, Monterey and Cambria), where it is restricted to the hilly ground near sea and in the Mexican Island of Guadalupe, Monterey Pine is still a wild tree but in Tasmania it is planted for its soft wood and is much used in the reforestation of New Zealand.

The Tasmanian forms are three leaved with asymmetrical cones, ovoid-conical when closed and almost spherical when open.

The karyotype of Tasmanian *Pinus radiata*, as revealed by the improved squash technique, is as follows:

(i) eleven pairs of long chromosomes with median or submedian constrictions: out of these, 5 pairs are characterised by secondary constrictions;

they show but little variation in length;

(ii) one pair of short chromosomes with distinctly submedian constrictions; this pair is a little more than half or a little less than $\frac{2}{3}$ of the longest chromosome in the complement.

Certain distinctive features of the karyotype of the Tasmanian forms require to be mentioned here. Among the long chromosomes, 7 or 8 pairs appear to be submedian and the rest median making the karyotype highly asymmetrical, although it is difficult to verify this fact from many metaphases due to the foreshortening of the chromosomes. On the whole, the chromosome complement presents a characteristic appearance with no satellites but with a rather high number of chromosomes having secondary constrictions. The position of the secondary constrictions is approximately the same in four pairs of chromosomes. It is located in a submedian position to devide the second arm of each of these chromosomes into two unequal parts ($\frac{1}{3}$: $\frac{2}{3}$) for the sake of descriptive convenience, this type is described as submedian secondary constriction. In the 5th pair, however, it is distinctly subterminal cutting off a small knob-like proximal part from the rest of the chromosome. This pair appears to be heteromorphic for the length of its chromosomes. It may be true for some other pairs of chromosomes as well but it could not be decided in them for want of distinctly recognisable morphological characters, as is the case with the 5th pair mentioned above.

The foregoing description of the karyotype of _P. radiata_ is a little different from that reported by Mehra and Khoshoo (1956). While the number and the relative lengths of the chromosomes have remained almost the same, they differ however in the number of secondarily constricted chromosomes. The Tasmanian forms show 5 pairs and the Indian forms only 2. These two pairs in the latter are all subterminal type unlike the Tasmanian forms. Although the original source of the Indian material is not mentioned by Mehra and Khoshoo (loc. cit.), it is a remarkable fact that such a divergence in the karyotype should occur within the same range of species population growing in two different geographic regions. The bearing of this karyological difference on the taxonomy of the species will be discussed later in this paper.

In some of the metaphases in root-tips treated with Phloroglucinol, the chromatids are seen to be subjected to a strain between the centromere and the point of separation, described by Darlington and Upcott (1941, Vide their text figure 7) as Klingstedt effect. This is obviously due to the early lapse of attraction at the centromeres and its delay in the chromatids. This is opposite of the C- mitotic effect, which results in X-shaped configurations. Curiously enough, a similar situation seems to prevail even in the untreated endosperm tissue of many Conifers as revealed by the figures of Sax and Sax (1933). Hence the possibility that it is due to the action of chemicals should be excluded. May be what is an abnormality with regard to the behaviour of the centromere in Angiosperms is of frequent occurrence in Gymnosperms. It is possible that the nature of centromere, to which the behaviour of the whole chromosome is intimately connected, is a little different from that of Angiosperms or that the chromatids are characterised by more specific attraction than the centromere in this group of plants. However, the breakage attributed to it by Darlington and Upcott (loc. cit.) has not been observed in the root-tip cells although it could be one of the plausible reasons for fragmentation of chromosomes in these plants, if it occurs at all. The spontaneous chromosome breakage in the cotyledons of 13 out of 20 species of Pinus reported by Tjio and Levan (1954) may be explicable on this basis. Nondisjunction as a possible source of variation among the genera of Pinaceae may perhaps be attributed to this attraction of chromatids. This will be discussed later on in this paper.

IV. DISCUSSION

The several facets of the outstanding phylogenetic and taxonomic problems relating to Tasmanian Conifers and the families to which they belong have been depicted in the introduction. The survey, although incomplete and sketchy in itself, nevertheless shows that while recognising the close relationship of Conifers as a whole, systematists have tended to make families and genera more and more compact and homogeneous by gradually rising their status in their respective schemes of classification. In 1887 all Conifers were known to belong to two orders (Pinoideae and Taxoideae) but as more and more knowledge about their comparative morphology accumulated they have been segregated into 7 families by Pilger (1926). In a more recent classification, Pulle (1937, 1938) recognised 7 families which were grouped into 5 orders namely, Araucariales, Podocarpales, Pinales, Cupressales and Taxales. According to him, this was done not without much hesitation as all the 7 families deserved the status of orders. In other words, what was considered as a tribe by Eichler in 1887 has now been given the status of an order by Pulle (loc. cit.). This point has a particular bearing on the ensuing discussion pertaining to the cytotaxonomy and phylogeny of Tasmanian Conifers and it has not been critically examined so far in the light of karyology.

That an elucidation of some of the cytotaxonomic and phylogenetic problems in Conifers is in sight stems from a variety of reasons. Comparative karyology has revealed that a correlation exists between the major taxonomic groupings and their chromosomes. The families are essentially monophyletic with a characteristic single base number. The few exceptions are the heterogenous Podocarpaceae, Psedotsuga and Psedolarix ($n = 11$) of Abietaceae ($x = 12$) and Sciadopitys ($n = 10$) of Taxodiaceae ($x = 11$). A time may come when Psedotsuga and Psedolarix will be removed from Abietaceae to make the family homogeneous, just as Hayata (1931) erected a family   for the endemic Sciadopitys verticillata and removed it from Taxodiaceae.

The differentiation of genera is to a large extent associated with differences in chromosome morphology due mostly to segmental interchange and to a lesser extent to other structural changes like fragmentation and fusion. The karyotypes within genera have remained constant within reasonable limits except in such large genera like Podocarpus and Pinus. This fact lends support to the view that speciation was entirely based on gene mutations in a vast majority of Conifer species. Polyploidy which tends to obscure the interrelationships of genera and species is rare or totally absent in some families even in such large genera as Podocarpus with wide range of chromosome numbers. Absence of polyploidy and the stability of chromosome numbers are functions in the lengths of their life cycle. The stability of the chromosome numbers in Conifers could be traced back to the Palaeozoic period. If so, chromosomes would certainly come to the aid of the taxonomists, whose ultimate aim is to seek phylogenetic relationships in Conifers. In the words of Darlington (1956): "The chromosomes provide us with a record of the past, a living record, significant in a surprisingly similar way to the dead record, which fossils provide to the paleontologist".

If the above mentioned cytological facts governing the evolutionary tendencies in Conifers and their pattern of past and present distribution (Florin, 1940) form the working hypothesis, the interrelationships of Tasmanian Conifers in the light of their cytology could be understood with a fair degree of certainty. It should however be remembered that modern genera mostly of a relic nature, with a maze of cross resemblances are likely to show complex relationship among themselves and that a certain broad-mindedness is necessary for any reasonable deductions of their phylogenetic tendencies. Positive assertions with regard to putative phylogeny of the Conifer families and genera are extremely difficult and sometimes even prove erroneous on account of parallel and convergent evolutions. Different organs have evolved at different rates in Conifers. Some of the genera retaining their primitive traits have become increasingly specialised in different ways. Not withstanding this fact, a critical evaluation of morphological, cytological and distributional data may go far towards solving the problems of interrelationships in Tasmanian Conifers. The following discussion is confined only to Podocarpaceae, Pinaceae, Taxodiaceae and Cupressaceae.

PODOCARPACEAE

Podocarpaceae, whichis mostly restricted to Southern Hemisphere, includes 6 genera, namely Saxegothaea, Microcachrys, Dacrydium, Pherosphaera, Phyllocladus and Podocarpus. It is generally regarded as a clearly defined family despite the fact that the different genera show considerable diversity in their morphological organisation.

In connection with its phylogeny and affinities with other Conifers, there are primarily two schools of thought. Thomson (1908), Tison (1909), Stiles (1908, 1912) and others envisage the origin of Podocarpaceae from Auracariaceae through such genera as *Saxegothaea* and *Microcachrys*, which bear a strong resemblance to them in the structure of the female cone, the vascular anatomy of the ovuliferous scale, the structure of the ovule and the development of the male gametophyte. According to this view, some species of *Dacrydium* and all species of *Podocarpus* are advanced. This view was accepted by Salin (1921) with minor changes in the interrelationship of the genera. On the other hand, Sinnott (1913) expressed a diametrically opposite view and regarded *Podocarpus* as the most primitive member of the family from which *Saxegothaea*, *Microcachrys* and *Pherosphaera* are derived and advanced. Strobuli as indicators of phylogenetic trends have been rejected by Sinnott who derived Podocarpaceae, Araucariaceae and possibly Taxaceae from Abietaceous stock some what on parallel lines of ascent. Other schemes of phylogeny of Podocarpaceae have been drawn (Hutchinson, 1924) and the interrelationship of the different genera have been discussed in the light of different types of data (Doyle and Looby, 1939; Doyle, 1945).

The overall cytological picture based on the already published results and those gathered during the present investigation no doubt repletes a unitary nature of Podocarpaceae. All the genera are characterised by medium-sized chromosomes with either median or submedian or terminal chromosomes. The presence of terminal chromosomes imparts a characteristic appearance to the karyotype of Podocarpaceae. In keeping with the great morphological diversity, the family exhibits a diversity in chromosome number and several base numbers representing different lines of evolution from a common ancestor have come into existence. In all these lines, *size* of the chromosomes has remained remarkably constant and hence it is a measure of phylogenetic relationship within the family. A change in size has resulted in catapulting a new genus and initiating a new line of evolution within the family as in *Microcachrys*.

When considered in the light of the broad cytological facts presented above, *Agathis* and *Araucaria* with 9 pairs of median and submedian and 4 pairs of terminal chromosomes approach Podocarpaceae in having the same chromosome types. It probably indicates that both were derived from a common ancestor. The chromosomes of Podocarpaceae do seem to disprove its Abitaceous descent (Sinnott, 1913) or its derivation from Taxaceae (Hutchinson, 1924).

The following discussion, which is an integration of morphological and karyological data on the Tasmanian Conifers clearly indicates that *Podocarpus*, *Dacrydium* and *Phyllocladus* constitute three main lines of evolution. *Phyllocladus* is remotely connected with the other two. The chromosomes of this genus not only provide unequivocal evidence towards this fact but also take us back to the ancestral stock from which Podocarpaceae and Taxaceae were diverged. Morphologically *Phyllocladus* resembles both these families. Furthermore cytology has clearly indicated that *Microcachrys* and *Pherosphaera* belong to the line of descent from which *Dacrydium* also took its origin. *Microcachrys* and *Pherosphaera* however diverged early from *Dacrydium* and developed as independent and parallel phyletic lines, each characterised by either a change in the size of the chromosomes due to genotypic changes or a change in the chromosome number. If one assumes the existence of a great southern land mass, where the primitive podocarp "plexus" flourished in the Mesozoic period, one could as well imagine the existence of one branch of this plexus, which gave rise to *Dacrydium*, *Microcachrys* and *Pherosphaera*. There appears to be no doubt that Tasmanian Podocarpaceae are the end products of long evolutionary change adapted to the alpine conditions. These old relics are endemic to Tasmania, just as some of the old vesselless dicotyledons occur as endemics in New Caledonia.

1. *Phyllocladus asplenifolius* (Labill.) Hook.f.

Phyllocladus is an aberrant genus with a peculiar external morphology, a restricted distribution and a strange juxtaposition of taxian and podocarpean characters.. It is but natural that the systematic and phylogenetic position of such a genus should give rise to a considerable dispute, although it is now generally realised by all modern taxonomists that it bears a natural relationship to the other Podocarpaceae. This controversy is closely linked with the systematic position of Podocarpaceae itself. Time was when Podocarpaceae and Taxaceae were associated together in a much larger family Taxaceae. Such a grouping which dates back to Endlicher (1847) was maintained by Coulter and Chamberlain (1901) and later on by Pilger (1903). Pilger in his monograph on Taxaceae placed the genus *Phyllocladus* in a sub-family of its own, intermediate between Podocarpoideae and Taxoideae. Robertson (1906, 1907) after a detailed consideration of several characters of *Phyllocladus alpinus* came to the conclusion that the view of Pilger more nearly expressed the true relationship of *Phyllocladus*, although it showed a greater affinity to Podocarpoideae. Considering the weight of evidence, Kildahl (1908) was inclined to assign *Phyllocladus* to Podocarpoideae and so Young (1910). The latter author considered *Phyllocladus* as a primitive member of Podocarpineae and that it branched from them at a comparatively short time after the separation of Podocarpoideae from Taxineae. Following this trend of thought, Pilger (1926) reclassified his former Taxaceae into Taxaceae proper and Podocarpaceae, placing *Phyllocladus* again in its own sub-family of Podocarpaceae.

The fundamental distinction between Podocarpaceae and Taxaceae extends to both male and female cones. In Taxaceae the male cones consist of peltate or subpeltate microsporophylls with 3 - 8 microsporangia in contrast to the simple bisporangiate microsporophylls of Podocarpaceae. The taxian ovule is erect and lateral with two symmetrical integuments while the ovule in Podocarpaceae is usually single, median and inverted with their inner integument and an outer integument partly enveloping the inner. It is here the dispute with regard to the systematic position of *Phyllocladus* arises. Its male fructification is typically podocarpean while its erect ovule with two symmetrical integuments allies it to Taxaceae. *Phyllocladus* is southern in distribution like Podocarpaceae and the range of distribution is like that of *Dacrydium* although more restricted.

Associated with these features of fundamental distinction, *Phyllocladus* bears a strong resemblance to Podocarpaceae in having two winged pollen grains which are four nucleate at the time of shedding unlike the taxian pollen, a well-developed megaspore membrane, which is typical of all Gymnosperms except Taxaceae. The protogonists of the view that it should be included in Podocarpaceae strongly emphasise the presence of evanscent prothallial tissue in the pollen grains, which is the primitive character encountered in all Podocarpaceae. On the contrary, the symmetrical arillus of *Phyllocladus* recalls *Taxus* except that it is not succulent. Also it originates at the base of the ovule as does in *Taxus*. The cladodes of *Phyllocladus* contain centripetal wood, which is more common in Taxaceae than in any other Conifer (Worsdell, 1897). It has been found in the leafy cotyledon of *Taxus* and *Cephalotaxus* (formerly included in

Taxaceae) and in the cotyledons of *Taxus* and *Cephalotaxus koraiana*. It is a primitive character still retained in *Phyllocladus*. In addition to this fact, the taxian pitting, which is a combination of boardered pits with spiral and scalariform thickening, occurs in *Phyllocladus*.

A cytological study has revealed that the chromosomes of *Phyllocladus asplenifolius* are not similar to any of the modern Podocarpaceae, the general features of which are clearly reflected in the karyotypes of *Podocarpus*, *Dacrydium*, *Microcachrys* and *Podocarpus*. They are usually characterised by medium-sized chromosomes, with median or submedian constrictions and a proportion of terminal chromosomes. On the other hand, the long chromosomes of *Phyllocladus asplenifolius* are very similar to those of *Taxus baccata* (Dark, 1932; Sax and Sax, 1933) and *T. cuspidata* (Sax and Sax, 1933) in having only median and submedian chromosomes and a pair of subterminal chromosomes. The only point of difference is number. The presence of chromosomes similar to those of Taxaceae in a genus with a greater proportion of Podocarpian characters would probably mean "harking back" to its hypothetical ancestor, which was the starting point of modern Taxaceae and Podocarpaceae. If Mrs. Arber (Miss Robertson, 1906) was impressed by certain features in which *Phyllocladus* seemed to approach Taxaceae rather than Podocarpaceae, it was due to its Taxus-like chromosomes.

Thus morphological, cytological, and distributional data tend to show that *Phyllocladus* is an aberrent genus representing an independent but parallel line of evolution, which requires an isolated position, if retained in Podocarpaceae (Pilger, 1926). Its cytology would even go further to indicate that it deserves a family of its own. It could be described as a podocarp with Taxus-like chromosome. To derive *Pherosphaera* from *Phyllocladus* (Stiles, 1912) or vice versa (Doyle and Looby, 1939) implying affinity between these two genera or to relate Dacrydium with Phyllocladus (Sinnott, 1913) would mean negation of cytological evidence presented in this paper.

2. Pherosphaera hookeriana Hook.f. non Archer.

The phylogenetic position of Pherosphaera hookeriana in Podocarpaceae has been an enigma to the systematists. Not only its relationship to the other genera in the family has been variously interpreted, its very inclusion in the family has been questioned. It was originally associated with Dacrydium but was separated by Archer in 1850 (cf. Groom, 1917). In his classification of Taxaceae (1903) and Podocarpaceae (1926) Pilger accommodated the genus in a subfamily of its own recognising several of its unique characters. Stiles (1912) regarded Pherosphaera as closely allied to Phyllocladus, in which the erect axillary position and the reduced strobulus are duplicated. He derived Phyllocladus from Pherosphaera. Lawson's work (1923) on the gametophytes of this genus has revealed a wealth of detail in which the most important characters like the absence of prothallial cells from the pollen grains and the lateral position of archegonia exclude it from the Podocarp allience. Lawson concluded: "the gametophyte structures and embryo of Pherosphaera, there are no structures which justify our classifying the genus among Podocarpaceae. It bears no essential resemblance to Podocarpus, Dacrydium, Microcachrys, Saxegothaea or Phyllocladus". In the light of such evidence, it was not surprising that Buchholz (1933) erected a new family Pherosphaeraceae containing only Pherosphaera. Saxton (1930) however considered it as a member of Podocarpaceae on the basis of anatomical characters and the presence of root nodules; but an account of its peculiarities in ovular development, the total absence of prothallial cells in the pollen grains and the erect axillary ovule with no epimatium, he thought that "the retention of Pilger's subfamily Pherosphaeroideae within Podocarpaceae to include Pherosphaera alone seems to be justified". Doyle and Looby (1939) regarded Pherosphaera as an aberrent genus in Podocarpaceae and "an advanced derivative of Phyllocladus line". Doyle (1945) subsequently advocated the view that it is closely related to Microcachrys. This view is similar to that of Sinnott (1913) who derived Pherosphaera from Microcachrys. Elliott (1948) brought forward mostly embryological evidence to show that it is phylogenetically related to Dacrydium-like forms and proposed a subfamily Dacrydioideae, which included Dacrydium, Pherosphaera, Microcachrys, Acmopyle, together with such sections like Dacrycarpus and Microcarpus and also P. vitiensis and P. minor. He was of the opinion that Pherosphaera does not deserve an isolated position in a subfamily equal in status to others.

The above mentioned controversy with regard to the systematic position of Pherosphaera hookeriana rests entirely on the fact that it is a curious admixture of Podocarpian characters as well as strong differences. Although inhabiting a region with good rainfall, the small triangular, imbricate, closely oppressed and highly reduced leaves of Pherosphaera show admirable adaptation to physiological xerophytism, with the stomata above and the palisade below, which is exactly a reverse orientation of a normal dorsiventral leaf. Saxton (1930) remarked: "No other Conifer is known where transpiration is hindered by a network of fungal hyphae over the stomatal area". The presence of root tubercles and the internal anatomy however indicate that it has much in common with the other Podocarpaceae.

In having a fertile branch system, which, according to Wilde's hypothesis (1943), is the most primitive, Pherosphaera allies itself not only with

Dacrydium but with sections Dacrycarpus and Microcarpus of the genus Podocarpus. The richly branched habit is closely associated with reduced leaves. Each fertile shoot has a proximal vegetative and a terminal fertile region.

The structure of the male cone is in line with the other Podocarpaceae. The pollen grains are small, smallest in the family, thin-walled, 3-winged and 2-nucleate at the time of shedding. In the exclusive ventral origin of their bladders, the pollen grains of Pherosphaera differ from the 3-winged pollen of Podocarpus dacrydioides and the 2-winged Dacrydium laxifolium (Wodehouse, 1935). A difference in number and nature of wings need not be an index to phylogenetic relationships in Conifers, since they were developed in different cycles of affinity (e.g. Podocarpaceae and Pinaceae). To add to this, the evanescent vegetative prothallial tissue, which is so characteristic of all Podocarpaceae is absent in Pherosphaera. The two male nuclei are unequal in size and are never separated by walls. The male nuclei are different in size in other Podocarpaceae too, such as Saxegothaea (Looby and Doyle, 1939), Microcachrys (Lawson, 1923) Podocarpus andinus (Looby and Doyle, 1944) and Phyllocladus (Young, 1910). In all these cases, there are two distinct male cells.

That an otherwise old Conifer like Pherosphaera should be characterized by a reduced female strobilus when compared with such genera like Saxegothea and Microcachrys is interesting. The small cone with its reduced cone axis consists of 2 - 5 futile sporophylls and many sterile ones (Pilger, 1926). According to Elliott (1948) there are only two fertile scales. The fertile branch is curved and the cone is pendulous. Such a reduced female cone approaches closely that of Dacrydium bidwilli, Dacrydium franklinii and D. cupressinus and Phyllocladus. The similarity between Pherosphaera and Dacrydium franklinii is of immediate interest, although the latter still retains some of the primitive characters like an elongated axis with internodes.

Each fertile sporophyll has a single, axillary, erect ovule as in *Phyllocladus* and some species of *Dacrydium*. Available evidence in Podocarpaceae shows that the erect position of the ovule is a recent acquisition in *Pherosphaera*. The megasporangium lacks the second integument or the epimatium which is most unusual to the other Podocarpaceae. It is, however interesting that all stages from an inverted to erect position and the presence or absence of epimatium are foreshadowed in several species of *Dacrydium*. In *D. bidwilli*, the inverted ovules are completely enclosed by an epimatium. In *D. cupressinum*, *D. intermedium* and *D. colensoi*, the young inverted ovules become erect at maturity and are provided with a thin rudimentary epimatium. Perhaps during the course of evolution the erection of the ovule is facilitated by the absence of epimatium and hence both must be correlated as in *Pherosphaera* and some species of *Dacrydium*.

There are a large number of features in the female gametophyte and embryo which throw *Pherosphaera* out of the family Podocarpaceae and do not justify its allience with *Dacrydium*. However, caution must be exercised at this juncture, since our knowledge of the gametophytes of *Dacrydium* is far from complete (Young, 1907; Stiles, 1911). Just as *Dacrydium* shows wide vide variation in its external morphology, it is possible that it may show equally wide variation in the structure of the gametophytes. When details become available, *Pherosphaera* may fit in the pattern of variation of the gametophytes of *Dacrydium*. For the present one should rest content that some of its features are unique to Podocarpaceae. They are: (1) An axial row of 3 megaspores, all of which germinate as in *Phyllocladus* (Young, 1910) and *Taxus* and become multinucleate;

(2) a very thin megaspore membrane as in Taxaceae and unlike Podocarpaceae like *Podocarpus*, *Phyllocladus*, *Dacrydium*, *Saxegothaea* and *Microcachrys* in which it is thick, 2 layered and highly suberised (Thomson, 1905; 1909; Young, 1910; Lawson, 1923); (3) lateral position of 3-4 archegonia, at least more markedly than *Callitris* (Saxton, 1910) and *Araucaria* (Seward and Ford, 1906); (4) the development of proembryo up to the binucleate stage resembles that of the other Podocarps particularly like *Dacrydium cupressinum* (Elliott, 1948) but the binucleate cells immediately form two-celled units, which is not primitive but derived.

The present cytological study of *Pherosphaera hookeriana* puts the whole controversy of its phylogenetic relationship in its proper perspective. The karyotype consists of 26 chromosomes of which two pairs are submedian and the rest terminal. There appears to be no doubt that it is reminiscent of the Podocarpian karyotypes and fits in very well in their general pattern and organisation. There is not even a semblance between the chromosomes of *Phyllocladus asplenifolius* and *Pherosphaera hookeriana*. The embryogeny of the former is in no way related to the latter (Elliott, 1948). Apparently the external similarities of both these genera in their reduced cones and erect ovules are not indicative of their relationship, as Stiles (1912) would have us believe, but show parallel trends in evolution. They do not even seem to belong to same line of descent and hence *Pherosphaera* cannot be regarded as an advanced derivative of *Phyllocladus* line (Doyle and Looby, 1939). Such morphological parallisms in evolution which do not rest on the foundations of karyology, are apt to give a false picture of phylogenetic relationships and introduce confusion in taxonomy.

The external similarities between *Microcachrys* and *Pherosphaera*, which in all probability are an outcome of their common environment, are associated with strong differences too. The same situation obtains in their comparative karyology. The chromosomes in both the genera bear a superficial morphological similarity. But by no criterion the 30 small chromosomes, smallest in Conifers so far known cytologically, can be related to the 26 medium-sized chromosomes of *Pherosphaera*. This is based on the tacit assumption that number and more particularly size of chromosomes in related genera of Conifers have remained constant during their phylogeny. Hence their general structural similarity taken by *itself* provides no cogent evidence of their relationship. The fundamental differences in *size* and *number* of chromosomes together with the prevailing morphological and embryological differences provide a negation of such a hypothesis. The relationship between them, if any, emphasised by Doyle (1945) and Sinnott (1913) should be remote and does not even warrent their grouping into one subfamily as proposed by Elliott (1948).

A morphological and embryological comparison would show that *Pherosphaera* has obvious resemblances to *Dacrydium*, the genus in which it was originally placed. The points of resemblance are indeed so numerous and far reaching. To a great extent it is reflected in the chromosomes of *Dacrydium franklinii* ($2n = 30$) and *Pherosphaera hookeriana* ($2n = 26$). In both these genera the chromosomes are medium-sized and submedian and terminal with no satellites. The only point of difference between them is the number. The presence of two pairs of submedian chromosomes in *Pherosphaera* as against five pairs in *Dacrydium franklinii* is closely associated with a change in the basic number from 15 to 13. On the whole the resemblances between their chromosome complements are more significant than existing difference in the number. The significance becomes increasingly apparent when it is remembered that Podocarpaceae, as it is constituted to-day, is [illegible] homogeneous with regard to its chromosome shape and size while the chromosome number shows considerable variability.

If the foregoing morphological and cytological similarity are agreed upon as indicating close affinity between Pherosphaera and Dacrydium, it is not improbable to think that Pherosphaera hookeriana might have originated directly from Dacrydium-like ancestor with 26 chromosomes or indirectly from an ancestor with 30 chromosomes as a result of reduction due to segmental interchange. That Dacrydium franklinii, which co-exists with Pherosphaera as a Tasmanian endemic is also characterised by 30 chromosomes and that it shows evidence of segmental interchange in the root-tip cells are facts of considerable interest supporting the second hypothesis.

Interestingly enough, a reduction in the chromosome number, if at all has occurred in the phylogeny of Pherosphaera, has progressed along with a reduction in such morphological structures like epimatium. It has been pointed out that epimatium dwindled to a rudimentary membranous structure in some species of Dacrydium. Perhaps the tendency towards reduction of the epimatium is a case of common inheritance to Dacrydium and Pherosphaera. What has been initiated by way of morphological specialisation in Dacrydium has reached an extreme expression in Pherosphaera. Associated with a reduction in the epimatium, Pherosphaera shows some specialised morphological and embryological features like reduced cone, 3-winged small pollen grains, the absence of male cells, the thin megaspore membrane, lateral archegonia, and the uninucleate embryo units. These are all specialised divesifications ever since its divergence from the Dacrydium-like ancestor. To add to this list, $2n = 26$ is unique in Podocarpaceae.

The above mentioned evidence seems to point to the conclusion that *Pherosphaera* is a genus of Podocarpaceae retaining relatively primitive characters and at the same time considerably specialised in many ways. Phylogenetically it seems to be off shoot from a Dacrydium-line of descent. Even if it has not taken its origin from *Dacrydium* directly, there is every reason to believe that *Pherosphaera* and *Dacrydium* have descended from the same primitive stock. Such a taxa with a curious juxtaposition of opposite characters requires immediate segregation into a sub-family of its own as was done by Pilger (1926). The erection of a new family following the suggestion of Buchholz (1933) would be a logical extension of the same idea. As already pointed out, the history of Conifer taxonomy is replete with instances, where a sub-family has been given the status of a family.

There is one more reason for the separation of *Pherosphaera hookeriana* to a new family of its own. The writer is strongly inclined to believe with Arnold (1948) that "likenesses are sometimes deceiving when questions of affinities are involved, especially likenesses that are accompanied by strong differences". This may be so with *Pherosphaera hookeriana*. Its chromosomes may resemble some Podocarpaceae. It does not mean that it should be retained in Podocarpaceae when it strongly deviates from the family in certain features. Its cytological resemblances with the family may only indicate that it should be traced back to the ancestral stock from which some other Podocarpaceae had taken their origin. Hence the creation of a new monotypic family Pherosphaeraceae which is closely allied to Podocarpaceae is in keeping with morphological and cytological data.

3. *Microcachrys tetragona* Hook. f.

The Tasmanian endemic genus *Microcachrys* with its fleshy and crimson coloured cones is certainly the most unique in Gymnosperms. It has been much confounded with such cognate genera as *Athrotaxis*, *Dacrydium* and *Pherosphaera* and therefore requires to be evaluated from a cytotaxonomic point of view. Its only species *M. tetragona* was first described by Sir. W.J. Hooker in 1843 (Tab. 560) and later on by Sir. J.D. Hooker in 1845, the female cones then described under that name being those of *Pherosphaera*. The male and female cones of this genus were described by Archer (1850) as *Pherosphaera*. The whole confusion in the nomenclature was ultimately cleared by Sir. J.D. Hooker (1860).

Highly restricted in its distribution and admirably adapted to wind-swept and snow covered mountainous regions of Tasmania, *Microcachrys* is specialised in its vegetative features. The whip-like branches are clothed with small, triangular and closely appressed leaves arranged in a decussate manner. The internal anatomy of stem and leaf respectively are characterised by the absence of resin canals in the former and a single foliar canal, restricted to leaf alone in the latter.

The cones are terminal on the leafy shoots. This is essentially a primitive character. Unlike other Podocarpaceae, in which the sporophylls are arranged in a spiral fashion, the microsporophylls in the male cones of *Microcachrys* are arranged in whorls of four, following the arrangement of the foliage leaves on vegetative shoots. The pollen grains are five nucleate at the time of shedding with 3, 4, 5 or 6 symmetrical wings. The variation in the number of wings is a character of recent acquisition. In many respects the male gametophyte has essential points of resemblance and differences to that of _Podocarpus_ (Burlingame, 1908). In _Podocarpus_ there is but one functional male gamete while in _Microcachrys_ both the gametes are functional (Lawson, 1923).

The organisation and structure of the female cone are indicative of its phylogenetic status. The female cones are ovoid-globular consisting of about 20 sporophylls, which are borne in alternating whorls of 4. The upper most of these are sterile. Each fertile tetragonal sporophyll bears a single median ovule, which is completely surrounded by the inner integument and partially by an epimatium. The ovule when young is situated near the tip of the scale with the micropyle facing upwards. The integument and epimatium are free from one another for about their upper half at this stage. At maturity the ovule is bent with its micropyle facing the cone axis. The fleshy cone scales do not coalase but retain their individuality. The seeds are small with a thin membranous outer and an inner sclerenchymatous coat.

The vascular supply to the cone axis is exactly similar to that of _Saxegothaea_ and _Dacrydium franklinii_. There is a ring of vascular bundles from which the sporophyll bundles arise with no resin canals. The ovular supply have xylem in the inverse orientation and they are not accompanied by resin ducts.

Lawson (1923) enumerated a large number of characters in the female gametophyte which are unique to _Microcachrys_. The presence of five or six archegonia as against one in _Phyllocladus_ (Kildahl, 1908) and eleven in _Podocarpus_ (Coker, 1902), the occurrence of four neck cells in a single tier, differing in this particular case from Podocarpus (Coker, 1902) and _Phyllocladus_, a thick megaspore membrane as in other Podocarpaceae like Phyllocladus, Dacrydium, Saxegothaea, the fertilisation of two adjacent archegonia by two functional male gametes of the same size as in Cupressaceae and the development of the embryo from 3 tiers of nuclei which in many details differs from Podocarpus are all interesting in themselves. Some of these unique characters, which resemble Cupressaceae, are parallel development in Microcachrys.

