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EXPERIMENTAL TAXONOMY OF OXALIS SECTION

ACETOSELLAE AND MAIANTHEMUM

A thesis submitted to the University of Durham for the degree
of Doctor of Philosophy
by Hassan Mustafa Hassan (B.Sc.)

June 1968



P331

ACKNOWLEDGEMENT

I would like to express my sincere thanks and gratitude to the Freedom From Hunger Campaign for offering me the Fellowship; to the World University Service for administering the Fellowship; and to Professor D.H. Valentine for suggesting the problem; for his wide collections of living plants from various parts of the geographical range; and for providing me with his experimental data on hybridization in Maianthemum, specially the hybrid seedlings. I am greatly indebted to him also for his extreme patience and keen interest in the process of the preparation of my manuscript.

I am also thankful to the University of Khartoum for approving the necessary extension required for the completion of this research; to Mr. R.H. Gregory for helping me with the photographic part; and to Mrs. J. Wilson who undertook the typing of this thesis.

H.M. HASSAN

ABSTRACT

The taxonomy and distribution of Maianthemum Web. (liliaceae) and Oxalis section Acetosellae, are discussed with reference to their variation and evolution.

In Maianthemum there are two main taxa: M.dilatatum (Wood) Nels. & Macbr., confined to the Pacific region; and M.bifolium (L.) Schmidt with a wider distribution. The latter consists of three subspecies, subspecies bifolium of Eurasia, and subspecies canadense and subspecies interius of N. America. All these have some populations or individuals with $2n = 36$, but all also have representatives with higher chromosome numbers, $2n = 54$ or $2n = 72$. It is not yet possible to evaluate the chromosome races taxonomically.

The taxa have similar karyotypes; the chromosomes are rather large and some are satellited. The evidence suggests that the plants with $2n = 54$ and 72 are autopolyploids based on $2n = 36$.

Hybridization experiments have produced good seeds and a few seedlings, but no hybrids have been raised to maturity; the experiments support the view of a close genetic relationship between the taxa.

M.dilatatum is morphologically variable. In Asia it has been modified as a result of contact with M.bifolium; hybridization and introgression between them may have resulted in a recognised Asian variety.

In Oxalis, little is known about the Asiatic taxa. Of the other northern hemisphere taxa O.acetosella L. of Eurasia and O.montana Raf. of N. America are best treated as subspecies of O.acetosella. Hybridization experiments, because of germination difficulties, have been inconclusive. O.oregana Nutt. of northwest America, is distinct from O.acetosella and hybridization experiments gave empty seeds. All three taxa have similar karyotypes.

Problems of phytogeography are discussed. In both genera the taxa along the Pacific coasts of N. America are different from those of C. and E.N. America, a possible [?] indication of their early isolation from the main stock, which perhaps originated in E. Asia.

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The genus

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INTRODUCTION

This thesis deals with the experimental taxonomy of the three species of the genus Maianthemum and of selected species of Oxalis section Acetosellae. In both genera, the group of species studied has a circumpolar distribution in the northern hemisphere; and this pattern of distribution raises many interesting problems of evolutionary and taxonomic importance.

Part one of the thesis deals with Maianthemum and part two with Oxalis. Problems common to the two genera are discussed in a final section at the end of the thesis.



Chapter oneTaxonomy

The genus Maianthemum is distributed over the Northern hemisphere and is almost circumpolar. In Europe and Asia it is represented by M.bifolium, in the Pacific region by M.dilatatum, and in north-eastern America by M.canadense.

Some systematists have thought that the genus consists of only one species. Farwell (1915) says, "a series of specimens ranging from Oregon to Hungary allows but one conclusion - that there is but a single species". Farwell's point is that the leaf characters are the only characters which distinguish canadense from bifolium. Our data, as will be seen later, show that this is not so. Hultén (1962) says that, "differences between these three species are rather obvious but it would perhaps be more rational to regard them as subspecies of M.bifolium". Ohwi (1965) extends the geographical area of bifolium to North America thus confusing it with the American taxa.

Accordingly this account begins with a description of the genus and the three species, and of their geographical distribution, taken from the literature. Following this is a detailed investigation of the plants themselves, based on both herbarium and fresh material, which seeks to clarify the differences between them, and express them, where possible, in quantitative terms.

In subsequent chapters hybridization experiments and cytological investigations are described; and finally an attempt is made to discuss the relationships of the taxa in both taxonomic and biosystematic terms.

We shall begin by giving a description of the genus and the species as it is given in current floristic works. This will give some idea of the characters which systematists have used. Notes on the geographical distribution of the taxa are then given, before proceeding to an account of our own examination of both herbarium and living material.

Maianthemum Web

Perennial herbs, with creeping rhizomes; stem erect, absent in sterile plants; leaves 1-to-3, alternate. Flowers white, in terminal racemes; perianth segments 4, spreading; stamens 4. Ovary globose, 2-celled, with 2 ovules in each cell; style usually equal to ovary; stigma 2-lobed. Fruit a 1-to-4-seeded berry; seeds subglobose to bean-shaped.

1. Flowers 4-7mm in diameter; styles stout; base of ovary truncate M.dilatatum
1. Flowers 2-4mm in diameter; styles slender; base of ovary rounded or turbinate ...
2. Lower cauline leaf petioled; sinus 4-11mm deep
M.bifolium
2. Lower cauline leaf sessile or subsessile; sinus 1-5mm deep.
3. Plant glabrous M.canadense
3. Plant pubescent M.canadense var.
interius

M.bifolium (L) Schmidt

Stem 10-25 (-30) cm, glabrous except in the upper part. Lower cauline leaf 3-6 x 2-4 cm. pilose, ovate, acute, cordate at base, sinus 4-11 mm deep; petiole 5-25 mm. Inflorescence 15-to-30 flowered; flowers 2 - 4 mm in diameter, in fascicles of 2-3; perianth segments 1-2 mm, white, pedicels 2-5 mm, spreading. Base of ovary turbinate; style slender, tending to split at summit. Berry 4-6 mm, red, speckled, 1-3 seeded; seeds 2.2 - 2.5 x 1.8 - 2.1 mm flattened.

M.canadense Desf.

Stem 10-20 cm, glabrous. Lamina of lower leaf 3-6 x 1-3 cm, ovate-lanceolate, glabrous, acute, cordate at base, sinus 1-5 mm deep; all veins including cross-commisures distinct in dried leaves; margin of leaf entire or slightly crenulate; petiole 0-3 mm. Raceme 15-to 30-flowered; flower 2-4 mm in diameter, white; perianth segments 1-2 mm, white; pedicels 2-6 mm, spreading. Base of ovary rounded, styles slender with less tendency to split at summit than in M.bifolium. Berry 4-6 mm, red, speckled, 1-to3- seeded; seeds 2-2.6 x 2 mm, nearly spherical.

M.canadense var. interius Fernald : According to Fernald (1914) this variety differs from the type in having a pubescent stem, and a different geographical distribution (see below). According to Butters (1926), the differences between the varieties of M.canadense are as follows (Table 1).

Table 1

Resemblances and differences in C. & E.N.American Maianthemum taxa (from
the literature).

<u>Character</u>	<u>Canadense</u>	<u>interius</u>	<u>Carolinianum</u>	<u>ovale</u>
<u>Indumentum</u>	glabrous	pubescent	glabrous	glabrous
<u>Lamina width</u>	2.95 cm	3.5 cm	2.5-4 cm	1.8-4 cm
<u>leaf form</u>	acute, cordate	obtuse cordate	obtuse to acute, cordate	ovate, scarcely cordate
<u>Petiole</u>	5-12 mm	-	1.5-4 mm	10 mm
<u>leaf margin</u>	entire	ciliated	entire	-
<u>No. of fls. per inflor.</u>	23.1	26.1	-	-
<u>Filaments</u>	subulate	clavate	-	-
<u>Habitat</u>	northern evergreen forest	southern deciduous forest	mountains of N.Carolina, occurring with the species	eastern N.America (Atlantic City, New Jersey Minnesota.)

Material of var. carolinianum Butters, and var. ovale (Pursh)

Butters has not been seen. The differences between the species and var. interius have been confirmed. This variety has been raised to a subspecies rank by Löve and Löve (1954) on ecological grounds.

M.dilatatum (Howell) Nels. & Macbr.

Stem 10-40 cm, glabrous. Lower cauline leaf 2-10 cm, cordate to sagittate, sinus 12-30 (-100) mm deep; petiole 2-8 cm. Raceme 15-to-40 flowered; flowers 4-7 mm in diameter, in fascicles of 2-4; perianth segments 2-3 mm; pedicels 2-5 mm, spreading. Base of ovary truncate; style stout. Berry 6-8 mm, red 1-to 4- seeded; seeds 2.5-3 x 3 mm, bean shaped or globose.

Varieties of M.dilatatum; Butters (1927) suggests the recognition of an Asian variety for which he recommends the use of the name var. kamtschaticum (Gmel.) Butters. Its range is on the Asiatic mainland, along the coast from latitude 44° to Kamchatka, and in the Asiatic islands from southern Japan to Bering Island. The differences between the type and the variety are as follows (Table 2).

Table 2Differences between the type and the variety of M.dilatatum

	American species	Asian variety
Anther	0.6 mm long, oblique	0.8 mm long, parallel
Connective	Wide	Narrow
Filament	Versatile	Non-versatile
Seed	2 x 2.5 x 3-3.5 mm	1 x 1.8 x 2.4 mm*
* One seed only		

We have looked into the Asiatic material of M.dilatatum and we have found many points of interest which will be discussed later. It is convenient here to point out that the character of the anther, described as consistent by Butters (Table 2), is found to be variable as shown in Table 4. Of the remaining characters it has not been possible to confirm the differences in the connectives and the filament, both of which are rather difficult to examine. As for the possible difference in seed size, lack of material prevented us from verifying this. It is interesting that other evidence supports Butters' view that the Asiatic material could be considered as a variety of M.dilatatum; this we will discuss in more detail later.

Sokolovskaya (1961) has found and described a forma of M.dilatatum in the Far East, which she called f.vegetior, the cytology of which will be mentioned later (p.). The forma is triploid and large in all its parts.

Geographical distribution: The geographical distribution of the three species and a variety is shown in the map, Fig.1, taken from Hultén (1962).

M.bifolium extends from Britain in the west (where it is very rare) across Europe to E.Asia. It is a common woodland plant in Europe and doubtless over much of its range. In the Far East, it is recorded in Japan (rare), north China, and Korea, also in Siberia, N.Szechuan, and C.Kamchatka, but is rare in Sakhalin and the Kuriles. In this area, it appears to overlap slightly with M.dilatatum, but it is not known if hybridization occurs. Hultén (1962) states that the "occurrence of transitional forms should be studied in the areas where the ranges overlap in eastern Asia and in British Columbia". Kawano (in correspondence) says that "M.bifolium and M.dilatatum are sympatric at several localities in Honshu and Hokkaido, and I have often discovered morphologically interconnecting forms, which may represent hybrids or hybrid derivatives".

M.dilatatum occurs along the shores of continental Asia, in Siberia (scattered localities), in Japan and Sakhalin, in Manchuria, Korea, Kamchatka, and in the Aleutian Islands. Hutchinson, remarking on a herbarium specimen collected from Attu Island says that, "the plant is not found on any of the Aleutian Islands (with the exception of Kiska) and is very common at Attu". This is similar to the remark of Turner (quoted by Hultén, 1937), that M.dilatatum is "not common throughout the Aleutian Islands" a remark considered by Hultén as incorrect. Hultén, however, states that

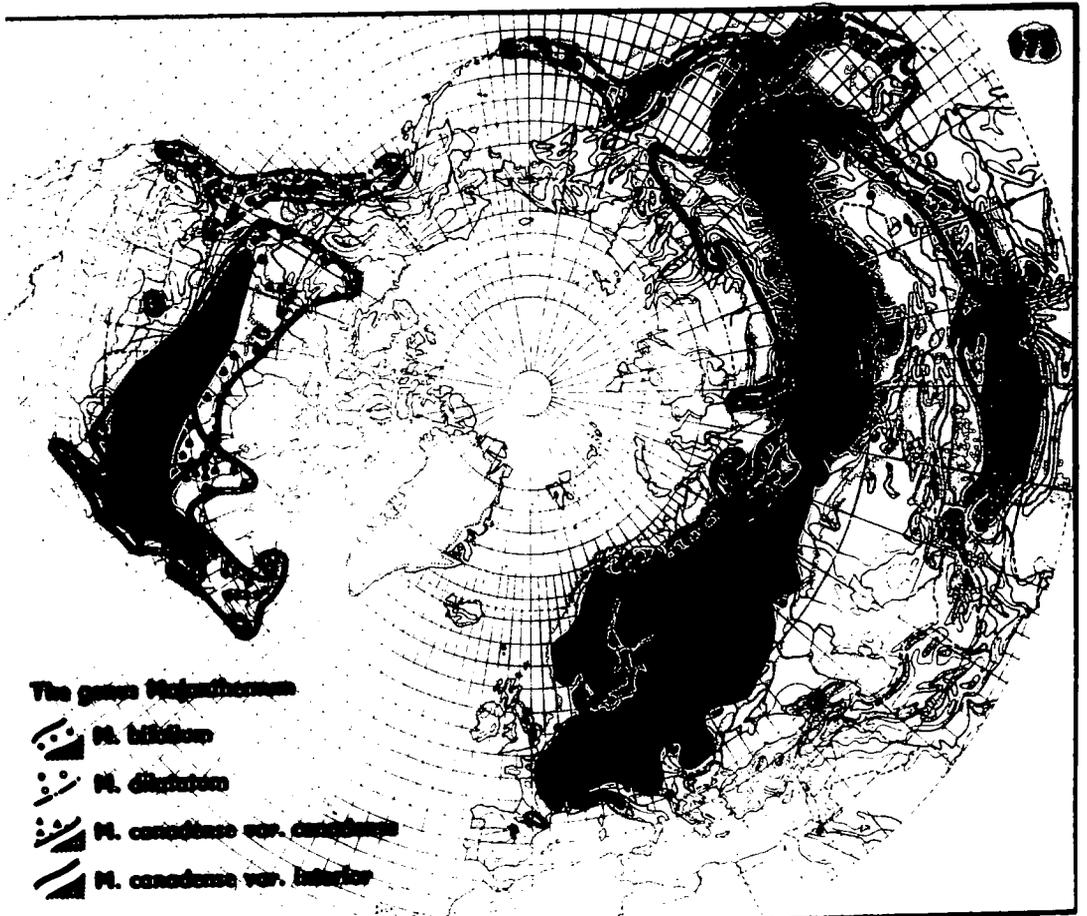


Fig. 1 : Distribution of the genus Maianthemum, (Hulten, 1962).

there is a gap in the distribution of M.dilatatum of about 3000 km. between the middle Aleutians and S.E. Alaska. The plant then extends southwards to California.

M.canadense extends across N.America from East to West, but it is separated from M.dilatatum in the west by the Rocky Mountains. The varieties canadense and interius have rather different but considerably overlapping distributions, and it would appear from the literature (Fernald, 1914 and Butters, 1926) that the legend on Hulten's map is incorrect. It is variety interius which extends farthest to the west.

Table 3 gives data on the extent of overlap between the various taxa, assembled from the literature.

Table 3Range of overlap between Maianthemum taxa

Main area and taxon	Overlapping zone	Main area and taxon
<u>M. canadense</u> E.N. America	Minnesota, Indiana N.York, Wisconsin	var. <u>interius</u> C.N. America
<u>M. bifolium</u> Eurasia	Japan, China, Korea, Kamtchatka, Manchuria	<u>M. dilatatum</u> Pacific area

It is very difficult to obtain accurate habitat information over the whole range of the three species. Kawano (in correspondence) says that in Japan "M. bifolium is strictly confined to temperate broad-leaved deciduous forests and boreal coniferous forests, but M. dilatatum is exhibiting a tolerance to diverse ecological conditions". A similar situation is reported by Butters (1926) in N. America where M. canadense is found in Minnesota only in the region of boreal coniferous forests, whereas its variety interius is found in deciduous forests.

Herbarium studies:

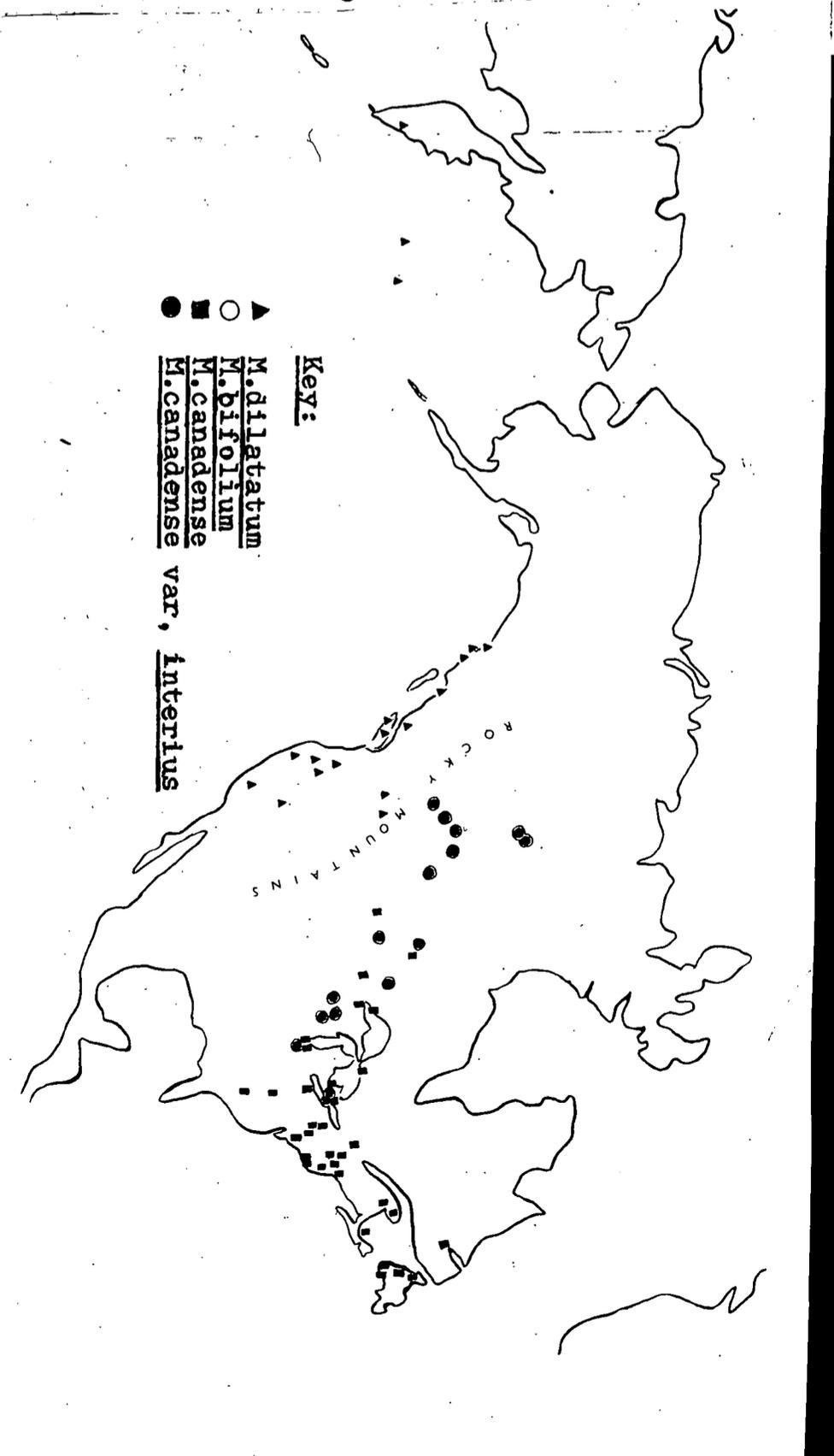
The materials used in this survey were from the herbaria of Durham University, Manchester University, the New York Botanic Garden, Harvard University, Munich University, and the British Museum (Natural History).

The specimens measured are listed in Appendix 1. It will be seen that those of M.bifolium cover most of the range outside the Soviet Union; the specimens seen of M.dilatatum and M.canadense cover a large part of the range of the 2 species, though the number of specimens seen of M.canadense var. interius is rather small. The localities for the species studied are plotted in the map Fig. 2. Unfortunately, the collections studied had very few specimens from the Soviet Union, although it is known from the flora that its distribution in this area is extensive. From the map it is also clear that M.canadense var. interius is separated from M.dilatatum in the west by the Rocky Mountains. It should also be said that some of herbarium sheets do not give the exact localities, this has made it very difficult for us to present such localities in the map, though they are listed in the Appendix.

On most herbarium sheets, several specimens of the plant are represented; and the method used has been to select the largest plant as a representative sample for that particular locality. Vegetative characters are mostly used, as floral characters have been difficult to assess due to their smallness added to the difficulty in preserving the many-flowered inflorescence in a reasonable condition.



Fig. 2



It has been possible to study some of the flowers more closely but we must admit that the material available was too limited. Characters of the seed and fruit were rarely available from herbarium specimens, and most of the study on seeds and fruits has been done on imported material through seed-exchange. The sources of the seeds are listed in Appendix 2.

After a preliminary survey, it was decided to measure the following characters; stem length (from ground level to apex of inflorescence), petiole length, width of lamina, length of midrib, depth of sinus, length of pedicel, length of anther, flower length, and number of flowers per inflorescence. The means, and the standard errors of the means of the measured characters are shown in Table 4. The data are presented in different ways. First, Fig. 3, shows a scatter diagram, in which data for individual specimens are given, this gives a good idea of variability. Along the abscissa is plotted the midrib length in Fig. 3-1, and the depth of sinus in Fig. 3-2. Along the ordinate is plotted the width of lamina in Fig. 3-1, and the petiole length in Fig. 3-2; in both it is evident that M.dilatatum has the longest midrib, the widest lamina, the longest petiole, and the deepest sinus.

Key for the characters enumerated in Table 4 (Facing page)

1. Stem height.
2. Petiole length.
3. Sinus depth.
4. Lamina width.
5. Midrib length.
6. No. of flowers per raceme.
7. Pedicel length.
8. Anther length.
9. Flower length.

Table 4

Morphological character in Maianthemum.Means and standard error of the means.All measurements in mms.

<u>Character No.</u> Taxon and area	1	2	3	4	5	6	7	8	9
<u>dilatatum:-</u>									
Pacific N. America	270 [±] 10.9	46.5 [±] 3.5	20 [±] 1	60 [±] 2.5	70 [±] 2.4	37 [±] 3	4.9 [±] 0.2	0.8 [±] 0.3	6.5 [±] 0.1
Alaska and Aleutian Is.	270 [±] 21.5	60 [±] 2.4	18 [±] 2.4	45 [±] 9	67 [±] 7	27 [±] 2.2	3.6 [±] 0.4	0.9 [±] 0.03	4.4 [±] 0.05
Asia	152 [±] 19	24 [±] 2.2	11 [±] 1.7	41 [±] 3.6	40 [±] 3.8	12.5 [±] 1.4	3.6 [±] 0.2	1.0 [±] 0	6.5 [±] 0.22
Japan	130 [±] 8.7	24 [±] 1.3	13 [±] 0.07	34 [±] 2.3	40 [±] 1.8	15 [±] 0.6	3 [±] 0.05	0.9 [±] 0.02	5.5 [±] 0.12
<u>bifolium</u>									
Asia	170 [±] 6.4	19 [±] 1.2	8 [±] 0.4	35 [±] 1.7	44 [±] 1.7	25 [±] 1	4.2 [±] 0.15	0.7 [±] 0.2	6.7 [±] 0
Europe	167 [±] 3.8	10.2 [±] 0.7	6.1 [±] 0.6	32 [±] 0.9	44 [±] 1.3	27 [±] 1.5	3.8 [±] 0.1	0.8 [±] 0.02	6.7 [±] 0.1
<u>canadense</u>									
E.N.America	130 [±] 3.5	2 [±] 0.2	1.7 [±] 0.1	20 [±] 0.6	25 [±] 2.3	21 [±] 0.54	4 [±] 0.15	0.8 [±] 0.05	6.7 [±] 0.08
C.and W.N.America (var. <u>interius</u>)	152 [±] 24.3	5.4 [±] 0.9	2 [±] 0.4	30 [±] 1	40 [±] 3.5	28 [±] 1.1	4.5 [±] 0.15	0.9 [±] 0.002	7 [±] 0.13

Fig. 3 Scatter diagrams, based on herbarium data.

3-1 Scatter diagram, variability of two
correlated characters i.e. lamina width
and midrib length.

3-2 Scatter diagram, variability of two
correlated characters i.e. depth of sinus
and petiole length.

Key to symbols

▲ M.dilatatum

• M.bifolium

x M.canadense

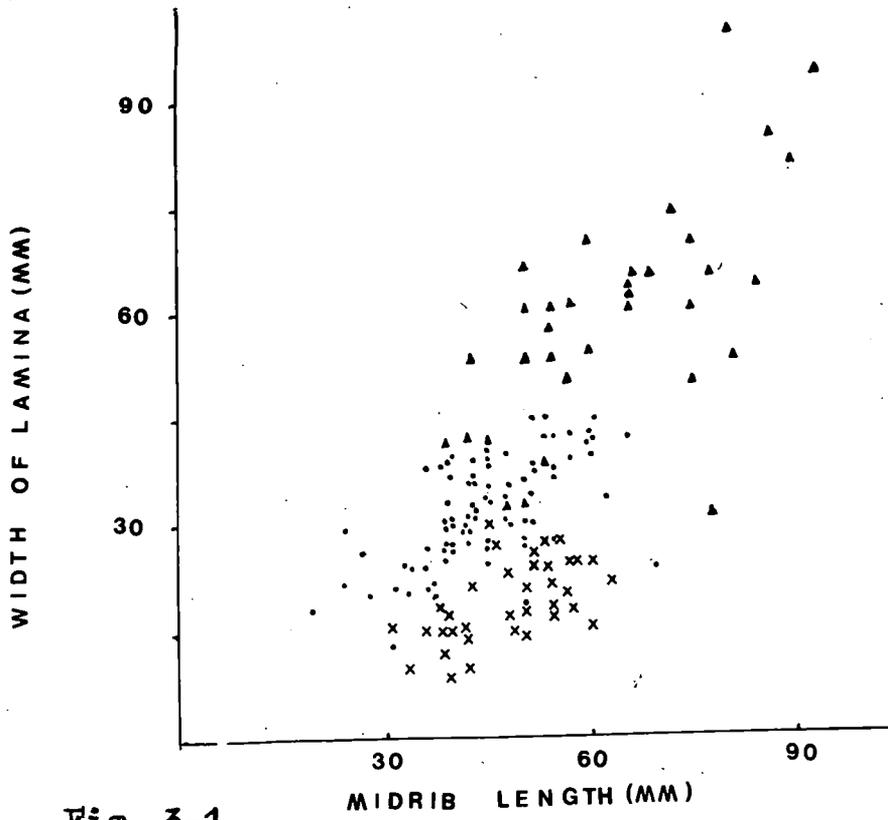


Fig. 3-1

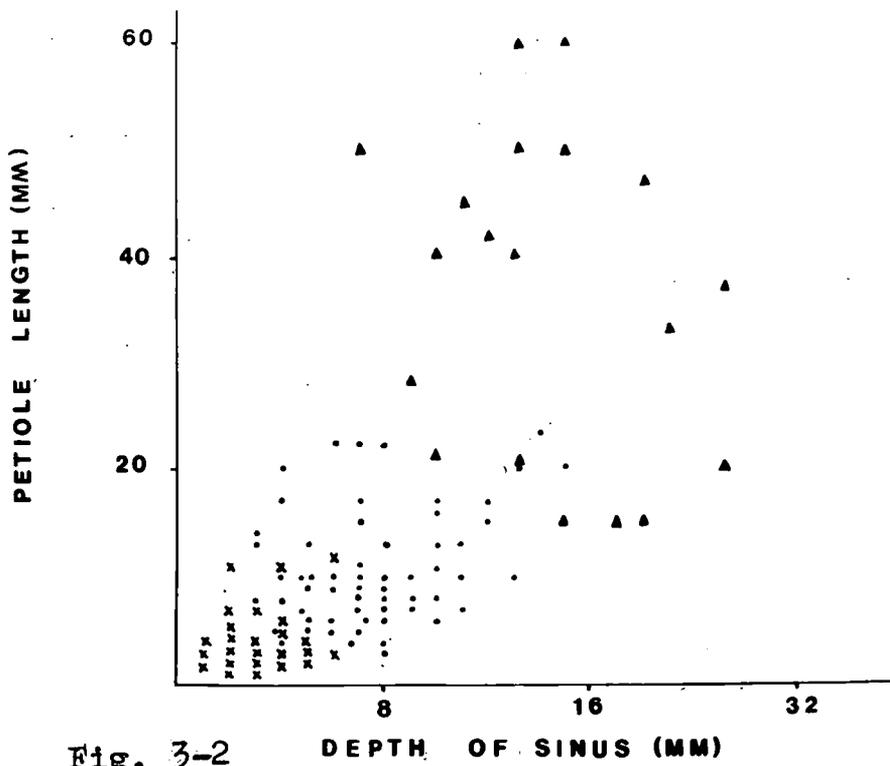


Fig. 3-2

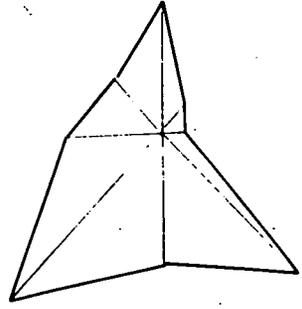
Secondly, the data are presented in polygon-form whereby the mean of each of the characters examined is plotted along one of the radii constituting the polygon. Sometimes the mean is so small for certain characters that it is necessary to enlarge it by a constant factor, or it may be so large to necessitate its reduction by a constant factor also. The reduction, or magnification, factor is stated in the key attached to the polygons.