The foregoing account of Microcachrys brings it close to Saxegothaea on one hand and Dacrydium franklinii on the other. It is believed by taxonomists that Saxegothaea has led to other Podocarpaceae through Microcachrys. Coulter and Chamberlain (1917) and Thomson (1908, 1909) thought Microcachrys with its variable wings is intermediate between wingless Saxegothaea and winged Podocarps, although Wodehouse (1935) doubts the idea. The points of resemblance between Saxegothaea and Microcachrys are: (1) Organisation of compact male and female cones with 20 megasporophylls; (2) the distribution of vascular structures in the cone axis; (3) the close connection between the integument and epimatium; when the ovules are young they are free from one another in their upper half; (4) nucellus free from the integument for most of its length; (5) the absence of resin canals in the wood.

They however respectively differ: (1) in the presence and absence of centripetal xylem in cone axis; (2) spiral and whorld arrangment of the megasporophylls; (3) in habit and (4) the smooth and winged pollen grains. The wings in podocarps are developed from the primitive furrow, which is absent in Saxegothaea (Wodehouse, 1935).

Microcachrys tetragona resembles Dacrydium franklinii in many morphological features except for the fact that they differ in habit and that the megasporangiate strobuli in the former consist of 20 spirally arranged megasporophylls forming compact cones, while the lax spikes in the latter consist of 8 to 9 sporophylls separated by conspicuous internodes. In other words, the strobulus of *Dacrydium franklinii* is much more advanced than *Microcachrys*. However, the large number of other similarities certainly indicate their common origin and early divergence. The two genera resemble each other in the following:

(1) The presence of resin ducts in the leaves and their absence in the stem; (2) the absence of resin canal in the secondary wood; (3) the occurrence of stomata on the upper surface of leaves; (4) the megasporophyll of *Dacrydium franklinii* is reminiscent of *Microcachrys* as it contains a resin cavity, which ends blindly in both the directions, resin canals being absent in the cone axis as in the vegetative stems; (5) the medianly placed single ovule is attached to the tip of the sporophyll on the upper surface; (6) the nucellus is free from the integument for most of its length and the epimatium partially surrounds it. Stiles (1912) differs from Tison (1909), who said that the epimatium completely surrounds the ovule in *Dacrydium*; (7) the tip of the sporophyll curves into a point just behind the epimatium giving the appearance of a third integument; (8) the bundles of the primary ovular supply have *inverse* orientation of xylem and phloem and they are not accompanied by resin ducts; (9) ovule is partially inverted.

The cytology of *Saxegothaea* is not known. But the external similarity and differences between *Dacrydium franklinii* and *Microcachrys tetragona* are manifestly reflected in their chromosomes, which are prototypes of other Podocarpaceae in their general appearance. Both the karyotypes are characterised by 30 diploid chromosomes with the same number of morphologically similar chromosomes. Five pairs of median or submedian and 10 pairs of terminal chromosomes out of which one pair is satellited are common to both the genera. The chromosomes in their idiograms show a gradation in lengths among themselves.

Equally significant are the two points of difference between Microcachrys tetragona and Dacrydium franklinii. While the chromosomes in Dacrydium franklinii are medium-sized, those of Microcachrys tetragona are the smallest in Podocarpaceae and perhaps Conifers in general known cytologically. Although the same number of morphologically similar chromosomes are present in both the genera, their relative positions in their respective idiograms are at varience (Figs. 6, 8, 9). It means that genotypic changes reducing the size of all the chromosomes in the complement have accompanied structural changes in the chromosomes during the course of phylogeny of Microcachrys without altering the number chromosome types.

The afore mentioned morphological and cytological facts clearly lead one to postulate that Microcachrys tetragona and Dacrydium franklinii are not only closely related but have originated from a common ancestor. This explains their taxonomic confusion. Curiously enough both are limited in their distribution to Tasmania. Microcachrys tetragona with 20 and Dacrydium franklinii with 8 megasporophylls are certainly at two different levels of morphological organisation. They are also at two different levels of chromosomal organisation as revealed by the relative size differences and positions of median and submedian chromosomes in their respective idiograms. Apparently Microcachrys tetragona diverged from the line of descent of Dacrydium franklinii very early in the history of Podocarpaceae probably in the Mesozoic period due to genotypic and structural changes. The assumption that it originated from an ancestor with medium-sized chromosomes like those of Dacrydium franklinii is supported by the fact that all other Podocarpaceae are charaterised by the same type.

In a group of plants like Podocarpaceae in which medium-sized chromosomes appear uniformly in most of the genera, a sudden mutation resulting in a reduction of the size of all the chromosomes in the complement would prove violent. Such is the change responsible for the origin of *Microcachrys*. The reasons behind a sudden reduction in the chromosome size other than mutation must be complex and must be due to an interaction of several factors. In Angiosperms reduction in size has been a sign of evolutionary advancement. Several morphological facts go to show that *Microcachrys tetragona* is primitive with a restricted distribution although Sinnott (1913) thought that it is advanced in several respects and could be derived from Podocarpus-like ancestors. Cytological facts go counter to Sinnott's views as the chromosomes of *Microcachrys* are most unlike any other species of *Podocarpus* so far reported. On the other hand the totality of morphological and cytological evidence goes to show that *Microcachrys* is closely related to *Dacrydium franklinii*.

Again in Angiosperms like *Crepis*, a reduction in the size of the chromosomes is correlated with a reduction in size of the organs, with a change into annual habit, especially those living under extremes of conditions (Babcock and Jenkins, 1943). In *Microcachrys tetragona* a change in the chromosome size has been adaptive and irreversible and is to be correlated with its shrubby habit. Its chromosome complement is a reduced and a slightly reshuffled replica of its close relative *D. franklinii*, which is a tree.

Avdulov (1931) postulated that a progressive increase in the size of the chromosomes in grasses was due to a progressive cooling of climate. Climate and chromosome size cannot be correlated in *Microcachrys* because *D. franklinii* with medium-sized chromosomes co-exists with it. On the whole, the evidence seems to point out that a reduction in the size of the chromosomes in Microcachrys is a gene determined physiological difference that has appeared only once in the family Podocarpaceae and it has resulted in the origin of a new line of evolution retaining the primitive male gametophyte and the female cone structure but becoming specialised in its female gametophyte and metabolism.

The foregoing discussion on the phylogenetic position of Microcachrys tetragona closely agrees with that of Sahni (1921, pp. 287). It also approaches the views of Stiles (1912, pp. 496) but for the fact that Dacrydium franklinii was derived from Microcachrys. It would perhaps be safe to assume that both these were derived from a common ancestor and that Microcachrys deviated from the Dacrydium-line due to the genotypic changes in the chromosomes. Because the pH of the leaf sap in Microcachrys did not fall within the range of Saxegothaea - Dacrydium, and an account of its epistomatic and monocyclic stomata (Florin, 1931) not found in any other Podocarp except Pherosphaera, Doyle and Looby (1939) regarded Microcachrys as "a special line taking its origin close to the stock from which Saxegothaea arose". That Microcachrys is special line is amply justified by its small chromosomes unknown in any other Podocarpaceae. That it originated at the base of the Dacrydium-line of descent cannot be denied in view of its primitive characters and the similarity between the karyotypes of Dacrydium franklinii and Microcachrys tetragona. Whether Saxegothaea is to be connected with this Dacrydium-line or not cannot be decided till the karyology of Saxegothaea becomes available.

4. Podocarpus alpina Hook.f.

Podocarpus with about 65-70 species is the most dominent Conifer in Southern Hemisphere having an extensive range of distribution which is paralleled to that of Pinus in the northern himisphere. Stiles (1912) remarked: "These two genera must be regarded both from the point of view of number of species and of wide geographical distribution, as the successful Conifers of the present day".

The whole range of morphological variation encountered in Podocarpaceae is easily discernable in the genus Podocarpus alone. It is also exceptional in showing several distinct types of embryogeny, where as the other genera in the family usually follow one type. Hence it is taxonomically very complex. All this evidence is sufficient to warrent its segregation into several genera (Buchholz, 1941).

Pilger (1926) divided the genus into 5 sections. Buchholz and Gray (1948) revised the genus with special reference to leaf anatomy and classified the genus into 8 sections, rejecting Pilger's sub-families as heterogeneous and not well founded. Wilde (1944) discussed the evolutionary trends in the female strobuli of Podocarpus.

On the basis of comparative morphology, P. andinus and P. spicatus (section Stachycarpus) are the most primitive species (Wilde, 1944). Embryologically a part of Stachycarpus and Nageia are most primitive (Buchholz, 1941). The section Eupodocarpus, which has an extensive distribution in southern hemisphere may well be called primitive. However it appears to be morphologically advanced in showing secondary clusters instead of primary as is the case with Stachycarpus. Within the section Eupodocarpus, P. alpina still shows transitions from primary to secondary clusters on the same individual and is thus regarded as most primitive (Wilde, 1944). It is perhaps the starting point of Eupodocarpus line.

Our meagre knowledge of the cytology of the genus is now confined to the sections Stachycarpus and Eupodocarpus (Flory, 1936; Tahara, 1941; Stiff, 1952; Mehra and Khoshoo, 1956 this paper). In the former, the haploid numbers are 20 and 12 and in the latter 20, 19 and 11 (Table 1). In both the cases, the diploid number 40 appears to be doubtful even according to Flory (1936) and a reinvestigation would pay. The highest numbers like 38 and 40 are shown by the most primitive species like P. andinus in Stachycarpus and P. alpina in Eupodocarpus. Both these species are characterised by terminal chromosomes. The morphologically advanced species have median or submedian and terminal chromosomes. If external morphology is a reliable criterion for judging the chromosomal evolution, a gradual reduction in the chromosome number by fusion is responsible for the origin of species in Podocarpus. With the available knowledge of the nonfusability of the chromosome ends, the mechanism of centric fusion would involve reciprocal translocation. Several basic numbers must have originated in Podocarpus in a similar manner. Drosophila psedoobscura with 5, D. melanogaster with 4 and D. willistonii with 3 chromosomes may have originated in the same way. Fusion of chromosomes has been inferred by Gates (1924) in Drosophila, Davie (1933) in Lavatera and Gossypium, Kostoff (1929) in Nicotiana and Lawrence (1931) in Cardamine pratensis. A parallel reduction in the basic number must have progressed simultaneously in several sections with the evolution of identical karyotypes.

A critical examination of the karyotypes like those of P. falcatus (2n = 24) P. glacilior (2n = 24) P. latifolia (2n = 22) as sketched by Mehra and Khoshoo (1956b) reveals that they are not wholly straight cases of fusion. For instance, a karyotype like that of P. latifolia with 4 terminal chromosomes can be derived from P. gracilior with 8 terminal chromosomes by simple fusion.

P. falcatus and P. gracilior with the same diploid number of 24 chromosomes are not similar, the former with no terminal and the latter with 8 terminal chromosomes (Figs. 1 and 2 of Mehra and Khoshoo, 1956b). This means that after fusion, some of the median or submedian chromosomes have undergone further structural changes, which may be of the nature of segmental interchange. When primary structual changes are superimposed by secondary changes, the end products would not be wholly median or submedian chromosomes. The whole picture of evolution would become apparent when many species of all the sections are morphologically, embryologically and cytologically become known.

Chromosome morphology and regular formation of bivalents during meiosis (as revealed by the figures of Mehra and Khoshoo, 1956b) in P. latifolia (n = 11) P. gracilior (n = 12) and P. macrophyllus (n = 19) exclude polyploidy as an agent of speciation in the genus.

It is interesting to observe that in two dominant Conifers like Podocarpus and Pinus structural changes with and without any variation in the chromosome number have played a prominent role in the origin of species, which are extensively distributed in southern and northern hemispheres respectively. In both the cases there is no evidence of polyploidy. That there is a parallelism in the mechanism of evolution associated with extensive geographic distribution in both the genera is remarkable.

PINACEAE.

Pinaceae is the largest and yet a highly natural assemblage of living genera constituting the major Conifer display in the Northern Hemisphere, rivalling Araucariaceae of the Southern Hemisphere in antiquity. Notwithstanding its homogeneous nature, early divergence, differentiation, reduction and independent development marked the evolutionary progress of the family. The winged dorsiventral pollen grains of Pinus, Cedrus, Picea, Abies and Psedolarix with a single long furrow, the wingless grains of Tsuga without a true furrow but resembling the winged graines of Pinus in the character of the exine, and the smooth wingless pollen grains of Larix and Psedolarix very well illustrate the point in question. The pollen grains of Larix and Psedolarix reveal but little of their phylogeny, as they independently represent highly reduced end products of evolution.

The karyology of the family reflects clearly its unitary nature at the same time revealing stages of early divergence. A single basic number 12, which is the predominating number binds the whole family as a monophylotic group with such exceptions as Psedolarix amabilis (x = 11; 2n = 44) and Psedotsuga taxifolia (n = 13), which replete many features of evolutionary advance in the sub-family Abietoideae. Psedolarix is an admirable instance to illustrate a change in the basic number from 12 to 11, associated with profound structural alterations in its chromosomes and tetraploidy. A change in the basic number is also correlated with its restricted geographic distribution in China. Its haploid set (n = 22) with 2 median and the rest with subterminal and terminal chromosomes is unique not only in Abiteneae but also in the whole group of Gymnosperms. Its tetraploidy with complete bivalency during meiosis and high pollen fertility is truly of a different kind and order when compared with tetraploidy in Larix decidua (x = 12; 2n = 48), which is highly sterile with multivalents of different types during meiosis as reported by (Christiansen,1950).

Hence *Psodolarix* and *Larix* belong to two distinct lines of evolution in Abietoideae, as shown in the preceding paragraph.

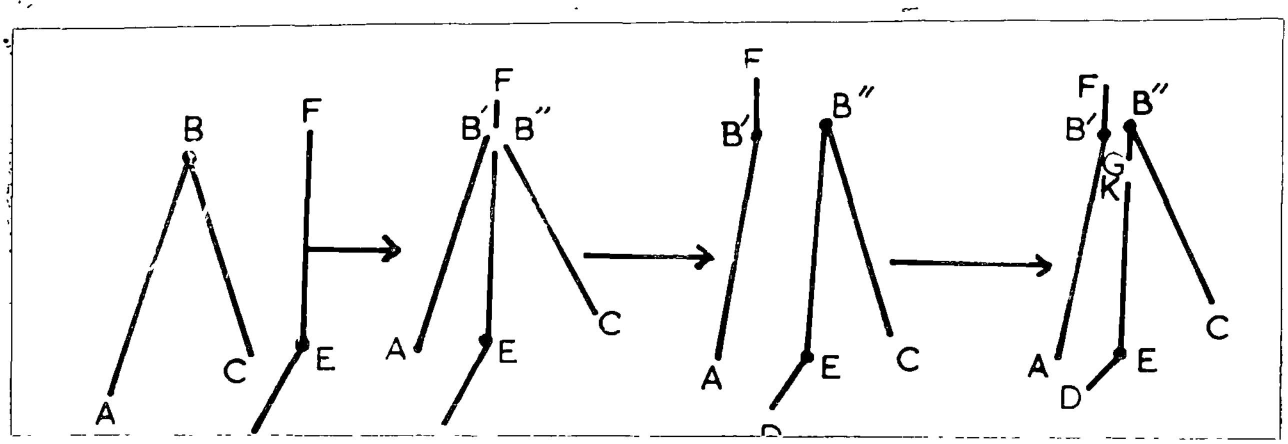
Chromosome fusion as a mechanism of reduction in the basic number from 12 to 11 is not tenable in *Psodolarix*. Comparative karyology negates such a hypothesis. Possibly *x* = 11 could be derived from *n* = 12 by a transference of all genetically active materials of one chromosome to the rest with a subsequent loss of the centromere, a process envisaged in a large number of Angiosperms, particularly by Babcock (1942) in *Crepis*, and which was experimentally proved later on by Tobgy (1943). An analagous situation prevails in the artificially produced 3-paired strain from a standard 4-paired strain of *Drosophila melanogaster* by Dubinin (1934, 1936). Babcock and Cameron (1934) assumed reciprocal translocation followed by meiotic irregularities resulting in the loss of a single chromosome. Darlington (1937, 1939) postulated genetic inertness of regions proximal to centromere and unequal reciprocal translocation. One of the products of translocation, which would entirely be made up of heterochromatic parts, could be lost without any deleterious effect on the plant and a concomitant reduction in the chromosome number would result. The preponderance of the basic number 12 and the relative infrequency of 11 shows that 12 is a stable number in Abiteae. "Evolutionary stability of basic chromosome numbers means activity of genes near the centromere and the ends. Instability means their inertness" (Darlington, 1937).

The derivation of *n* = 13 as in *Psodosuga* from *n* = 12 is difficult, not being based on unequivocal evidence. The following are some of the suggestions.

(1) Fragmentation of a median or submedian chromosomes resulting in two terminally constricted chromosomes, which regenerate a new centromere in the first one or two mitoses, so that they survive as normal chromosomes. A regeneration of a centromere in an acentric fragment is possible (Darlington, 1929) although acentric fragments formed by inversion or deficiency invariably degenerate.

(2) Nondisjunction of a pair of chromosomes resulting in an irregular distribution of the chromosomes, due to accentuated attraction of the chromatids and failure of separation.

(3) The thrid suggestion is the breakage of a centromere of a V or J-shaped chromosome to form two terminal chromosomes, followed by reciprocal translocation with another chromosome leading to the formation of a dicentric chromosome. When this breaks two new chromosomes with submedian or subterminal chromosomes are formed, as shown in the following diagrams:



The serious objection, however, is the breakage of the centromere, which is possible in light of our knowledge of the quadriple structure of the centromere (Tjio, and Levan, 1950) and such parallel instances as the severence of the nucleolar organiser (McClintock, 1934) may also serve to illustrate the point in question.

The monotypic Pineae, the second subfamily of Pinaceae with its fused ovuliferous and bract scale, is the highest evolved in the family (Hutchinson, 1924). *Pinus* is uniformly characterised by the haploid number 12. That the highest evolved genus has the most stable number is interesting. The constancy of the chromosome number illustrates "one of the most conservative properties of the genetic system" (Darlington, 1939) in *Pinus*, as is the case with large sections of Orthoptera, the Gramineae and Rosaceae. If the smallest pair of chromosomes could be used as chromosome markers, comparative study of the karyotypes of a dozen species of *Pinus*, reported by Mehra and Khoshoo (1956a) would at once reveal that extensive repatterning of chromosomes is in main responsible for the origin of species in *Pinus*. While the smallest pair is seen in most of the species *P. gerardiana* is an exception to the same. That structural changes are responsible for the evolution is also indicated by a variation in the number of chromosomes with secondary constrictions. There are all gradations between species like *P. halepensis*, *P. nigra*, *P. pinaster* and *P. ponderosa* having no chromosomes with secondary constrictions and such species as *P. gerardiana* with 8, *P. lambertiana* with 6 such chromosomes. The location of the secondary constrictions varies in different species. It is submedian or subterminal as in Tasmanian *P. radiata* or very near the primary constriction imparting a characteristic appearance to the karyotype.

The same type of variation in the position and the number of secondary constrictions, which are now known to differentiate species is encountered within the range of the same species population of *P. radiata*. As pointed out already, in having 5 pairs of secondarily constricted chromosomes, which is the largest number so far reported in the genus, the Tasmanian *P. radiata* has no parallel in in the twelve species of the genus, including its Indian counterpart reported by Mehra and Khoshoo (1956a). It is interesting to note that the same mechanism of isolation that is responsible for the origin of new species also serves as a means of isolating the two geographical races, which are now included under P. radiata. This is a strong reason to think that Tasmanian P. radiata is a new species. A close scrutiny would suggest that this cytological character could be correlated with the morphological differences. Already an attempt has been made to classify Monterey Pine on the basis of external morphology (refer Fielding, 1953). A critical karyotype analysis will provide a basis for the morphological classification.

Geographical races with different karyotypes are known in literature. The several species of Iris illustrate the point in question. I. psedopumila, I. mellita, I. pallida and I. variegata not only show karyological differences but correlated morphological differences too (Mitra, 1956). Morphology of chromosomes and the size of the satellites are known to differentiate geographical forms of I. aguria (Westergaard, 1938). Similar geographical races have been reported by Yamamoto (1933) in Rumex acetosa. In a collection of 50 individuals of Scilla peruviana, coming from 6 sources, Battaglia (1949) discovered 6 biotypes with different karyotypes, all known to have arisen as a result of structural changes. Apparently a divergence in the karyotype is possible in a population without effecting much the external morphology. This is the starting point of speciation within a population, since these groups of plants develop independently due to isolation and ultimately form new species. Such processes are obviously at work in Pinus radiata.

III. TAXODIACEAE AND CUPRESSACEAE.

There is a great unanimity of opinion among the taxonomists that Taxodiaceae and Cupressaceae are closely related to each other. Of the two, Cupressaceae is relatively more evolved. That they have a modern aspect when compared with other Coniferales and that they are relatively younger and could be derived from Pinaceae were emphasised by Jeffrey (1917) Coulter and Chamberlain (1917), Hutchinson (1924). In this connection it is interesting to note that Saxton (1913) considered both the families under Cupressaceae and regarded Sciadopitys as a connnecting link between Abietoideae on the one hand and Cupressoideae on the other thereby connecting Cupressaceae with Pinaceae like all the earlier authors. In the light of the structure of the pollen grains, Wodehouse (1935) corroborated these ideas of their close affinity amounting to identity. He affirmed that they are the highest evolved in Conifers on account of the absence of the prothallial cells in their pollen grains. Unlike the preceding authors, he however suggested antiquity in origin for both the families from Cordaitalean stock quite independently of Pinaceae on account of "the remarkable persistence and stability of such characters as the thin, flecked exine and greatly thickened intine". He derived them from the archeic open-furrowed type by the reduction of furrow.

A summation of cytological characters of Taxodiaceae and Cupressaceae, although not decisive of their relative evolutionary status, certainly allies them as one natural and closely related assemblage of Conifers. The present cytological study of Athrotaxis (Taxodiaceae) and Callitris and Diselma (Cupressaceae) when considered in conjunction with the previous cytological results on the other genera clearly shows that both the families are characterised by 22 fairly long chromosomes in the somatic cells, out of which one pair shows secondary constrictions. In a few Cupressaceae a pair of chromosomes are satellited instead of having secondary constrictions. On the whole both the families are similar cytologically with one basic number reflecting their common origin. In this connection, Athrotaxis ($2n = 22$) is interesting since it is the only genus of Taxodiaceae in the Southern Hemisphere, which has either spirally or decussately arranged leaves and subspirally arranged staminate scales, characters somewhat intermediate between Taxodiaceae and Cupressaceae. Its chromosomes are similar to both the families. It is therefore intermediate between the two families not only in its external morphology but also in its chromosomes. Maybe it is the only surviving member of a stock, from which Taxodiaceae and Cupressaceae have diverged at comparatively recent times. Its anamalous geographic distribution today could perhaps be explained on that assumption.

Cytology not only brings the two families together but affords critical evidence for the systematic position of some genera in their respective families. Whenever a genus showed a departure from the general cytological features of the families mentioned above, it required to be isolated into a family of its own. To cite one instance from Taxodiaceae: When Hayata (1931) erected a new family Sciadopityaceae to accommodate Sciadopitys, little did he realise that 20 slender diploid chromosomes, which appear to be median or submedian with no secondary constrictions (Tahara, 1940) would justify his contention. The chromosomes of Sciadopitys are certainly most unlike any other

Taxodiaceae. The genus *Tetraclinis* illustrates again the same point in Cupressaceae. It is intermediate between the southern and northern genera but in having basically valvate cone-scales, it is closer to the southern forms. There are two pairs of cone-scales of equal size but of different shape. The young scales are fleshy. In its vegetative characters, it closely approaches *Heyderia* and *Thujopsis* of Thujopsideae and in having three to five cotyledons it recalls *Juniperus* of Junipereae. Li (1953) however placed it in a tribe of its own, namely Tetraclineae of the subfamily Callitroideae. It is more or less isolated in the family Cupressaceae. Hayata (1932) went a step further to create for it a new family Tetraclinaceae. Its cytology supports its isolated systematic position in Cupressaceae and even the formation of new family to accommodate it. While the typical Cupressaceae have 22 long diploid chromosomes with one pair of secondarily constricted chromosomes or a pair of satellited chromosomes, *Tetraclinis* has 22 *short* median or submedian chromosomes, none of which show either the secondary constrictions or satellites. To a large extent the chromosome complement is similar to *Juniperus procera* (2*n* = 22) reported by Mehra and Khoshoo (1956a). The only point of difference is that the pair of subterminal chromosomes found in *Juniperus procera* are absent in *Tetraclinis*. In this respect the former appears to be more highly evolved cytologically than *Tetraclinis* (cf. Hutchinson, 1948, pp. 48).

The above mentioned instances run parallel to the cytological situation obtained in *Diselma archeri*, which again deserves an isolated position in

Cupressaceae on cytological characters alone. The systematic position in Cupressaceae has been variously interpreted. Pilger (1926) placed it in the subfamily Thujoideae. This view was shared by Moseley (1943) who made minor changes in the generic composition of the same subfamily. Li (1953) in his classification of the subfamily Callitroideae included *Diselma* in the tribe Libocedreae and also indicated that it probably originated from *Widdringtonia*. The present cytological study of *Diselma archeri* does not support any of the above mentioned views, particularly the probable origin of *Diselma* from *Widdringtonia*. There are 22 fairly long chromosomes in the root-tips of *Diselma* with median or submedian constrictions. Out of these two chromosomes show intercalary satellites and the chromosomes simulate those with secondarily constrictions. They originate as a result of inversion of a satellited chromosome, fusion of the satellite with distal end and the breakage of the chromosome in the middle of the short proximal arm. The subsequent healing of the broken ends will result in a chromosome with a "secondary constriction" showing the structural details as those of *Diselma*. When considered in this light, the most probable ancestor of *Diselma* appears to be a species like *Cupressus torulosa* (2n = 22) with a pair of satellited chromosomes, whose inversion and breakage would give rise to chromosomes with intercalary satellites. *Widdringtonia cupressoides* (2n = 22) with a pair of normal secondary constrictions (Mehra and Khoshoo, 1956a) could never be the possible progenitor of *Diselma* as was thought by Li (1953).

By the way it may be remarked that secondary constriction was first described by Delaunay (Lewitsky, 1931) as a triarticulate body. Heitz (1931) established its function as a region where nucleoli were organised. In this respect, they have a function similar to those of satellited chromosomes, although Sato (1937) claimed that the secondary constrictions have no connection with the nucleoli. But in recent literature cases are known in which the chromosomes with secondary constrictions are both nucleolar as well as non-nucleolar. Perhaps in the absence of satellited chromosomes they are nucleolar in function. From a morphological point of view there is little difference between a satellited and a secondarily constricted chromosome. The only difference is in the length of the segment distal to the constriction. Perhaps a satellited chromosome is more recent and represents a higher evolutionary type. The case of secondary constriction described in Diselma is different from an ordinary secondary constriction described above. While the ordinary secondary constriction is primitive the intercalary trabant simulating a secondary constriction is recent and perhaps highest evolved.

Closely associated with its unique cytological characters, Diselma shows advanced morphological features. There are two pairs of cone scales of equal size, one sterile and one fertile, the fertile ones bearing two or three winged seeds. If Tetraclinus with the same number of cone scales could be regarded as highly advanced (Hutchinson, 1948), Diselma too appears to have the same evolutionary status. With its intercalary trabant, it is extremely doubtful whether it could be derived from a genus like Widdringtonia. On the basis of karyology alone Diselma archeri is to be given an isolated position in Cupressaceae as is the case with Tetraclinus. Its actual systematic position in a particular tribe and a subfamily of Cupressaceae can be decided only after a thorough cytological survey of the whole family.

V. SUMMARY

(1) Chromosome studies, with a view to ascertaining taxonomical and phylogenetic relationships of the Tasmanian Conifers was undertaken for the first time. The present study includes five species of Podocarpaceae, three species of Cupressaceae, three species of Taxodiaceae and one species of Pinaceae.

(2) *Phyllocladus*, as represented by *P. aspleniifolius* (2n = 18), is an aberrent genus representing an independent but parallel line of evolution which requires an isolated position, if retained in Podocarpaceae. It could be described as a Podocarp with *Taxus*-like chromosomes and this would probably mean "harking back" to the hypothetical ancestor, which was the starting point of modern Taxaceae and Podocarpaceae.

(3) *Pherosphaera hookeriana* (2n = 26) is curious juxtaposition of primitive and advanced characters. It is phylogenetically an off-shoot from a Dacrydium-like descent or both *Dacrydium* and *Pherosphaera* have descended from the same primitive stock. When compared with other Podocarpaceae, it has likenesses accompanied by strong differences. Considerable evidence has been presented to show that it deserves a separate family.

(4) Morphological and cytological facts clearly postulate that *Microcachrys tetragona* (2n = 30) is not only closely related to *Dacrydium franklinii*

(2n = 30) but both are probably descendants from a common ancestor. The chromosome complement of Microcachrys tetragona is a reduced replica of Dacrydium franklinii. In the origin of Microcachrys tetragona genotypic changes leading to a reduction in the size of the chromosomes was an important factor. This is correlated with its shrubby habit.

(5) Podocarpus alpina (2n = 38) is considered as the starting point of Eupodocarpus line and hence 2n = 38 is the most primitive number in Podocarpus. Reduction due to fusion has been the most probable line of evolution within the genus. Morphologically complex and highly evolved species are characterised by lower chromosome numbers. Polyploidy is absent.

(6) Tasmanian Pinus radiata (2n = 24) with its five pairs secondarily constricted chromosomes is different from its Indian counterpart with only two such pairs. It is suggested in the light of the cytological data that it is a new species. A reclassification of Pinus radiata is recommended.

(7) The genus Athrotaxis (2n = 22) is regarded as connecting link between Taxodiaceae and Cupressaceae. Both cytology and morphology supports the same contention.

(8) Diselma archeri (2n = 22) has probably an isolated position in the family Cupressaceae in having of a pair of chromosomes with intercalary trabants, which are unique in the family. It seems to have originated from an ancestor like Cupressus torulosa (2n = 22) with a pair of satellited

chromosomes. Inversion of a SAT-chromosome, end-to-end fusion and subsequent breakage in the middle of the short proximal arm will lead to the formation a chromosome with intercalary trabant. It is one of the highly advanced genera of Cupressaceae.

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Figs. 1 - 9.

Explanation of the text figures illustrating "Cytotaxonomy and phylogeny of the Tasmanian Conifers".

Figs. 1 and 2. Phyllocladus asplenifolius. Fig. 1. Somatic metaphase, $2n = 18$; note F^{I} and F^{II} chromosomes with unequal centromeric constrictions. Fig. 2. Idiogram. Figs. 3 and 4. Pherosphaera hookeriana. Fig. 3. Somatic metaphase, $2n = 26$. Fig. 4. Idiogram. Figs. 5 - 7. Microcachrys tetragona. Fig. 5. Somatic metaphase, $2n = 30$; note the 2 SAT-chromosomes. Fig. 6. Idiogram. Fig. 7. A root-tip cell showing "reduction groupings". Figs. 8a - 9. Dacrydium franklinii. Fig. 8a. Somatic metaphase, $2n = 30$; note A^{I} and A^{II} for their unequal lengths. Fig. 8b. Somatic metaphase showing thirty two chromosomes. Fig. 9. Idiogram.

Figs. 10 - 19.

Figs. 10 - 12. *Podocarpus alpina*. Fig. 10. Somatic metaphase, $2n = 38$; note two SAT-chromosomes, one at 3 o'clock and the other in the centre. Fig. 11. Somatic metaphase, $2n = 38$; showing the minute second arms of all the chromosomes except the SAT-chromosomes. Fig. 12 Idiogram; note the gradual gradation in the chromosomes. Figs. 13 - 19. *Callitris oblonga*. Fig. 13. Somatic metaphase, $2n = 22$. Fig. 14. Somatic metaphase, $2n = 22$ to show a pair of heteromorphic chromosomes with secondary constrictions and a third chromosome which has lost a piece; the effected chromosomes are blackened. Fig. 15. Somatic metaphase with twenty one chromosomes and a fragment; the pair of chromosomes with secondary constrictions is heteromorphic; the other two chromosomes showing structural alterations. Fig. 16 - 18. Showing the variation in the length of the heteromorphic pairs of chromosomes. Fig. 19. Idiogram; the pair G is unequal just like the pair H.

Figs. 20 - 26.

Figs. 20 - 21. Callitris tasmanica. Fig. 20. Somatic metaphase, $2\underline{n} = 22$. Fig. 21. Idiogram; note the gradual gradation in the chromosomes. Figs. 22 - 24. Diselma archeri. Fig. 22. Somatic metaphase, $2\underline{n} = 22$. Fig. 23. Idiogram showing the SAT-chromosome (C), which shows a granular body in the secondary constriction. Fig. 24. Somatic metaphase with ninteen chromosomes. Figs. 25 - 26. Athrotaxis selaginoides. Fig. 25. Somatic metaphase $2\underline{n} = 22$. Fig. 26. Idiogram; note two short chromosomes.

Figs. 27 - 31.

Figs. 27 - 28. A. laxifolia. Fig. 27. Somatic metaphase with twenty two chromosomes. Fig. 28. Idiogram; note the gradation in the length of chromosomes except the single last pair (K). Figs. 29 and 30. A. cupressoides. Fig. 29. Somatic metaphase showing twenty two chromosomes; the homologous chromosomes A^{I}, A^{II} and G^{I}, G^{II} are unequal in length. Fig. 30. Idiogram; note the single short pair of chromosomes (K). Fig. 31. Pinus radiata. Somatic metaphase showing twenty four chromosomes.

(All figures are drawn at x 2,500 and reduced to the page size in the photographs).

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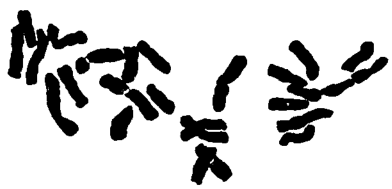

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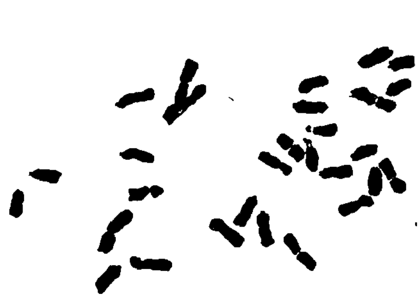

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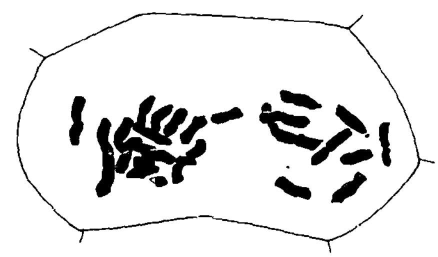

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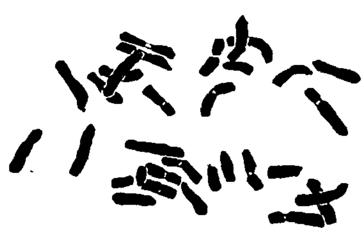

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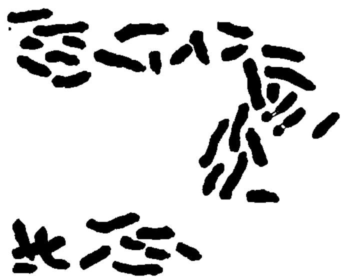

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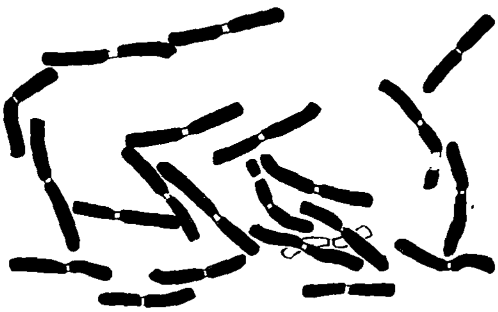

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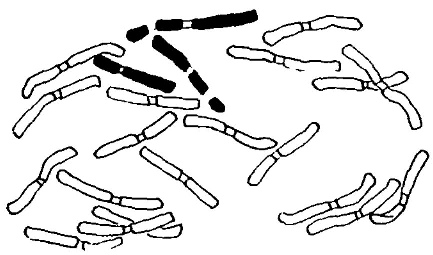

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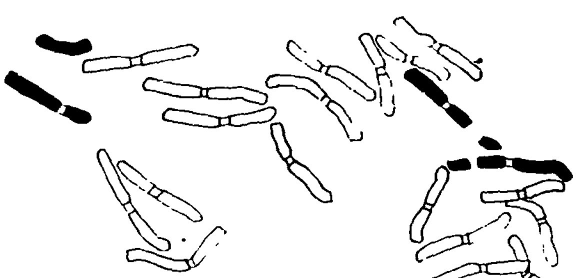

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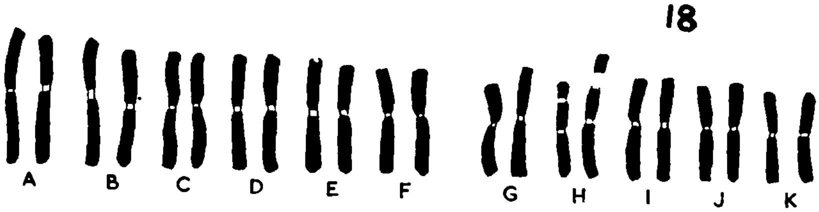

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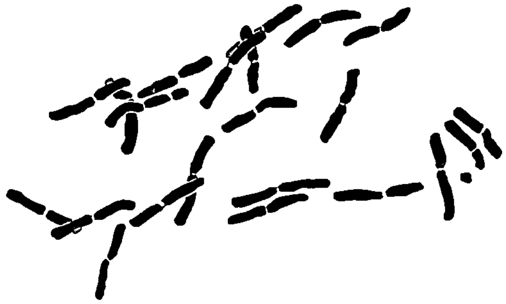

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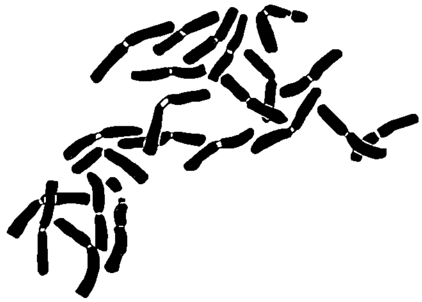

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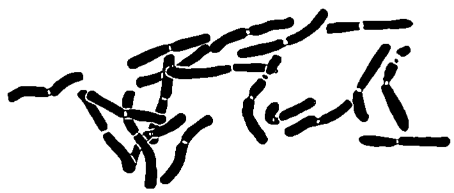

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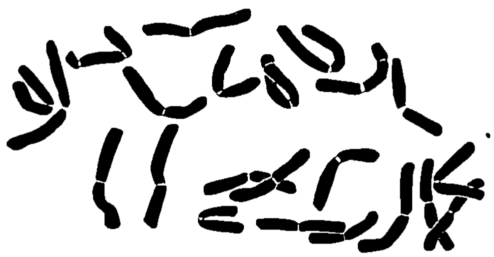

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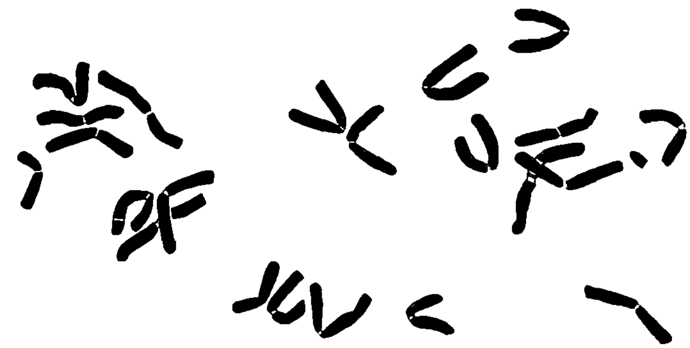

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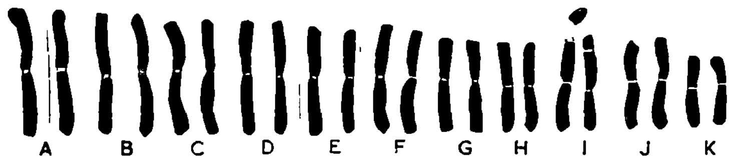

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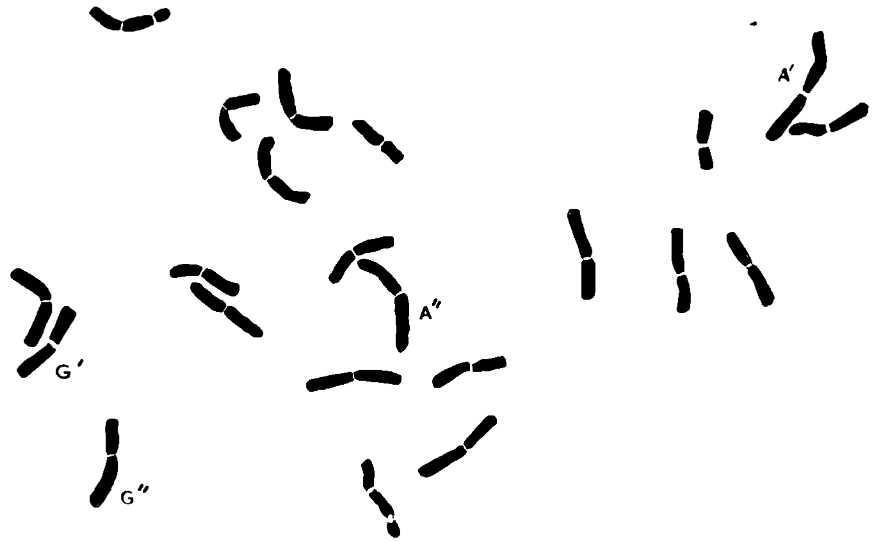

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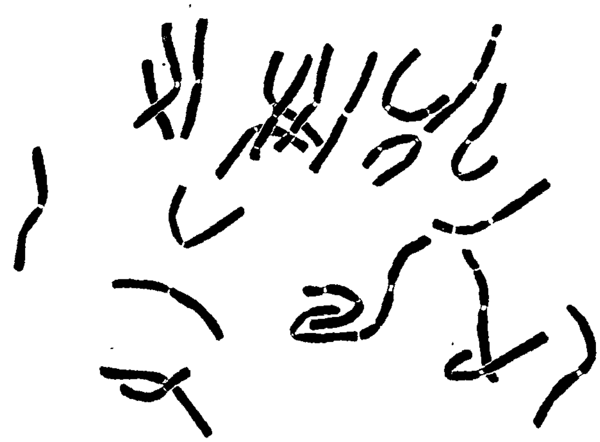


Dacrydium franklinii (2*n* = 30)

somatic metaphase; same as fig. 8a.

x 1250

Dacrydium franklinii (2*n* = 32);

same as fig. 8b. x 1250

Callitris oblonga (2*n* = 22); one chromosome at about 11 o'clock showing structural change. x 1250

Callitris oblonga (2*n* = 22) showing heteromorphic chromosomes with secondary constrictions (about 6 o'clock and 8 o'clock) x 1250

Callitris oblonga (2*n* = 22); metaphase with one fragment and one structurally altered chromosome; same as fig. 15.

x 1250

Diselma archeri ($2n = 22$); somatic metaphase note the two chromosomes with tandem satellites at about 12 O'clock.

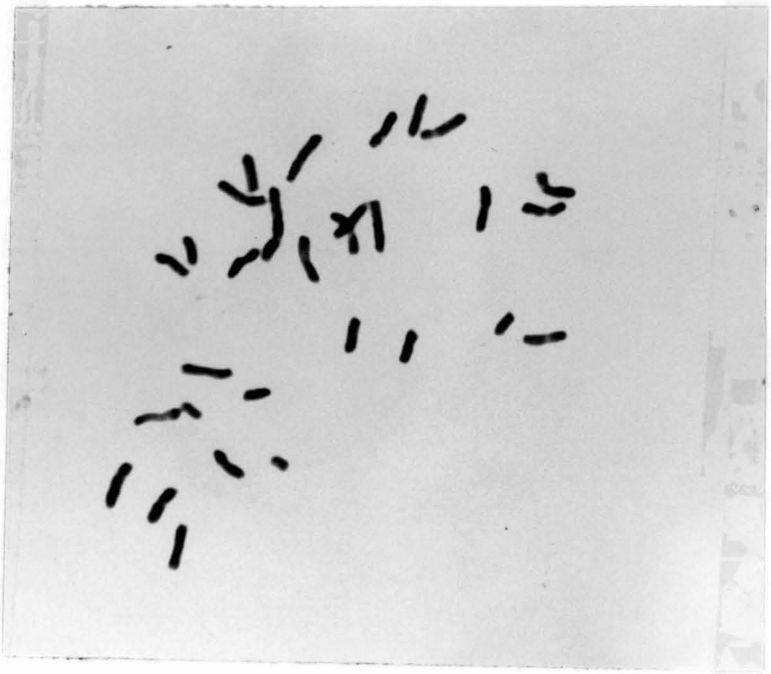
x 1250

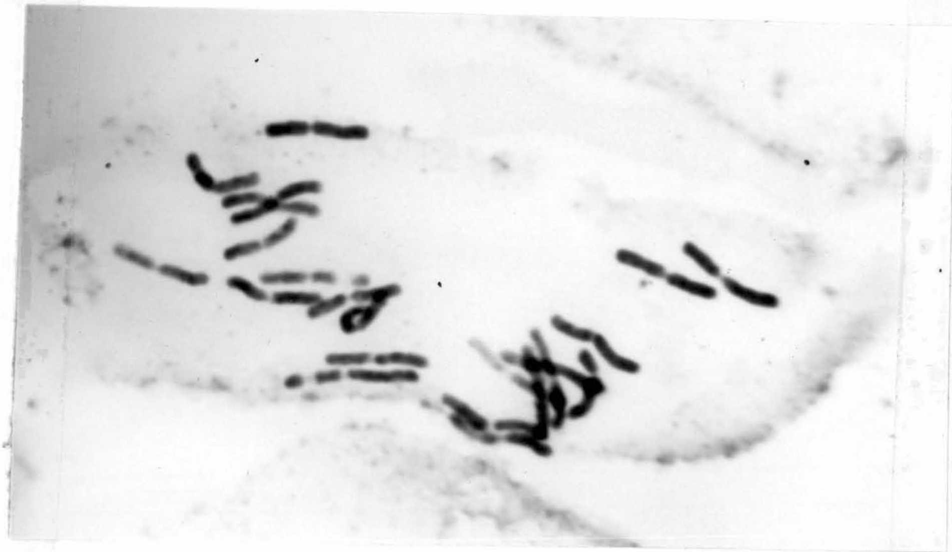
Diselma archeri ($2n = 19$); an abnormal metaphase; same as Fig.24.

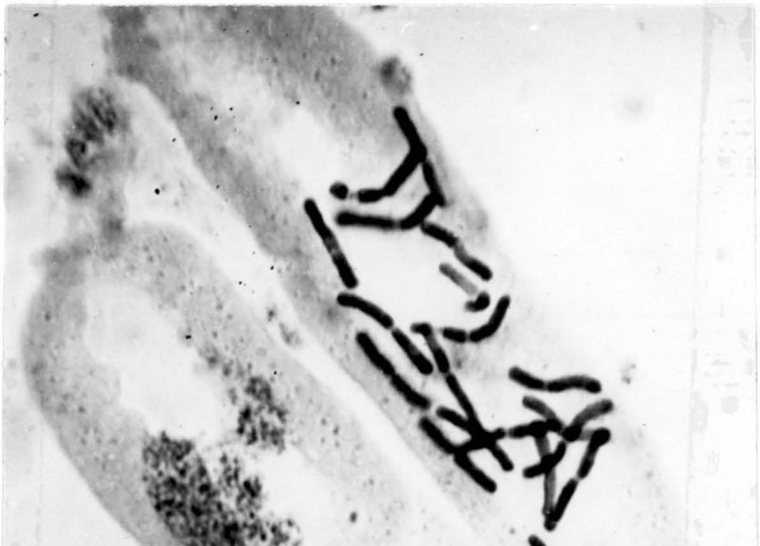
x 1250

Athrotaxis cupressoides (2n = 22) somatic
metaphase; same as fig. 29 x 1250

Athrotaxis selaginoides (2n = 22) somatic
metaphase; same as fig. 25. x 1250







IMPROVED SQUASH TECHNIQUES FOR CONIFERS

IMPROVED CHROMOSOME SQUASH TECHNIQUES FOR CONIFERS.

Abstract

For a critical karyotype analysis in Conifers, two improved squash methods are outlined. One utilises 0.5% aqueous colchicine solution (2 hours) and 0.003M 8-hydroxyquinoline (2 hours) as prefixatives, acetic-alcohol (1 : 3) with 5% concentrated hydrochloric acid as a fixative, prolonged cold hydrolysis in a macerating fluid containing concentrated hydrochloric acid and 95% alcohol (1 : 1) and feulgen staining coupled with acetocarmine. This method is most useful for all Conifers. The second method involves the use of concentrated aqueous solution of α-Bromonapthaline in tap water as a prefixative (3-4 hours), osmic fixative like Benda with 0.003M 8-hydroxyquinoline (5 : 1), hydrolysis in hot 1N hydrochloric acid for 45 - 60 minutes at 60°C and feulgen squashing. This method gives good results with *Pinus radiata* and *Podocarpus alpina*. The preparation could be made permanent by any well known method.

Obviously in *Pinus radiata* and *Podocarpus alpina* the tannin could be hydrolysed into soluble sugars, while it is not possible in others. The following two methods are based on this fact.

There has been a great paucity of literature relating to critical karyotype analysis in Conifers, which figure prominently in all evolutionary and phylogenetic discussions. One of the most important contributory causes, apart from others, is the lack of facile techniques for a study of their chromosomes. The pioneer work of Sax and Sax (1933), important though it may be in many ways, falls short of any modern standard of karyotype analysis. Sax and Sax (loc. cit.) employed mostly acetocarmine squashes of the endosperm tissue rejecting totally root-tips as unsuitable for a study of somatic chromosomes. Flattening of chromosomes was achieved mostly by physical pressure. Flory (1936) subsequently extended the work of Sax and Sax. It was also based entirely on the acetocarmine method of Warmke (1935). They were precolchicine days.

Even after the advent of colchicine, no serious attempt was made to improve the techniques for Conifers. The rapid squash technique of Johnson (1945) was meant to provide a method for counting chromosomes in Pinus, Abies, Picea, Psedotsuga, Thuja and some angiosperms. The author himself did not claim that it was designed for any critical observation of chromosomes. The only new feature of his method was the use of n-propyl acetate as a dehydrating agent, which was considered as less toxic than dioxane and it absorbed no moisture from air thereby proving superior to absolute ethanol. All the earlier and later workers like Dark (1932) and Baldwin (1945) used root-tip materials fixed in osmic or formalin fluids and stained sections with crystal violet. When dealing with such genera as Conifers, some of which like Athrotaxis are characterised by exceptionally long chromosomes, squash methods give a very definite advantage over the sections, since squashes eliminate forshortening and facilitate the study of relative lengths of chromosomes. Such methods are well-nigh indispensable to test the age old hypothesis that the chromosomes in Conifers are highly stable and are liable to little or no variation.

One of the outstanding facts connected with the above-mentioned problem is the occurrence of abundant tannin or tannic acid or tanniniferous substances in the alpine and subalpine Tasmanian Conifers, serving to diminish the danger of desiccation in the absence of snow and protect them from assaults of animals and frost. They form reserve food or products of excretion in some Conifers. Constituting a mixture of a variety of substances, whose chemistry is imperfectly known, they are probably of the nature of glycocides or derivatives of protocatechuic and gallic acids. Glycocides yield dextrose on hydrolysis while the rest do not. They react with heavy metalic ions, like iron, in acetic solutions. Ordinary reagents like chromic acid, osmic acid, Pot. dichromate etc., give brownish to black precipitates with tannins. Heavy metalic ions are introduced by the iron instruments when used in conjunction with acetic alcohol. Hence during the whole manipulations described below, particularly in the initial stages of fixation, care should be exercised to avoid iron needles and forceps and to use plated or plastic instruments (Li, 1954). Their precipitation in the initial stage would seriously interfere with squashing. Flattening, which is so essential for obtaining clear pictures of chromosome morphology, would become an impossibility.

Precooling: In the case of small plants of the Conifers with root tips, cold treatment was tried with success. If the plants with their roots immersed in tap water were left in a cold room at 0°C overnight, the frequency of metaphases in the root-tips considerably increased. Chromosomes were probably held at metaphase due to the suppression of the spindle. Gerstal (1949) however thought that cold treatment, effective at 5°C, considerably reduced the number of metaphases in Scorzonera tau-saghyz. The effect of cold treatment was first studied in detail by Delaunay (1930) in _Crepis_ and it has been used in the cytological schedules formulated by Hill and Myers (1945), Warmke (1946) and Dowden (1949). Barber and Callan (1942) have adopted freezing as a means of obtaining metaphases with super-contracted chromosomes in _Triton_. It is now realised that freezing not only precipitates some of the nuclear proteins but also brings about fixation of the chromosomes.

Prefixation: The number of chemical substances for obtaining well preserved, well scattered and straightened chromosomes when the cells with thin solidified plasma are pressed under a cover glass is rapidly increasing. The use of Colchicine, 8-hydroxyquinoline, Paradichlorobenzene, Coumarine, α-Bromonapthalene as prefixatives, either singly or in combination, is a valuable step forward in improving the squash techniques. Ever since the discovery of colchicine as a potent tool for inducing polyploidy, it is widely used in cytological techniques for destroying the spindle, for contracting chromosomes and for obtaining well-spaced chromosomes (O'Mara, 1939, 1948; Burrel, 1939; Bhaduri, 1940; Rosen, 1946 a, b; Gerstel, 1949). It exaggerates the size differences by the optical foreshortening of the smallest chromosomes (O'Mara, 1939). It seems to have some specific action on the centromere too (Karpechenko, 1940).

During the present investigation on Tasmanian Conifers, mitotically active root-tips of sufficient length were soaked in several concentrations of pure colchicine (Pal chemicals Ltd., London and British Drug House, Poole, England) in distilled water (0.1%, 0.25%, 0.4% and 0.5%). Although it is generally understood that at lower concentrations the only effect of colchicine is to destroy the spindle mechanism with little or no perceptable change in the chromosomes, practically no visible inhibiting action of the spindle has been observed in Tasmanian Conifers at lower concentrations even after a treatment extending over six or seven hours. At high concentrations, the desired effect was produced only after a prolonged treatment. Well straightened and scattered chromosomes were obtained after a treatment in 0.5% aqueous solution for 3 hours in the case of most of the Conifers. However, the period of exposure and the strength of the solution varied to some extent depending on the nature of the species. For instance, in the case of Podocarpus alpina 0.25% solution for three hours or 0.5% solution for two hours were found sufficient. In all these experiments exposure to light (100W. lamp) resulted in hastening the action of colchicine. The development of the spindle is perhaps arrested due to heat and this would naturally augment the colchicine action in the same direction.

The tolerance of the Tasmanian Conifers to high doses of colchicine is indeed very remarkable. As is the case with Colchicum which contains colchicine in its cells and therefore resistent to the drug (Levan and Steinegger, 1947), Tasmanian Conifers may presumably have within their cells substances that are C-mitotically active. It is also possible that the tannins present in their cells may physically and chemically react with colchicine thereby reducing its efficacy and delaying its action.

Colchicine alone did not produce the desired effect of clarifying the primary and secondary constrictions as in Diselma archeri and Athrotaxis species. Hence other chemicals either singly or in combination were tried. The specific action of 8-hydroxyquinoline in bringing about changes in viscosity and accentuating the primary and secondary constrictions is a timely discovery (Tjio and Levan, 1950). Treatment of the excised root-tips of Tasmanian

Conifers in 0.003M 8-hydroxyquinoline alone at 10 - 14°C was found to produce the desired effect only after 8 - 10 hours, a duration which was unduly long for any pretreatment in a squash technique. In recent years, saturated solutions of Paradichlorobenzene (Meyers, 1945; Derman and Scott, 1950) and Coumarine (Cornman, 1946)were accredited with C-mitotic action. They did not prove useful for the Conifers in question as they also required prolonged treatment like oxyquinoline.

To achieve a rapid distruction of the spindle and a mild initial contraction of the chromosomes, the root-tips were first treated with 0.5% colchicine for 2 hours at room temperature or before an electric lamp. It was then followed by 2 hours' treatment with 0.003M 8-hydroxyquinoline at 10 - 14°C for 2 hours resulting in a further contraction of chromosomes, solidification of the plasma and above all in clarifying the primary and secondary constrictions. In such a schedule the rapidity of the action of colchicine and the efficacy of 8-hydroxyquinoline in bringing out the constrictions prominently have been combined and exploited.

The adoption of α-Bromonapthalene as a chemical for pretreatment of the somatic chromosomes has been frequently used in recent cytological techniques. Schmuck and Kostoff (1939) first employed it as a polyplodising agent. Later on, O'Mara (1948) used mono-bromonapthaline and monobromobenzene to secure well scattered and straight chromosomes. In the case of Tasmanian Conifers, it took 4 hours to attain the initial mild contraction of chromosomes and the suppression of the spindle. When this treatment is coupled with the use of 8-hydroxyquinoline in Benda or a chromo-osmic fixative in the proportion of 1 : 6 and 1 : 5 respectively, well clarified and highly transparent chromosomes were obtained. The effect was pleasing, the outline of the chromosomes was smooth and a wealth of new details were revealed, as in *Pinus radiata*. Consistent application of this method alone should orientate our knowledge of the karyotype evolution of *Pinus* and other genera of Conifers in a new perspective.

Fixation: When fixation was minimised to 10 minutes, acetic-alcohol (1 : 3) was found to be most useful and gave consistent results. From the point of view of tannin and maceration, a slight increase in the acetic acid (i.e. 1 part of glacial acetic acid and 1 part of absolute alcohol) gave better results. However, the swelling produced was so great as to distort the sharpness of the primary and secondary constrictions and hence acetic-alcohol with this formula was discarded. Repeated trials have revealed that the addition of 5% concentrated hydrochloric acid to acetic-alcohol (1 : 3) helped in overcoming the resistence offered by tannin and helped in the dissolution of the middle lamella. HCl alone as a fixative was first used by Gerstel (1949) combining fixation and hydrolysis at 60°C for 10 - 14 minutes. This method cannot be used with any advantage in the case of Tasmanian Conifers. The use of 45% acetic acid as a fixative suggested by Schreiber (1951) for *Bromus* which strongly resists maceration and hence the separation of cells was tried in vain during the present study.

Osmic fixatives were in general found most unsatisfactory during the present investigation for the Tasmanian Conifers. They precipitated tannin in their cells as dark brown crystalline masses. No matter how long the hydrolysis time was extended, the major problem of effective maceration and squashing still remained in these cases. It hindered all efforts to obtain clear images of the metaphase chromosomes. It is possibly due to the fact that tannin contained in these Conifers cannot be hydrolysed into sugars. The only two exceptions to this rule are *Pinus radiata* and *Podocarpus alpina*. In both these cases, osmic fixatives like Benda, with and without acetic acid, and chrome-osmic fixatives have proved pre-eminent. As pointed out earlier in this paper, the addition of 8-hydroxyquinoline to these fluids (5 : 1) and fixation at 10 - 14°C have given all the advantages of 8-hydroxyquinoline to the resulting effect. Prolonged hydrolysis is the only prerequisite for successful squashing. In this respect these two Conifers are most unlike other Tasmanian forms. Perhaps in these cases, the tannins are hydrolysed into sugars.

Hydrolysis: Prolonged cold hydrolysis of root-tips in a macerating fluid containing equal parts of concentrated hydrochloric acid and 95% alcohol has given consistently good results. The time of hydrolysis varied with the species depending on the nature and amount of tannin contained in their cells. For instance, in the case of *Diselma archeri* and *Pherosphaera hookeriana*, the time of hydrolysis could be prolonged to 35 minutes with advantage. Normally, 20 - 25 minutes hydrolysis was found sufficient. Hydrolysis 1N Hydrochloric acid for the same period was unsatisfactory. A macerating fluid with 3 parts of concentrated hydrochloric acid and 1 part of 95% of alcohol was used in the case of *Microcachrys tetragona*, *Callitris* species, *Diselma archeri* and *Pherosphaera hookeriana*. Although it occasionally gave good results, corrosions appeared on the chromosomes and hence they presented a distorted appearance. No doubt this treatment interferred with the subsequent feulgen reaction also.

In the case of *Pinus* and *Podocarpus* which contain either Glycosides or tannins allied to them, hydrolysis at 60°C in 1N hydrochloric acid for

1 hour resulted in a breakage of tannins into soluble sugars like Dextrose. Further staining, separation of cells and squashing presented no difficulty at all, when such a hydrolysis was proceeded by proper bleaching in a mixture of hydrogen peroxide and concentrated aqueous ammonium oxalate. Ammonium oxalate brings about a break down of the pectic compounds and it is completed by hydrolysis.

Staining: After hydrolysis, the hot acid is replaced by cold 1N Hydrochloric acid and the root-tips are left for 5 minutes in it. Then they are rinsed thoroughly in distilled water for 5-10 minutes. Decolourised basic fuchsin prepared according to de Thomasi (1939) is poured into the tube. It took 2-3 hours to stain the tips properly. If the working time necessitates, the material could be left overnight in basic fuchsin without any damage to it. In the latter case, the material may sometimes need subsequent bleaching in freshly prepared SO_2 water. Generally, this has been avoided because SO_2 water toughened the tissues to some extent.

Feulgen reaction alone has not resulted in intense staining of the chromosomes, due to hydrolysis much beyond the optimum period. The stain could be intensified by washing the tips repeatedly in tap water. Brightly stained meristematic region is cut off and then dissected in a drop of acetocarmine on a slide. A small portion of this dissected tissue is put in a fresh drop of acetocarmine on another slide, albuminised cover slip is added and squashed without moving the cover slip side ways. A sharp needle is applied on those parts of coverslip where the tissue is located and further flattening is effected. During these stages, gentle heat over the spirit flame was applied 2 or 3 times. In some Conifers like Callitris, bringing the acetocarmine to the boiling point facilitated squashing. Excess carmine is then blotted out holding the cover glass in position, the preparation is sealed with wax and left for 2 or 3 days before they are made permanent. Generally drawings and photomicrographs of various chromosome complements are best made from the temporary preparations, in which the constrictions remain crisp.

Dehydration, clearing and mounting:

When the chromosomes are sufficiently stained, wax is carefully removed from the edges of the cover glass and the preparation is made permanent by any of the well-known methods. If Butyl alcohol and acetic acid mixture (1 : 1) is used for separating the coverslip from the slide, treatment of the preparation in a mixture of redistilled turpentine and Butyl alcohol (1 : 1) clears the cytoplasm from osmic stain. The preparation is finally mounted in canada balsam after giving one or two changes in Butyl alcohol.

If cytoplasm stains with acetocarmine, the most effective way of clearing it is to separate the cover glass from the slide in 45% acetic acid, which destains cytoplasm. The preparation is then made permanent by any method given by Darlington and La Cour (1942).

Summary of the squash techniques

Schedule I.

(1) Precool the root system in tap water at 0°C for 20 - 24 hours in a cold room. If young plants with roots are not available this step could be avoided.

(2) Bring the water to the room temperature, cut off the root-tips and pretreat them in 0.5% aqueous colchicine solution, 2 hours.

(3) Wash in running water, 2 hours.

(4) Treat the tips in 0.003M 8-hydroxyquinoline at 10 - 14°C, 2 hours.

(5) Wash again in running water, 2 hours.