A polygon, therefore, stands for a group of characters measured on several specimens, representing a certain area of distribution; it gives a visual representation of the average plant. On examination of the series of polygons shown in Fig. 4, the similarity is evident between the two North American taxa. Another line of similarity is between the Eurasiatic bifolium - dilatatum populations. Both the American and the Eurasiatic taxa are distinctly different from the Pacific northwest population of dilatatum.

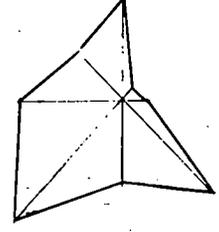
Thirdly in Fig. 5, leaf characters of the specimens studied are drawn on basis of their means. The two extremely different taxa are those of Pacific Northwest American dilatatum and Eastern North American canadense. The remaining taxa, i.e. Eurasiatic bifolium Asiatic and Japanese dilatatum, and var. interius are rather similar.

- Fig. 4 Morphological characters in Maianthemum.
Polygon representation. Based on the
data in Table 4. Key for the characters
plotted is as follows:
1. Stem length x $1/4$
 2. Petiole length x 2
 3. Sinus depth x 4
 4. Lamina width x 2
 5. Midrib length x 1
 6. No. of flowers per raceme x $1/4$
 7. Flower length x 5
 8. Pedicel length x 5

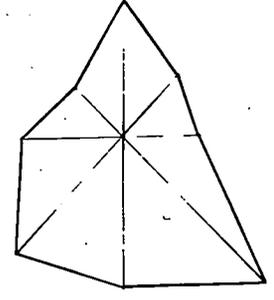
WESTERN & CENTRAL
N. AMERICAS-
CANADENSE VAR.
INTERIUS (●)



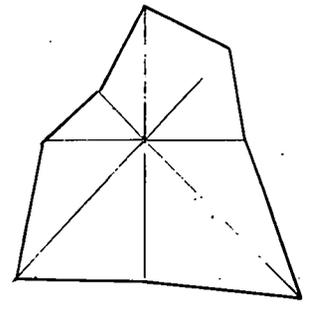
EASTERN
N. AMERICAS-
CANADENSE (■)



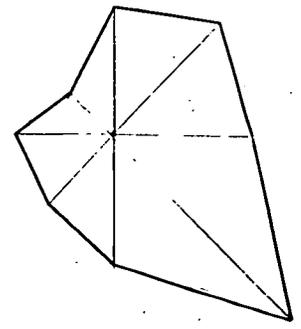
EUROPE :-
M. BIFOLIUM (○)



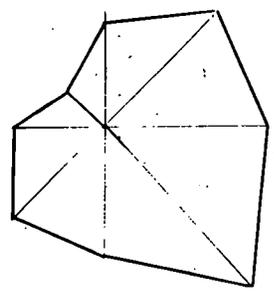
ASIA :-
M. BIFOLIUM (○)



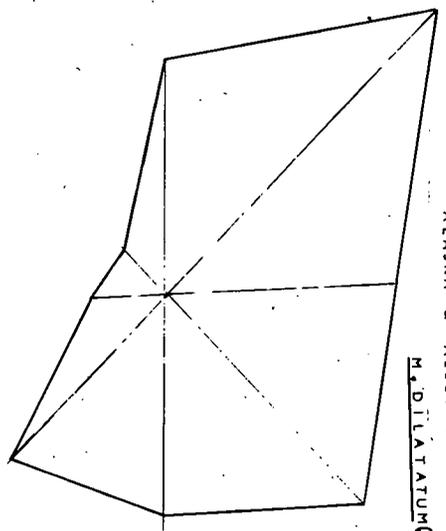
ASIA :-
M. DILATATUM (▲)



JAPAN :-
M. DILATATUM (▲)



ALASKA & ALEUTIAN ISLES :-
M. DILATATUM (▲)



NORTH AMERICAN PACIFIC AREA :-
M. DILATATUM (▲)

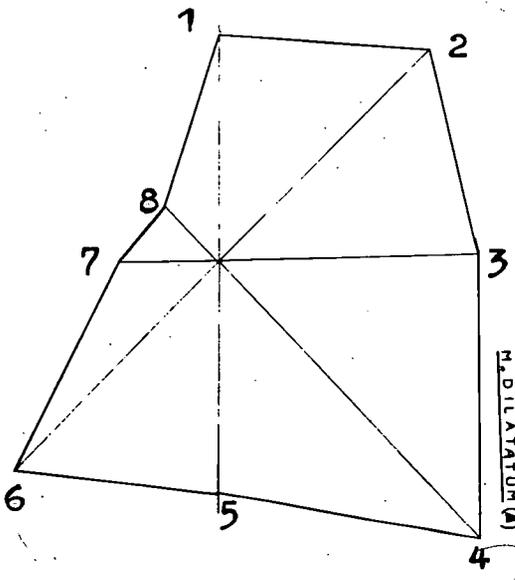


Fig. 4

Fig. 5

Lower cauline leaf in Maianthemum. Based on
the means stated in Table 4.

- A dilatatum Pacific Northwest America.
- B dilatatum Alaska and Aleutian Is.
- C dilatatum Asia.
- D dilatatum Japan.
- E bifolium Europe.
- F bifolium Asia
- G var. interius W. and C. North America.
- H canadense Eastern North America.

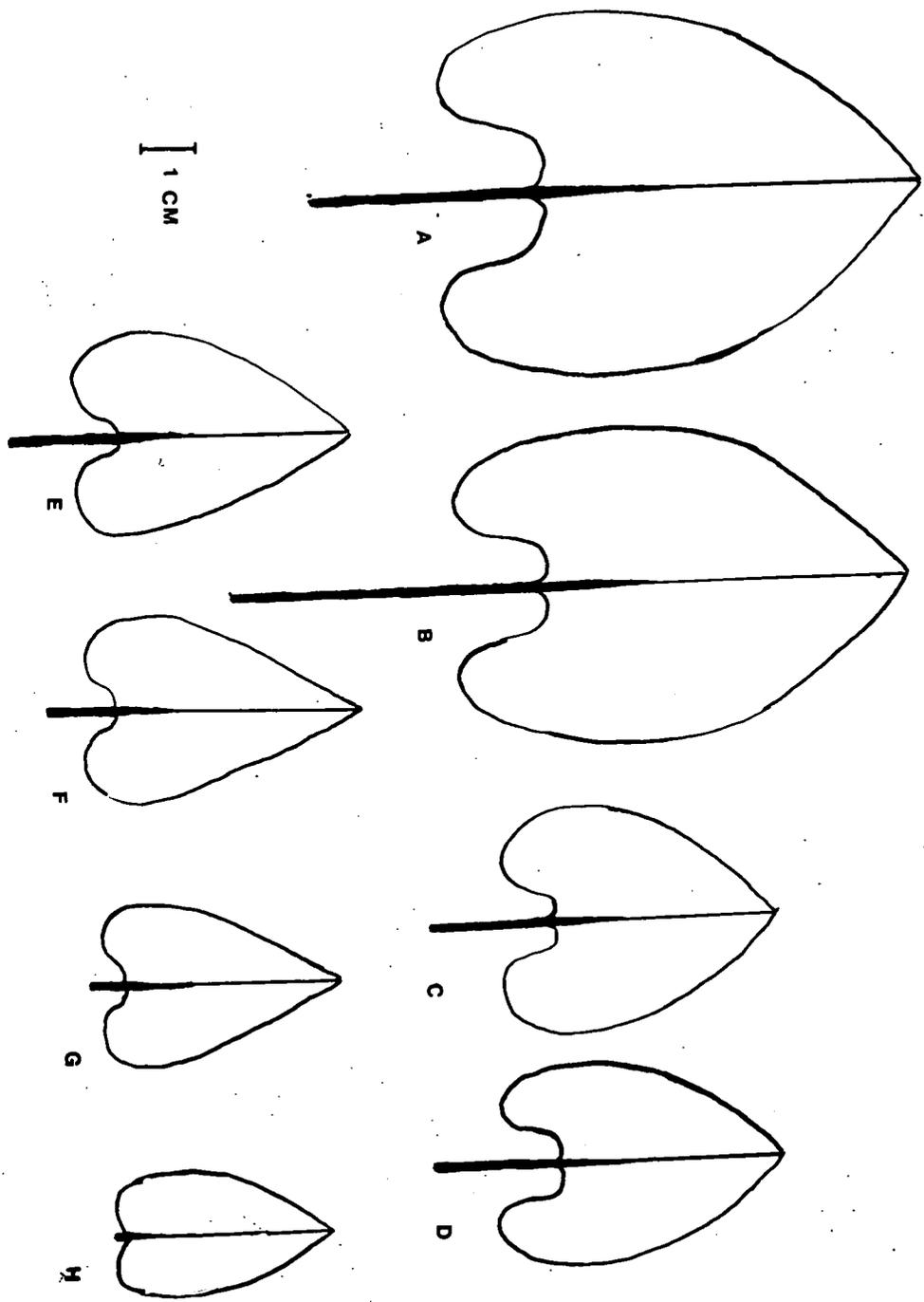


Fig. 5

It is interesting to note the difference in the stature of dilatatum plants on both sides of the Pacific. The plant seems to attain a higher stature along the American Pacific northwest coast, becoming reduced in size as it spreads along the Asiatic Pacific coast. On scoring the various localities on the basis of plant height, the results were obtained and listed in Table 5. It is evident from the Table that there are marked differences in plant height in dilatatum populations. In Asia most of the localities are represented by short plants. In Japan, there are short and medium-sized plants. In Alaska and the American Pacific Northwest coast, all the specimens are very tall. Butters (1927) says that "Southwards the stature of the plant (i.e. dilatatum) diminishes on both sides of the Pacific, but much more notably so on the Asiatic side". This is similar to the statement of Kawano (in correspondence) that "M. dilatatum is enormously variable in gross morphology and reveals a clinal variation in several characters from the populations in northern Pacific regions to those in the southern districts. The variation seems to be genetically fixed, and not environmentally induced". Fig. 6 illustrates this variation in stature. To the left are shown two specimens from California; in the centre from Siberia and parts of Asia; and to the right, from Japan.

This Asiatic population presents us with an interesting problem of evolutionary, phytogeographical and taxonomic importance. This will be discussed later.

Table 5

Variation in plant heightin Maianthemum Number of localities examined

Taxon and area	Less than 5 cms.	6-10 cm.	11-15 cm.	16-20 cm.	over 21 cm.
<u>M. dilatatum</u>					
Japan	3	7	5	8	2
Asia	1	5	2	2	2
Alaska and Aleutian Islands.	0	0	1	2	3
American Pacific Coast	0	0	1	0	15
<u>M. bifolium</u>					
Asia	0	0	6	9	3
Europe	0	0	8	6	2
<u>M. canadense</u>	0	11	25	11	1
var. <u>interius</u>	0	2	7	5	2

Specimens from some of these localities have only been measured for plant height as most of the leaf and inflorescence characters are either lacking or completely spoiled on drying.

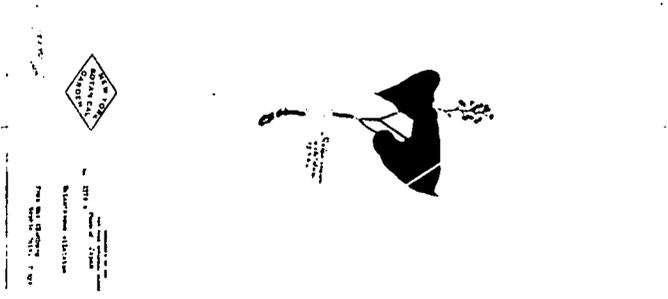
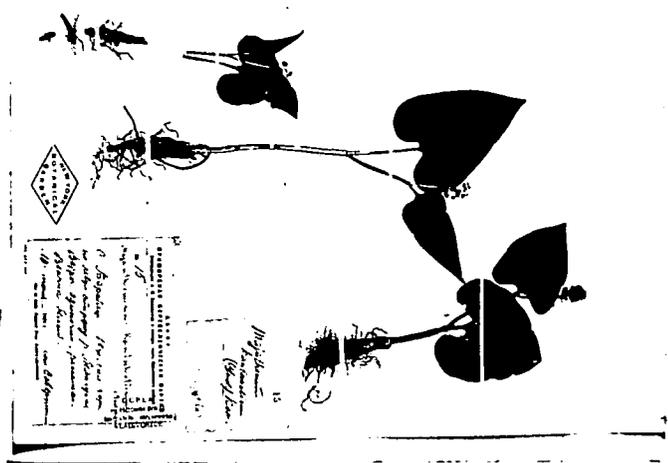
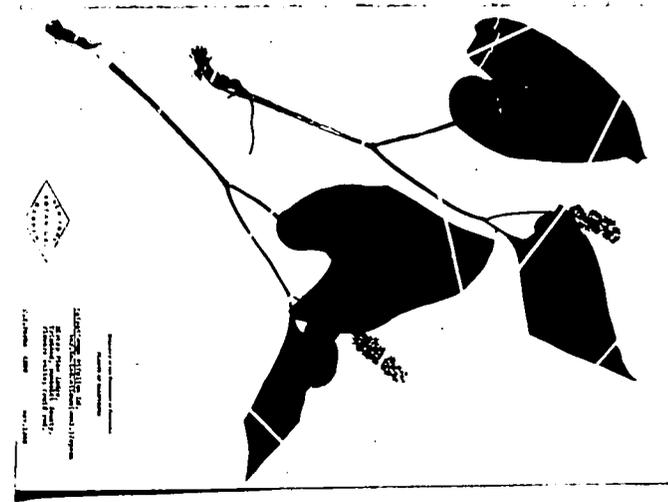


Fig. 6

Characters of the fruit, seed and flower

Maianthemum berries are speckled, reddish and fleshy on maturity. It has been reported that some species of birds eat them (see p. 27) The number of seeds per berry is rather variable and for this reason we have thought it of interest to examine the berries received through seed-lists. The localities of the seeds are shown in appendix 2. In most cases it is clear that the collection represents a good number of fruiting plants. In the following Table (Table 6) the various localities are examined for single seeded, 2-, 3-, or 4- seeded berries, thus:

Table 6
Number of seeds/berry in Maianthemum.
(imported material)

Taxon	Locality reference	1-seeded berries	2-seeded berries	3-seeded berries	4-seeded berries
<u>M. bifolium</u>	(3)	37	20	5	0
"	(5)	40	6	0	0
"	(6)	120	51	4	0
"	(7)	8	2	0	0
"	(8)	11	7	1	0
"	(11)	14	8	3	0
"	(12)	5	7	1	0
<u>M. canadense</u>	(9)	15	12	4	0
	(13)	22	21	4	0
<u>M. dilatatum</u>	(10)	33	12	3	1

It is apparent from the table above that all the taxa produce 1-3 seeds per berry, rarely 4. Two taxa, viz., dilatatum and bifolium

produce most of the seeds in uni-seeded berries, whereas in canadense single-seeded and two-seeded berries seem to occur at the same frequency. The seeds in all cases vary in shape, depending on whether there is one seed, or several, in the berry; in the latter case they become flattened at the point of contact.