(6) Fix the tips in a freshly prepared acetic-alcohol (1 : 3) to which 5% concentrated hydrochloric acid is added, 10 minutes.

(7) Transfer the root-tips to 75% alcohol and run them down to water; treat the tips in 1% sulphuric acid, if the plant is known to contain oily and fatty cell inclusions, 10 - 15 minutes. Sulphuric acid dissolves the oily cell inclusions.

(8) Rinse thoroughly in distilled water, 10 - 15 minutes. If the plants do not contain oily cell inclusions, steps 7 and 8 could be ommitted.

(9) Hydrolyse the tips in a macerating fluid containing equal parts of concentrated hydrochloric acid and 95% alcohol, 25 - 35 minutes. according to the species.

(10) Drain off the macerating fluid and wash the tips in distilled water.

(11) Stain the tips in leuco-basic fuchsin (de Thomasi, 1936), 2 - 3 hours; if the working time necessitates, the material could be left overnight in basic fuchsin without causing any damage to it.

(12) Intensify the stain by washing repeatedly in tap water.

(13) Bleach, if necessary, in SO_2 water, 3 changes, 10 minutes each.

(14) Rinse in distilled water.

(15) Cut off the brightly stained meristematic region, put it in a drop of acetocarmine on a slide, tease it with plated needles.

(16) Put a small piece of the tissue in a fresh drop of acetocarmine on another slide, apply an albuminised cover slip.

(17) Heat gently over the spirit flame; if necessary bring acetocarmine under cover glass to the boiling point; squash by applying the pointed end of the needle on those parts of the coverslip where pieces of tissue are localised; avoid side way movement of the coverslip.

(18) Blot out the excess carmine and seal the edges of cover slip with wax.

(19) Store the slides, 2 - 3 days.

(20) Scrape the wax, invert the slide in a mixture of glacial acetic acid and n Butyl alcohol (1 : 1); keep the preparation in this mixture till the cover glass separates from the slide.

(21) Pass the slide and the coverslip in 2 changes of n Butyl alcohol.

(22) Mount in neutral Balsam.

N.B: If cytoplasm takes stain, it is best to separate the cover glass from the slide in 45% acetic acid, which destaines the cytoplasm. Preparations could be made permanent by any well known method and Euparal could be used as a mounting medium as given by La Cour, 1947.

Schedule II

(1) Precool as in schedule I.

(2) Prefix in a freshly made concentrated solution of α-Bromonapthalene in tap water, 4 hours.

(3) Wash in running water, 2 hours.

(4) Fix in Benda and 0.003M 8-hydroxyquinoline (6 : 1) or chrome-osmic fixing fluid with 0.003M 8-hydroxyquinoline (5 parts of 1% chromic acid, 1 part of 2% osmic acid, 1 part of 0.003M 8-hydroxyquinoline) for 1 hour at 10 - 14°C.

(5) Rinse in distilled water and then lukewarm water, 5 - 10 minutes.

(6) Treat the tips in 1% sulphuric acid for 10 - 15 minutes.

(7) Rinse in distilled water, 10 - 15 minutes.

(8) Hydrolyse in 1N hydrochloric at 60°C, 45 - 60 minutes.

(9) Replace hot acid with cold acid, 5 minutes.

(10) Rinse in distilled water, 5 - 10 minutes.

(11) Stain in leucobasic fuchsin, 2 hours.

(12) Intensify the stain by rinsing in tap water, 5 minutes.

(13) Squash in a similar way as in Schedule I.

The rest of the procedure is same as in the Schedule I, except that if bleaching is necessary in equal parts of turpentine and Butyl alcohol, it is to be done after the separation of the cover glass from the slide.

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Athrotaxis cupressoides ($2n = 22$);
colchicine-oxyquinoline pretreatment
and acetic-alcohol fixation. x 1250

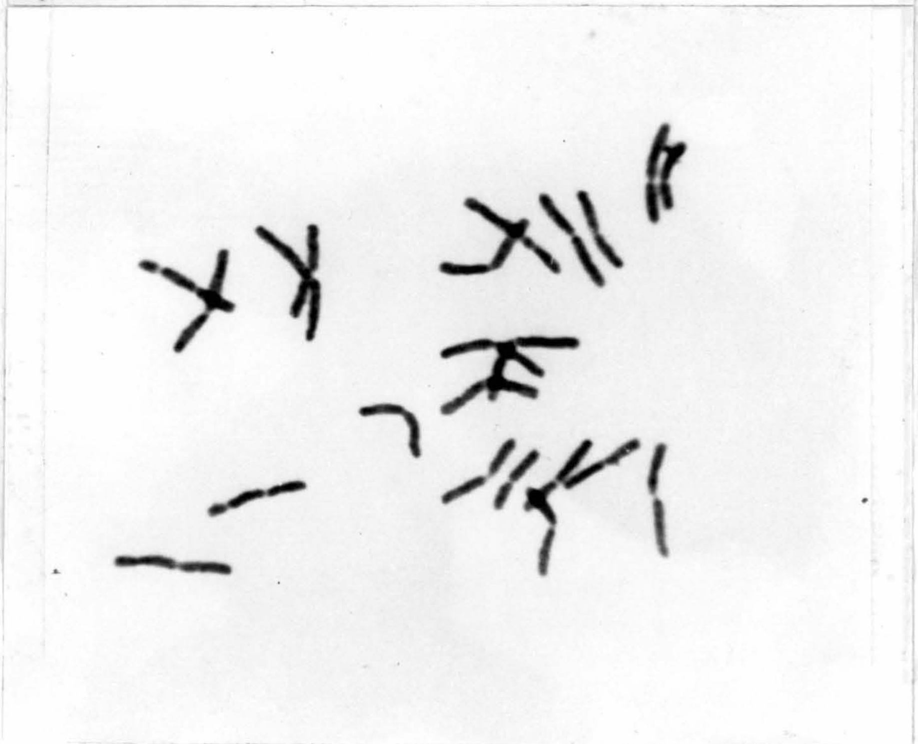
Microcachrys tetragona ($2n = 30$);
treatment same as above. x 1250

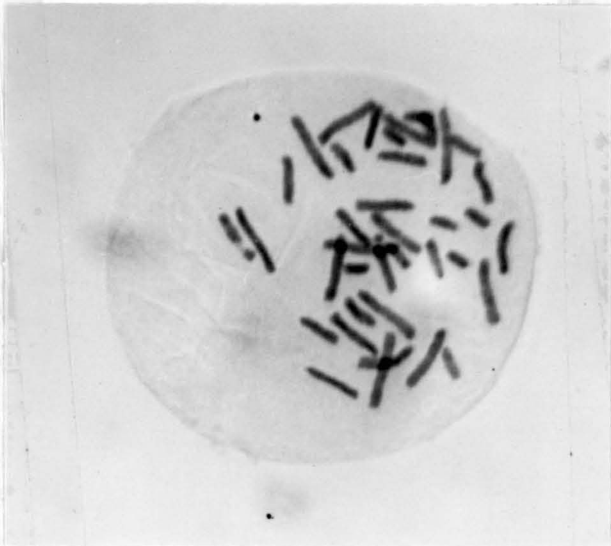
Podocarpus alpina ($2n = 38$);

-Bromonapthalene pretreatment;

Benda-oxyquinoline fixation.

x 1250



CYTOLOGICAL STUDIES IN THE INDIAN SPECIES OF *DIPCADI*

CYTOLOGICAL STUDIES IN THE INDIAN SPECIES OF DIPCADI

Contents.

I. INTRODUCTION

The large liliaceous genus *Dipcadi* with about 110 species forms a well-defined and a highly natural assemblage of plants. Many species show a considerable intergradation of the morphological characters. This is particularly true to most of the Indian species. Even a casual perusal of the descriptions of these species (Hooker, 1894; Blatter, 1928) will at once reveal that overlapping of characters has obviously introduced an element of uncertainty in taxonomy rendering the species delimitation extremely difficult. In such critical genera recognition of cytological differences would not only prove a useful adjunct to the morphological classification of the systematist but may also give a considerable insight into at least some of the evolutionary mechanisms at work in the genus. With this end in view a chromosome survey of four Indian species was undertaken.

Further-more, *Dipcadi* presents interesting problems with regard to its geographic distribution, which is wide and at the same time highly discontinuous. Several of its species are mostly African. About 30% of the species are endemic, either occurring on islands or growing in restricted areas, as is the case with eight Indian and a few African species. It would be a matter of considerable interest to correlate the cytological characters of the species with their endemism.

II. PREVIOUS WORK

Dipcadi has but scant cytological literature. *D. serotinum* has received the greatest attention due to its cytological variability. Sato (1942) was first to determine its chromosome number as $2n = 8$. Later on, Levan (1944) not only confirmed the chromosome number reported by Sato but also described the chromosome morphology (refer also Tjio and Levan, 1950) and meiosis. Levan observed a high frequency of ring bivalents at metaphase I despite the fact that all the chromosomes were subterminally attached. There were occasional univalents due to failure of metaphase pairing. In a group of 11 plants collected from Lisbon, Resende and da Franca (1946) isolated two plants with 2 – 16 supernumerary fragments in addition to the 8 normal somatic chromosomes. They were of the opinion that the extra fragments were hetero-chromatic in nature and further postulated that they might have originated from the euchromatic parts of the normal complement and had become gradually "heterochromatinised". In a population of the valley of Falagueiro (Portugal), Fernandes, Garcia and Fernandes (1948) observed a single plant of D. serotinum var fulvum (2n = 8 + 1B) with a small supernumerary heterochromatic chromosome, which was of a similar type described by Resende and de Franca. La Cour (vide Darlington and Wylie, 1955) recorded 18 diploid chromosomes in D. glaucum. Battaglia (1954) adduced cytological evidence to show that D. serotinum var fulvum (2n = 34) collected from Ain-Sebba (French Marocco) should be separated as a new species from D. serotinum. On the basis of comparative karyology, he also explained the probable hypertetraploid mode of origin (2n = 4X = 32+2) of this highest polyploid known so far in the genus.

III. CHROMOSOME NUMBERS IN DIPCADI

The following are the chromosome numbers of Dipcadi arranged in their ascending order. No attempt has been made to follow any particular phylogenetic tendency within the genus, but the species are grouped according to Baker (1871). The Botanical nomenclature and cytological terminology of the respective authors have been mentioned.

Table 1.

Sub genus Tricharis (Salisb.).

Species	n	2n	Author	Remarks
Dipcadi serotinum Medic.	-	8	Sato, 1942	
" "	4	8	Levan, 1944	
" "	-	8	Resende and da Franca, 1946	Plants with 2 - 16 supernumerary fragments in root-tip cells.
" "	-	8	Tjio and Levan, 1950.	

Species	n	2n	Author	Remarks
D. serotinum (L.) Medic. var. fulvum Webb & Berth.	-	8	Fernandes, Garcia and Fernandes, 1948.	one plant with one small supernumerary heterochromatic chromosome.
D. saxorum Blatter	6	12	this paper.	
D. hydsuricum Baker	6	12	this paper.	
D. montanum Baker	10	20	this paper.	
D. fulvum Webb.	-	34	Battaglia, 1954.	Baker (1871, pp. 397) described it as a robust form of *D. serotinum*: vide Battaglia for further information.
		Sub genus Uropetalum (Burchell, Salisb.).		
D. glaucum Baker	-	18	Le Cour (cf. Darlington & Wylie, 1955).	

IV. CYTOLOGICAL TECHNIQUE

Root-tips of adult plants were fixed in Benda's fluid, Benda's fluid with 0.002 M 8-hydroxyquinoline (6 : 1), 2 BD, Lewitsky's chromic-formalin (1 : 1), (1 : 2), (6 : 4) and chromic-formalin with 0.002 M oxyquinoline (6 : 3 : 1). When oxyquinoline was used as a component in the fixing fluid, fixation was done at 10 - 14°C for the first 2 hours and at room temperature for the subsequent 22 hours. The addition of 8-hydroxyquinoline to some of the above mentioned fixatives and increasing the formalin content in Lewitsky's fluid proved useful for revealing the constrictions in the chromosomes. The root-tips were left overnight in the fixative after adding equal quantity of 1% chromic acid. It prevented excessive blackening in the case of osmic fixatives and increased the staining capacity of the chromosomes after the formalin fixatives. Transverse sections (14 - 16μ thickness) were cut and crystal violet was used as a stain.

Observations on the somatic chromosomes were also made on Feulgen squash preparations of root-tips. Benda's fluid with oxyquinoline and a chrome-osmic-oxyquinoline mixture (1% chromic acid 5 parts, 2% osmic acid 1 part, 0.002 M oxyquinoline 1 part) gave excellent results after a pretreatment of root-tips with concentrated aqueous solution of - Bromonaphthalene in tap water for $1\frac{1}{2}$ - 2 hours.

For a study of meiosis, flower buds were fixed in acetic-alcohol (1 : 3) for 10 minutes, hardened overnight in 90% alcohol and permanent acetocarmine squash preparations of PMCs were made.

V. MITOSIS AND MEIOSIS

The study of the somatic chromosomes in *Dipcadi* was fraught with considerable difficulty in spite of the fact that the chromosome number is small and that they show distinctive morphological features. This is because critical fixation, which reveals all the structural details in the chromosomes, is difficult to acheive. It necessitated the use of several fixatives. One of the most important facts that emerged out of the present study is the presence of stained and unstained regions in the somatic chromosomes of *D. saxorum* and *D. hydsuricum* (*cf.* Brown, 1949, in Tomato). Such regions are more prominent in *D. hydsuricum* than in *D. saxorum*. In the former, the unstained regions near the subterminal centromere are exceptionally long and at these regions, the chromosomes appear to be attenuated and drawn out chromosomes a, b, c in Fig.24). In sufficiently destained preparations, these regions seem to be differentially charged with nucleic acids and hence react differently to basic fuchsin and crystal violet. However, in a properly fixed and stained preparation their position is always constant, no matter whether the fixative is an osmic or a formalin type. Hence for the sake of convenience the unstained regions other than the primary constrictions are described as secondary constrictions in the following account. In a closely related genus *Lachenalia*, Moffett (1936) described chromosomes with multiple constrictions, which are similar to those of *Dipcadi*. The arctic species of *Ranunculus* show numerous constrictions in the chromosomes (Flovik, 1936).

Secondary constrictions have been interpreted as heterochromatic regions (Darlington and La Cour, 1940). Cold treatment (0°C for 24 hours) has revealed that these unstained regions in *Dipcadi* are not heterochromatic (in its restricted usage. *cf.* Darlington, 1941) and this fact is correlated with the absence of pycnotic knots in the resting nucleus. That secondary constrictions are not heterochromatic has been shown in *Polygonatum* and *Smilacina* (Therman-Suomalainen, 1949). It is not improbable that the so-called secondary constrictions in the chromosomes of *Dipcadi* are specialised regions, which are differentially charged with nucleic acid. The unstained regions immediately below the primary constrictions appear narrow as they are less spiralised. Perhaps spiralisation is not effected on account of the fact that there is little or no attachment of thymonucleic acid in these regions of chromosomes. (Darlington and La Cour, 1945).

It is on account of the difficulty of revealing these regions that very little attention has been paid by the previous workers on these points, although species like *D. serotinum* have been subjected to critical study by Sato (1938), Levan (1944) and Tjio and Levan (1950). It is pertinent to remark here that all these authors have described the chromosomes of *D. serotinum* as subterminally attached but their figures consistently indicate more than one constriction, which in all probability is the case. This point is of far reaching significance because *D. serotinum* is taxonomically related to both *D. saxorum* and *D. hydsuricum* and the presence of morphologically similar chromosomes provides strong evidence towards the same.

(a) *Dipcadi saxorum* Blatter. ($2n = 12$; $n = 6$)

Bulbs of *Dipcadi saxorum* have been collected from the typical locality of the species namely, Kanheri Caves in Salsette Islands, Bombay State, India. They usually flower in August - September every year. The material for the present investigation was collected from 30 plants, which conform to the description of Blatter (1928).

Mitosis: The somatic cells are uniformly characterised by 12 chromosome (Fig.1). No variation in the chromosome number has been encountered in different cells save for five which showed 13 chromosomes (Fig.3). The somatic chromosomes could be classified as long, medium and short. The complement consists of:

(i) Two pairs of long satellited chromosomes (a, b) with subterminal and submedian constrictions; one pair (b) has distinctly smaller proximal arms than the other (a);

(ii) one pair of long chromosomes (c) with one subterminal and two submedian constrictions;

(iii) one pair of medium-sized chromosomes (d) with one subterminal and one submedian or median constrictions;

(iv) one pair of medium-sized chromosomes (e) with one submedian and one subterminal constrictions; unlike the previous pair, the arm cut off by the submedian constriction is proximal and the short arm cut off by the subterminal constriction is distal;

(v) one pair of small chromosomes (f) with subterminal constrictions.

It is difficult to differentiate the primary from the secondary constrictions in the karyotype of D. saxorum and hence they have not been designated as such in the foregoing description. No anaphase could be studied from this point of view. Judging from the metaphase orientation however it is possible that the subterminal constrictions of all the long and short chromosomes and the submedian constrictions of the medium-sized chromosomes (iv category) are the primary constrictions. During C-mitosis, these primary constrictions appear to be more prominent than the secondary ones, which are scarcely visible, if at all.

The long, medium and short chromosomes do not as a rule conform to a particular pattern of arrangement on the metaphase plate. The characteristic pattern of arrangement that is generally observed (Fig.1) and the sporadic formation of "hollow spindle" (Fig.2) are facilitated by the characteristic chromosome morphology.

Although their relative size and the available space on the spindle allows the movement and the orientation of the small chromosomes in the centre of the spindle, accumulated data have revealed that it is not always so. In 50% of the metaphases they tend to orientate at the periphery of the spindle,

Table 2 Showing the position of the pair of small chromosomes on somatic metaphase.

Both inside	Both on the periphery	one outside and one inside	Total
4	13	9	26

Meiosis: Due to relatively small number of PMCs and difficulties inherent in their fixation (cf. Levan, 1944) earlier stages in meiosis of D. saxorum could not be studied in detail. During diplotene stage, 6 bivalents could be counted. Some of them show unstained regions in acetocarmine squash preparations fixed in acet-alcohol (1 : 3). These are apparently similar to the unstained regions observed in the somatic chromosomes of D. hydsuricum.

At diakinesis 6 bivalents are arranged at the periphery of the nucleus. Two fairly big bivalents are always attached to the nucleolus in the early stages. As the diakinesis progresses, the nucleolus gets smaller and the nucleolar bivalents are detached. Due to the movements of the bivalents at diakinesis, the nucleolus is seen to be pulled apart and at the point of attachment the bivalents are greatly stretched. Rupture of the nucleolus at this stage consequent upon such movements of the bivalents may account for the presence of 2 nucleoli with bivalents attached to them at this stage. Two nucleoli have never been observed in stages earlier than diakinesis.

In a majority of cases the bivalents are attached to the nucleolus by both the chromosomes and the satellites are not visible. At the point of attachment to the nucleolus, the 2 chromosomes of a bivalent are close together or apart from each other. If chiasma is near the attachment, they are close together. If the chiasma is distal to the attachment they are away from each other. Evidently, their position at the point of attachment is dependent on pairing and chiasma formation. It seems nucleolus sometimes interferes with all these processes. In some cases, one of the chromosomes of the bivalent was slipped off the nucleolus while the other was still attached to it. Upcott (1936) found in _Eremurus_ that when one chromosome was attached the other had the appearance of being repelled from its homologue and it was attributed by her as probably due to the nucleolus having the same surface charge as the chromosomes and hence exerting a repulsive force. A similar explanation may hold good in _D. saxorum_ too.

Fig.12 represents a metaphase I with 6 bivalents. The smallest bivalent shown in outline always remains relatively understained in acetocarmine squashes. An observation of sixty seven metaphases I showed no deviation from the normal haploid number six. However, in one giant pollen-mother-cell about 19 chromosomes could be counted showing the configurations $6_{II} + 7_{I}$ (Fig.13). Metaphase pairing was complete in cells with 12 chromosomes and bivalent formation was regular with a single exception, where 3 univalents were noted (Fig.15). This is unlike the behaviour of _D. serotinum_ which according to Levan (1944) showed 25% of metaphases with univalents.

There is a preponderance of rod over ring bivalents at metaphase I. This is in harmony with the morphology of chromosomes, as all the 6 pairs of chromosomes are subterminally attached with short proximal arms, so that the chances for the chiasmata to slip off are more in the short arms than in the long ones. The result would be the formation of a rod bivalent. However, in the case of long chromosomes there appears to be a localisation of chiasmata near the centromere and perhaps very little movement of chiasmata takes place in them during diplotene to metaphase I. If terminalisation is arrested in the distal arms long before the chiasmata are slipped off in the proximal arm, the result would be a ring bivalent. A greater frequency of rod bivalents over the rings herein reported for _D. saxorum_ is contrarary to that reported by Levan (1944) in _D. serotinum_. The latter is a species with 4 pairs of chromosomes, which are subterminally attached like D. saxorum and yet it shows a high frequency of ring bivalents at metaphase I. Levan (1944) suspected that a proportion of them were false rings with two chiasmata in the long arm, one close to the centromere and another at the distal ends. If such a bivalent is stretched at the proximal arms, it simulates a ring bivalent. Unlike this, most of the bivalents of D. saxorum appear to be true rings, in which connections in the short arms are clearly visible.

Another interesting feature observed at metaphase I was the delay in the orientation of certain bivalents at the equator. According to Darlington (1937) repulsion between centromeres of a bivalent is the effective agent in orientation. Delay in orientation is due to increased distances between the centromeres of a bivalent with a decrease in the repulsion. In D. saxorum the smallest bivalent has the centromeres close to each other with a repulsion at its maximum. Still this bivalent appears to remain unorientated. Hence the factors responsible for nonorientation must be sought otherwise. The stages between diakinesis and metaphases I are usually marked by a series of movements of bivalents leading to the formation of metaphase plate. Some of them bring the axis of the bivalent in line with the axis of the spindle. Such an alignment is the first step towards anaphase I since "repulsion is relatively ineffective in any direction other than the axile one". (Darlington, 1936). The second set of movements responsible for bringing the bivalents to the equator are due possibly to the repulsion between the centromeres and the poles. The third type of movements that space the bivalents at the equatorial region are the body repulsion. All these act together to orientate the bivalents at the metaphase. Those bivalents which are parallel to the long axis of the spindle before the onset of the movements are first to be orientated. If on the other hand the bivalents are disposed at random and are to exhibit considerable movements before the bivalents are orientated they are delayed. Size appears to be no factor in increasing the velocity of movement, because the sixth bivalent which is the smallest is sometimes delayed. In one cell (Fig.22) it was last to separate leading to the conclusion that it was also last to orientate itself. The difference in the staining capacity of the smallest bivalent and the rest cannot be correlated with its occasional delay in orientation because the fifth bivalent stains normally and is sometimes delayed.

Regular anaphase I separation of bivalents, absence of lagging chromosomes, the normal occurrence of 6 chromosomes at metaphase II show that the delayed bivalents are orientated properly and regular anaphase I separation is ensured. Forty eight cells showing anaphase I amply demonstrated this fact (Fig.21). During the telophase I 2 nucleoli were regularly formed. The diad cells showed rarely micronuclei and their origin could not be traced. Two nucleolar chromosomes are attached to the nucleolus during the prophases of the second division. On the whole, regular cell division results in a tetrad of pollen grains.

(b) D. hydauricum Baker ($2n = 20$).

The bulbs of D. hydauricum collected from Taxilla, near Rawalpindi, Pakistan provided material for the present investigation. The species is highly localised in its distribution and thrives in the limestone on the southern hilly slopes near Rawalpindi at 2000 ft. It constitutes an element of the ephemeral flora of Pakistan, which makes its appearance in March soon after rains, dries up and disappears by the middle of April. It was identified as such at Kew but Dr. R. Stewart was definitely of the opinion that the plants under investigation belonged to D. serotinum Baker. Hooker (1894) recorded the species from Ludhiana, the Punjab, India and according to him it closely resembled D. serotinum but for their short and long bracts respectively.

The somatic chromosome number in this species has been determined as $2n = 12$ (Fig.22) and the complement consists of long,medium and short chromosomes. There are:

(i) two pairs of long chromosomes with subterminal primary constrictions, which cut off the short, rounded proximal arms from the long distal ones (a, b); the distal arms show a differentiation into 3 stained and unstained regions; the unstained region immediately below the primary constriction is exceptionally long and as a consequence the chromosomes appear to be narrow and attenuated at this region; the proximal arms are furnished with satellites; the chromosomes are heteromorphic for a size variation of satellites in different individuals;

(ii) one pair of long chromosomes (c), almost identical with (i) in morphology and size but are devoid of satellites.

(iii) one pair of medium-sized chromosomes (d) with subterminal primary constrictions and an unstained region in the middle;

(iv) one pair of medium-sized chromosomes (e) with a subterminal primary constriction and a submedian secondary constriction; the narrow unstained region in this chromosome pair looks more like a constriction;

(v) one short pair with subterminal primary constrictions (f).

On the whole, D. hydauricum closely resembles D. saxorum, both in number and gross morphology of chromosomes. If the chromosome complements of these two species is compared with that of D. serotinum ($2n = 8$) reported by Sato (1942) and Levan (1944) a remarkable similarity is revealed. The 3 long and one medium-sized chromosomes in all the three species are almost identical in morphology indicating a common origin and close relationship. It amply justifys their grouping into one subgenus namely, Tricharis.

During prophases in D. hydauricum 4 chromosomes are found attached to the nucleolus (Fig.23) corresponding to the 4 nucleoli of different sizes in telophases (Fig. 30). The chromosomes with secondary constrictions are not attached to the nucleolus. It means they do not take part in the organisation of nucleoli. Non-nucleolar chromosomes with secondary constrictions were reported by Stewart (1947) in Lilium.

(c) D. montanum Baker ($2n = 20$).

D. montanum is a common Indian species occurring in Concan and Western parts of Deccan. It has a more extensive distribution than D. ursulae. It again resembles D. serotinum in having lanceolate and accuminate bracts, which are as long as pedicles but with a stipitate ovary unlike the latter.

The species is characterised by 20 somatic chromosomes (Fig. 35). The karyotype is unique in showing distinct size variation, which have been found in other related species too with lower chromosome numbers. It also resembles them in having chromosomes with secondary constrictions, which are difficult to reveal even after critical fixation and feulgen staining. From this point of view even the homologous chromosomes in the same metaphase are sometimes different (Fig. 35). The chromosome complement consists of (Fig. 37):

(i) one long pair of chromosomes (a) with distinct submedian primary constrictions and median secondary constrictions on the short arm (vide Fig. 35);

(ii) one pair of long chromosomes (b) with submedian primary constrictions and a secondary constriction on the long arm very close to the primary constriction; this is definitely shorter than (a);

(iii) two pairs of long chromosomes (c, d) with subterminal primary constrictions and secondary constrictions close to the primary dividing the distal arm into two very unequal parts; out of these, the longer pair (c) is satellited;

(iv) one pair of medium-sized chromosomes (e) with median or sub-median primary constrictions;

(v) one pair of medium-sized chromosomes (f) with subterminal primary constrictions and submedian secondary constrictions;

(vi) one pair of medium-sized chromosomes (g) with subterminal primary constrictions; this pair is a little different from the rest of the subterminal types in having a slightly longer proximal arm, representing a distinct segment of a chromosome and not appearing as a knob;

(vii) three pairs of short chromosomes (h, i, j) one pair (h) a little longer than the rest, all with subterminal primary constrictions; out of these, the smallest pair (j) is satellited.

Meiosis progresses with a remarkable regularity forming 10 bivalents of different sizes, which is in keeping with the size variations of the chromosomes (Fig. 38). The number of chiasmata in the bivalents range from 3 to 1 and in some of the large bivalents, the chiasmata are localized near the centromere and consequently with no terminalization at metaphase. In general, terminalization is incomplete in the large bivalents whether they show localization or not.

Formation of the bivalents is the rule. However as a departure from it, two univalents were sometimes observed. Also a quadrivalent was observed in one cell. This is apparently due either to the segmental interchange or autosyndesis. Its rare occurrence indicates that very short segments are involved in the interchange or the homology of the chromosomes in the parental species is remote and hence does not permit frequent pairing. The more remotely the parental species are related the more is the fertility of the resulting amphiploid. All these facts support the hypothesis that D. montanum is an amphiploid, which originated from perfectly diplodised parents and the possibility of segmental interchange having played a part in the evolution of the species apart from amphiploidy is suggested.

The I and II divisions pass quite regularly except for an occasional variation in the chromosome number at anaphase (II) due possibly to nondisjuction either during the first division or in the premeiotic divisions. Fig. 39 represents a anaphase II with 8 + 10 chromosomes at the two poles. Fig. 40 shows a pollen mother cell at anaphase II. In one half, 10 chromosomes at one pole and 11 chromosomes at the other, making a total of 21 chromosomes could be counted clearly. In the other half of the same cell, 20 chromosomes are irregularly distributed. This is an instance where a premeiotic irregularity is perpetuated up to the end of second division resulting in pollen grains with 10 or 11 chromosomes. Such occasional irregularities are known in amphiploids. (Goodspeed and Bradley, 1942).

(d) *D. ursulae* Blatter (2n = 20).

This species occurs on the Tableland of Panchgani, W. Ghats, Bombay Presidency. It is more restricted in distribution than *D. montanum*, which it resembles in having 20 somatic chromosomes. A similar amphiploid origin as that proposed for *D. montanum* could be inferred for this species also.

VI. DISCUSSION

The large genus *Dipcadi* with about 110 species has attained a great tempo in evolution and a wide geographic distribution. Some of them have undergone genotype changes during the course of their evolution owing to the inherent property of the genes themselves, which is "mutable productivity" (*cf.* Snell, 1938) and also in reaction to the environment. On the other hand, isolation between some species may be purely external and spatial. They are perhaps geographically and ecologically isolated as in *D. hydsuricum* and *D. saxorum*. The former flourishes on limestone rocks of the Punjab and Rawalpindi, forms an element of the ephemeral flora of those regions and flowers in March - April. The latter occurs in the rocky places above the Kenheri Caves in Salsette, Bombay State, about 1,000 ft., and blooms in August - September. In these cases, there seem to be no reason why complex genetic differences should account for speciation.

(or numerical changes)

In conjunction with such genic and genotypic changes many cycles of chromosomal changes, both numerical and structural, seem to have had a profound influence in directing the course of evolution within the genus. It seems probable that structural changes resulted in genetic barriers between the species at the same level of polyploidy. In fact, both must have progressed simultaneously, one augmenting the other and culminated in the ultimate genetic differences between the species.

Changes in the number of chromosomes in related species may be generally in multiples of a basic set (polyploidy) or may involve the reduplication of some of the chromosomes of a set, both in diploids and polyploids (Polysomy and secondary polyploidy). The lowest diploid chromosome number reported so far in the genus is 8. It is encountered in Dipcadi serotinum. Whether four is the basic number or not is difficult to say with the available data. It is possible that several secondary basic numbers might have evolved within the genus and one of those appears to be 6. On this basis, D. saxorum (2n = 12) and D. hydsuricum (2n = 12) show aneuploid change in the chromosome number when compared with D. serotinum (2n = 8). Both the karyotypes with 2n = 8 and 2n = 12 are highly heteromorphic in having only subterminal chromosomes. Another equally significant fact is that the 3 pairs of long chromosomes and one pair of medium-sized chromosomes characteristic of D. serotinum are also found in the karyotypes of D. saxorum and D. hydsuricum indicating their close taxonomic relationship. The morphology of these long chromosomes is almost identical in all the three species.