Seed measurements have been done on the seeds produced by the single-seeded berries; this makes the measurement more easy as the seed is more or less spherical. The results are shown in Table 7.

Table 7

Seed measurements in *Maianthemum*.
(imported material)

Taxon	Locality reference	Seed length mm.	Seed width mm.
<u>M. bifolium</u>	(6)	2.6 ± 0.02	2.2 ± 0.02
<u>M. canadense</u>	(9)	3.0 ± 0	2.7 ± 0.05
<u>M. canadense</u>	(13)	2.6 ± 0.03	2.2 ± 0.04
<u>M. dilatatum</u>	(10)	2.8 ± 0.04	2.8 ± 0.015

There are some significant differences between dilatatum (10) and bifolium (6), and dilatatum and canadense (9); in both cases seeds of dilatatum are larger. There is also a difference between the seeds of bifolium (6) and canadense (9) those of the latter being larger. It is interesting that a strain of canadense, now in cultivation, i.e. M4, 1, produced on shelving 7 seeds measuring on the average 2.9 x 2.5 mm. Thus similar to strain (9) above, but the strain in cultivation is found to be a triploid. On the whole, seeds do not give us any

differences of taxonomic value and will not be discussed further.

Flower characters have been difficult to analyse in the dried plants; the flowers are very small and badly preserved on the sheets. On the living plant we have employed most of the buds in cytological and hybridization experiments, but it has been possible for us to examine the specimens made available for us by the Curator of the Manchester University herbarium, who kindly allowed us to remove some of the flowers for the purposes of our work. The results are shown in the following Table (Table 8) and are presented diagrammatically in Fig. 7. It is clear from both the table and the figure that the flowers are generally very similar, but we have observed some differences. The anthers in M.canadense are seen to be joined to the connective at either the midpoint or the base (see Fig. 7). The stylar split at the summit is more marked in M.canadense var. interius and M.bifolium than in the other species (Fig. 7), and the style is stouter and thicker in M.dilatatum. We have been unable to confirm the characters of the ovary base pointed out by Butters and incorporated in the key page 3 and in our opinion the ovary base does not provide any marked differences of taxonomic importance. There is only one way in which the whole ovary seems to be different, viz. in M.canadense var. interius where it is wider than in any of the other taxa (Fig. 7).

Characters of the flower other than thickness of the pedicel and style and width of perianth, will not be discussed further as they are not of much taxonomic value.

Table 8

Flower character in MaianthemumMeans and standard error of means.All measurements in mms. Herbarium data.

<u>Taxon Character</u>	<u>Interuis</u>	<u>canadense</u>	<u>bifolium</u>	<u>dilatatum</u>
<u>Ovary length</u>	1.0 ± 0.01	1.0 ± 0.01	1.0± 0.01	0.94± 0.01
<u>Ovary width</u>	1.35± 0.02	1.0 ± 0.02	1.0± 0.01	0.94± 0.01
<u>Style length</u>	1.1 ± 0.01	1.3 ± 0.02	1.0± 0.1	0.9 ± 0.01
<u>Stigma width</u>	0.55± 0.02	0.46± 0.02	0.5± 0.02	0.6 ± 0.01
<u>Filament length</u>	1.8 ± 0.02	1.4 ± 0.03	1.8± 0.02	1.5 ± 0.03
<u>Anther length</u>	0.9 ± 0.01	0.7 ± 0.01	0.7± 0.01	0.6 ± 0.01
<u>Length of largest perianth segment</u>	3.0 ± 0.03	2.8 ± 0.04	2.6± 0.03	2.7 ± 0.03
<u>Width of largest perianth segment</u>	1.6 ± 0.04	1.2 ± 0.03	1.2± 0.03	1.8 ± 0.03
<u>Number of flowers studied</u>	4	16	12	8

Fig.

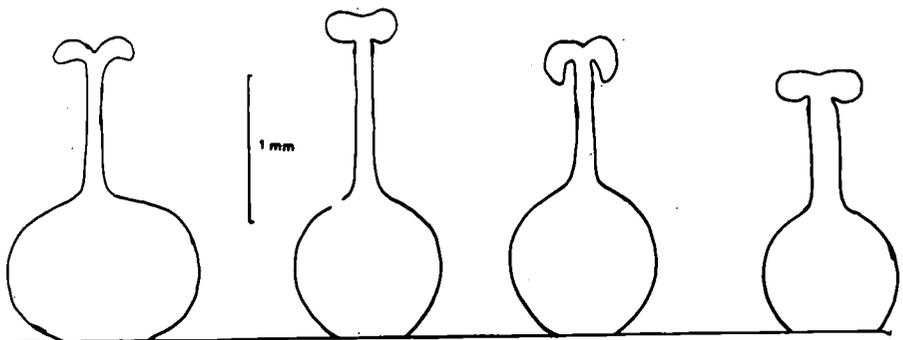
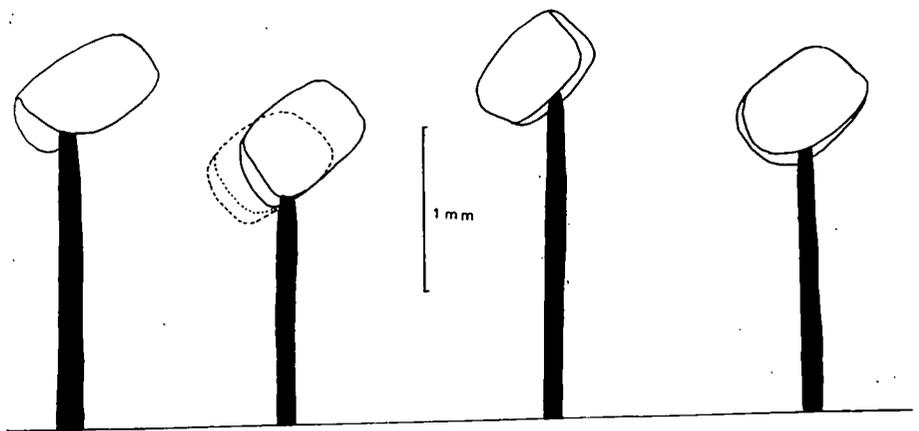
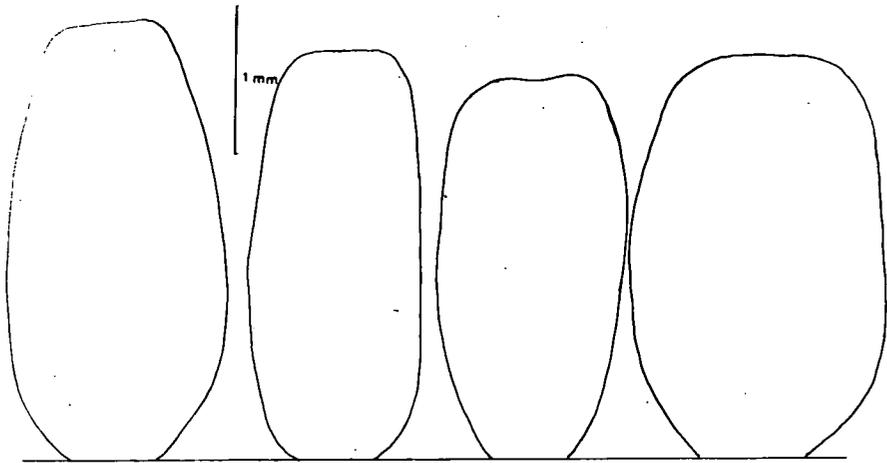
Flower characters in *Maianthemum*.

Based on the means. Herbarium data.

Top: Largest perianth segment.

Middle: Stamens.

Bottom: Ovaries and styles.



<u>M. canadense</u> var. <u>interius</u> (MEAN OF 4)	<u>M. canadense</u> (MEAN OF 16)	<u>M. bifolium</u> (MEAN OF 12)	<u>M. dilatatum</u> (MEAN OF 8)
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Fig. 7

Survey of fresh material

The sources of the living plants used in this work are given in Appendix 3. Up to the present, it has not been possible to investigate floral characters as all flowers have been used either for examination of pollen mother cells or in hybridization experiments. However, the epidermal characters of the leaf were studied, using strips of epidermis stained in aceto-carmin and some interesting differences were found.

Data for representative plants of the three species and the variety are given in Table 9. It will be seen that as well as stomatal length, width and aperture, it was possible to measure the nuclei of the guard and subsidiary cells, which were very well stained. In each preparation, 25 stomata from a single leaf were measured, and means and standard errors calculated. The leaves were at comparable stages of development and had been grown under similar conditions. The means are shown diagrammatically in Fig. 8.

Table 9

Means and standard error of means
of epidermal characters in species of
Maianthemum. All measurements in microns.

	<u>M. canadense</u> <u>var. interius</u> <u>(Mv4.1)</u> <u>Tetraploid</u>	<u>M. canadense</u> <u>(M1.1)</u> <u>Triploid</u>	<u>M. dilatatum</u> <u>(American)</u> <u>(L5.1)</u> <u>Diploid</u>	<u>M. bifolium</u> <u>(N5.1)</u> <u>Diploid</u>
<u>Stomatal</u> <u>length</u>	45.7 ± 1.3	37.2 ± 0.78	35.1 ± 0.6	34.8 ± 0.4
<u>Stomatal</u> <u>width</u>	38. ± 0.5	38.9 ± 0.6	35.3 ± 0.6	33.7 ± 0.5
<u>Larger diameter</u> <u>of guard cell</u> <u>nucleus</u>	11.5 ± 0.2	11 ± 0.3	9.9 ± 0.2	8.26 ± 0.2
<u>Narrow diameter</u> <u>of guard cell</u> <u>nucleus</u>	11.6 ± 1.1	7.8 ± 0.15	7.6 ± 0.1	7.2 ± 0.17
<u>Large diameter</u> <u>of subsidiary</u> <u>cell nucleus</u>	13 ± 0.0	9.3 ± 0.36	9.9 ± 0.28	8.5 ± 0.26
<u>Narrow diameter</u> <u>of subsidiary</u> <u>cell nucleus</u>	13 ± 0.0	8.16 ± 0.3	8 ± 0.4	8.2 ± 0.3
<u>Stomatal</u> <u>aperture</u>	27.4 ± 0.7	17.36 ± 0.5	14.5 ± 0.4	19.3 ± 0.8

It will be noted that there is a significant difference in both stomatal and nucleus size between M. canadense var. interius and the other three taxa. There are other significant differences too, but these should not be emphasized, as only a single set of samples has been taken. It is noteworthy that var. interius is tetraploid, as will

be shown below, and this fact may well be correlated with stomatal and nuclear size.

These characters need to be investigated in the dried material; until this is done, they cannot be used in the general taxonomic^{9, 0} discussion.

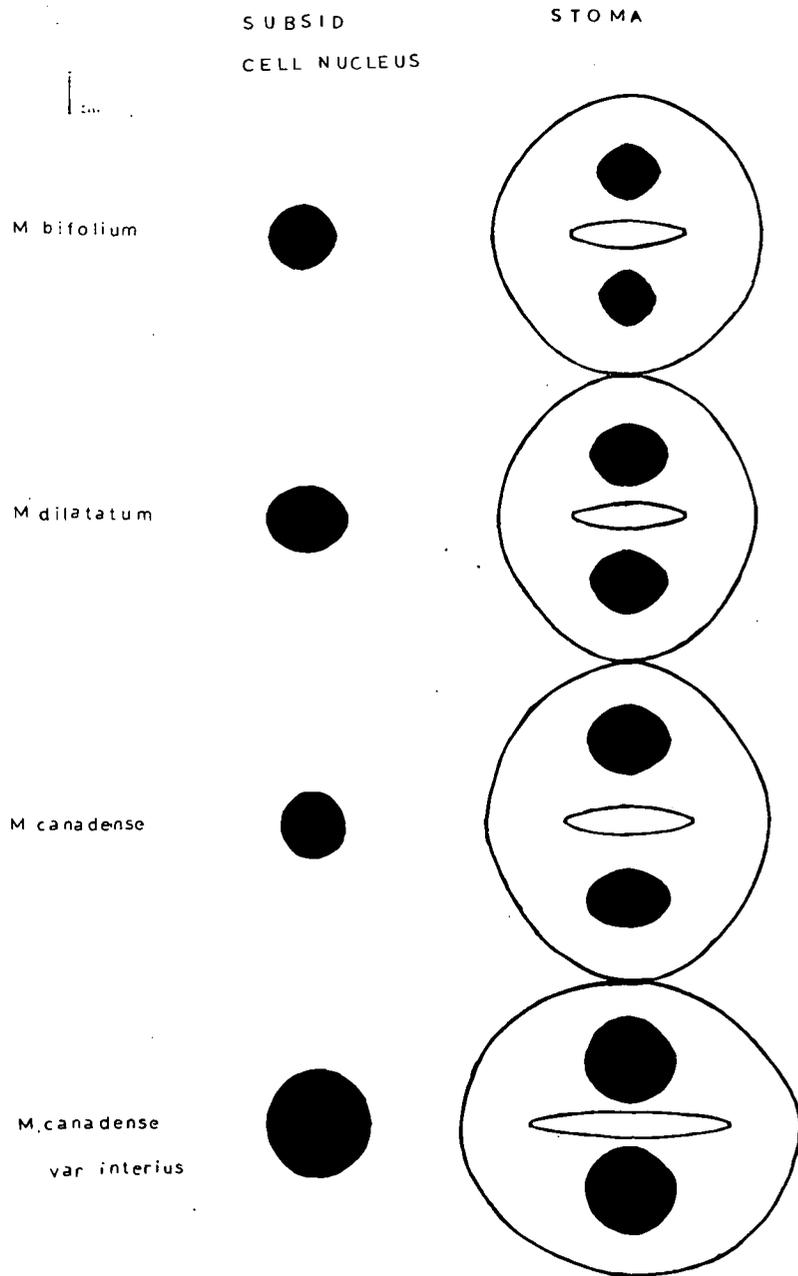


Fig. 8: Diagrammatic representation of epidermal characters in the leaf of Maianthemum. Based on means shown in Table .