Such aneuploid changes in the chromosome number could be effected not only by the loss or duplication of a centromere after a segmental interchange between two chromosomes but also by the fixation or loss of supernumerary or B chromosomes. In a spectrum of chromatin material, heterochromatin and euchromatin should occupy the two end regions. A reversible but a gradual change, one from the other, is possible during the course of evolution. If so, B chromosomes could get fixed in the karyotype and behave like normal chromosomes due to gradual "euchromatinisation" (assuming for the moment that B chromosomes are heterochromatic: cf. Östergren, 1947 for a full discussion). White (1957, pp. 112) however would have us believe that fixation of supernumerary chromosomes (largely, but not entirely hetero-chromatic) as regular members of a karyotype never seem to have been attained in grasshoppers, due to their inherent irregular behaviour during meiosis and mitosis. According to him, B chromosomes could only undergo fixation if they are translocated to the normal chromosomes endowed with a regular behaviour. If the difference in the behaviour of A and B chromosomes could be accounted for on the basis of the difference in their respective nucleic acids, a gradual change of heterochromatin into euchromatin would remove the difficulty of White (loc. cit.). What ever may be the mechanism, B chromo-somes do seem to play a part in changing the basic number of a species, a type of variation and evolution, which may not be morphologically perceptable to start with. Polymorphism of the karyotype achieved through B chromosomes was reported by Fernandes (1952) in Narcissus bulbocodium.

For such changes leading to aneuploidy within the genus Dipcadi, the evidence is three-fold namely, the occurrence of 1-16 supernumerary chromosomes in D. serotinum, the presence of the so-called secondary constrictions which are specialized segments which are differentially charged with nucleic acids, and the existence of a species like D. hydsuricum with almost similar morpho-logical features as those of D. serotinum but for their short and long bracts respectively. In fact it is believed that the bracts only differentiate the two species, which otherwise resemble each other (Hooker, 1894; pp. 347). It has been found during the present investigation that the length of bracts is variable even in the same inflorescence of D. hydsuricum. Hence this alleged difference between the two species should vanish. The chromosome number alone distinguishes them. It is possible that D. hydsuricum ($2n = 12$) (if the identification of the species is correct) has originated from D. serotinum ($2n = 8$) as a result of the fixation of the heterochromatic B chromosomes in the latter. These two species co-exist or exist in two contiguous areas like Rawalpindi and the Punjab lending support to the view. At any rate, this is one of the many alternative suggestions for the origin of aneuploid, morphologically similar and hence closely related species within the genus.

Much more spectacular than aneuploidy is the numerical variation in the chromosome number due to amphiploidy. D. montanum and D. ursulae with $2n = 20$ could be derived from putative parents like D. serotinum ($2n = 8$) and D. saxorum ($2n = 12$) or D. hydsuricum ($2n = 12$). It has been pointed out in the preceeding paragraph that morphologically D. hydsuricum ($2n = 12$) is very similar to D. serotinum ($2n = 8$) and cytologically almost similar to D. saxorum ($2n = 12$). Presumably D. hydsuricum represents a transitory stage between D. serotinum and D. saxorum or a case of convergence. This apparent cytological similarity between the chromosome complements of these 2 sets of species with $2n = 8$ and $2n = 12$ their close proximity of growth make hybridisation possible. Most of the hybrids could successfully pass through the "bottleneck" of the resulting sterility on account of the fact that like most of the bulbous Liliaceae, Dipcadi are propagated by vegetative means for at least the first few generations till the chromosome number is doubled and fertility is restored. All these made possible for speciation through amphiploidy.

A similar situation seems to prevail in D. fulvum (2n = 34) investigated by Battaglia (1954), who predicted a hypertetraploid origin for it. The 34 somatic chromosomes could be classified into 8 sets of four each and 2 small chromosomes, presumed to be supernumerary in nature, forming a category of their own. He went a step further to assume the occurrence of forms of D. serotinum with 2n = 16 + 1B. Granting that a mode of origin is true on purely morphological grounds, it seems equally true that D. fulvum might have had an amphiploid origin like D. montanum and D. ursulae. A diploid gamete of a putative parent like D. serotinum (2n = 8) when fused with a haploid gamete of a parent like D. glaucum (2n = 18) would give rise to a triploid sterile hybrid (8 X 9 = 17) which on doubling will form a fertile amphiploid with 34 somatic chromosomes. In such a species, 16 chromosomes are contributed by a parent like D. serotinum and 18 by the other. In all probability, it is an auto-allopolyploid and the formation of multivalents is not altogether precluded.

Amphiploidy, which has no doubt accompanied speciation in Dipcadi, has brought in its wake a reticulate rather than dendritic pattern of evolution. This has resulted in morphologically more or less similar species and introduced a taxonomic complexity. Three more factors might result in a closer convergence that is normally not anticipated by amphiploidy alone. A hybrid resembles more strongly the parent contributing a larger number of chromosomes than the other. Sometimes a new polypoid on account of its different ecological properties or wider range of tolerance when compared with its diploid progenitors, may migrate by itself and colonise new areas where it may overlap in distribution with another closely related species of the same ploidy. At other times still, exigencies in the environment like glaciation may force a polyploid species into a new ecological niche, in which its diploid progenitors may not survive and where it may encounter an interfertile polyploid species. In either case, the polyploid species escapes the spatial and genetical isolation of its parents and has a chance of crossing with another species, as illustrated by Saxifraga (Skovested, 1943) and Veronica (Scheerer, 1937). If the polyploid meets more than one species a hybrid complex would result as, is the case with Vaccinium corymbosum, a tetraploid species complex, which contains in all combinations and in different frequencies the genes of the Ozarkian V. arkansanum, the Applachian V. simulatum and the costal V. australe (Camp, 1942). In all these cases, the divergence brought about polyploidy, has lead to convergence after crossing with a related polyploid.

A third and a much more important factor of convergence in Dipcadi appears to be the systematic elimination of characters of one parent assuming for the moment that the new amphiploid is intermediate between the two parents to start with. In all such cases the hybrid would tend to overlap in characters more with one parent than the other. This is one of the possible explanations for the prevailing confusion in the taxonomy of the Indian species. This is perhaps the only way out of the confusion that existed between D. serotinum ($2n = 8$) and D. serotinum var. fulvum ($2n = 34$) investigated by Battaglia (1954). This endemic of Morocco, a new possible amphiploid with the highest chromosome number so far known in the genus has come to resemble so closely D. serotinum as to compel Baker (1871) to describe it as a more robust form of the latter. This logically proves that D. serotinum is one of the putative parents of D. fulvum and that the elimination of characters of the other parent has taken place during the course of its phylogeny. Whether the characters of D. serotinum are of adaptive significance or not they are retained in D. fulvum. In addition to this fact, *D. fulvum* is yet to lose its initial gigantism an account of the characteristic 'mutations' that would ensue in a new polyploid due to loss of parts of chromosomes, or to a variation in their number or to a mere recombination. A critical appraisal of these facts one after the other would immediately show why it was described as a mere robust form of another species, despite the fact that their chromosome numbers are widely different.

The mode of origin of a group of Tetraploid roses in Central Oregon, which bear a strong resemblance to the hexaploid *R. nutkana* may prove interesting at the present context. According to Erlanson (1931), they might have originated as a result of interspecific hybridisation between the hexaploid *R. nutkana* ($2n = 42$) and the tetraploid *R. californica* ($2n = 28$) in the southern region of Oregon, where their ranges overlap. Such a cross would give rise to an unbalanced pentaploid F_1 with 14 bivalents and 7 univalents in PMCs. Many of the F_1 gametes would receive 14 chromosomes, since the univalents are liable to get lost during meiosis and almost fertile tetraploids would result resembling one of the parents but exhibiting the characters of both the parents. Similar cases are known in crosses between two polyploid species like *Nicotiana tabacum* X *N. sylvestris* (Goodspeed and Clausen, 1917) and *Triticum dicoccum* X *T. vulgare* (Thompson and Hollingshead, 1927). While admitting that such a hypothesis is tenable in *Dipcadi* too, it must however be pointed out that there is at present no evidence to show that hybridisation has taken place between species with different degrees of ploidy and that the ranges of diploid and highly polyploid species overlap. Moreover species originating in such a manner would always exhibit a certain amount of pollen sterility and surely would not be as fertile as any amphiploid species. Since most of the naturally occurring polyploid *Dipcadi* are highly fertile, amphiploidy alone would explain their origin and the fact of convergence should be related to any one of the 3 factors mentioned above. Introgression as one of the possible mechanisms of evolution in *Dipcadi* should be ruled out (vide Saunte, 1958).

(b) Structural changes:

In addition to the numerical changes structural changes seem to have played an equally important role in the evolution of *Dipcadi*. Accumulated evidence shows that fragmentation, translocation leading to the fusion of chromosomes, inversions and to some extent elimination of chromosome segments are few such readily detectable changes. Fragmentation of chromosomes was frequently observed in the root-tip cells of all the species studied during the present investigation. The size of the fragments varies considerably depending on the position of the break. Breakage occurs at or near the centromere (Fig. 9) or the so-called secondary constrictions (Figs. 10, 11) or at random anywhere in the chromosome. Breakage has also been observed in the SAT-thread reducing the satellite to a short filament with no satellite (Fig. 5). Breakage has simultaneously occurred at the centromere and in the SAT-thread resulting in small fragments with filamentous threads (Fig. 8). When fragments arise from the distal parts of the chromosomes or as a result of a break at the primary constriction, they are always small and are devoid of centromere. If the break occurs at or near the secondary constriction, a relatively short proximal fragment with a centromere (assuming that the centromere is located at a subterminal position) and a long distal fragment without a centromere would result (Figs. 10, 11).

They have no fixed position on the spindle with reference to the other chromosomes. Sometimes they are included within the spindle (Figs. 4,5,6,9).

At other times they are at the periphery of the spindle (Fig.8). In most of the cases, they are away from the metaphase plate and are not properly orientated. When the breakage occurs near or at the so-called secondary constriction, the proximal fragment with the centromere is in a state of equilibrium with the rest of the chromosomes in the complement (Fig. 10).

Fig. 7 represents a cell with 13 chromosomes. Judging from its size, one of it is apparently a fragment. The chromosomes are not properly congressed and orientated to form the normal metaphase plate characteristic of *Dipcadi*. The spindle appears to have remained "*unattached* and *inexact*" (*cf.* Darlington and Thomas, 1937). The co-operation between the external organisers of the spindle and the chromosomes so essential for a regular cell division seems to have been upset due possibly to the unbalance created by fragmentation, since such a phenomenon was not observed in a cell without fragments. It is also possible that the movement of the chromosomes with the extra fragment might have been delayed and hence indirectly fragmentation might have introduced a timing unbalance resulting in a scattered arrangement of the chromosomes.

The fate of the acentric fragments, whatever may be their size, has not been followed in detail. But any one of the following could happen to them:

(i) Proximal acentric fragments may acquire a new centromere and behave like normal chromosomes as shown by Darlington (1929) in *Tradescantia*. Their survival and subsequent behaviour was dependent on repeating mitosis and their ability to form chiasmata during meiosis.

(ii) They may be translocated to the homologous or a nonhomologous chromosome and persist during the subsequent stages of mitosis. The fusion of a portion of Y chromosome to the X chromosome in *Drosophila* illustrates the point in question (Stern, 1926).

(iii) They may be lost owing to their not fusing with a proximal fragment or a normal chromosome in the complement. Such a deletion would mean impairing the viability of a cell, or the whole organism depending on the nature and amount of the material lost.

Translocation in the region of the satellite and the consequent fusion of two SAT-chromosomes was observed in D. hydsuricum as shown in Fig. 34. (cf. Sato, 1936, Scilla; Resende, 1937, Aloe). Such a translocation would involve a simultaneous breakage of the SAT-threads in the two chromosomes and their reunion at the broken ends due presumably to their close proximity to each other during the prophases. It would also involve elimination of a portion of thread and terminal satellites belonging to the effected chromosomes. Since it is now known that the satellite stalk contains genes (Anderson, 1934), such a deletion would result in genetic deficiency and fusion would bring in "position effect". The behaviour of these dicentric chromosomes at anaphase is not known and it cannot be said with certainty whether they survive and persist as such. Perhaps such abnormal chromosomes behave like monocentrics, as is the case with dicentric chromosomes having two centromeres close to each other. Or they may again break at anaphase due to tension imposed on the SAT-thread by the chromosomes going to the opposite poles.

A few cases of lateral satellites have been noted in D. saxorum (Fig. 6). They are similar to those reported by Darlington (1929) in Tradescantia, Levan (1932) in Allium, Mather and Stone (1935) in Crocus etc. They have been found in the cortical region of the root. Levan (1932) described that the lateral trabants divided normally into two and each divided half passed to the opposite pole along with their respective chromatids. Such a behaviour of the lateral satellites however has not been observed in Dipcadi. They might have originated on account of inversion or due to lateral translocation to a homologous or nonhomologous chromosome. Lateral attachment of fragments may sometimes give a false appearance of a satellited condition (cf. Beal, 1939) but such an explanation in Dipcadi appears to be improbable, as the satellites are much smaller than the smallest fragments observed so far during the present investigation. The normal function and the organization of the nucleolus is in no way impaired, if a portion of the chromosome subtending the satellite with the nucleolar organising body translocated laterlly.

Variation in the length of the SAT-thread has been an interesting phenomenon in D. hydsuricum. In one plant, the thread was short and was terminated by a small scarcely visible satellite (Fig. 31). In three others, the thread was long and the size of the satellites varied in accordance with the length of the thread (Figs. 32, 33). There are all gradations from a satellite as large as a segment of a chromosome itself with a short filament to a small satellite with a long thread (Fig. 33). It is however interesting to note that a short filament with minute satellite has never been observed in these 3 plants.

Such a chromosomal heteromorphism involving unequal size of the satellites has been reported from time to time by other workers, Gates (1942) has reviewed the literature. It leads one to think that satellites can appear or disappear bringing about a numerical variation in a population of a species. Sometimes satellites not found in the parents could appear all of a sudden in their hybrid (Levan, 1937) or vice versa as in amphiplasty. There are also constant differences in the size of satellites and the threads subtending them. These differences are maintained in their presumed hybrids as in Alisma plantago aquatica (unpublished observations).

Sato (1937) studied in detail single and double flowered races of *Galanthus nivalis* and found all conditions ranging from a long connecting thread to those with no connecting thread. Mensinkai (1939) recorded the same in *Allium*. Contrary to these observations, Resende (1937) discovered 10 different types of satellites in *Aloe*, which according to him were fixed. It is indeed a remarkable fact that both these extremes have been observed within the limits of the same species like *D. hydsuricum* and that within individuals these two types of variation are a fixed character. More extensive investigation certainly reveal hybrids between these two sets of individuals differing in the satellites.

Any reasonable explanation of variation in the length of the SAT-thread would at once call forth a proper conception of the SAT-thread itself. It is now more or less definitely established that the thread is feulgen positive; it is a continuation of the chromonema of chromosome;it is devoid of sheath and outer wrapping of chromonema. It has a spiral of lower order than the minor spiral possesed by the chromosome (Mensinkai, 1939). According to thid idea, the satellite is a mere rolled end of the chromonema. While such a concept explains satisfactorily variation in the long SAT-thread in *D. hydsuricum*, it cannot go a long way towards the understanding of the short thread and a minute knob observed in one individual. To think that the satellite thread has permanently rolled towards the chromosome proper to give rise to a short filament and a minute knob is improbable. This would disturb the position of the nucleolar organising body, which is always fixed in a chromosome. On the other hand, a reasonable explanation would be that such a condition could be attained due to permanent structural change like translocation in the satellite thread and that such a change has become stabilized in certain individuals of the species. It would not be far wrong to seek such an explanation since structural changes involving the SAT-thread have been recorded during the present investigation.

(c) Geographic distribution and cytology:

A word need be said about the geographic distribution of the genus in relation to its cytology. It finds an extensive distribution in Africa, Europe and extends eastwards up to India. The area of D. serotinum covers Europe, where no other species abounds, boreal Africa and extends upto the temperate parts of India. There is no other species in the genus with a comparable range of distribution. That wide variability of a species accounts for its wide distribution is an accepted dictum. Variability of D. serotinum is reflected in the diversity of its karyotype reported from different localities.

The presence of 1 - 16 supernumerary chromosomes in its karyotype is but another factor of the same variation. After all when it is realised that the B chromosomes have a part of their own to play, when they accentuate cell division, when they lead a parasitic existence with a mitotic mechanism of their own to perpetuate themselves, when plants with and without B chromosomes exist side by side in equilibrium without eliminating each other and above all when they are even known, according to Darlington (1956), "to boost one or two nuclei which accomplish fertilization", and hence control fertilization will be too much to postulate that their accumulation up to a certain stage would mean a resulting effect analogous to that of polyploidy in a rapid spread of a species at any stage in its history? Could a postulate of this type, though not universally applicable, be true in the case of D. serotinum, which has a wide range of distribution because it is armed with a set of B chromosomes?

On the other hand, the amphiploid species of Dipcadi have a limited range of geographic distribution when compared with the area of the genus, and some of these species are highly localized and endemic. There appears to be no doubt that they are phylogenetically younger and hence recent arrivals to the stage of evolution when compared with their diploid progenitors. Although these amphiploids are supposed to have combined the ecological amplitudes of their parents and capable of extending their range of distribution, their population size is precariously small at present and is confined to a small area. Naturally occurring amphiploids, which have not spread far from the point of origin have been reported by Ownley (1950) in Tragopagon. The amphiploid Dipcadi have remained endemic due to any one of the following reasons apart from being phylogenetically recent in origin. They are perhaps incapable of forming biotypes either by a recombination of their existing genes or by new mutations. In the case of recessive or imperfectly dominant mutations the visible mutation rate is very much reduced, in polyploids. (cf. Haldane, 1930). From this point of view alone any polyploid system tends to be a closed system. Furthermore, close in breeding resulting in a random fixation of nonadaptive characters may account for their inability to occupy even slightly different environments other than those now occupied by them. What is of immediate interest is that such amphiploids have probably originated simultaneously in different regions and have been thrown into new, suitable and highly restricted ecological niche beyond which they are unable to migrate any further. The gaps between the areas of these species is far and wide. Hence an evolution by sudden jumps possible by amphiploidy alone has made the distribution of the genus discontinuous. Thus cytological study not only elucidates the mechanism of evolution but also illuminates the nature of endemism and the disjunct pattern of distribution of the whole genus.

VII. SUMMARY

(1) The paper deals with mitosis and meiosis of four species of *Dipcadi*, namely *D. saxorum* ($2n = 12$), *D. hydsuricum* ($2n = 12$), *D. montanum* ($2n = 20$) and *D. ursulae* ($2n = 20$).

(2) The study leads to the following conclusions with regard to the mechanism of evolution and speciation in the genus:

(a) *D. saxorum* ($2n = 12$) and *D. hydsuricum* ($2n = 12$) are aneuploid when compared with *D. serotinum* ($2n = 8$), whatever may be the direction of change. Apart from gain or loss of centromere in normal chromosomes, aneuploid changes in chromosome numbers in more or less morphologically similar species may be also due to fixation of supernumerary B chromosomes, when such chromosomes are known to occur in species with low diploid number.

(b) *D. montanum* ($2n = 20$), *D. ursulae* ($2n = 20$) and *D. fulvum* ($2n = 34$) might have had amphiploid origin. It has led to reticulation in evolution and hence taxonomic complexity.

(c) Species with widely different chromosome numbers like *D. serotinum* ($2n = 8$) and *D. fulvum* ($2n = 34$) show more convergence in morphological characters than what is expected of amphiploidy alone. After a detailed discussion it is concluded that gradual elimination of characters of one parent is the most important factor for such a convergence in the genus.

(3) Structural changes like fragmentation, inversion, translocation and fusion of chromosomes are described and discussed.

(4) Amphiploidy has not resulted in extending the range of distribution in *Dipcadi*. The localized amphiploid species are regarded as recent endemics. Simultaneous evolution of new species by amphiploidy in different parts of the area is the most plausible reason for the disjunct distribution of the genus.

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Figs. 1 - 11.

Explanation of the text figures illustrating the paper on "Cytological studies in the Indian species of Dipcadi".

Figs. 1 - 11. Somatic mitosis in *Dipcadi saxorum*. Fig.1. Polar view of somatic metaphase ($2n = 12$) to show four SAT-chromosomes and the usual arrangement of chromosomes at this stage. Fig. 2. Metaphase showing the arrangement of chromosomes round the spindle ("hollow spindle"). Fig. 3. Metaphase with thirteen chromosomes; one small chromosome (blackened) duplicated. Fig. 4. Metaphase with twelve chromosomes and one fragment. Fig. 5. Metaphase with twelve chromosomes and one fragment; in one chromosome, the satellite thread is broken and the satellite is lost. Fig. 6. Metaphase showing one chromosome having a lateral satellite; one fragment is also present. Fig. 7. Metaphase with thirteen chromosomes ($2n = 12 + 1f$) showing scattered arrangement. Fig. 8. Metaphase with a fragment showing a minute SAT-thread. Fig. 9. Metaphase with two fragments of unequal sizes; the chromosome at 12 O'clock is lightly stained unlike others in the complement; the chromosome at 6 o'clock is structurally altered. Fig. 10. Metaphase showing fragmentation at the region of secondary constriction. Fig. 11. Same but with two chromosomes effected in the same way.

Figs. 12 - 22

Figs. 12 - 22. Meiosis in *Dipcadi saxorum*. Fig. 12. Metaphase I with 6 bivalents, the smallest bivalent (shown in outline) lightly stained. Fig. 13. Metaphase I showing $6_{II} + 7_{I} = 19$. Fig. 14. Early Anaphase I, the early disjunction of fifth and the sixth bivalents. Fig. 15. Metaphase I with three univalents. Fig. 16. Early Anaphase I, the fifth bivalent disjoined and the sixth bivalent obliquely orientated and hence not disjoined. Fig. 17. Early Anaphase I, the sixth bivalent disjoined (shown in outline). Fig. 18. Metaphase I with bivalents irregularly orientated but the sixth bivalent showing precocicus separation. Fig. 19. Early Anaphase I, the fifth bivalent disjoined and the chromosomes almost reached their respective poles (à later stage than that represented in fig. 16) the sixth bivalent still oblique and not disjoined.

Figs. 12 - 22

Figs. 12 - 22. Meiosis in <u>Dipcadi saxorum</u> cont. Fig. 20. An abnormal PMC showing spontaneous chromosome breakage, the chromosome being as long or even longer than the somatic chromosomes. Fig. 21. Normal Anaphase I. Fig. 22. Anaphase I, the sixth bivalent disjoined last and hence the chromosomes lagging.

Figs. 23 - 34.

Figs. 23 - 34. Somatic mitosis in *Dipcadi hydsuricum*. Fig. 23. Prophase showing the attachment of four chromosomes to the nucleolus. Fig. 24. Somatic metaphase, (Bende-oxyquinoline fixation, feulgen squash preparation) 2n = 12 with four satellited chromosomes. Fig. 25. Somatic metaphase (2BD fixation sections), the chromosomes showing the stained and unstained regions; the pattern of their arrangement is almost similar in the homologues. Fig. 26. Somatic metaphase, one long chromosome broken near the secondary constriction. Fig. 27. Metaphase with thirteen chromosomes, one chromosome (in outline) duplicated. Fig. 28. Metaphase chromosomes, some not showing the secondary constrictions and one chromosome at 11 o'clock broken into three pieces. Fig. 29. Somatically doubled metaphase with twenty four chromosomes. Fig. 30. Telophase with four nucleoli in the lower and three in the upper nuclei.

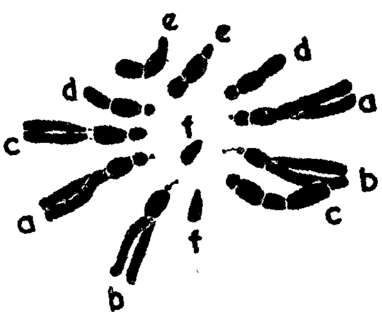
Figs. 23 - 36.

Figs. 23 - 34. Somatic mitosis in Dipcadi hydsuricum Cont.
Fig. 31. Four SAT-chromosomes from a metaphase, the satellites are small and the threads are short. Fig. 32. Four SAT-chromosomes, three with long threads and big satellites and the fourth with a small satellite and a short thread. Fig. 33. Three SAT-chromosomes showing the correlation between the length of the thread and the size of the satellite; two chromosomes with very long threads and small satellites and one with a very large satellite and a very short thread.
Fig. 34. Two SAT-chromosomes fused by their satellite threads.
Fig. 35 - 40. Mitosis and meiosis in Dipcadi montanum. Fig. 35. Somatic metaphase, 2n = 20. Fig. 36. Somatic metaphase, 2n = 20 + 1f).

Figs. 37 - 40.

Figs. 37 - 40. Mitosis and meiosis in *Dipcadi montanum* Cont.
Figs. 37. Idiogram. Fig. 38. Metaphase I, showing ten bivalents.
Fig. 39. Anaphase II, seven chromosomes at one pole and ten at the other with one chromosome lagging (the second cell omitted).
Fig. 40. Anaphase II, twenty chromosomes irregularly distributed in one cell; the other showing ten chromosomes at one pole and eleven at the other, making a total of twenty one chromosomes.

(All figures x 2,500 except 24, 31-35 and 37 x 3,400 reproduced almost to the same magnification in the photographs).



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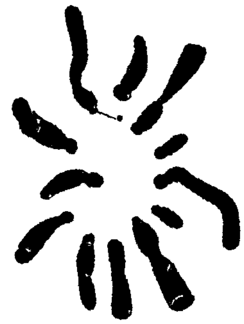

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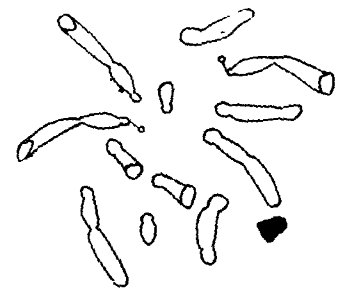

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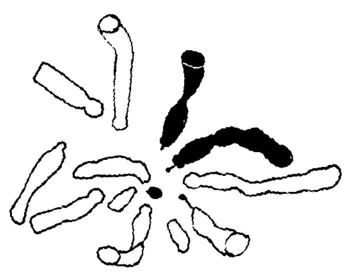

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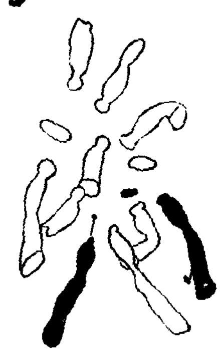

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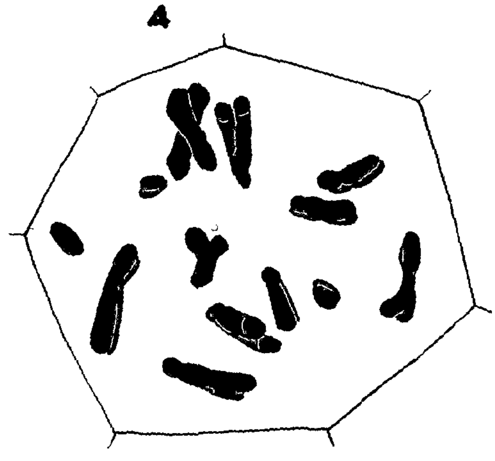

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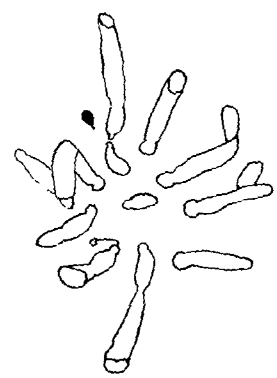

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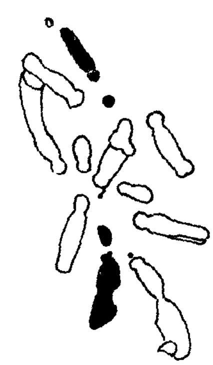

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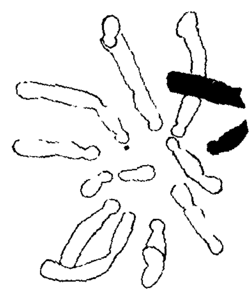

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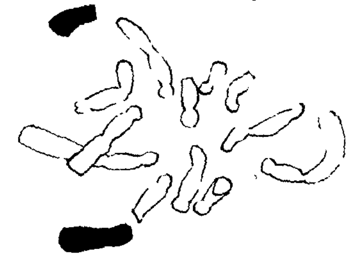

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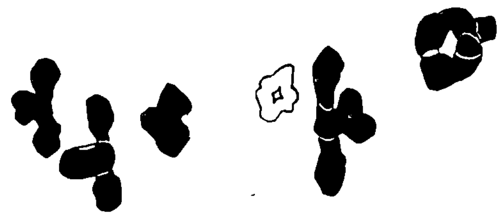

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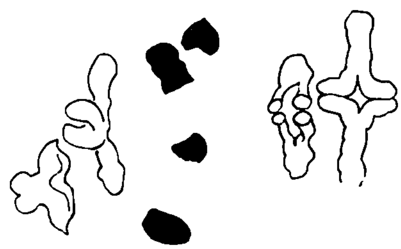

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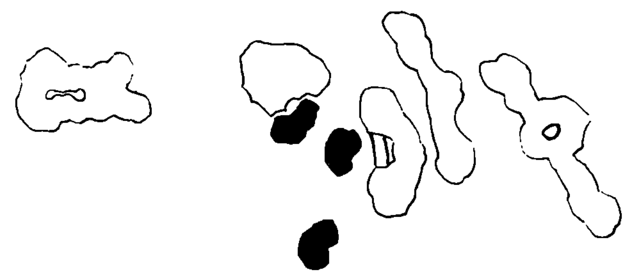

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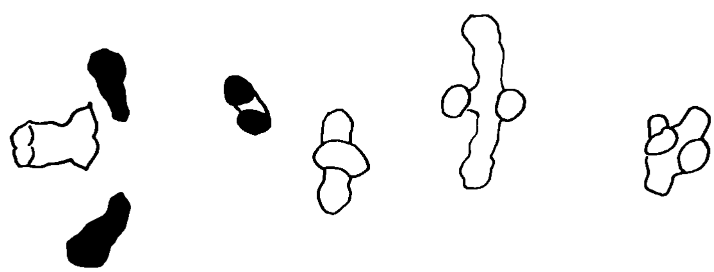

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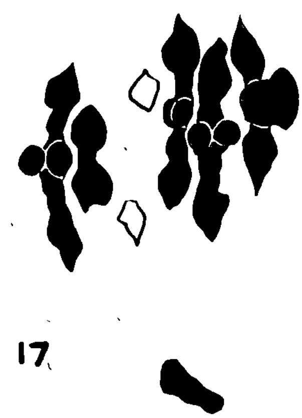

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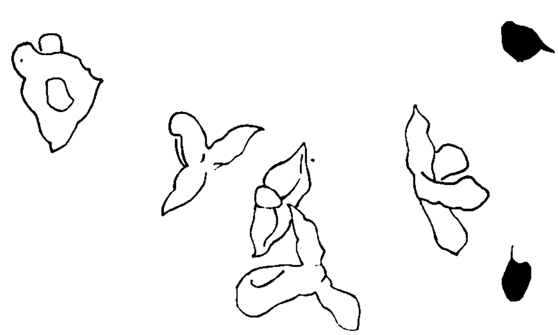

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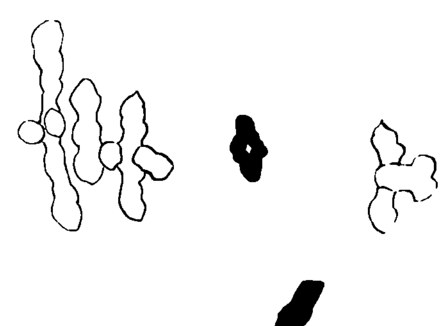

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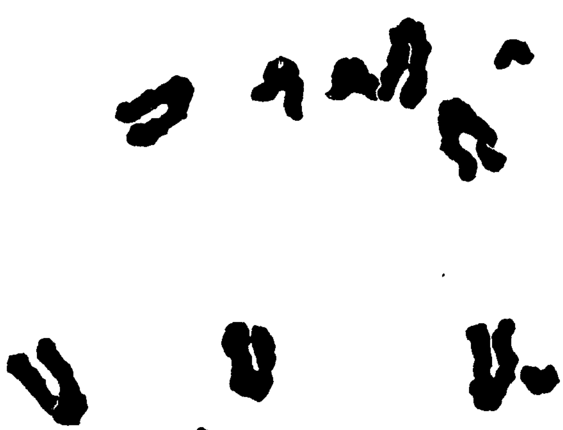