Anatomy of the rhizome in species of *Maianthemum*

Butters (1927) gives a number of anatomical differences in the rhizomes of *Maianthemum* species. He does not, however, specify the age of the material used, and there must be, therefore, doubt about the significance of these differences. We have looked into the anatomy of the young rhizomes of all the species, and our findings differ from those of Butters. In one case he describes the endodermis of *M. canadense* as made up of thick cells that are relatively narrower than those of the variety, and forming two complete layers, with scattered thickened cells in the third layer. We have not been able to confirm this. The endodermal cells of this species are wide, with very little thickening and forming only one layer. He describes the endodermis of the variety *interius* as relatively wider than in the species, more shallow and with no second layer of thickened cells or at least incomplete, and no thickened cells in the third layer. We have found this also untrue. There are in this variety 1-2 fibrous layers below the two-layered endodermis. In short, we do not consider the anatomy of the rhizome as being of much importance in the taxonomy of *Maianthemum*.

Chapter twoHybridization experiments in *Maianthemum*

It was thought of interest to see if the three species of *Maianthemum* could hybridize when brought together under greenhouse conditions. We have also thought it necessary to do some experiments on self and cross-compatibility. Clapham, Tutin and Warburg (1962) report that the species may be self-fertilized. Knuth (1909) reports that self-pollination is possible should insect-visits fail.

Several factors have operated to restrict our hybridization programme. It has already been mentioned that some of the flowers have been used for cytological work. Another difficulty is that in all three species of *Maianthemum* there is a tendency to flower only in alternate years. This has the disadvantage of preventing the repetition of a particular cross. Butters (1927) reported that *M.bifolium* and *M.canadense* appear to flower every other year, whereas *dilatatum* flowers continuously over several years. We have noticed that all three species tend to flower in alternating seasons, though even this tendency is not consistent (Table 10). Visits to a *bifolium* population at Scarborough in the summers of 1965 and 1966 suggest that the plant is flowering every year, but no fruits have been seen. Lousley (1950) says that the Scarborough population "in some years produced only very small numbers of flowers". This confirms our observation that flowering is not consistent.

Table 10

Flowering habit in Maianthemum

Species and code	1964-65	1965-66	1966-67	1967-68
<u>M. canadense</u>				
(M1, 1)	Fertile	Sterile	Sterile	Sterile
(M4, 1) 3x	Fertile	Sterile	Fertile	Sterile
(M6, 1) 2x		?	Fertile	Fertile
<u>M. canadense var. interius</u>				
(Mv6, 1) 4x	?	Fertile	Sterile	Fertile
<u>M. bifolium</u>				
(N1, 1)	Sterile	Sterile	Fertile	Fertile
(N2, 1)	Fertile	Sterile	Fertile	Sterile
(N2, 2)	Fertile	Sterile	Fertile	Fertile
(N2, 3)	Sterile	Fertile	Sterile	Fertile
(N3, 1)	Sterile	Fertile	Fertile	Sterile
(N4, 1)	Sterile	Fertile	Fertile	Sterile
(N5, 1)	?	Sterile	Fertile	Fertile
(N5, 4)	?	Sterile	Fertile	Sterile
<u>M. dilatatum</u>				
(L5, 1)	Fertile	Sterile	Sterile	Sterile

The sign "?" indicates that the plant was not available to us in the season concerned and little is known about it.

Absence of fruits raises the question of compatibility. It is true that some species of birds eat the Maianthemum berries, e.g. Pica rustica (the Magpie) - reported by Ridley (1930); and the absence of fruits can be explained on this basis; but several plants were seen with the whole inflorescence, still attached to the plant, completely dry. Knuth (1909) reported that small flies were observed on Maianthemum, and therefore the lack of fruit is not due to lack of suitable pollinators. We therefore thought it necessary to do some experiments on self-compatibility in M. bifolium. The programme was as follows:

- (a) The whole inflorescence is left intact.
- (b) The whole inflorescence is shaken to facilitate pollination.
- (c) Several flowers are selfed repeatedly, each time using the same pollen parent, in most cases the same flower.
- (d) Several flowers from one strain are pollinated from another strain.

The results are shown in Table 11.

It will be seen that repeated self-pollination sometimes leads to development of fruits, but these are apparently subnormal in size and are empty. The cross (d) between different plants was much more successful and set normal seeds.

Table 11

Self-compatibility in M.bifolium

Strain code	Ref. for method used	No. of fls. poll.	No. of fls. fail- ing	No. of fls. success- ful	Size of berry	No. of seeds per berry	Size of seed mm.	
N5, 1	a	20	20	0	-	-	-	
N5, 1	b	7	7	0	-	-	-	
N4, 1	b	12	12	0	-	-	-	
N4, 1	a	12	12	0	-	-	-	
N5, 1	c	9	9	0	-	-	-	
N5, 4	c	13	10	13	3 x 2mm	Empty	-	
N4, 1	d	8	3	5	a. 7x6mm	2	3.5x3.0	
								3.0x3.0
					b. 8x6mm	2	3.0x3.0	
								3.0x3.0
					c. 5x5mm	1	3.0x3.0	
					1	3.0x3.0		
N1, 1	d	4	2	1	5x5mm	1	3.5x3.0	

The other factor restricting our hybridization experiments is the late flowering-time of dilatatum (L5,1). This plant flowered early in June 1965, whereas all the other taxa flower in May or sometimes as early as March, as in 1967. We have tried to preserve some pollen of both bifolium and canadense for purposes of crossing with dilatatum, but the pollen did not keep for long.

Even in interspecific crosses involving bifolium and canadense, which have a considerable period of overlap in their flowering season, another difficulty arose. The stigmas of both are only receptive, or seem to allow pollen dust to stay on them, when they are covered with a small, colourless droplet. When this disappears, the stigmatic surface, and part of the style, become brownish and dry up.

The method of pollination used is to emasculate the flowers shortly before they open. Emasculated flowers usually receive a second dose of pollen whenever possible. It takes an ovary slightly over two months to reach maturity, after which time it becomes reddish.

Hybridization results are shown in Table 12.

Table 12

Hybridization results in *Maianthemum*

Date	Seed parent pollen parent	No. of pollin flowers	No. of flowers setting seed	No. of seeds germination
1962	(Prof. Valentine's experiments)			1964 65 66 67
	<u>canadense</u> bifolium	8	6	5/9
1965	(Own)			
	<u>bifolium</u> canadense	18	5	0/8 0/8
1965	<u>canadense</u> bifolium	15	2	0/4 0/4
1965	<u>canadense</u> bifolium	20	4	0/4 2/4
1966	<u>bifolium</u> canadense	6	2	0/2
1967	<u>bifolium</u> canadense	8	6	(6 seeds not sown)
1968	<u>interius</u> canadense	6	6	(berries developing)
1968	<u>canadense</u> interius	4	4	(berries developing)
1968	<u>bifolium</u> interius	8	6	(berries developing)

All the canadense plants, except in 1967 and 1968, were triploid, and the only crosses in which germinable seeds was obtained were those in which canadense was the seed parent. The fertility of the triploid seed-parent

in relation to its meiotic behaviour will be commented on later in the thesis (p.79). It is enough to say here that the chromosome numbers of the hybrids produced in 1963-64 varied from $2n = 41$ to $2n = 43$.

The plants are quite vigorous, but have not yet flowered.

It is unfortunate that we were unable to obtain diploid canadense until 1967 (M6, 1).

Natural hybrids in *Maianthemum*

There is very little information in the literature about natural hybrids. As regards *M.dilatatum*, Hultén (1962) suggests that transitional forms should be studied in the areas where the ranges overlap, in eastern Asia and British Columbia. Hybrids between this species and *M.bifolium* have been described, with reservations, by Butters (1927) from Korea, Manchuria and Japan. Kawano (in correspondence) reports the occurrence of interconnecting forms in Japan (p. 8).

In eastern North America, several "puzzling forms" have been found by Butters (Butters 1927). These he found to combine the characters of *M.canadense* and *M.canadense* var. *interius*. Butters considers them as intergrading forms; but they may be hybrids. We will refer to these later after the cytology has been dealt with.

Chapter threeSeed germination in Maianthemum

The berries and seeds of Maianthemum have been described earlier (p.18) and no differences of taxonomic importance have been found.

For purposes of germination experiments, seeds have been obtained through seed exchange and the list of the localities are given in Appendix 2.

Seeds have been sown in loam in 4 inch pots, and left in the open over winter. The results of germination of seeds sown in 1964 are shown in Table 13.

Table 13Germination of Maianthemumseeds in 1965, 66, and 67.All seeds sown in 1964.Localities referred to by numbers.

Taxon	Locality reference	No. of seeds sown	No. germinating in			
			1965	1966	1967	Percentage
<u>M.bifolium</u>	(1)	40	6	0	2	20
	(2)	20	2	0	2	20
	(3)	30	3	0	3	20
	(4)	40	0	0	10	27
	(5)	60	12	0	5	28
	(6)	60	21	0	0	35
	(7)	60	6	0	6	20
	(8)	60	6	0	0	20

The average germination is 17 per cent.

More seeds were sown in the summer of 1965; the results of germination are shown below in Table 14.

Table 14

Germination of Maianthemum seeds sown in 1965.

Taxon	Locality Reference	No. of seeds sown	No. germinating in		
			1966	1967	Percentage
<u>M. bifolium</u>	(11)	10	0	0	0
	(12)	70	0	5	7
	(13)	34	0	3	9
	(14)	15	0	0	0
	(15)	22	0	2	9
	(16)	30	0	0	0
	(17)	45	3	0	7
	(18)	40	0	0	0

Adams (1927) says that "germination of some species of seeds is greatly accelerated by exposure for a time to low temperature". His experiments on the germination of seeds of Actaea, Aralia racemosa, various Liliaceous plants including Convallaria, Maianthemum, Smilacina, two species of Ribes, etc., show that exposure to winter conditions is necessary. His results on M. canadense are shown below in Table 15.

Table 15

Adams' results on the germination of M. canadense seeds.

Environment	No. of seeds sown	No. germinating	
		1st. year	2nd. year
(a) Sown in soil out of doors.	100	60	18
(b) Sown in soil in greenhouse indoors.	100	28	0
(c) Kept in packets outdoors for first winter then sown in soil.	100	3	8
(d) Kept in packets in heated room for first winter then sown in soil	100	2	3

Our results are probably most closely comparable to (b) in that the winter temperatures in Durham would not be so cold as in North America. In the first experiment (Table 13) the average germination in the first year was 17 per cent, which is of the same order as Adams' (b) figure, and there was no germination in the second season. In the second experiment (Table 14), very low germinations were recorded in the first season.

Some experiments were done on the dormancy of seeds of M.bifolium. All the seeds received cold treatment, but some were soaked in a solution of Gibberellic acid (1000 ppm); the testa of others was opened with a scalpel. All the seeds, irrespective of the treatment given, showed 11-14 per cent germination, the period being twelve weeks. This result is similar to that obtained in seeds sown in soil and exposed to the cold over winter (Table 13); the reason for the low percentage germination remains obscure, though it is possible that very low winter temperatures would have an effect. This is indeed suggested by the results of Adams' (Table 15) where he obtained 60 per cent germination for seeds sown out of doors in the North American winter.

Chapter FourCytology of Maianthemum1. Review of the literature and current chromosome counts:

The literature on the cytology of Maianthemum is difficult to harmonise into one pool of information, for purposes of comparison and criticism. There are several difficulties, the first of which is the tendency of most authors to quote other reports uncritically. The second is concerned with naming of the species examined; this is very annoying as the plant named is sometimes given a geographical origin outside its normal range, and not even a brief description of the plant is given. Moreover, it is not enough to adopt the label names of imported seeds; the origin of the seeds should always be stated, whatever the nature of the work. This leads us to the third difficulty which is the nature of the investigation. Some workers tend to produce an extensive piece of work on a few species, others deal with a whole tribe, genus or family. Thus the details of the information vary in depth and value.

In the following pages we shall discuss in detail the cytological reports on Maianthemum. To start with, all the reports on M.bifolium are listed in Table 16.

Table 16

Chromosome counts on *M. bifolium*

Locality	Country	2n	n	Author
-	-	-	14	Lawson 1913
Uppsala				
Uppland				
Flottsund	Sweden	-	15-18	Stenar 1935
-	Japan	54		Sato 1942
-	Denmark	42*		Löve ^V and Löve ^V 1942
-	-	36		Löve ^V and Löve ^V 1954b
Helsinki	Finland	36	18	Therman 1956
Mustila	Finland	36	18	Therman 1956
Gander	Newfoundland	36		Therman 1956
Piikio	Finland	36	18	Therman 1956
Lepaa	Finland	36	18	Therman 1956
Hornbeak	Denmark	36	18	Therman 1956
Far East		36		Sokolovskaya and Strelkova 1960
	Sakhalin	36		Sokolovskaya 1960
Vladivostock	U.S.S.R.	36		Sokolovskaya 1961
		64-70		
Yamanashi (four different localities)	Japan	36		Kawano et al 1967
Oze, Gunna	Japan	36		Kawano et al 1967
Ibaraki	Japan	36		Kawano et al 1967
Tochigi	Japan	36		Kawano et al 1967
Nagano	Japan	36		Kawano et al 1967

*N.B. Löve^V (personal communication) confirms to us that a count of 2n = 42 for *M. bifolium* is inaccurate and therefore no longer valid.

Lawson (1913) has been quoted by several authors as the reference for the haploid count of $n = 14$ given to M.bifolium. In some cases (Matsuura and Suto 1935) the same count is given to M.dilatatum, also under the same author. We have looked into the paper of Lawson, and failed to find any reference to Maianthemum. Lawson's work is based on Smilacina without even specifying the species.

Stenar (1935) has been also quoted by many authors and in each case the somatic number for bifolium covers a wide range i.e. $2n = 30, 36, 38$ and 42 . The counts of $2n = 38$ and $2n = 42$ do not exist in the original paper of Stenar. The other counts are a possible misunderstanding of the findings of Stenar. Stenar does not state how many plants she examined, but she records counts of $n = 15$ and $n = 17$ in egg mother-cells and $n = 18$ in a pollen mother-cell. She also notes stickiness of the chromosomes and says that she cannot be sure of the exact count. It seems likely that some chromosomes were missed in the count of $n = 15$ and that a quadrivalent was mistaken for a bivalent in the count of $n = 17$.

Sato (1942) has produced an excellent piece of work on the cytology of Liliaceae and allied families. The material used by him is obtained from pot plants raised from imported seeds. For the species named, Sato states that the label names on the bags imported were adopted in most cases. This, though unavoidable, sometimes leads to error as the seeds of Maianthemum are very similar

(p.19). The technique used by Sato, i.e. paraffin-wax, can lead, on sectioning, to loss of parts of the chromosomes, or to the reconstruction of several chromosomes from bits and parts of a single chromosome. The karyotype formulae given by him to M.bifolium (in fact to all species of Maianthemum studied by him) is as follows:

$$2n = 54 (4b - 2) = 4L + 4s + 46 s$$

Of the 25 small pairs, two are satellited, and there are four chromosomes classified as large. Sato thinks that this material is a secondary polyploid, having 14 as a base ($4b - 2$); thus he suggests the existence of two base numbers for Maianthemum i.e. 14 and 16. The former of these has already been discussed above, and the latter will be discussed later for it was originally given to M.dilatatum by Matsuura and Sato (1935).

Therman (1956) gives a more detailed study on the tribe Polygonatae. Her findings for M.bifolium confirm the occurrence of two satellited chromosomes. Most of the counts given are in agreement with ours; but the origin of the botanic garden material is unknown. The other interesting, or rather annoying, plant is the material said to be collected from its natural habitat in Gander, Newfoundland, Canada. It is generally considered that the geographical range of M.bifolium does not reach that part of Canada, and that the species is mainly Eurasiatic (though Ohwe 1965) gives the range of bifolium as extending to N. America). Therman does not give any description for the material and, therefore, we think of the identification as unreliable.

Sokolovskaya (1961) gives an interesting count for M.bifolium. The material was collected from the neighbourhood of Vladivostock. Typical plants were found to have $2n = 36$, but plants having slightly larger dimentions in all parts were found to have $2n = 64 - 70$. The exact number of the metaphase chromosomes is stated to be difficult to establish. In our opinion, the number could reach 72, or at least very possibly. We have found a parallel situation in America with M.canadense and its variety interius and this will be described below.

Kawano et al. (1967) working on Japanese material produced very interesting results that match, to a great extent our own. We will discuss these later when we describe our own findings.

Our own somatic counts, based on root-tips, are given in Table 17.

Table 17Own somatic counts on M.bifolium

Origin	No. of cells of root-tip counted	Distribution of counts		
		35	36	37
S. Germany (single plant)	13	3	9	1
Switzerland (single plant)	12	1	11	
Czechoslovakia (single plant)	10	0	10	0
Denmark (single plant)	5	1	4	0
England (single plant)	4	0	4	0
Poland (single plant)	4	0	4	0

In view of these results, the counts for the far East material identified as *M.bifolium* of $2n = 54$ (Sato, 1942) and $64 - 70$ (Sokolovskaya 1961) may represent triploid and tetraploid chromosome races of *M.bifolium*, analogous to those of *M.canadense*. It would be of interest to examine their meiosis.

The literature on chromosome counts on *M.dilatatum* are given in Table 18.

Table 18

Counts on M.dilatatum

Locality	Country	2n	n	Author
Sapporo, Prov.Isikari	Japan		16	Matsuura and Sato 1935
	Japan	54		Sato 1942
	Japan	54		(var. <u>nipponicum</u>) Sato 1942
Botanic Garden Gothenburg		36	18	Therman 1956
-	Kamtchatcha	36		Sokolovskaya 1961
-	"	54		(f. vegetior) Sokolovskaya 1961
Yamanashi (5 different localities)	Japan	36		Kawano et al. 1967
Tochigi (4 different localities)	Japan	36		Kawano et al. 1967
Miyagi	Japan	36		do
Nagano	Japan	36		do
Saitama	Japan	36		do
Iworte	Japan	36		do
Aomori (2 different localities)	Japan	36		do
Nara	Japan	36		do
Kamanoto	Japan	36		do
Kagoshima	Japan	36		do
Hokkaido (5 different localities)	Japan	36		do
Isl. Ullung	Korea	36		Lee (as in Kawano et al. 1967

Matsuura and Suto (1935) give a haploid number of $n = 16$ and the following description of the karyotype as seen in the meiotic metaphase: "of the 16 bivalents, four are distinctly larger than the rest": and they note that the same count has been given to the same species by Lawson (1913). We have already discussed the count of Lawson; our concern now is the validity of the count of $n = 16$. This count has not been confirmed by any other worker. In their introduction to the paper, the authors say that they fear that in earlier works uncertainty often prevails as to the number of chromosomes, causing much confusion and rendering the matter very obscure. It may well be the case that there is an error of miscounting in a count of $n = 16$. As to the material used, identification was done in the University of Hokkaido, and there seems no doubt as to its correctness. The only possible source of error is the sectioning technique used, which we have criticised above (p.38).

Sato (1942) gives for the species, counts similar to those reported by Sokolovskaya for her forma vegetior. But he does not describe his material and it cannot be compared with that of Sokolovskaya.

Sokolovskaya (1961) gives not only the counts but also the origin, and description of her plants. There is no doubt about the accuracy of the results, which we accept. Sokolovskaya, however, is of the opinion that the hexaploid form is an auto-polyploid arising as a result of fusion of reduced and an unreduced gametes. She describes this polyploid as being different from the species in having a

"taller stem (20 - 40 cm), by the dimensions of the leaves, which impress by their vigour and attain a length of 6 - 15 cm and a width of 4 - 12 cm. with a wider cordate base to the leaf lamina, with semicircular lobes, and also by the dimensions of the flowers, the perianth of which reaches a length of 4 mm." The forma is certainly very close to the species and very different from M.bifolium. For this reason Sokolovskaya rules out the possibility of it being a result of hybridization between the two species, which meet in that part of the geographical range. Alternatively, we can think of the hexaploid form as being the result of hybridization between two chromosome races of the same species, one with $2n = 36$ the other with $2n = 72$. We are thus assuming the possibility of the existence of the higher polyploid ($2n = 72$) somewhere in the Far East.

Kawano et al. (1967) observe that despite the extraordinary polymorphism of the species, its karyotype is identical to that of bifolium also examined by them (p.55)

Our own count on material from California is $2n = 36$.

Counts on M.canadense are given in Table 19.

Table 19

Chromosome counts on *M. canadense*

Locality	Country	2n	n	Author
	Canada	36		Löve 1954
Maskwa Rapids, Manitoba	Canada	36		Löve and Löve 1954
Bot. Garden, Gothenburg		36	18	Therman 1956
Alpine Garden	Canada	36		Löve and Löve 1958
Between Clarenceville and Venice, Quebec	Canada	36		Hedberg 1959
Trout Lake Point, Vilas Co., Wisconsin	U. S. A.	36		Iltis and Kawano 1965

Löve (1954) introduces his paper by saying that the chromosome numbers listed are either original counts or confirmation of those listed in various chromosome manuals. No details are given. The material of Therman (1956) came from a botanical garden, and its exact origin is also unknown. The other counts listed above, Hedberg (1959) and Löve and Löve (1954) and Iltis and Kawano (1965) are properly localized and agree in giving $2n = 36$. The Manitoba plant was identified as var. interius Fernald.

Our own counts of M. canadense and its variety interius are given in Table 20.

Table 20

Own counts of M. canadense and its variety interius.

For details of localities see Appendix 3

Locality	Code	Taxon	No. of cells counted	2n	n
Near Hull, Quebec, Canada.	(M6, 1)	<u>canadense</u>	10		18
Nova Scotia	(M4, 1)	<u>canadense</u>	11	54	
Near Montreal, Canada		<u>canadense</u>	5	54	
Minnesota, U.S.A.	(Mv4, 1)	<u>interius</u>	5	72	
Alberta, Canada.	(Mv6, 1)	<u>interius</u>	15		36

It would appear from these results that there are three chromosome races here, with $2n = 36, 54$ and 72 . It is interesting to note that the 54 -chromosome race is fertile, and that its hybrid with M.bifolium has numbers of $2n = 41-43$ (Fig.17-1). The fertility and other characters of these races will be discussed below after the chromosome morphology and meiosis have been considered.

CHAPTER FIVEThe determination of the karyotypes

Healthy root-tips have been collected from all three species of Maianthemum in the spring and early summer of 1965, 66 and 67. Root tips are pre-treated in cold water (5 degrees) in a cold room for a period of at least 24 hours; then fixed in acetic alcohol in the ratio of 1 : 3. This pre-treatment method has been used by Dr. M.J. Harvey and Professor Valentine for Viola, hence its use for Maianthemum. Following fixation, storage, if required, is in 70 per cent alcohol. An ^a acetic-orcein squash technique is employed and the best results are obtained by applying the following procedures:

One root-tip at a time is placed on a clean slide containing a small drop of the stain. With the aid of a flattened needle and the dissection microscope, the very tip is removed and the unwanted tissue thrown away. The growing meristematic part of the root-tip can be seen even with the naked eye, being whitish in colour (root-tips stored for very long periods turn brownish and are useless). The root-tip is then placed in the centre of the drop and gently pressed. The soft tissue can be seen oozing out. At this stage the extra tissue is removed and placed on another slide (B) and squashed hard. In most cases good metaphase plates are most frequent in slide (A). It sometimes seems necessary to tap the slide to overcome overlapping of chromosomes, and in this case pressure must be applied evenly. Slides are made permanent using the dry ice technique. At this stage the slide can be examined under the low and high powers, but not under oil, as the Euparal is still wet.

Data on the karyotype have been obtained from several plants of M. bifolium, canadense, dilatatum, and var. interius. Unfortunately the somatic chromosomes of the diploid canadense have not been studied as it was only discovered amongst recent strains received from Canada, but it has been possible to look at its meiosis.

Method of determining the karyotype:

1. Chromosome length

The chromosomes of the somatic complements of each species have been measured. Metaphases are used, but it is difficult, even where the roots have been pre-treated, fixed and squashed in a standard way, to be sure that cells from different roots are comparable as regards chromosome length. For this reason, we have adopted the following procedure.

The chromosomes are photographed, cut out and arranged in pairs on the basis of length and morphology (Figs. 9 - 15). The two arms of each chromosome are then measured separately; and for each pair of chromosomes, means for total length and arm length are obtained. The length of the largest is taken as unity, and of the remainder as a fraction of the largest. This fraction is the index of length.

For triploid and tetraploid material, the chromosomes are grouped in threes and fours.

2. Position of the centromere.

Chromosomes are also characterised by the centromeres and other features such as secondary constrictions and satellites. The ratio

between the short and the long arm is calculated to give an index of symmetry. The median chromosomes will give an index of 1.0, those that are submedian will be in the range 0.5 - 0.9, and those that are terminal or sub-terminal will fall below 0.45.

The karyotypes are analysed in three different ways. The first is the visual way, in which pictures of the chromosomes are shown, arranged in pairs, trios, or quartets of diploid, triploid or tetraploid plants respectively (Figs. 9 - 15). The second is the graphical way; here, along the abscissa, is plotted numbers of chromosome pairs (or trios or quartets), from 1 to 18; and along the ordinate the indices of length and symmetry. Length is shown as a continuous curve, from the largest to the smallest, and symmetry by a series of lines (Figs. 17 and 18). The third way is by idiograms where, as in the graphs, the means of length and symmetry of every individual pair, trio or quartet is calculated and a drawing of the chromosome is then made. The satellited chromosome is shown by an open square replacing the short arm (Fig. 16).

In the following pages we are going to describe the somatic complement of each of the taxa in question, beginning with M.bifolium.

Somatic complement of M.bifolium

Material collected from Scarborough, England, has been analysed cytologically. The details are shown in Figs. 9, 16a - 1, and 18 - 1.

It is clear from all three figures that there are seven large-medium pairs with a length index in the range of 0.65 - 1.0. Pair one is usually the largest and is metacentric. Of the remaining six pairs three are sub-median and the rest are sub-terminal.

The small chromosomes all have a length index ranging from 0.33 to 0.55. Eleven pairs are involved, of which one (pair 10) is satellited. In this group of small chromosomes, two pairs are mediocentric, five are sub-median and four sub-terminal.

The absolute length of the chromosomes in this material is 4-14.4 microns.

Specimens from Poland are similar in their karyotype to those just mentioned. The details of the Polish material are shown in Figs. 9, 16a - 2 and 18 - 2. Here again we find that the large-medium pairs are seven in number, and the small pairs are 11; one of these, pair 10, is satellited. The absolute length is more or less the same as in the English material i.e. 4.4 - 13.2 microns.

German material is here studied on two analyses from two plants, a and b, the details are shown in Figs. 10 and 18 -3. In both cases the number of the large-medium pairs is the same as found above, i.e. 7. It is also observed that the small pairs are 11 as before. The satellited pair is present in all material examined. The absolute

length is slightly shorter i.e. 3.2 - 11.4 microns, due to the high degree of contraction.

Material from Czechoslovakia (Figs. 16a and 18 - 4) is identical in its karyotype to those just described; there are the seven large-medium pairs, the 11 small pairs, and the satellited pair. The range of length is very much the same i.e. 3.7 - 12 microns.

Danish material has the same characteristics as above, Figs, 16-a 3 and 18 - 5.