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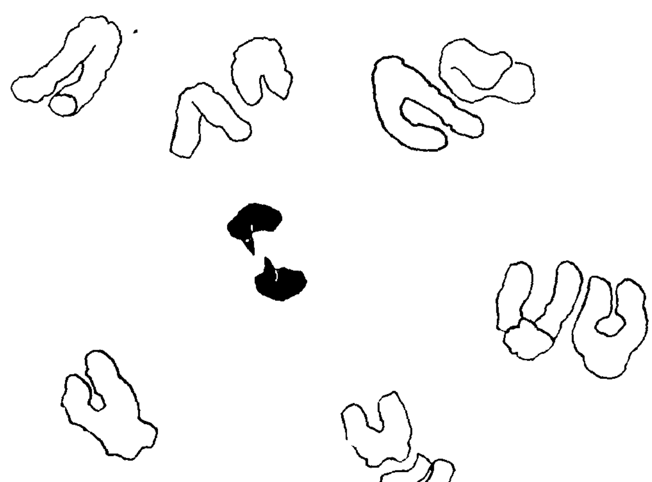

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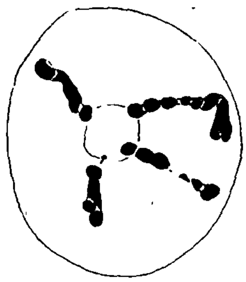

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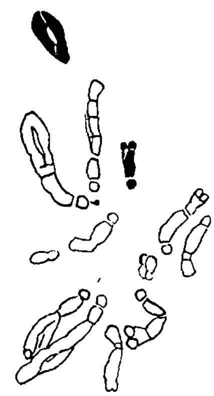

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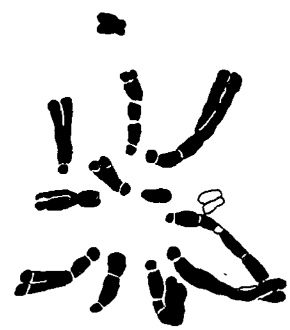

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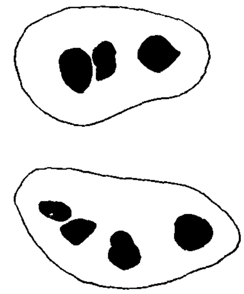

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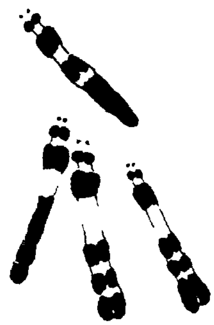

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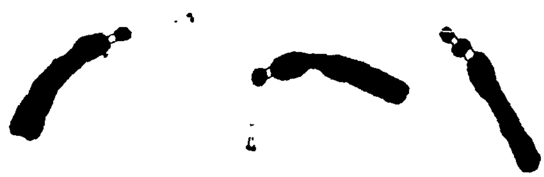

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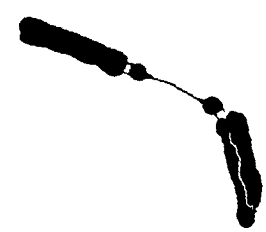

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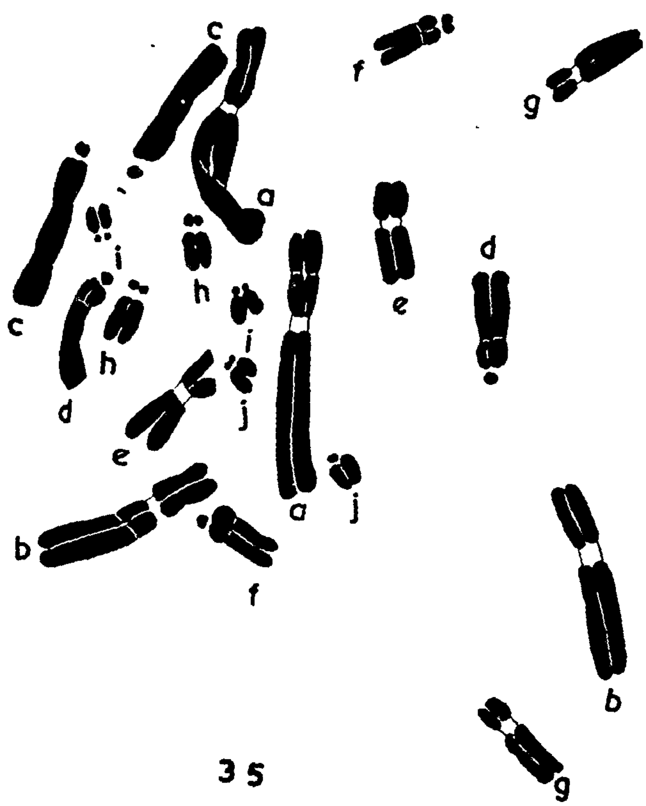

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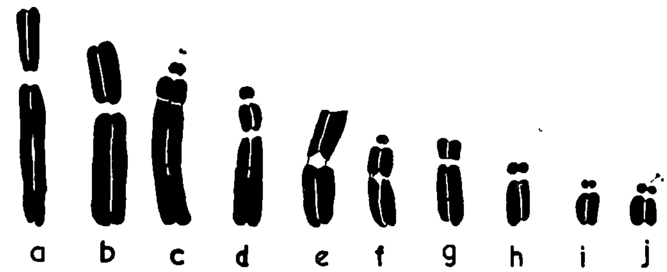

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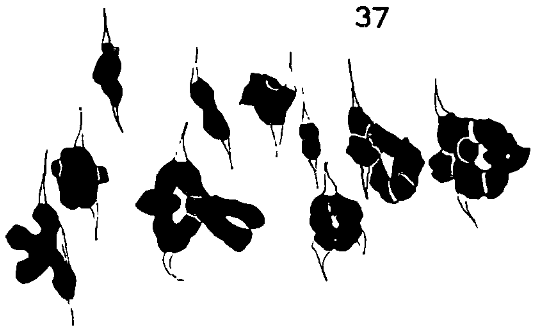

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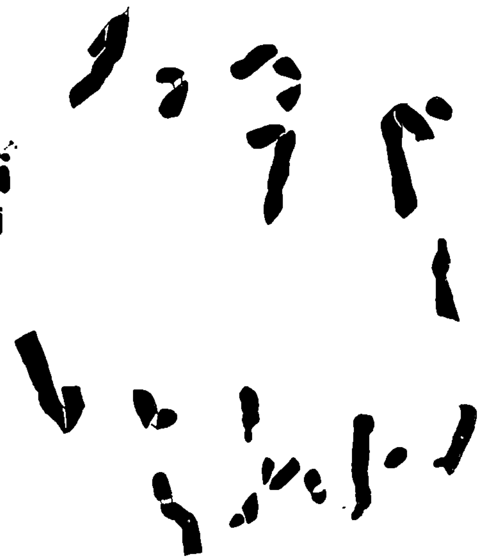


39

40

Dipcadi saxorum Diakinesis ($n = 6$); two bivalents attached to one nucleolus.

same as above 2 bivalents attached to 2 nucleoli.

D. saxorum Metaphase I; $n = 6$; noncongression of one bivalent.

D. saxorum Metaphase I: the smallest bivalent understained.

D. saxorum Early Anaphase I; the sixth bivalent oblique and the fifth disjoined

D. saxorum. Early Anaphase I: a later stage than the previous one.

D. saxorum Anaphase I; both fifth and sixth bivalents disjoined.

D. saxorum Normal Anaphase I.

D. saxorum Anaphase I; the chromosomes of the sixth bivalent lagging

D. montanum Metaphase I $n = 10$

all photomicrographs x 570

D. saxorum

A normal ($n = 6$) and abnormal

($6_{II} + 7_{I} = 19$) PMCs

same as fig. 13.

D. saxorum

Early second Prophase with

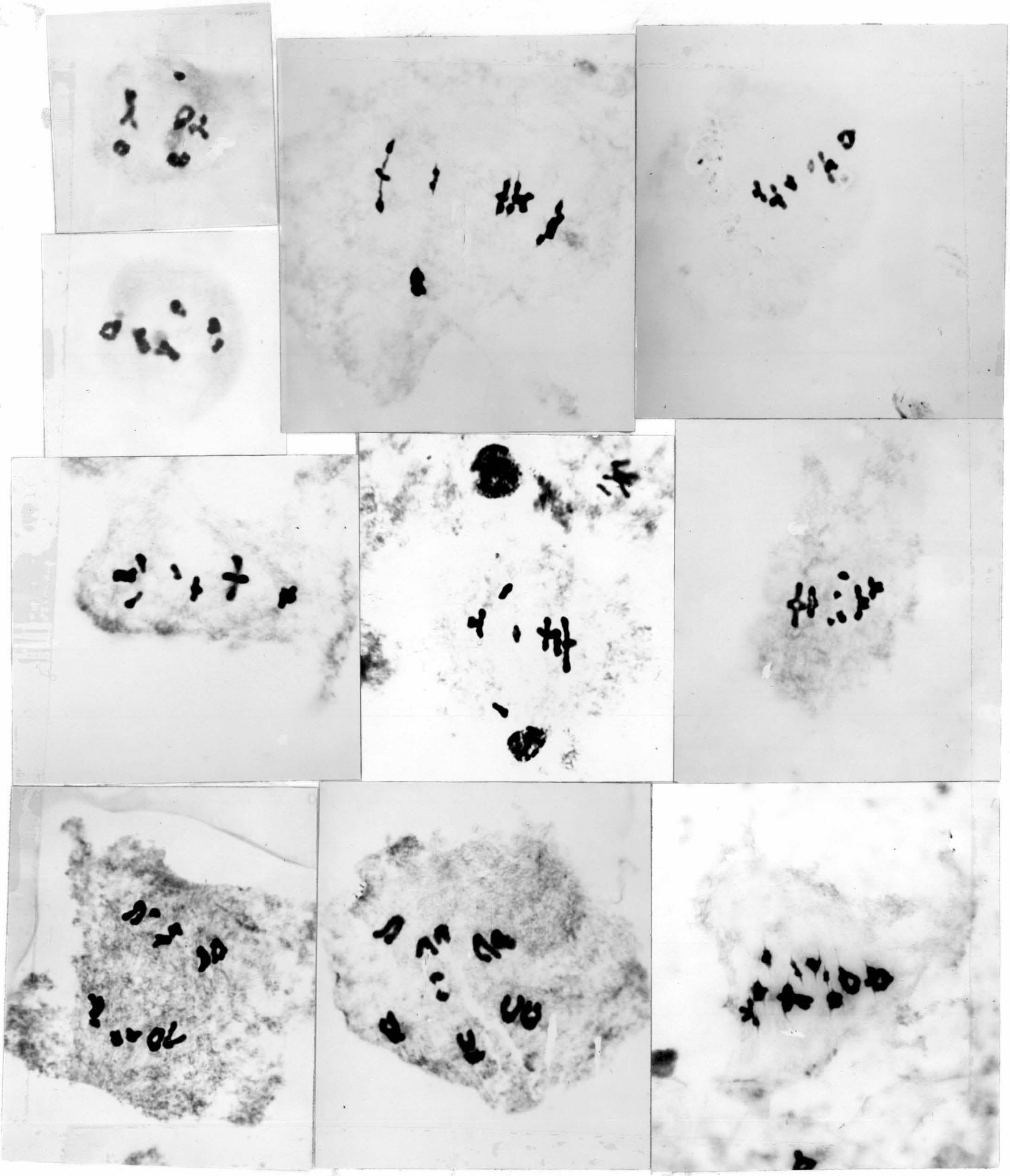
2 chromosomes attached to

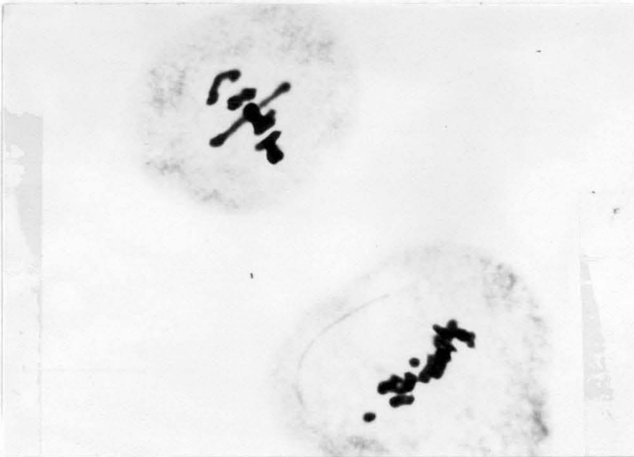
the nucleoli.

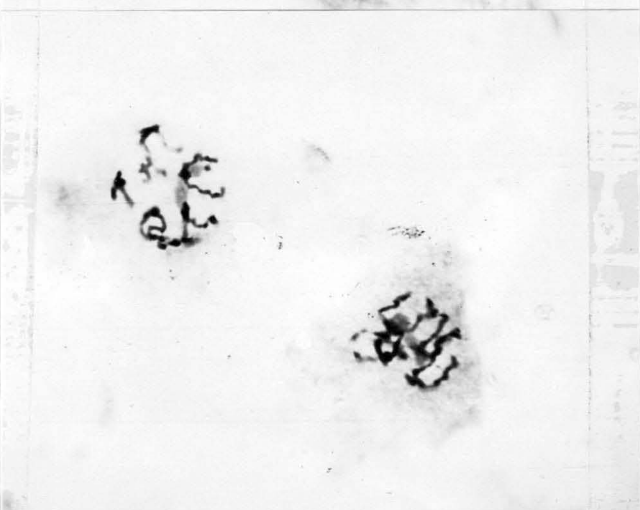
D. saxorum

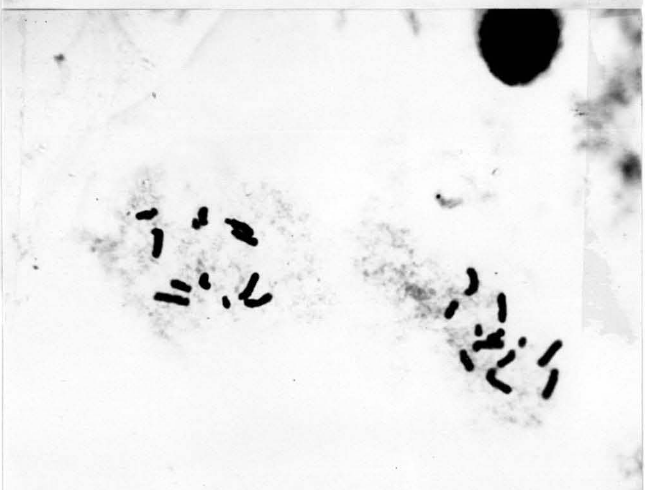
Anaphase II.

All photomicrographs x 570









CYTOLOGICAL STUDIES IN THE INDIAN *SCILLA*

CYTOLOGICAL STUDIES IN THE INDIAN SCILLA

Contents.

I. INTRODUCTION.

The genus *Scilla* is geographically one of the most widely distributed, ecologically one of the most varied groups in habitat, and horticulturally one of the most popular in the gardens. It consists of about 90 species which are mostly distributed in the temperate parts of the Old World. A few of them however have intruded into the tropical regions like India where it is represented by *Scilla hohenackeri* and *S. indica*. What is known of the distribution patterns of these two species would indicate that they were mutually exclusive, *S. hohenackeri* being confined to the temperate regions and *S. indica* penitrating the tropical parts of India and Ceylon.

A preliminary chromosomes survey has revealed that the Indian species do not also overlap in the nature of their cytological variation. The different individuals in *S. hohenackeri*, vary in the heterochromatin content. This is not manifested in their external morphology except perhaps in the vigour of the plants to a small extent. *S. indica* on the other hand shows a remarkable polyploid and aneuploid variation in the chromosome number which is no doubt responsibe for the origin of more or less morphologically similar forms with a characteristic geographic distribution in India. Furthermore, diploid and triploid cytotypes show adaptive morphological divergence, growing as they do in different environments. All these forms have successfully escaped the attention of the systematists so far.

The study of mitosis and meiosis would certainly give an inkling of the nature of evolution within a species complex like *Scilla indica*. The position of the spindle attachment region, which determines the arms of the chromosomes, the secondary constrictions and satellites indicate the chromosome type and all changes observed in these would lead to the inference of translocation, fusion and fragmentation. Much more important is the study of meiosis, where changes not detectable during mitosis manifest themselves owing to the special conditions of pairing and crossing-over. Translocation, inversions, deletions, duplications could be detected. Sometimes failure of pairing not only indicates segmental non-homology but also gene controlled processes leading to the same effect (Beadle, 1930). Presence and absence of multivalents during meiosis in species with double the diploid number of chromosomes would sometimes indicate the nature of polyploidy within certain limitations. So far, these tests have not been applied to the various cytotypes of *Scilla indica* with a view to assure factual assessment of the chromosomal variation within the species population and the consequent discontinuities that would ensue resulting in a clevage of population and subsequent speciation.

II. PREVIOUS WORK.

From a karyological stand point, *Scilla* has been a favourable genus. Most of the relevent literature is summarised in Table 1. Cytological studies in the past have been practically confined only to karyotype analysis and very little attention has been paid to the behaviour of chromosomes during meiosis. The following survey has been undertaken with a view to estimating, whether the pattern of variation exhibited by the Indian species falls in line with the trend of evolution in the whole genus.

Darlington (1926) described the somatic chromosomes of *Scilla nutans* ($2n = 16$). Later on, Dark (1934) reported eight morphologically distinct pairs of chromosomes during mitosis and four types of bivalents during meiosis of *S. italica* ($2n = 16$). The study of meiosis in these species amply demonstrated that chiasma frequency was a function of the length of the chromosomes. This fact, however, could not be verified by Sato (1934) in *S. peruviana* ($2n = 16$), which, on the other hand illustrated the phenomenon of interference in chiasma formation as in *Yucca recurvifolia* ($2n = 30$). In 1935, Sato described the karyotypes of 7 species of *Scilla* namely, *S. sibirica* ($2n = 12$), *S. nutans* ($2n = 16$), *S. peruviana* ($2n = 16$), *S. bifolia* ($2n = 18$), *S. hyacinthoides* ($2n = 20$), *S. chinensis* ($2n = 34$) and *S. japonica* ($2n = 34$). He considered *S. sibirica* ($2n = 12$) as the most primitive species and ascribed the origin of aneuploid species to elimination, fragmentation, and hybridisation of forms with those structural changes.

Polymorphic species of *Scilla* like *S. peruviana* ($2n = 16$), *S. peruviana* var. *alba* ($2n = 16$), *S. permixta* ($2n = 16, 15, 14$) and *S. uchii* ($2n = 17, 19, 20, 22$), some of which showed aneuploid variation in the chromosome numbers of different individuals also received the attention of Sato (1936a, b; 1944). In both *S. uchii* and *S. permixta*, the new karyotypes have originated by the fusion of the satellited chromosome (S_3) with another chromosome (M_4), supporting Navashin's dislocation hypothesis of the evolution of the chromosome complements. However, his work revealed no correlation between the satellited chromosomes and the nucleoli. Nucleoli were sometimes observed by him at telophase when the corresponding satellites were absent in the chromosome complement.

In his publication on the cytology of Liliaceae, not only did Sato (1942) extend the work on the above mentioned species but observations on other species like *S. lingulata* ($2n = 16$), *S. pratensis* ($2n = 26$), *S. autumnalis* ($2n = 29$) were added. Organisation of nucleoli at the primary constriction in *S. sibirica*, the lack of correlation between the number of nucleoli in *S. nutans*, the similarity between the karyotypes of *S. nutans* and *S. lingulata* were some of his important observations.

Following the work of Sato, who apparently contradicted Heitz's hypothesis, Bhaduri (1944) studied in detail the chromosome-nucleolus relationship in some species and varieties of *Scilla* after the application of his differential staining technique. He could establish a numerical relationship between chromosomes and nucleoli and constant and specific differences between nucleoli due to structural hybridity. He also showed that nucleoli had originated from secondary constrictions very close to the primary ones in *S. sibirica* and not from primary constrictions as was thought by Sato (1942). Bhaduri (1940) concluded that *S. sibirica* ($2n = 12$) was the oldest in the genus and that species like *S. nutans* originated on account of structural changes like fragmentation and translocation and possibly after hybridisation. According to him, *S. sibirica* var. *taurica* not only differed from *S. sibirica* in having an extra pair of fragments but also in having two pairs of heteromorphic chromosomes. *S. sibirica* var. *atrocoerula* was reported by him as an autotriploid. *S. peruviana* showed eight secondary constrictions and a pair of chromosomes with tandem satellites. Some of these conclusions, particularly on the chromosome-nucleolus relationship were later on confirmed by Bhaduri and Sharma (1949).

Working on the flora of Braganda Fernandes, Garcia and Fernandes (1948) reported the karyotype of *Scilla ramburei* var. *intermedia* ($2n = 20$) which provided cytological evidence to show that it was no longer a variety of *S. verna*, as was formerly thought by the systematists. The karyotype of *S. italica* ($2n = 16$) recorded by them was similar to that observed by Dark (1934). From the flora of Sardinia, Martinoli (1949) recorded the chromosome numbers of *S. obtusifolia* ($2n = 8$) and *S. autumnalis* ($2n = 28$). Both these species were characterised by chromosomes with a secondary constriction close to the primary one.

With a view to understanding the phylogenetic tendencies in the genus, a karyotype analysis of *Scilla* was undertaken by Battaglia (1952, a, b; 1953; 1955). *S. obtusifolia* var. *intermedia* ($2n = 8$), *S. autumnalis* ($2n = 14$), *S. numidica* ($2n = 18$) and *S. villosa* ($2n = 20$) received his attention. In a series of publications Battaglia (1949, a, b; 1950) recorded a large number of biotypes of S. peruviana which showed an aneuploid change in the chromosome number associated with profound structural alterations, in both nucleolar and nonnucleolar chromosomes.

Gopal-Iyengar (1957) working on the meiosis of S. sibirica (2n = 12) encountered two types of bulbs: (i) "asynaptic" exhibiting a high proportion of univalents during Diakinesis and Metaphase I; (ii) apparently normal with a high degree of inversion hybridity having a variable number of I and II division bridges. Fragmentation, ameiosis, formation of restitution and micronuclei, suppression of some of II division processes were some of the abnormalities were observed by him. He concluded that S. sibirica was a structural hybrid.

The literature on the Indian species of Scilla is very scanty. Raghavan and Venkatasubban (1939) recognised plants with 44, 45, 46 somatic chromosomes described the irregular meiosis of the hypotriploid form with 44 chromosomes and discussed the hybrid origin for this cytotype. According to them, the somatic chromosome complement of Scilla indica (2n = 44) was composed of a diploid set of some species with 20 chromosomes and a haploid set of a species with 16 chromosomes. Sheriff and Murty (1946) reported the diploid karyotype with 30 chromosomes for the first time. Bhaduri (1944) confirmed the observations of Raghavan and Venkatasubban on the somatic chromosomes of cytotype with 44 chromosomes. The present writer conducted a preliminary study of the two Indian species. S. hohenackeri (2n = 10) showed a variation in the heterochromatin content in the different bulbs. The polymorphism of S. indica was accounted for on the basis of its cytotypes with a characteristic pattern of geographic distribution in India. It was believed that the penninsular India below the Vyndhyas and the Sathpuras was a region of great evolutionary activity for *Scilla indica*.

III. CHROMOSOME NUMBERS IN THE GENUS SCILLA.

TABLE I.

The following chromosome numbers in Scilla are arranged according to their ascending series with the exclusion of *Endymion* species, which were formerly described as *Scilla*.

Species	n	2n	Author
Scilla obtusifolia	-	8	Martinoli, 1949
" "			
var. *intermedia*	-	8	Martinoli, 1949; Battaglia, 1952
forma. *stricta*	-	8	Martinoli, 1949
forma. *lata*	-	8	" "
forma. *brevis*	-	8	" "
var. *typica*	-	8	" "
var. *glauca*	-	8	" "
forma. *nana*	-	8	" "
S. villosa	-	10	Battaglia, 1955
S. hohenackeri	-	10	Sundar Rao, 1956
S. sibarica	-	12	Sato, 1935, 1936, 1942.
" "	-	12	Bhaduri, 1944
var. *alba*	-	12	Sato, 1942; Bhaduri, 1944

Species	n	2n	Author
var. *taurica*	-	12 + 2B	Bhaduri, 1944
var. *atrocoerula*	-	18	" "
S. siberica	-	12	Bhaduri and Sharma, 1949
" "	6[1]	12	Gopal-Iyengar, 1957
S. permixta	-	14 + 0 - 2B	Sato, 1936
" "	-	16, 15, 14	Sato, 1942
S. autumnalis	-	14	Battaglia, 1952b
" "	-	24$^{(-28)}$	Heitz, 1926
" "	-	28, c. 42	Maude, 1939, 1940
" "	-	28	Martinoli, 1949
" "	-	29	Sato, 1942
S. italica	-	16	Dark, 1934
" "	-	16	Fernandes, Garcia & Fernandes, 1948
S. hughii	-	16	Maugini, 1953
S. lingulata	-	16	Sato, 1942
S. peruviana	8	16	Sato, 1934, 1935
" "	-	14, 16, 22, 23, 28	Battaglia, 1949a, c: 1950b
" "	-	16	Bhaduri, 1944
" "	-	16	Sato, 1942
" " as *hughii*	-	15, 17, 19, 20, 22	Sato, 1942
S. nutans	-	16	Darlington, 1926; Sato, 1935, 1942

Species	n	2n	Author
S. nutans	-	16	Bhaduri, 1944
" " var. alba	-	16	" "
var. rosea major	-	16	" "
S. pratensis	-	16	Sato, 1942
" "	-	28	Maude, 1940
S. bifolia	-	18	Sato, 1935
" "	-	18	Bhaduri and Sharma, 1949
S. chinensis	-	18, 26, 34, 35	Sato, 1942
S. numidica	-	18	Battaglia, 1953
S. hyacinthoides	-	20	Sato, 1935, 1942
S. monophylla	-	20	" "
S. ramburei var. intermedia	-	20	Fernandes, Garcia & Fernandes, 1948.
S. verna	-	22	Maude, 1940
S. pratensis	-	26	Sato, 1942
S. japonica	-	26, 34, 36, 42, 44	Morinaga, 1932; Sato, 1935

Species	n	2n	Author
S. japonica	17[2]	-	Sato, 1935
S. indica	-	30	Sheriff and Murty, 1946
" "	22[3]	44, 45, 46	Raghavan & Venkatasubban, 1939
" "	-	44	Bhaduri, 1944
" "	15, 22, 30[4]	30, 44, 58, 60	Sundar Rao, 1953, 1956

1. "Asynaptic" and apparently "normal" with inversion hybridity.
2. They are seven bivalents.
3. Irregular with trivalents, bivalents and univalents.
4. The diploids with bivalents: triploids with trivalents, bivalents and univalents; the tetraploids with tetravalents, trivalents, bivalents and univalents.

IV. CYTOLOGICAL TECHNIQUE.

The bulbs of *Scilla hohenackeri* and the various cytotypes and ecotypes of *S. indica* were grown in pots containing equal parts of garden soil and sand. In Hobart weather, the pots needed a lot of basal heat in a hot box for proper growth. Root-tips were collected from vigorously growing bulbs and were fixed in Navashin's chrome-acetic-formalin, Benda with low acetic acid, chromic-formalin (1 : 1, 1: 2, 6 : 4, 4 : 6), chromic-formalin (3 : 6) to which 0.003M 8-hydroxyquinoline was added in the proportion of 9 : 1. A little maltose was added to help spreading of a large number of chromosomes in relatively small cells. An exhaust pump was invariably used. The root-tips fixed in formalin fluids were thoroughly washed in running water for 8 - 10 hours and those fixed in osmic fluids were rinsed in tepid water for 15 - 30 minutes, graded up through a series of alcohol-chloroform grades and embedded in paraffin wax. Sections of 14 - 16 μ thickness were cut and stained in crystal violet.

A more efficient way of studying the somatic chromosomes is by means of feulgen squashes of root-tips fixed in Benda with 0.003 M 8-hydroxyquinoline after a pretreatment of the roots with α-Bromonapthaline for 2 hours. This method afforded a means of studying the relative lengths of chromosomes with ease.

The study of meiosis was exclusively made from acetocarmine squashes of the pollen-mother-cells prepared following the method of Marks (1952). Squashes proved invaluable and superior over sections of flower buds fixed in Navashin's fluid and stained according to Newton's crystal violet method.

V. OBSERVATIONS ON MITOSIS AND MEIOSIS.

(1) Scilla indica

(a) Diploids

(i) General: Plants collected from Dharwar, Bombay, Tiruchurapalli and Ceylon provided material for the present investigation. The importance of this random sampling within the diploid population consists in the fact that Dharwar and Bombay are close to each other and these in turn are far away from Tiruchurapalli. Ceylon forms are away from all their Indian counterparts. This would allow comparisons between nearby units with those at a distance. This would also mean a fair random sampling of the habitats to which the diploids are adapted. Extensive investigation on all these groups has revealed no cojent differences in their somatic chromosomes. The presence of morphologically similar chromosomes in all the diploid local populations would justify the assumption that they form a phylogenetically closely knit group despite the fact that they show slight morphological variation. Although meiosis is also broadly similar in all these morphologically divergent local populations, small but significant differences in the behaviour of chromosomes exist between them. They speak of the prevailing genetical differences. Unfortunately the Ceylon forms did not flower at Hobart just like their Indian relatives. Hence they did not permit any comparison with the Indian forms in their meiotic behaviour. Such a comparison would prove useful because the diploid Ceylon forms resemble some of the

Indian triploids in their external morphology and both are characterised by vegetative propagation. The following account of mitosis and meiosis of the Dharwar forms is equally applicable to the others with the same chromosome number. The differences, if any, will be mentioned in the end.

(ii) Mitosis: The somatic chromosome complement consists of 30 chromosomes. It exhibits the characteristic bimodality. The chromosomes show distinct size variation. They fall into long, median and short types. The distinction between the median and short types is not always easy. The chromosome complement consists of:

Long chromosomes:

(i) Two pairs of chromosomes with submedian primary constrictions; one pair is a little shorter than the other: the longest pair has a median secondary constriction in the short proximal arm and the other very close to the secondary constriction: they are non-nucleolar.

Medium and short chromosomes:

(ii) Eleven pairs are medium-sized and two pairs of short chromosomes with median, submedian and subterminal constrictions; as already show gradation in length among themselves: two pairs are secondarily constricted and two pairs are satellited; both these types are nucleolar.

Spontaneous structural changes were sometimes observed in the somatic tissue. One somatic chromosome complement of the Bombay form consists of a "false dicentric" and a telocentric chromosome. Judging from the morphology of the rest of the chromosomes in the complement, the long chromosome with a median secondary constriction in the proximal arm and a short or medium-sized chromosomes are involved in their origin. Interchange of segments near the centromeres of both these chromosomes would account for the fusion and the origin of "false dicentric" and a telocentric chromosome. The chromosome with two constrictions is described as false dicentric because one of its constrictions does not contain centromere.

A similar structural change but with a different effect was observed in the Dharwar forms. In most of the metaphases in a few foot-tips the second long pair of chromosomes was unequal in length. This heteromorphism for length of these chromosomes could be explained on the basis of segmental interchange between homologous or non-homologous chromosomes. It may be a insertional or mutual translocation.

(iii) Meiosis: Stages prior to Diakinesis could not be observed during the present investigation Diakinesis shows 15 bivalents well spaced in the nucleus (Fig. 1). There are well marked differences in the size of the bivalents, two being the largest. This is in keeping with the size variations encountered in the somatic chromosome complement. Four bivalents corresponding to the 2 pairs of satellited chromosomes, and 2 pairs of chromosomes with secondary constrictions in the somatic chromosome complement are in contact with the two nucleoli, which are unequal in size. Apparently, the long chromosomes showing the secondary constrictions are not nucleolar in function. Such chromosomes were reported by Stewart (1947) in Lilium.