Somatic complement in *M.canadense* var. *interius*

The chromosome number of this material is found to be $2n = 72$. The absolute length of the chromosomes is the same as that seen in *M.bifolium* i.e. 4 - 12 microns. Here the chromosomes have been grouped into quartets. The number of large-medium quartets is 7 (*M.bifolium*); the number of the small quartets is 11, and one of these, quartet 10 is satellited. These details can be seen in Figs. 12; 17-b, and 19-6. It is evident that the only difference between this material and *M.bifolium* lies in the degree of ploidy; in the case of *M.bifolium* each class consists of two chromosomes, whereas it consists of four in this American variety of *M.canadense*. In fact if we compare the figures for *M.bifolium*, where the complements of two different strains are represented together, e.g. Fig. 10, with those of var. *interius*, the striking similarity is evident. The only slight difference is seen in the number of small, mediocentric chromosomes; in the variety we find four classes, compared with 2-3 such classes in *M.bifolium*.

Somatic complement of *M.canadense*

The chromosome number of some plants of this species is found to be $2n=54$. This count is based on two different plants (M1, 1 and M4, 1). The details are shown in Figs. 13, 14, 16b and 17. Here the chromosomes have been grouped in trios. There are seven large-medium sized trios, and eleven small-sized trios, of which one, Number 10, is satellited. The absolute length tends to be rather small in M1, 1. The analysis shows that the species is very similar to *M.bifolium* and *M.canadense* var. interius in possessing the same number of large-medium classes, but more like its variety in possessing four small metacentric classes.

Somatic complement in *M.dilatatum*

The data on the karyotype of this Californian material are shown in Figs. 15 and 17-2. This material has not been pre-treated. Nevertheless, there are seven large-medium pairs and eleven small sized pairs of which one, pair 10, is possibly satellited.

The above karyotypes are summarized in Table 21.

Table 21

Formulae for the karyotypes of Maianthemum

Fig. No.	Taxon	Large	Small	2n
15	<u>dilatatum</u> (as pairs)	1M+1Sm+4St	3M+5Sm+4St	36
	<u>bifolium</u> (as pairs)			
16-1	British	1M+1Sm+4St	3M+5Sm+4St	36
16-2	Polish	1M+1Sm+4St	3M+5Sm+4St	36
10	{ German -a	1M+2Sm+4St	4M+4Sm+4St	36
	{ German -b	1M+1Sm+5St	4M+4Sm+3St	36
16-4	Czechoslovakia	1M+1Sm+4St	4M+3Sm+5St	36
16-3	Denmark	1M+1Sm+3St	4M+5Sm+4St	36
	<u>canadense</u> (as trios)			
16-6	M4, 1	1M+1Sm+4St	4M+3Sm+5St	54
16-7	M1, 1-a	1M+1Sm+4St	4M+4Sm+4St	54
16-8	M1, 1-b	1M+1Sm+4St	5M+3Sm+4St	54
	<u>interius</u> (as quartets)			
16-5		1M+1Sm+4St	4M+5Sm+3St	72

The general similarity of the karyotypes of all taxa is evident. In all cases satellited chromosomes are present; two in the diploids, three in the triploids and four in the tetraploid.

It will be seen from Fig. 15 and Fig. 16 that the idiograms for all taxa, reduced to a basic minimum of 18, based on the means of pairs, trios and quartets, are generally very similar. It is also evident from Fig. 17 and Fig. 18 that the size range in clones of all species is more or less of similar distribution. As regards symmetry, there is a slight variation in the complements of strains of the same species, possibly within the individual range of variation.

From the data presented above, it is clear that we have not been able to establish any significant difference between the karyotypes of different strains of M.bifolium and between the different species whatever their level of ploidy. It must be admitted that the number of plants examined is small, and that it is difficult to obtain very precise measurements. It is indeed possible that there are structural differences between the chromosomes of the various races and species; but if these exist, they are probably fairly small.

Note on Figs. 9 to 15

The somatic chromosomes are arranged in pairs, trios, or quartets and numbered 1 to 18 in order of length as explained in the text.

- Fig. 9 Somatic complements of two different German strains of M.bifolium ($2n = 36$) from Scarborough and Poland arranged as pairs.
- Fig.10 Somatic complements of two different German strains of M.bifolium ($2n = 36$) arranged as pairs.
- Fig.11 Somatic complement of a single strain of M.canadense var. interius ($2n = 72$) arranged as quartets.
- Figs. 12, 13 and 14 Somatic complement of strain of M.canadense ($2n = 54$) arranged as trios.
- Fig.15 Somatic complement of M.dilatatum ($2n = 36$) arranged as pairs. In the figure the corresponding idiogram is also shown.

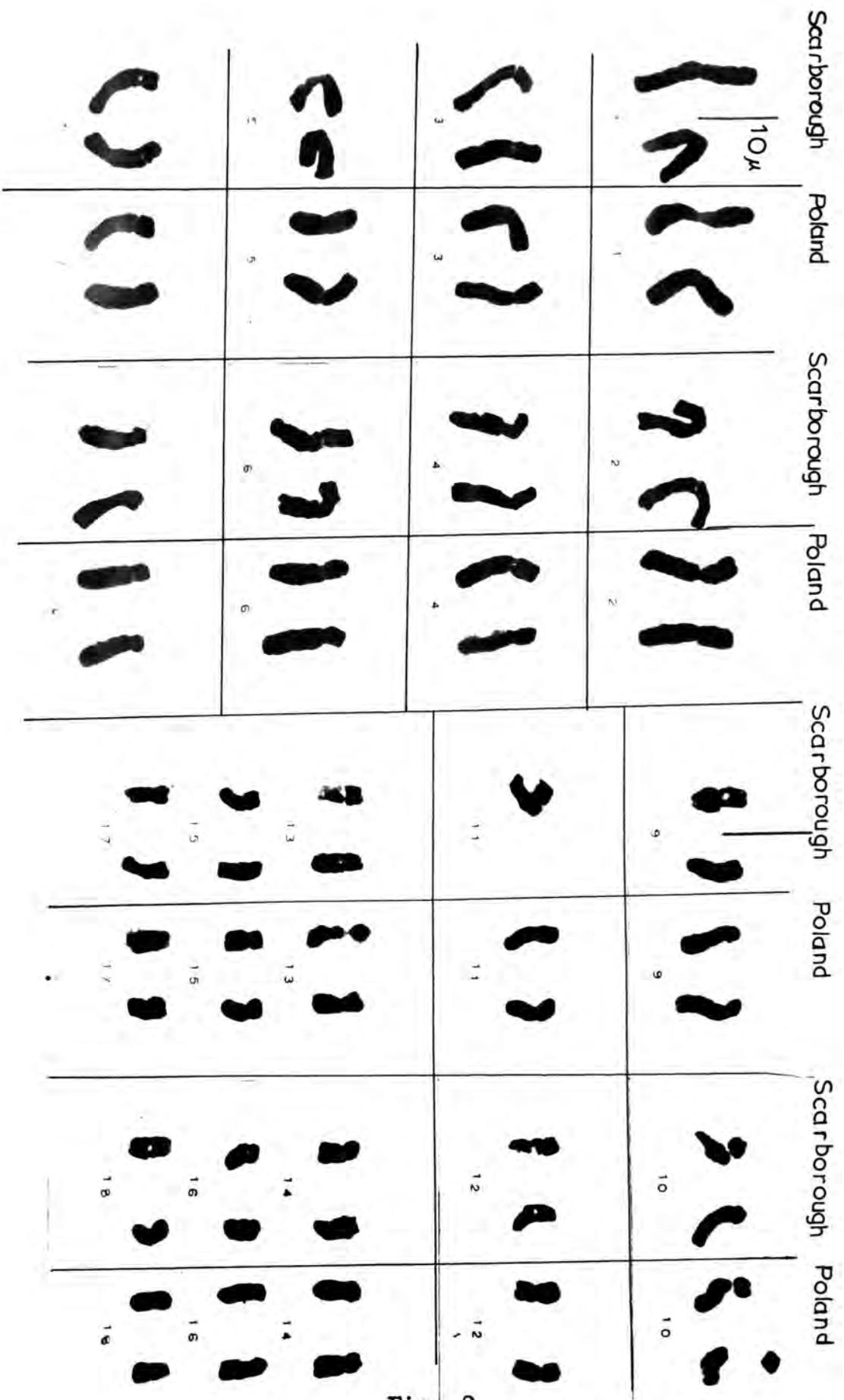


Fig. 9

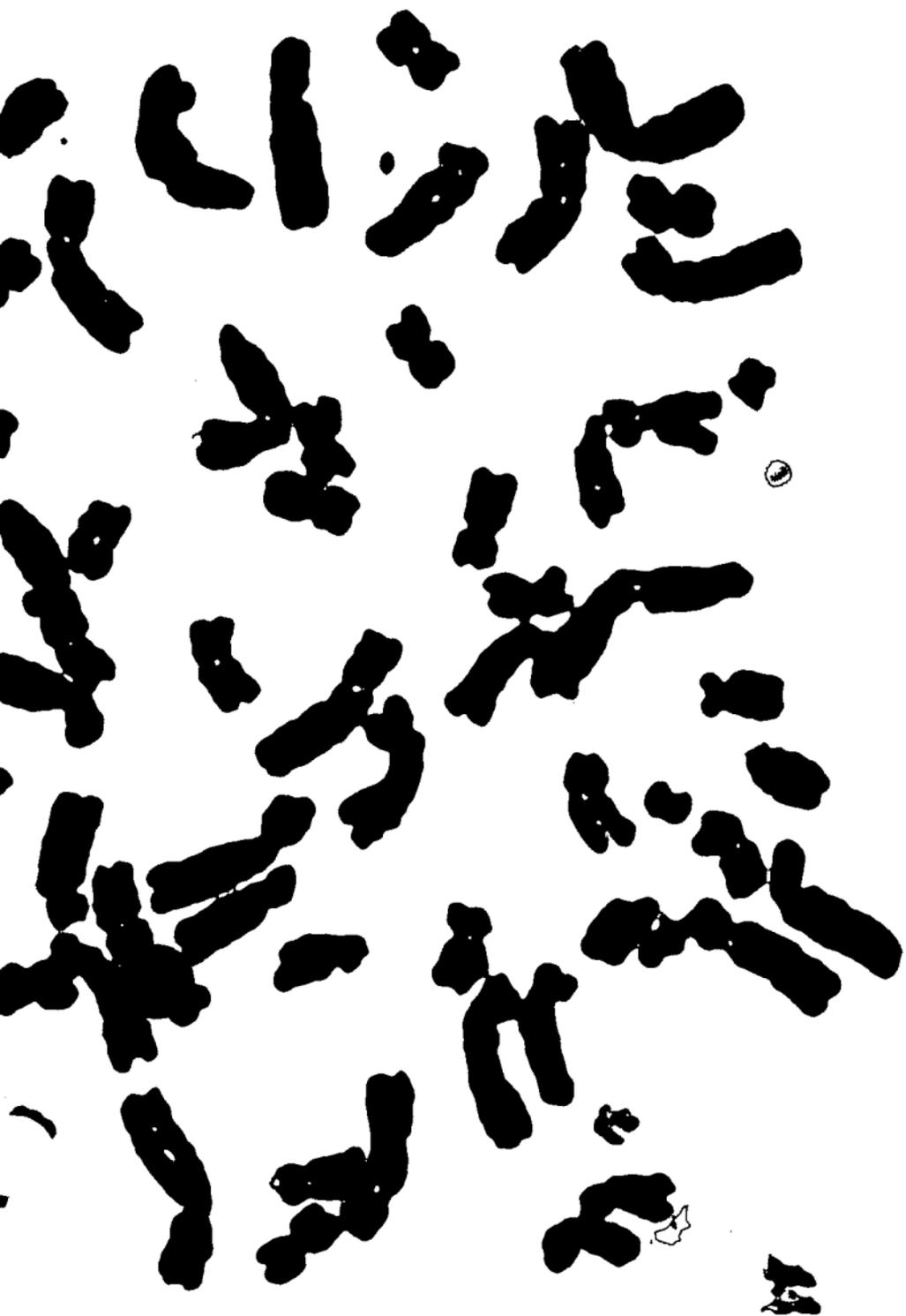


M. bifolium ($2n=36$)-see Fig.10-b
x 1500

M. canadense var. interius ($2n=72$) -see

Fig. 11. x 1500

N.B.: The two photographs are for one and the same somatic cell. Part
Some chromosomes are contained in both photographs and allowance must be made for these.