Neither multivalents of any order nor univalents were observed at the Diakinesis stage, despite the fact that a large number of nuclei were scored. Just as there is a wide variation in the size of the bivalents, there is also equally wide variation in the number of chiasmata per bivalent. The large bivalents are characterised 4 and 3 chiasmata respectively and the rest with 2 or 1 chiasmata at this stage.

In pollen-mother-cells fixed on unusually warm days (May-June) in India, the long bivalents showed distal differentiation, which consisted in reduced stainability of major part of the chromosomes (Fig. 2). This has been observed at Diakinesis and sometimes even at Diplotene stage. These bivalents are perfectly synchronised in every other respect with the normal bivalents of the same nucleus. There is neither a change in the number of chiasmata nor a change in the degree of spiralisation. The differential condensation that is usually ascribed to the differential reaction has not been noted. The length of the bivalents also remains unaltered when compared with the normal ones. The constant position and length of these differential regions must result from a special genetic property of the locus concerned, as in nucleic acid starvation (Darlington and La Cour, 1940). This is analogous in effect to the differential reactivity of chromosomes described by Darlington and La Cour (1938). In the case Scilla indica, it probably manifests itself due to the influence of external factors like high temperature. It is however not intelligible why all the cells in the same anther loculus do not behave in a similar manner. Perhaps these cells differ in their capacity of reacting to the external variable factors. The threshold values of these cells may be also different.

The differential staining reaction is sometimes associated with clumping of other bivalents in the same nucleus, the number of bivalents included in the clump being variable. Both do not accompany each other since clumping without differential staining reaction sometimes occurs and vice versa. Chromosome clumping was produced after irradiation (Catcheside, 1947). Sherman Walters

(1957) found the clumped chromosomes almost always associated with nucleolus and it was attributed to an alteration in the usual metabolic relationship between the nucleolus and the chromosome complement. This is not a plausible explanation in the case of Scilla indica since the clumped chromosomes were never found in the proximity of the nucleolus. Probably it is again a case of nonsynchronised reaction of some of the chromosomes in a nucleus to the external factors like temperature (cf. Jain, 1957 in Lolium).

Fig. 3 represents the side view of Metaphase I with 15 bivalents. Two big bivalents could be distinguished from the rest which vary but little in size. This fact is in keeping with the size variations in the somatic chromosomes. There is less marked size difference in the medium and small chromosomes, just as 13 bivalents formed by these chromosomes show no great size variation at Metaphase I. Although elaborate statistical data are not available, the conditions of chiasma formation and its frequency at Metaphase I are interesting. In the diploid Dharwar forms, the average number of chiasmata per cell is 25.4. The chiasma

Average number of chiasmata per cell - 25.4.

Chiasma frequency per bivalent - 1.70.

Chiasma frequency of medium and short chromosomes - 1.4

Chiasma frequency of first long trivalent - 3.3.

Chiasma frequency of second long bivalent - 2.3.

frequency per bivalent is 1.7. The chiasma frequencies of the two long bivalents are 3.3. and 2.3 respectively. Whenever there is a reduction in the number of chiasmata in the second long bivalent, there is an increase in the number of chiasmata in the longest bivalent. Assuming that the developmental and environmental conditions of different cells in the same anther loculus are same, this may illustrate the law of negative correlation postulated by Mather and Lamm (1935), Mather (1936) and Lamm (1936). That it is confined sometimes to the two long pairs in S. indica is interesting.

The presence of 30 chromosomes which form 15 bivalents is the normal feature in most of the pollen-mother-cells. Sometimes 14 bivalents and 2 univalents (Fig. 5) or 13 bivalents and 4 univalents were observed. Univalents have never been noted during the diakinesis or even at the earlier stages of meiosis. In all probability, the observation of univalents at Metaphase I should be attributed to the precocious separation of bivalents with single terminal chiasmata. This fact is to be correlated with a reduction in the total number of chiasmata in such cells. While the total number of chiasmata in normal cells with 15 bivalents range from 23 to 28, the total number of chiasmata in cells with 2 univalents falls down to 20. In Scilla indica this reduction in the chiasma frequency of certain cells is in all probability due to the high temperature in the environment at the time of flowering (cf. Dowrick,1957). The presence of univalents at Metaphase I is also to be related to a deviation in the chromosome number. Fig.4 represents a cell with 29 chromosomes forming 14 bivalents and a univalent. When there are 31 chromosomes, the odd chromosome remains unpaired.

Much more interesting abnormality is the formation of univalents, due to either partial asynapsis or partial desynapsis effecting all the bivalents in a cell except the two long ones (Fig. 6) and very rarely one of the big bivalents too (Fig. 7). The univalents formed as a consequence of these phenomena are scattered irregularly and at other times univalents of the same size as secondarily associated. This is clearly visible in the case of big chromosomes also (Fig.7).

It is extremely difficult to decide whether it is a case of asynapsis or de-synapsis because no univalents were observed at Diakinesis. Pending contrary observations at this stage, it may be tentatively concluded that the formation of univalents in these cells is due to partial failure of pairing. A whole series of genetical and environmental factors have been inferred by several workers to influence the conjugation of chromosomes. Pairing of chromosomes is now known to be gene controlled in Drosophila (Gowen, 1928) Zea (Beadle, 1930), Rice (Ramanujam and Parthasarathy, 1935) and Datura (Bergner et al. 1934). External agencies like temperature have been demonstrated by several workers to affect conjugation of chromosomes (Katayama, 1931; Stow, 1926, 1927; Heilborn, 1930; Sax, 1931). Sometimes genic homology alone is not sufficient to bring about pairing. In a diploid Crepis capillaris, which originated as a result of doubling in a haploid, all gradations from complete bivalency to complete univalency exist (Hollingshead, 1930). Variation in pairing of chromosomes in different cells of the same anther was attributed to a variation in the nutritional conditions in Rubus (Meurman, 1928). Genetical, nutritional and environmental factors may be attributed to explain the failure of pairing in Scilla indica. Just as proximal differentiation and clumping are attributed to high temperature in the environment, formation of univalents may be due to the same cause. Assuming that there was normal pairing in these cells, a condition simulating partial asynapsis or desynapsis could be attained by nonsynchronisation and timing unbalance in spindle formation and anaphase separation.

In most of the cells, the bivalents disjoin synchronously at Anaphase I resulting in a distribution of 15 chromosomes at each pole (Fig. 11). Rarely a slight departure from the normal division may occur causing an unequal distribution of the chromosomes at the poles. Fig. 8 represents a pollen-mother-cell with 15 chromosomes at the upper pole and 16 at the lower. This is obviously a cell with 31 chromosomes at Metaphase I and the single unpaired chromosome has passed to one pole without division at Anaphase I. Sometimes the big bivalents are delayed and lag at Anaphase I due presumably to the delay in their congression or persistence of interstetial chiasmata (cf. Catcheside, 1934 in Brassica). As a consequence, they are last to disjoin. One chromosome of such a large bivalent may move quickly to one pole and the other may lag at the equator (Fig. 9). If such chromosomes are not ultimately included in the daughter nuclei, gametes with unbalanced numbers are formed. The reverse of what may happen to the large bivalents may sometimes happen to the small bivalents, which divide precociously. One such precociously separated chromosomes may reach the pole earlier than its partner and hence it may not be included in one of the daughter nuclei. Such a chromosome which is of the upper group is shown in Fig. 10. Fig. 12 illustrates a small lagging chromosome at late Anaphase I. It is very likely that it would not be included in either of the daughter nuclei, which are already unequal in size.

In an anther loculus, a few cells showed an interesting abnormality of chromosomes at Anaphase I. The chromosomes in these cells were very much smaller in size when compared with those of the normal cells (Fig. 13). There was also a similar reduction in the volume of the cell as a whole. Their exact location in the anther loculus with referenced to the tapetum could not be decided in these squashes. Cases of such a marked reduction in the size of the chromosomes in the same preparation are rare. Darlington (1936) in his studies of Fritillaria found two isolated abnormal pollen-mother-cells at meiosis. In one pollen-mother-cell of F. pluriflora, the chromosomes were more condensed and more separated on the plate than those of the normal ones. In one pollen-mother-cell of F. meleagris the chromosomes were as long as those at mitosis and the nucleolar constrictions were still visible in them. He thought that the genotypic differences in these cells might have been responsible for these abnormal chromosomes. In a similar manner, the mutation in these cells of Scilla indica resulted in a change of the genotype, which was responsible for a reduction in the size of the chromosomes and also for any irregular behaviour that these chromosomes might show at Anaphase I. Since adjacent cells showed normal behaviour, no difference in the environment could be inferred. The significance of these genotypic changes in the evolution of the different races of S. indica will be discussed later on.

Fig. 14 shows an abnormal pollen-mother-cell with a chromatid bridge and no associated fragment. In the formation of this bridge two long chromosomes are involved. In addition to them, there are eleven chromosomes at the upper pole and thirteen at the lower. One chromosome which could be interpreted as a large fragment is close to the bridge. Its configuration reveals that in all probability it is a whole chromosome and not a fragment. The origin of such a bridge without a fragment could be explained on the assumption that the union of the homologous ends of the sister chromatids in the two chromosomes of the bivalent has taken place. Such an assumption is justified in the light of such observations of terminal unions in bivalents independent of chiasmata by Taylor (1949) in Tradescantia and by Matsurra and Haga (1942) in Trillium. Bridges without fragments have been reported in the pollen grains of Kniphofia rufa ($n = 6$) and Paeonia veitchii ($n = 5$) by Barber (1938), in the pollen grains of Hyacinthus orientalis var. "William Mansfield" ($2n = 16$) by Upcott (1937) and in Allium margaritaceum by Mensinkai (1939). There has been a considerable controversy with regard to the fusibility of the unbroken ends of the chromosomes. Irradiation experiments go counter to such a concept and they amply demonstrated the fundamental difference between the broken and unbroken ends of the chromosomes with regard to fusibility. Nevertheless under exceptional circumstances when the cells are unbalanced with a decreased number of chromosomes as in the abnormal cell of S. indica under discussion, fusion of chromosomes in a bivalent leading to the formation a bridge is perhaps a plausible assumption. This is similar to ageing in pollen (Barber, 1938) or the adverse external circumstances influencing the physiology of the nucleus leading to denaturation of chromatin in particular chromosomes resulting in the fusion of sister chromatids (Mensinkai, 1939).

The fundamental distinction between the Dharwar and Bombay forms on the one hand and the Tiruchirapalli forms on the other is the presence of tetraploid cells in the former and their absence in the latter. Their distribution in the anther loculus is haphazard as the dwarf pollen grains in Tradescantia (La Cour, 1949). They were never localised in the centre like the abnormal cells in Scilla (Rees, 1952) or in the peripheral part of the anther close to the tapetum. These tetraploid cells co-exist with the normal diploid cells and exhibit perfect synchronisation in the cell division. Meiosis progresses exactly in the same manner as those of the tetraploid plants. Multivalents have been identified during prophases and Metaphases I and the quadrivalents occur with about the same frequency as in the tetraploid plants. At Metaphase I and Anaphase I the spindle is divergent as in Maize (Clark, 1940). Anaphase I leads to a fairly regular disjunction and distribution of 30 chromosomes at each pole. Durigg Telophase I, the two nuclei at the poles are double the size of the normal. Although the second division stages have not been observed, there is every reason to believe that it progresses in a fairly regular manner and that diploid gamete formation is assured. Giant pollen grains have been observed during the present investigation.

The tetraploid cells in Scilla indica do not show any tendency towards the formation of plasmodial masses reported by Smith (1942) in Barley with multiploid sporocytes. In view of the cell size and in the absence of indic- ations of any fusions between the normal cells, it is reasonable to trace the origin of these cells to premeiotic disturbances. Their presence in Dharwar and Bombay forms and their absence in the Tiruchurapalli forms show that their origin is genetically controlled, just as many premeiotic errors are now known to be determined by (Rees, 1952). In the original diploid population of Scilla indica, segregation and recombination of genes affecting the penetrance of these premeiotic errors would ultimately result in the differentiation of the population into 2 groups one with the error and the other without the error. This seems to have happened during the course of evolution of the diploid population in S. indica. This is but one type of evidence to show that the different diploid local populations are genetically different. This phenomenon is analogous to the presence of reduced cells in one strain of autotetraploid rye (O'Mara, 1942) and their absence in the other (Müntzing, 1951) due to differences in the gene combinations.

(b) Triploids

(i) General: For the present study naturally occurring hypotriploid cytotypes have been collected from Madras (Madras State, India) and Masulipatam (Andhra State, India). They have been found to occur only in these areas and nowhere else in India. Apparently they show a preference to sea shore areas. Although Madras and Masulipatam do not markedly differ in their climatic conditions, the two forms collected from these localities however show significant cytological differences associated with great morphological variation. The leaves of Masulipatam form are thin, narrow, light green with blotches scarcely perceptable. On the other hand, the Madras form is characterised by thick, coriaceous, darkgreen leaves with blotches, which are almost black. Obviously they are two ecotypes growing in two different edaphic conditions. Raghavan and Venkata Subban (1939) studied narrow-leaved plants with 44, 46 somatic chromosomes and a horticultural variety with 45 chromosomes. The cytological behaviour in all these forms is broadly similar and the following account of the hypotriploid forms with 44 chromosomes is applicable to all. The points of difference, if any, will be mentioned in the end.

(ii) Mitosis: Due to small size and a relatively high number of chromosomes when compared with the size of the cells, a critical study of the karyotype was not possible. However, 44 chromosomes were clearly counted in the root-tip cells. As is the case with the diploids, the chromosomes are sharply distinguishable into long, medium and short types. They are as follows:

Long chromosomes:

(i) Six chromosomes, out of which two show secondary constrictions;

Medium and short chromosomes:

(ii) The rest of the chromosomes are of this type: some of the medium-sized chromosomes show secondary constrictions and their exact number could not be decided during the present investigation. Four small chromosomes are satellited, which are in themselves very minute and could not be observed in all preparations consistently.

Some of the above-mentioned observations are in agreement with those of Raghavan and Venkatasubban (1939).

At Metaphase I most of the pollen-mother-cells showed 44 chromosomes, which is also the somatic number. While the chromosome number in the root-tip cells is fairly stable, it is indeed remarkable that there is a considerable variaton in the chromosome number in the pollen-mother-cells. All aneuploid numbers ranging from 37 up to 46 have been observed in them, the peak being at 44. In one singular instance 51 chromosomes were observed. Notwithstanding their differences in number, they show perfect synchronisation and divide within the same anther loculus. Pairing in cells with decreased or increased chromosome number is in no way impaired despite the unbalance that is set in more due to the decrease than to an increase in the chromosome number. In all respects, they are comparable to the normal cells with 44 chromosomes in their meiotic behaviour. A cell with 37 chromosomes showed 10 trivalents, 2 bivalents and 3 univalents. Fig. 20 illustrates a cell with 40 chromosomes forming 13 trivalents and 1 univalent.

The origin of these aneuploid cells in triploid is parallel to the origin of tetraploid cells in the diploids. The underlying cause for both is also same. In all probability, the origin of aneuploid cells in triploids must be due to the irregular disjunction and spindle abnormalities in the premeiotic divisions. A large number of cases are known where spindle abnormalities are gene controlled. They are sometimes attributed to single gene differences (Smith, 1942). Multipolar spindles are known to arise due to certain gene combinations (Vaarama, 1949), which is the case with the split spindles (Darlington and Thomas, 1937). Perhaps a new gene combination in the triploid Scilla indica has brought in spindle abnormalities in the premeiotic cells initiated the origin of aneuploid cells.

In "normal" cells with 44 chromosomes, all stages between no pairing (Fig. 27) and complete pairing (Fig. 19) were observed. Trivalents, bivalents and univalents in different proportions are formed. For instance, Figs. 15-19 represent respectively: $10_3 + 4_2 + 6_1 = 44$; $4_3 + 10_2 + 12_1 = 44$; $8_3 + 6_2 + 8_1 = 44$; $6_3 + 5_2 + 16_1 = 44$; $14_3 + 1_2 = 44$. A maximum number of 14 trivalents have been observed during the present investigation (Fig. 19). The trivalents, bivalents and univalents line up at the equator with some of the univalents off the equatorial region. Sometimes there is a great irregularity in their orientation, the great bulk of the spindle being filled with chromosomes from one end to the other. Such an irregular distribution of the chromosomes is characteristic of most of the triploids (McClintock, 1929; Collins, 1933; Morinaga and Fukushima, 1935; Darlington, 1936; Karasawa, 1932). It is no doubt due to the asymmetrical nature of the trivalents, in which the presence of three centromeres sets in mechanical difficulties and interferes with their proper orientation.

The shapes assumed by the trivalents at Metaphase I are various, viz. fryingpan, the Y, a chain or irregular shapes like those represented in Figs. 30, 32, 33, 35, 37 etc. In this respect it is most pertinent to remark that the trivalents formed by the long and short chromosomes are similar in all essential respects, except for the fact that chains are rarely formed by the long chromosomes. Furthermore a definite association of any three homologous chromosomes, giving a constant morphologically recognisable trivalent does not seem to exist in the triploid Scilla indica. The constant formation of two chain trivalents has been reported by Mather (1935) in his triploid wheat hybrid and their formation has been explained by him as due to autosyndesis. Their appearance in a triploid like S. indica is not unexpected since the chromosomes in such a hybrid show different degrees of homology.

Association of more than three chromosomes to form multivalents of a higher order than trivalents has been encountered in a few cases. In Fig. 21 a chain of five chromosomes is illustrated. The rest of the chromosomes in the complement appear as trivalents, bivalents and univalents. This phenomenon of the formation of a pentavalent in a triploid is similar to the occasional quadrivalents in diploids (Iyeugar, 1939) and bivalents in haploids (Morinaga and Fukushima, 1935). It certainly indicates the presence of related chromosomes within the haploid complement of the triploid Scilla indica and that its parents were in themselves polyploid in origin. Its rarity implies distant homology of the chromosomes involved in the pentavalent Darlington and Moffett (1930) reported a maximum association of nine chromosomes in the triploid

Pyrus malus, corresponding to a maximum of six in the diploid. Ichijima (1934) and Ramanujam (1937) observed autosyndesis in triploid rice.

As is the case with triploids, the occurrence of univalents ranging from 0 - 44 has been observed in Scilla indica (Figs. 22-27). Metaphases I showing 44 univalents are rare. Sometimes whole anthers were found to contain only univalents. Such a complete asynapsis is generally ascribed to the action of genes, to the loss of chromosomes, to the external conditions like temperature, to apomixis, to the mechanical chromosomal conditions or to hybridity. In S. indica it is due to its hybrid origin. The orientation of the univalents on the metaphase plate was highly irregular, sometimes on the plate and usually away from it. Whenever univalents occur in triploids they conform to a particular behaviour. In his triploid wheat, Thompson (1926) found univalents in the plate, which arranged themselves regularly and divided equationally after the division of the bivalents. Sax (1922) recorded univalents which never moved to the equator in his triploid wheat.

According to the expectation, the haphazard orientation of the asymmetrical trivalents at the equator caused the Anaphase I to be irregular, leading to an unequal distribution of the chromosomes at the poles (Fig. 46). The two groups are not usually separate in most of the cells with a large number of lagging chromosomes bridging them (Fig. 47). The spindle at Anaphase I usually presents a characteristic appearance on account of the scattering of the chromosomes from pole to pole. The trivalents disjoin, as a rule, to form three univalents, two going to one pole and one to the other. In Fig. 65, the univalents of the two big trivalents show position correlation. The bivalents separate normally and the two chromosomes go to the opposite poles. Majority of the univalents migrate at random to the two poles without division. They behave normally at the second division and do not lag. Those that are away from the equator during the Metaphase I, move to the equator at this stage, orientate themselves properly and divide, the split halves going either to the same pole (Fig. 48) or to opposite poles (Fig. 51). The division and the migration of the split halves take place usually very late at Anaphase I. They are tardy in movement due presumably to their weaker centromere charge (Richardson, 1936). Rarely do the long lagging chromosomes align themselves on the spindle and show signs of division (Fig. 49) . It is difficult to ascribe any particular reason for this differential behaviour of the two types of chromosomes. The split halves of a long chromosome show stickiness. In spite of considerable lagging during Anaphase I, only two nuclei were found in majority of cells, indicating that the lagging chromosomes were included in the daughter nuclei. Considerable elimination and degeneration of the chromosomes was noted (Fig. 66). The straying chromosomes when not included in the daughter nuclei, form a membrane round themselves and organise micronuclei (Fig. 67), whose number and size vary considerably. Apparently the size of the micronucleus depends on the number of chromosomes included in it. (Fig. 70 and 71). In Fig. 68 is illustrated a spindle-shaped micronucleus with its lower part attached to one of the daughter nuclei. Presumably a row of univalents arranged end to end have taken part in its organisation.Formation of micronuclei has been reported by Ramanujam (1939) in rice and Levan (1936) in _Allium schoenoprasum_. The fate of these micronuclei has not been traced and in all probability they undergo degeneration.

Irregular Anaphase I leads to the formation of restitution nuclei in some allotriploids. As a consequence unreduced gametes are formed. Although considerable number of irregularities have been noted no restitution nuclei were observed in the triploid Scilla indica. Perhaps triploids differ in the frequency of occurrence of the restitution nuclei and there are all gradations between their total absence as in triploid rice (Morinaga and Fukushima, 1935; Ramanujam, 1937) and its frequent occurrence as in triploid Narcissus (Nago, 1929). Scilla indica is similar in this respect to the triploid rice.

Formation of bridges and fragments at Anaphase I is a phenomenon of frequent occurrence in Scilla indica. Sometimes the bridges are fine strands connecting the two anaphase groups and they are invariably as deeply stained as the chromosomes themselves (Fig. 53). At other times, they are as stout as the chromosomes themselves (Fig. 52 and 54). It only indicates that the triploid is heterozygous for an inversion. Inversion may involve a reversal of a segment of a chromosome or a chromatid and may arise during the early stages of prophases, when the chromosomes form loops. They may be distal or intercalary. Bridges are formed when the relatively inverted segment involves a sufficiently large region to allow the formation of chiasmata in relation to the centromere Richardson (1936) has analysed the consequences of chiasmata formation in the inverted region. The bridges and fragments arise when one or two chiasmata are formed in the inversion and in the latter when one chromatid is involved in both the cross-overs. The chiasmata responsible for the formation of bridges at Anaphase II are much more complex but they have never been found in the triploid S. indica.

Fragments are usually associated with bridges. Bridges without fragments have never been noted in S. indica while fragments without bridges were sporadically found both during Anaphase I and Anaphase II. A comparative study of figures 52, 53, 54, and 55, would at once reveal the same fact. The size of the fragment is a fair measure of the size of the inversion (Darlington, 1937).

If the inversion is very near the centromere, the fragment is larger in size. The fragments probably degenerate. But in Fig. 57 it is found attached to a lagging univalent, which as a consequence presents a false appearance of a satellited chromosome Trivalents are sometimes involved in the bridge formation (Fig. 62). When bridges are thin or show thick and thin portions (Fig. 53), it indicates that it is under a great tension, probably due to the axile stretching of the spindle, which have been regarded by Belar (1929) and Darlington (1937) as an important factor in causing anaphasic separation. Owing to this tension the bridge may break at any point, unequally (Fig. 53) or in the middle (Fig. 69). The latter is an instance of a bridge and fragment configuration which is still persisting at early Telophase I.

Univalent bridges have been observed rarely in the triploid S. indica (Figs. 56, 57 and possibly 60). Each one of them is associated with a big or a small fragment. Being made up of only two chromatids, they are always short and do not join the poles. Univalent bridges arise from lagging members of trivalents or multivalents which form chiasma in the inversion and one proximal to it with one of their partners and one chiasmata at least with the other partner since they are all parts of a trivalent. Lagging members may arise in a triploid when the co-orientation of a trivalent is linear or indifferent (Darlington, 1937). The arm which was a loop then forms a bridge. Upcott (1937) remarked that univalent bridges are rare and occur only in triploids with high inversion frequency, as is the case with the triploid Scilla indica.

At the end of first division a wall is generally formed (Fig. 20). It is as irregular as the first division and is simultaneous in both the cells. Figs. 72-80 are various Anaphases II showing a few abnormalities met with at this stage. Apart from the fact that the chromosomes are uniformly distributed on spindle from pole to pole as shown in the Figs. 78 and 73, the chromosomes at the poles are unequal in number. Fig. 72 illustrates 20 chromosomes at one pole and 24 at the other. Random assortment of chromosomes and the unbalance between the time of the division and spindle formation account for these abnormalities. Fragmentation of chromosomes was extensive. They divide normally and move to the opposite poles (Figs. 74, 76 and 77). The size of the fragment varies. That the fragments are endowed with the capacity of division like normal chromosomes is interesting. When a chromosome is broken at the centromere the two fragments are still connected to each other by a tenuous thread-like structure (Fig. 75). A tendency towards division at the centromere is shown in Fig. 79. Sometimes isochromosomes were observed. (Fig. 80).

Tetrapaloid Cytotypes

(i) General: The tetraploid Cytotypes are uniform in their external morphology. As far as the present investigation goes, they occur only in Madhya Pradesh.

(ii) Mitosis: In the root-tip cells, 60 chromosomes were clearly counted. The exact chromosome morphology could not be worked out due to the small size of the chromosomes. However, as in diploids and triploids, distinct bimodality exists in the chromosome complement. There are eight long chromosomes and the rest are either medium and small.

(iii) Meiosis: The study of meiosis in the tetraploids proved extremely difficult, partly on account of large numbers of small chromosomes and to some extent due to the stickiness of the chromosomes. With these limitations however a careful analysis of meiosis was made, as far as the material permitted.

Observations on meiosis were confined to Metaphase I and later stages. Sixty chromosomes were counted in most of the pollen-mother-cells at Metaphase I. A departure from this normal number was found though rarely and PMCs with 58-63 chromosomes have been recorded. The sixty chromosomes form quadrivalents trivalents, bivalents and univalents in variable proportions. Among these, the frequency of bivalents is high. For instance fig. 81 shows twenty five bivalents and ten univalents. The regularity with which the bivalents are formed is illustrated in fig. 83 with twenty eight bivalents and one quadrivalent.

The arrangement of these multiple bodies on a bipolar spindle is *ipso facto* irregular. The co-orientation and hence the mode of disjunction of the multivalents composed of small chromosomes could not be worked out in detail. The quadrivalents are chains and trivalents are either chains or Y-shaped. The shapes assumed by the large tetravalents and their co-orientation at Metaphase I are illustrated in Figs. 84-90. They are mostly parallel and a few are convergent and indifferent. Orientation is determined by the distances apart of the centromeres in the quadrivalent at the time metaphase begins and whether the chiasmata are terminal or interstitial. In a majority of cases, the ring of 4 chromosomes show complete terminalisation of chiasmata. Hence it becomes pliable in such a way as to accommodate itself on a crowded plate and yet maintain co-orientation. Otherwise co-orientation fails altogether when the centromeres are farther apart than they can be in bivalents.

In spite of the multivalent formation, Anaphase I proceeds with a fair degree of regularity resulting in a fairly equal distribution of the chromosomes at the poles (Fig. 92). Due to the uneven division of the multivalents, differences may ensue in the two daughter nuclei (Fig. 93). The second division was not studied for want of material.

(2) S. hohenackeri Fisch. and Mey. (2n = 10).

The karyotype of this species has already been reported (Sundar Rao, 1956). It consists of long, medium and short chromosomes.

(i) Two pairs of long chromosomes, one with median and the other with submedian constrictions;

(ii) Two pairs of medium-sized chromosomes of variable lengths, each with subterminal primary constriction and a secondary constriction in the short arm very close to the primary one;

(iii) One short pair of chromosomes with subterminal primary constrictions and two secondary constrictions in the long distal arms.

A study of 13 bulbs showed a variation in the heterochromatin content. The nature of the heterochromatin is exactly similar to that reported in the previous paper.

VI. DISCUSSION

Evolutionary trends in Indian species of Scilla.

From the foregoing cytological survey of the two Indian species, S. indica and S. hohenackeri it would appear that each shows a pattern of variation which is unique to itself. Just as they do not overlap each other in their geographic distribution, the nature of their variation is also different. Both of them are admirable instances to show the efficient use of the available chromosomal and genetic materials for evolutionary changes, which have made possible for a predominently temperate genus to migrate into the tropical parts of India through such species like *Scilla indica*.

S. indica could not only migrate into India but could spread far and wide due to its great polymorphism. Polyploidy is like a great pillar on which the superstructure of this morphological variation rests. It is significant to note that while polyploidy is absent in all the large chromosomal species of the genus, species like *S. indica* with small chromosomes are differentiated into chromosome races. It looks as though the general reduction in the size of the chromosomes due to its genotypic changes has favoured intraspecific polyploidy, taking for the moment the whole population of *S. indica* as one unit. In other words, the small chromosomes of this species are eminently preadapted for a duplication of the chromosome sets. This feature is equally time of *Scilla japonica*, which is also characterised by intraspecific polyploidy. Some parallel instances from Liliaceae, which illustrate the same relationship between chromosome size and polyploidy could be cited. The hexaploid *Narcissus bulbocodium* and the pentaploid *Tulipa clausiana* are the only species in these genera with small chromosomes (Darlington, 1956). In *Lilium* and *Fritillaria* with long chromosomes triploidy is the limit. Tetraploid *Lilium* is known only under cultivation. Evidence for genotypic change has already been presented in the diploid *Scilla indica*. It seems to have had a profound significance in the origin of polyploidy within the species.

The nature of the duplicated sets determines the kind of polyploidy (Müntzing, 1936: Stebbins, 1947, 1951). The difficulties inherent in the recognition of the different kinds of polyploids was discussed by these preceeding authors. The available evidence shows that within the range of the species population of S. indica, both allo- and intervarietal autopolyploids exist as is the case with Allium schoenoprasum L. (Levan, 1935). The diploid forms are wide spread and perhaps more successful than the rest. They are in all probability allopolyploids of amphiploid origin. Chromosome morphology regular formation of bivalents, high fertility tend to lead to the same conclusion. The origin of a basic number 15 through allopolyploidy is a major evoultionary step in Scilla. It is the highest so far known in the genus.

Furthermore the hybrid nature of the diploid is revealed by the regular formation of a few tetraploid pollen-mother-cells in the diploid anthers (cf. Kniphofia nelsonii, Moffett, 1932). Failure of wall formation in the premeiotic divisions would be the first step in their formation and as shown already such errors are probably genetically controlled. A clevage of such a population into those with and without tetraploid PMCs in the diploid anthers due presumably to the segregation of genes determining the premeiotic errors is the starting point of a series of polyploid forms within the species.

There appears to be no doubt that the triploid S. indica is allopolyploid in origin. The most crucial evidence is afforded by its somatic chromosomes. Of the six long chromosomes, the first two are characterised by secondary constrictions in the middle of the short proximal arms. The remaining four chromosomes, approximately equal in length, are a little shorter than the first two; These are devoid of secondary constrictions. Since these six chromosomes form two trivalents, heteromorphic chromosomes are apparently involved in their formation. On this basis alone, the genomic formula of the hypotriploid S. indica should be ABB, with one chromosome less from any one of those sets. A maximum number of fourteen trivalents were observed during the present investigation. It is certainly a high number for an allotriploid. It is more or less definitely established that pairing in species hybrids is a measure of the taxonomic relationship between the parents involved. If so, the high frequency of trivalent formation in S. indica is due to the close relationship of its parents. In fact, its pairing conditions alone indicate autotriploid origin, as postulated by Müntzing (1933) for Solanum tuberosum. But its chromosome morphology negates such a hypothesis.

A review of the available literature shows that in triploids there is a gradual transition between complete associations of all chromosomes into trivalents and complete absence of chromosome aggregation. Complete trivalency is generally attained in autotriploids. Most of the triploids of hybrid origin, however, exhibit a Drosera type of pairing, since they acquire two sets of chromosomes from one parent and one from the other, the two parents belonging to two different species or even genera. Under such circumstances, there can be very little association of the chromosomes into trivalents. To this question of homology determining pairing in triploids, a whole series of genetical (Beadle, 1930) nutritional (Meurman, 1928) and environmental factors (Katayama, 1931) should be added. Darlington (1931) found in Hyacinthus that homology is not only a factor in the formation of trivalents, but that size also is an important factor, since short chromosomes form trivalents much less frequently than do the long ones. Apparently then, complete association of homologous chromosomes in triploids is rarely possible.

The greatest possible deviation is shown by those allotriploids like *Scilla indica*, in which morphologically dissimilar chromosomes pair and exhibit complete trivalency. Pool (1931) reported pairing between *Crepis rubra* x *C. foetida*, both diploid and allopolyploid. Although the somatic chromosome morphology of the parents was different, there was often complete bivalent formation in diploid hybrid, and complete quadrivalent formation in allotetraploid. The triploid hybrid *Lolium rigidum* var. *strictum* x *L. loliaceum* (Jenkins and Thomas, 1939) forms a high frequency of trivalents and rarely complete trivalent association. Amongst the triploids of AAB type, Steere's Petunia hybrids (1932) are very good instances showing complete trivalency. Giles (1941) found in a triploid *Tradescantia* hybrid, numerous cells in which there was complete trivalent formation indicating that the chromosomes were largely homologous. All these instances are clearly indicative that complete trivalent formation is not a proof of autopolyploidy as in *S. indica*. Its trivalency is probably due to autosyndesis.

The structural hybridity as revealed by bridges and fragments in triploid *S. indica* is but another line of evidence to show its hybrid origin. Since fragments of different sizes are formed, triploid *S. indica* appears to be heterozygous for several inversions. It is a well known fact that the size of the fragments is dependent on the size of the inversion and its distance from the end of the chromosome. Similar structural hybridity was reported by Richardson (1936).

Although the mode of origin of the tetraploid cytotype is not clearly understood for want of uniquivocal evidence, it is reasonable to think that it is an intervarietal autopolyploid according to the terminology of Stebbins (1951). It is in all probability of hybrid origin from two diploid ecotypes which differ in their genetic constitution. Sufficient evidence has been presented that the diploid forms of Bombay and Dharwar on one hand and the forms at Tiruchurapalli on the other are different at least in the presence and absence of genes controlling the premeiotic errors. Since diploid pollen grains are known to be formed in them, it is reasonable to think that the fusion of a diploid pollen grain with a diploid egg will lead to the origin of a tetraploid. It is characterised by a low frequency of tetravalents, regular disjunction at Anaphase I and a fairly equal distribution of chromosomes at the poles. All genetic factors that are now known to control bivalent formation or a reduction in the chiasma frequency may explain the greater preponderance of the bivalents over the quadrivalents. As already explained, the completely terminal chiasmata in the quadrivalent favours its co-orientation and hence 2 + 2 disjunction. In other words, terminal chiasmata favours autopolyploidy and its survival in nature as, in American Tradescantias (Anderson and Sax, 1936). Although the tetraploid S. indica is not as fertile as the diploid, it is certainly more fertile than the triploid. The differentiation of the genomes during the course of evolution (Giles and Randolph, 1951) may bring about the same effect of reducing multivalent frequency and increasing fertility. In view of reduced number of bivalents, it is possible that the tetraploid S. indica may be a segmental allopolyploid also. It is difficult in fact to distinguish intervarietal autotetraploids from segmental allopolyploids. There is at present no evidence to show the different diploid ecotypes differ from each other by a large number of chromosomal segments or gene combinations so that free interchange between them is barred by partial or complete sterility at a diploid level.

Apart from polyploidy, aneuploidy at different levels of chromosome organisation has promoted weediness of the species and wide spread geographic distribution in India. It is absent in diploids but very frequent at triploid level. Plant with 44, 45 and 46 chromosomes have been reported within the species. The direction of the change is yet uncertain but it is highly probable that these aneuploid forms have originated due to acquisition of alien chromosomes during hybridisation. Aneuploids are found in all the morphologically indistinguishable types of the triploids and also tetraploids. Observations on the relative vigour and fertility of the aneuploids at the triploid level are under observation.

Whatever may be the mode of origin of these diploid, triploid and tetraploid forms of Scilla indica, they are in perfect equilibrium with the environment. Each cytotype is admirably adapted to the local conditions. The local populations belonging to the same cytotype exhibit functional specialisation and physiological differences. In other words, they show adaptive divergence leading to the formation of ecotypes. The diploid cytotypes of Bombay and Tiruchurapalli differ in the time of flowering. The triploid forms that occur in Madras and Masulipatam differ in their external morphology. They also differ in their chromosome behaviour, however small it may be in its magnitude. For instance, the triploid cytotypes (Madras and Masulipatam) differ in the frequency of inversion. That inversions differentiate naturally occurring races of Drosophila was inferred by Sturtevant, (1926) and Koller, (1935). The presence of inversions therefore is a useful adjunct in the classification of the races on a cytological basis. The direct factors concerned in effecting the organism due to inversions, may be said to be (1) the length of reversed segment (2) and the frequency of cross-over in it.

Long inversions merely reduce the fertility of the F_1 hybrids, so that the species must resort to clonal method of propogation. Short inversions on the other hand will not reduce fertility appreciably but produce genetic isolation of the reversed segment. If gene mutations arise in such segments, they will become a centre of discontinuity in the species (Darlington, 1936). When viewed in this light, the triploid cytotypes of S. indica are themselves evolving at a microscopic level, each in its own way, each according to its genetic system. They already show signs of genetic isolation. These local populations are potential species.

From the foregoing account it is clear that numerical, structural and genotypic changes in the chromosomes mark the evolutionary progress in Scilla indica. There is a great parallelism between the evolutionary tendencies within S. indica and the major discontinuities that led to the origin of species within the genus. It is now increasingly realised that apart from changes in the chromosome structural changes have played a prominent role in the origin and differentiation of several species of Scilla. All these evolutionary processes could be traced in S. indica alone when considered in its entirety.

VII. SUMMARY.

(1) A cytological survey of the two Indian species of Scilla, namely S. indica and S. hohenackeri has been the subject of the present paper. Details of mitosis and meiosis have been presented.

(2) S. indica is differentiated into diploid (2n = 30), triploid (2n = 44,45,46)

and tetraploid (2n = 60) cytotypes and hence exhibits considerable polymorphism. S. hohenackeri (2n = 10) shows variation in the heterochromatin content.

(3) The small chromosomes of S. indica in contrast to the long chromosomes of the other species within the genus favoured the incidence of polyploidy within the species. In other words, genotypic changes reducing the size of all the chromosomes in the complement have played a prominent role in the evolutionary processes within the species. The formation of tetraploid cells within the diploid cytotypes is the starting point of a series of polyploid and aneuploid forms.

(4) The diploids with a basic number 15 are considered as allopolyploids of amphiploid origin. It is the highest basic number in the whole genus and it favoured the migration of a predominently temperate genus into tropical parts.

(5) The triploid S. indica is considered as an allopolyploid, which shows the maximum number of 14 trivalents contrary to expectation. It is believed that in this allotriploid pairing between morphologically dissimilar chromosomes is responsible for high frequency of trivalents. Furthermore it is an inversion heterozygote.

(6) The tetraploids are either intervarietal autopolyploids or segmental allopolyploids. There is at present no evidence to show that they belong to the latter category. However, the fairly regular behaviour associated with partial sterility shows that they could be either.

(7) The cytotypes show local differentiation leading to the formation of ecotypes. Their importance is discussed.

(8) The structural numerical and genotypic changes within S. indica parallel the evolutionary trends in the whole genus.

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Figs. 1 - 14.

Explanation of the text figures illustrating "Cytological studies in the Indian Scilla".

Figs. 1 - 14. <u>Scilla indica</u>. Dharwar form. Fig. 1. Diakinesis showing 15 bivalents, of which 4 are attached to two nucleoli. Fig. 2. Late diakinesis with one long bivalent showing a [illegible]distal differentiation. Fig. 3. Metaphase I. Side view showing 15 ring and rod bivalents. Fig. 4. Metaphase I showing 14 bivalents and 1 univalent. Fig. 5. Metaphase I with 14 bivalents, and 2 univalents. Fig. 6. Metaphase I illustrating asynapsis. There are two bivalents and twenty six univalents. Fig. 7. Same. One bivalent and twenty eight univalents. Some of the univalents show secondary association. Fig. 8. Anaphase I. 15 + 16 making a total of 31 chromosomes. Fig. 9. Anaphase I. Irregular with one long chromosome lagging. Fig. 10. Anaphase I. 14 + 16. One chromosome is away from the two groups, probably due to its reaching the pole early. Fig. 11. Anaphase I. 15 + 15 normal disjunction. Fig. 12. Late Anaphase I or early Telophase I with one chromosome lagging. Fig. 13. Irregularly distributed chromosomes at Anaphase I. There is a general reduction in the size of all the chromosomes due to genotype control. Fig. 14. Anaphase I with a chromatid bridge and no fragment. The bridge is formed apparently by the long chromosome pair.

Figs. 15 - 45.

Figs. 15 - 45. Scilla indica: meiosis in the triploid cytotype: Madras form. Fig. 15. Metaphase I. $10_3 + 4_2 + 6_1 = 44$. Fig. 16. Metaphase I. $4_3 + 10_2 + 12_1 = 44$. There is one extra body in this metaphase. It is lightly stained and perhaps the persisting nucleolus. Fig. 17. Metaphase I. $8_3 + 6_2 + 8_1 = 44$. Fig. 18. Metaphase I. $6_3 + 5_2 + 16_1 = 44$. Fig. 19. Metaphase I. $14_3 + 1_2 = 44$. Fig. 20. Metaphase I. $13_3 + 1_1 = 40$. Fig. 21. Side view of Metaphase I, showing a pentavalent. $1_5 + 8_3 + 5_2 + 6_1 = 45$. Fig. 22. Metaphase I, side view showing a greater frequency of univalents. $6_3 + 7_2 + 18_1 = 44$. Fig. 23. Metaphase I. $1_3 + 1_2 + 39_1 = 44$. Fig. 24. Metaphase I. $2_3 + 5_2 + 29_1 = 45$. Fig. 25. Metaphase I. $2_3 + 9_2 + 21_1 = 45$. Fig. 26. Metaphase I. $1_2 + 42_1 = 44$. Fig. 27. Metaphase I showing all univalents irregularly distributed, and the big chromosomes exhibiting secondary association. Figs. 28 - 45. The different types of trivalents. The long chromosomes show a tendency towards the formation of ring or (Figs. 35, 36) frying pan type (Figs. 28, 31) and the small chromosomes either chains or Y-shaped configurations.

Figs. 46 - 57.

Fig. 46 - 57. Scilla indica. Meiosis of the triploid cytotype cont. Madras form. Fig. 46. Anaphase I showing the irregular disjunction resulting in unequal distribution of chromosomes, 20 + 24. Three chromosomes (not blackened) show a tendency towards division. Fig. 47. Irregular Anaphase I, with eight chromosomes lagging at the equator. Fig. 48. Anaphase I with five univalents lagging and dividing. The sixth body appears to be a fragment. Fig. 49. Anaphase I. one big and one small univalents lagging and dividing. Fig. 50. Anaphase I. Eight univalents lagging out of which two are completely divided. One divided univalent shows interchromatid stickiness. Fig. 51. Anaphase I showing five univalents lagging out of these two univalents are completely divided; note the migration of the split halves to the opposite poles in the case of one univalent. Fig. 52. Anaphase I showing a chromatid bridge and a fragment. Fig. 53. Anaphase I with slender chromatid bridge broken unequally and a fairly large fragment; one long chromosome is broken at the centromere the distal half forming an isochromosome and the proximal part lying close to the fragment formed as a result of the chromatid bridge. Fig. 54. Anaphase I with irregularly distributed chromosomes, a stout and short chromatid bridge and two fragments of unequal sizes. Figs. 55. Anaphase I showing five dividing univalents, four split halves of univalents, a chromatid bridge and fragment. Fig. 56. Anaphase I. Two small chromosomes forming a bridge and two fragments, one small and the other large. Fig. 57. Anaphase I. A bridge and fragment configuration. The fragment is fused with one lagging univalent to give a false appearance of a SAT-chromosome.

Figs. 58 - 71.

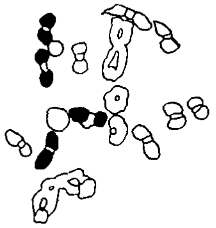
Figs. 58 - 71. <u>Scilla indica</u>. Meiosis in the triploid cytotype cont. Madras form. Fig. 58. Anaphase I. Four lagging univalents show signs of division. There is one univalent bridge and a fragment. Fig. 59. Anaphase I, showing two dividing univalents, one disjoining trivalent and one univalent bridge and a fragment very close to it. Fig. 60. Anaphase I to show a trivalent bridge and a fragment; two lagging and dividing univalents. Fig. 61. Anaphase I. A small fragment dividing at the equator. Fig. 62. Anaphase I. A trivalent bridge. Fig. 63. Late Anaphase I. A sinuous univalent bridge broken unequally and the fragment is far off from it. Three univalents or split halves of univalents lagging. Fig. 64. Anaphase I. Two univalents undivided and two univalents split. Fig. 65. Anaphase I. The two long chromosomes show position correlation during disjunction. If two of one trivalent go to one pole, two of the other go to the opposite pole. Fig. 66. Late Anaphase I. A small univalent bridge and a fragment, a micronucleus, a lagging split half of a univalent. Fig. 67. Anaphase I or Telophase I showing three micronuclei formed by the lagging univalents. Fig. 68. Telophase I. A long spindle-shaped central micronucleus formed by the lagging chromosomes. It is in continuation of the lower daughter nucleus. Fig. 69. Late anaphase I with the persisting fine chromatid bridge broken in the middle and a small fragment. Fig. 70. Telophase I after the wall formation. One cell shows two nuclei and the other a single nucleus. Fig. 71. Telophase I before wall formation with three micronuclei of different sizes.

Figs. 72 - 80.

Figs. 72 - 80. Meiosis in triploid cytotype cont. Madras form. Anaphase II to show the irregular distribution of chromosomes at the two poles; 23 + 1 + 20. Fig. 73. Irregular Anaphase II; note the chromosmes, which bridge the chromosomes at the two poles. Figs. 74. Anaphase II. The chromosomes are irregularly distributed; 20 + 3 + 21. Note the two small fragments (marked f), which are at the opposite poles. Fig. 75. Anaphase II. One univalent broken at the primary constriction but both of them are still attached by slender thread due to stickiness with one univalent close by. Fig. 76. Anaphase II. One of the long chromosomes shows structural alteration due probably to segmental interchange with another small or medium sized chromosome to form a rod shaped extra long chromosome. The two fragments (marked f) are at the opposite poles. Figs. 78. Anaphase II with no structurally altered chromosomes; note the three pairs of long chromosomes with submedian constrictions. The chromosomes are irregularly scattered. Fig. 79. Anaphase II. Two daughter halves of a medium-chromosome showing signs of division at the centromere. Fig. 80. Anaphase II to illustrate structural alterations in the medianly constricted long chromosomes leading to the formation of chromosomes with subterminal constrictions, one isochromosome which is a later stage shown in the previous diagram, and two fragments (marked f) at two poles.

Figs. 81 - 93.

Figs. 81 - 93. Scilla indica. Meiosis in the tetraploid cytotype. Sagar form. Fig. 81. Metaphase, I. $25_2 + 10_1 = 60$. Fig. 82. Metaphase I. $1_3 + 24_2 + 9_1 = 60$. Fig. 83. Metaphase I. $1_4 + 28_2 = 60$. Figs. 84 - 86. The ring-shaped quadrivalents and their orientation at Metaphase I in such a way as to ensure 2 + 2 disjunction. Fig. 87. A quadrivalent at Metaphase I. It is likely to disjoin in such a way as to ensure 2 + 2 distribution. Figs. 88 - 90. Quadrivalents, chain, ring and other types. Fig. 91a. A trivalent and a univalent formed by the medium-sized chromosomes. Fig. 91b. Different types of quadrivalents formed by the small chromosomes. Fig. 92. Anaphase I. Normal type showing a distribution of 30 + 30. Fig. 93. Anaphase I. 29 + 31 respectively at the two poles.
(All figures are drawn at an initial magnification of x2,000 and reduced to the page size in the photographs).



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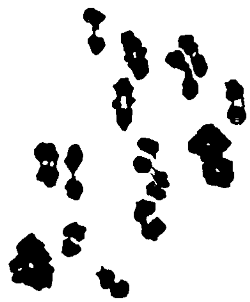

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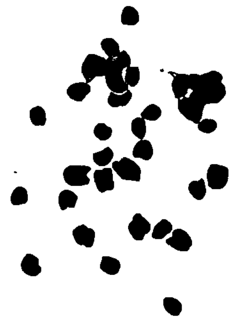

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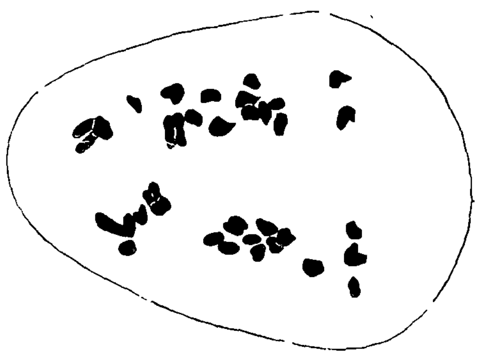

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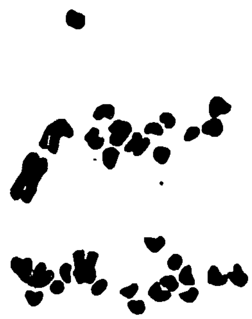

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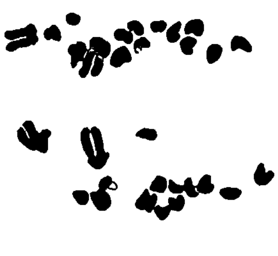

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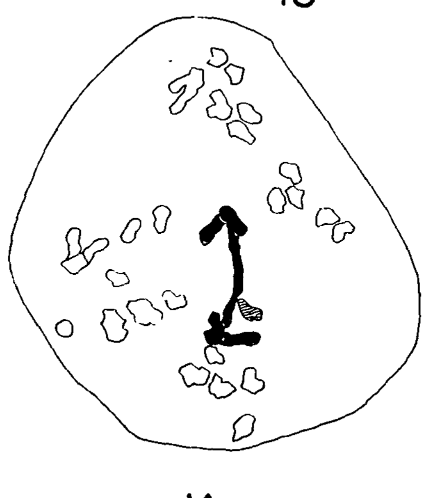

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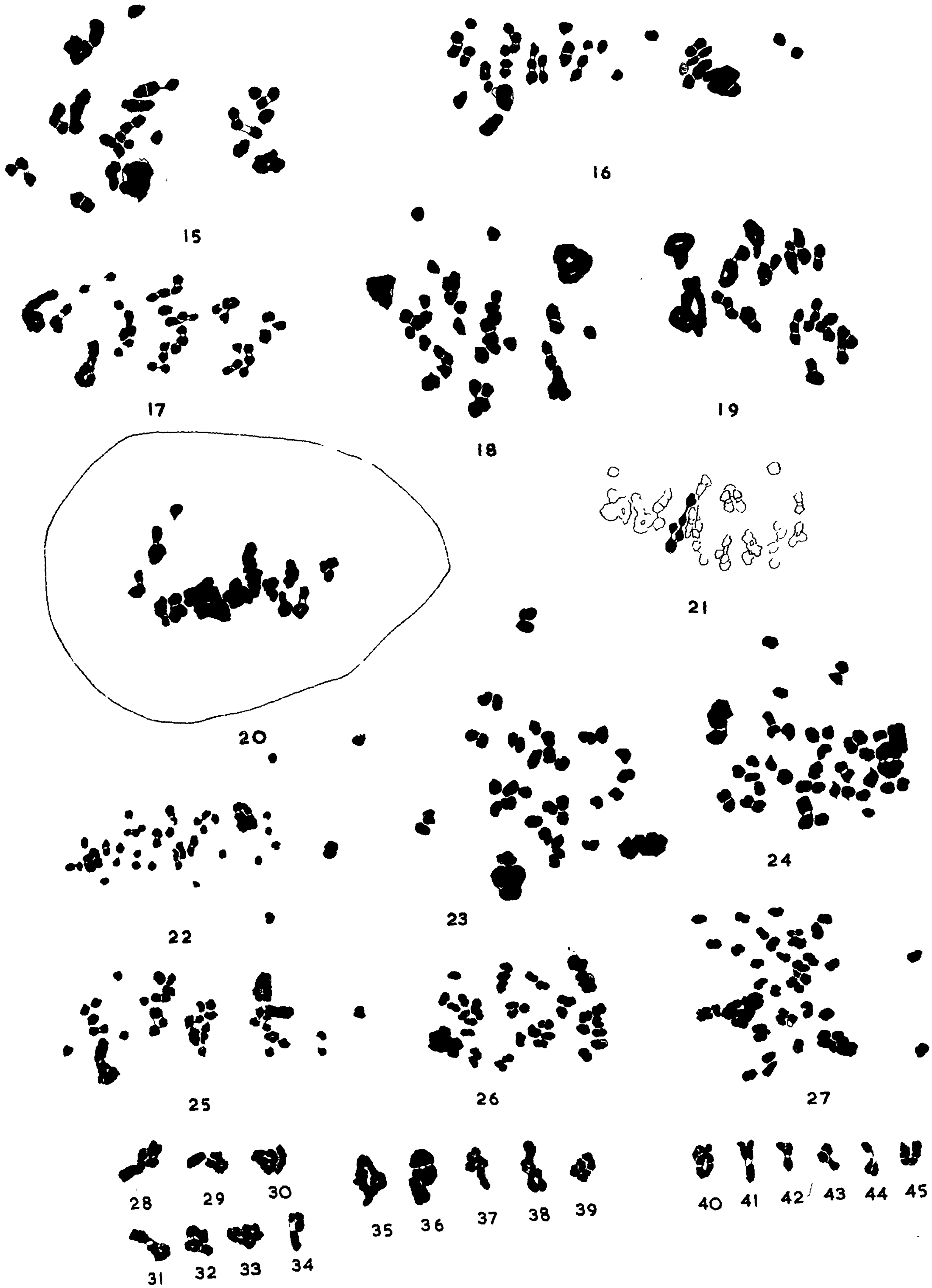

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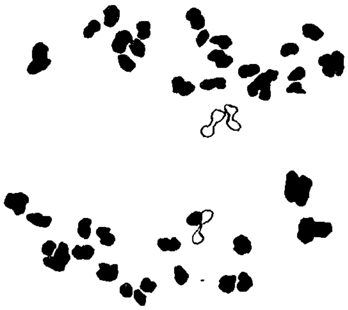

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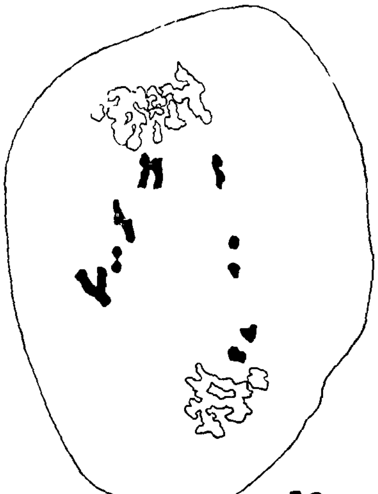

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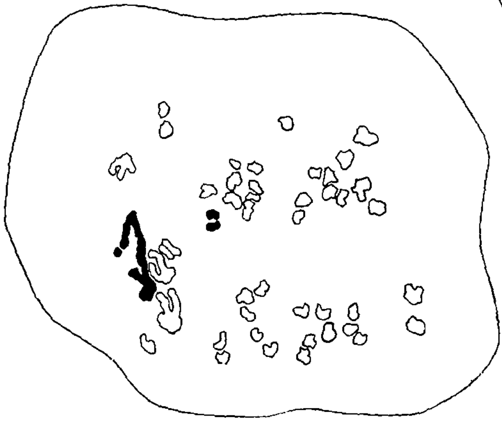

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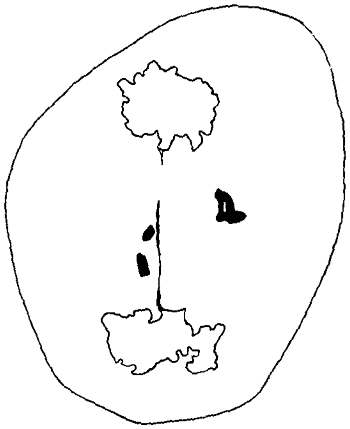

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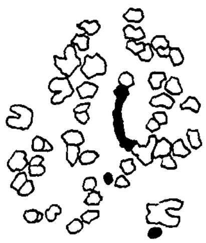

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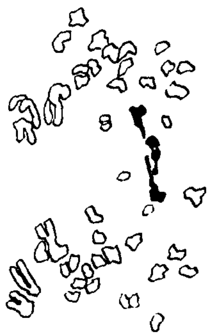

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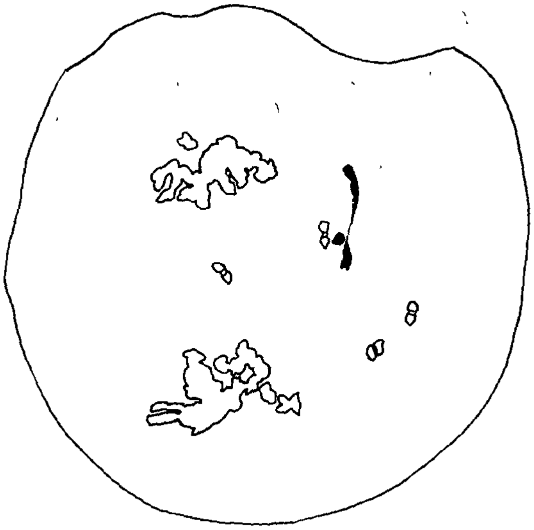

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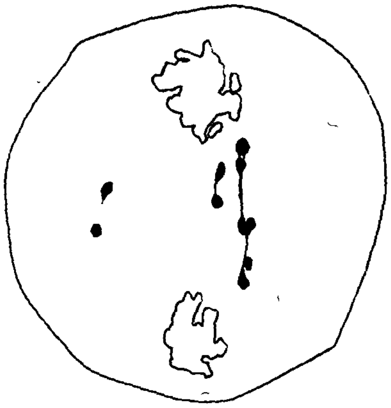

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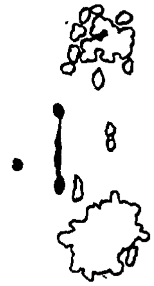

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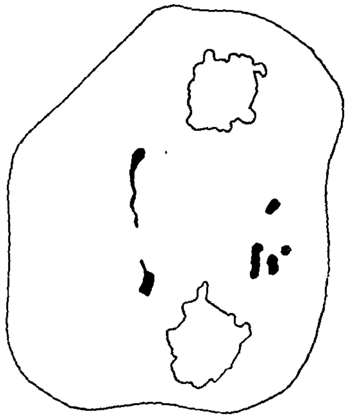

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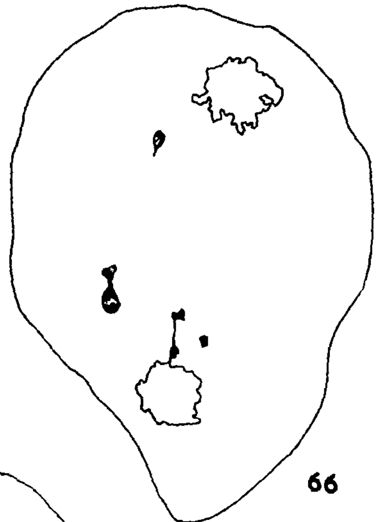

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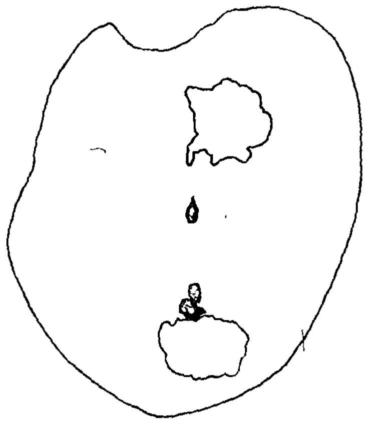

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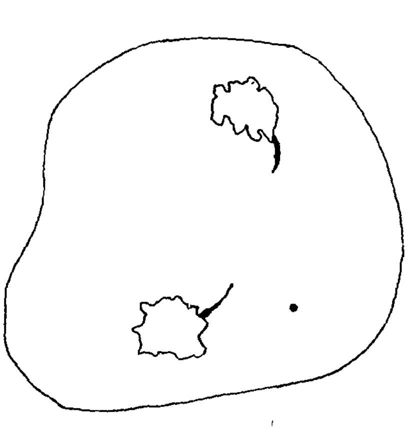

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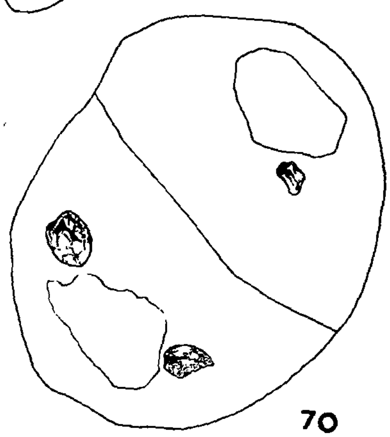

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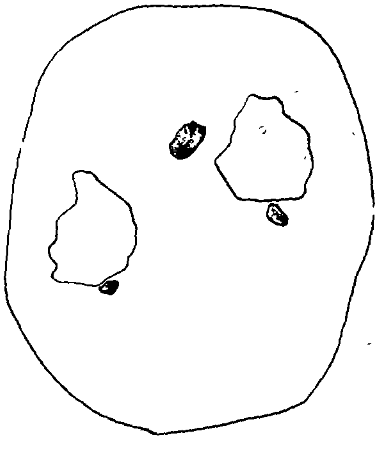

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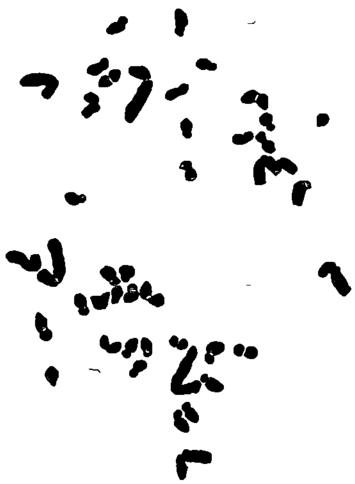

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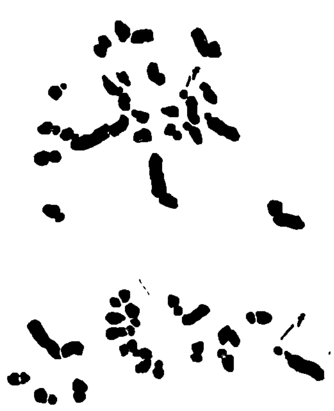

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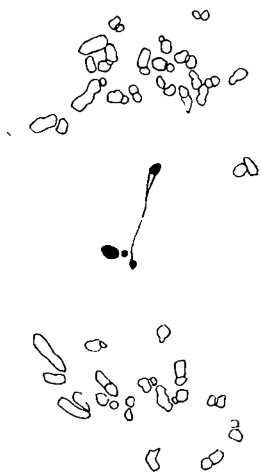

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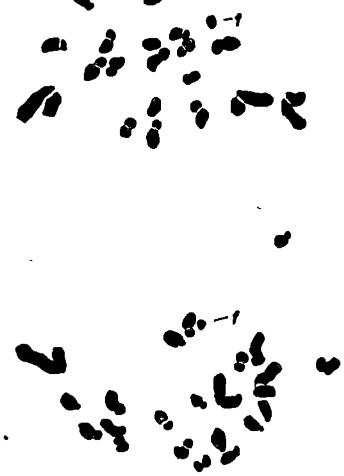

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