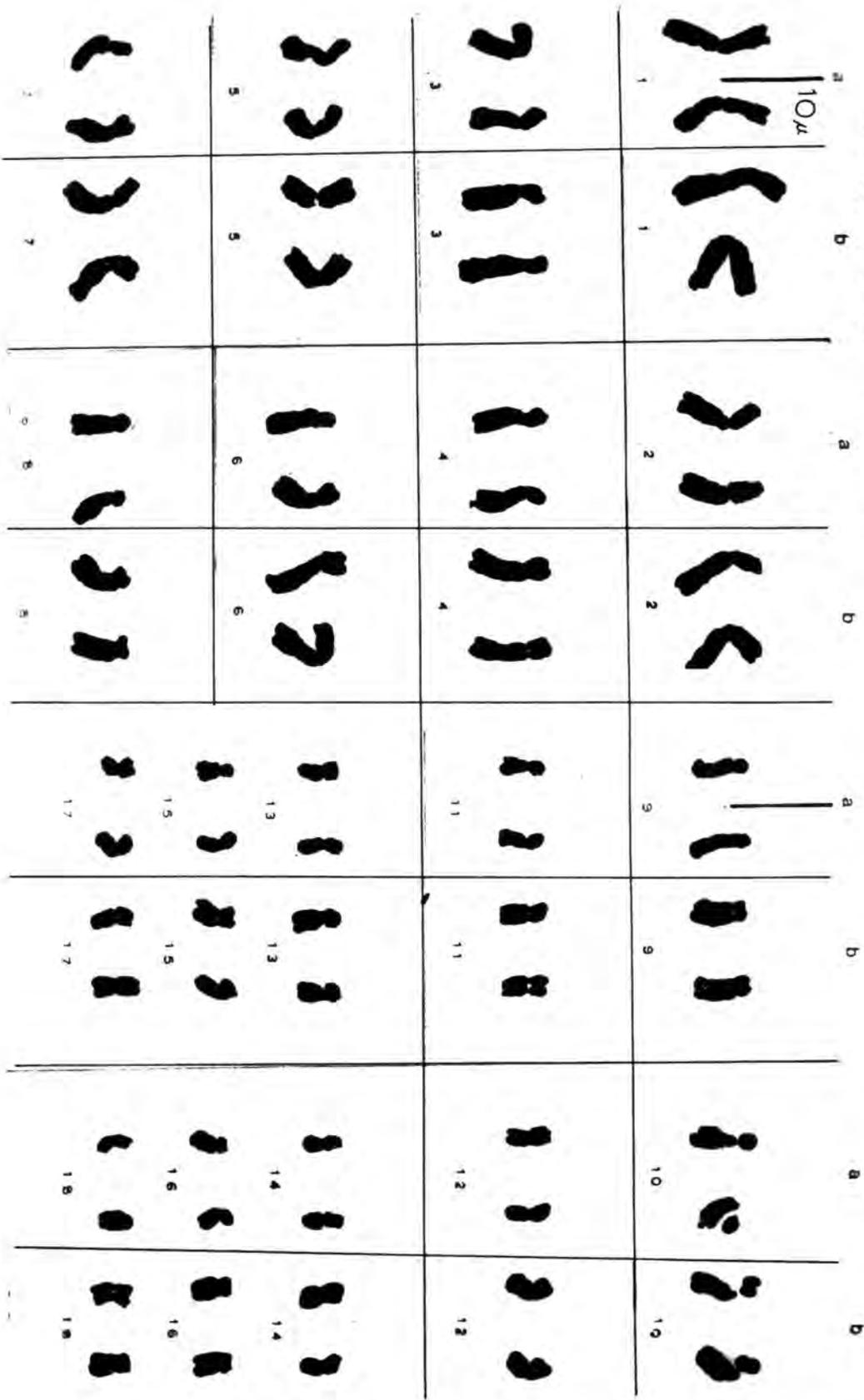
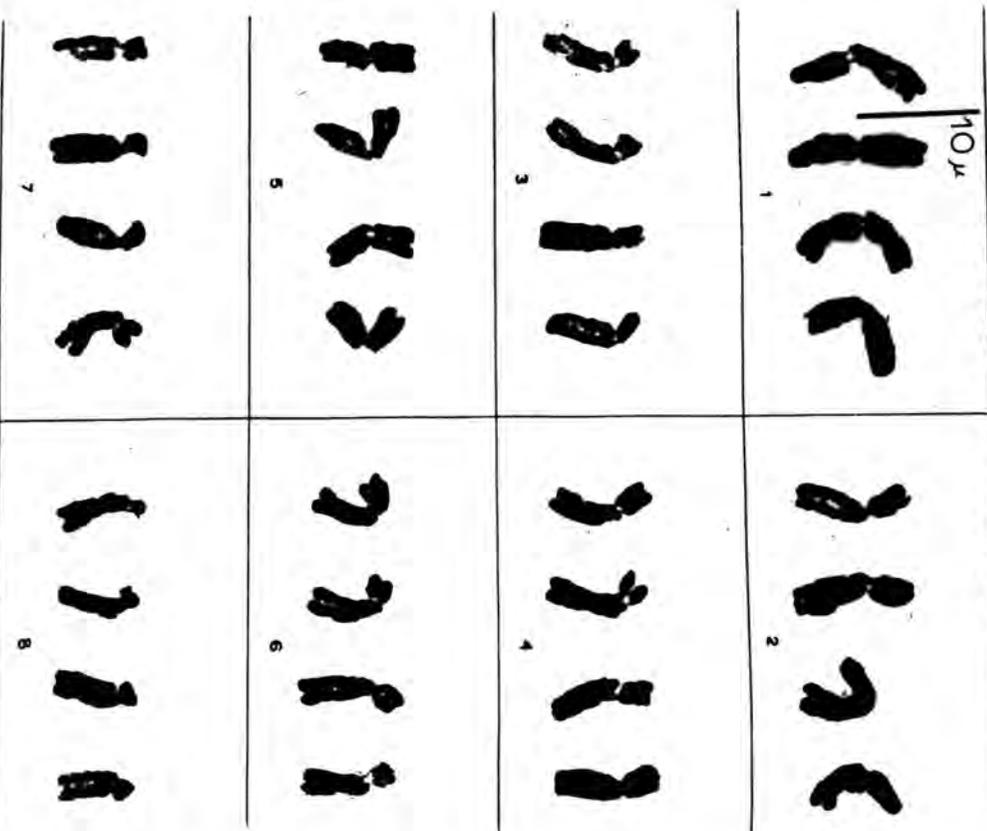


Fig. 10

M. canadense var. *interius*



M. canadense var. *interius*

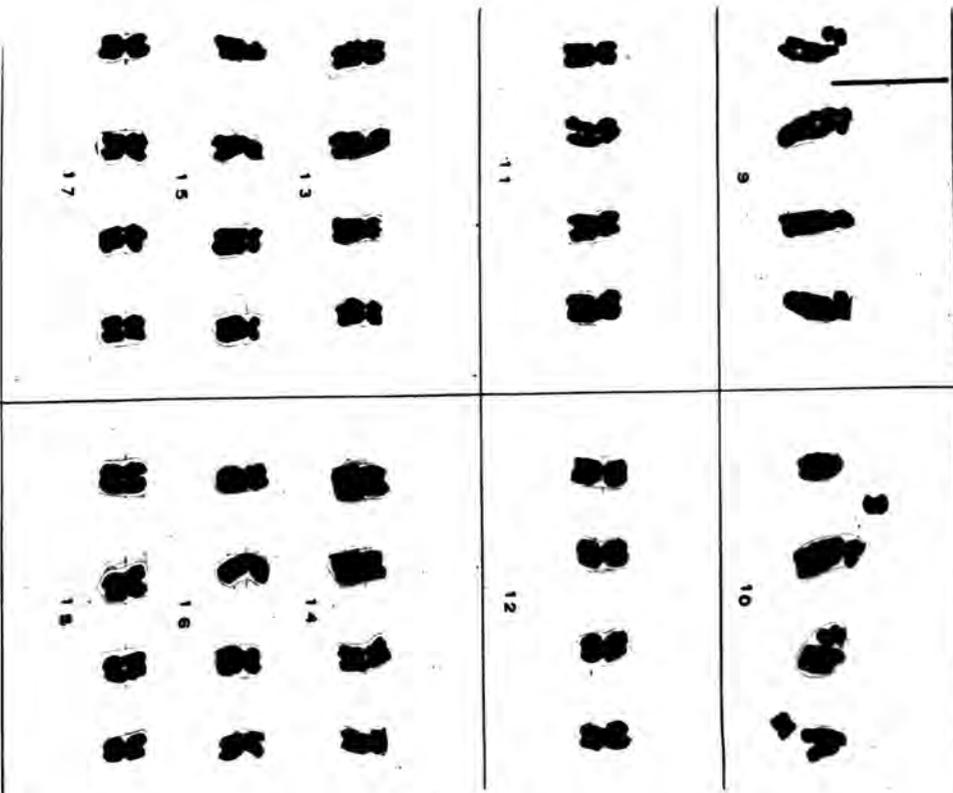


Fig. 11



M. canadense ($2n=54$)- see Fig. 12
x 1500

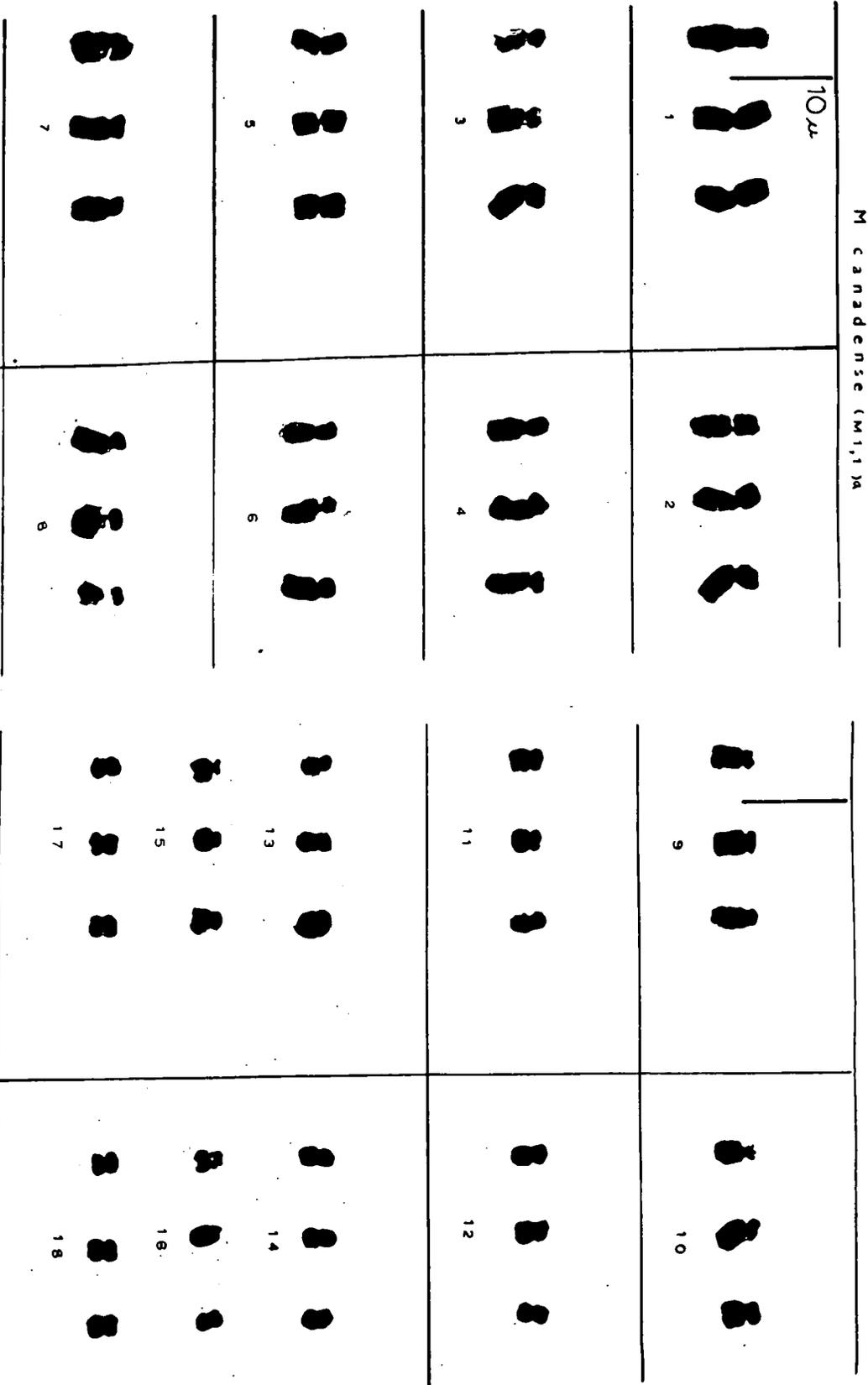


Fig. 12

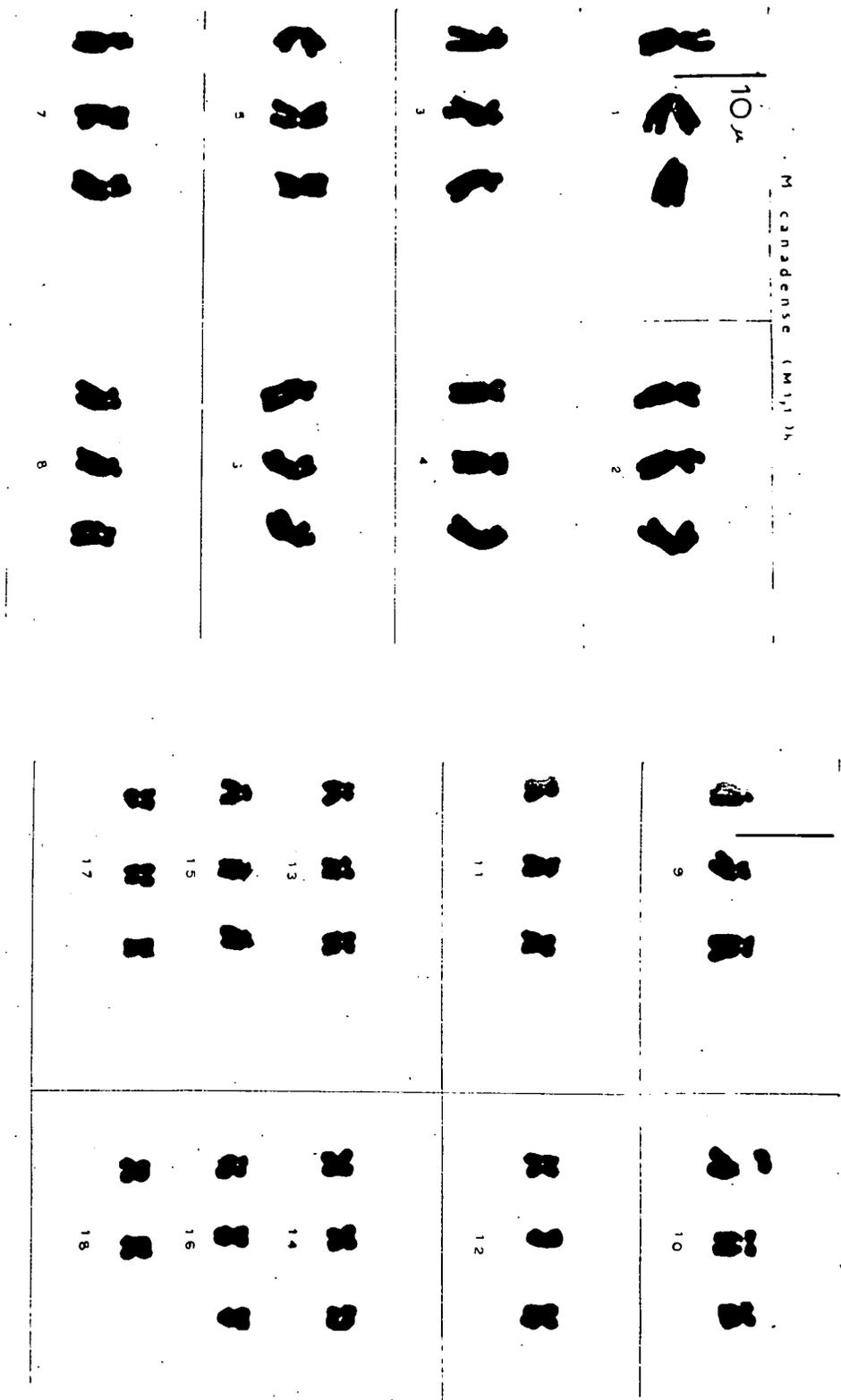


Fig. 13

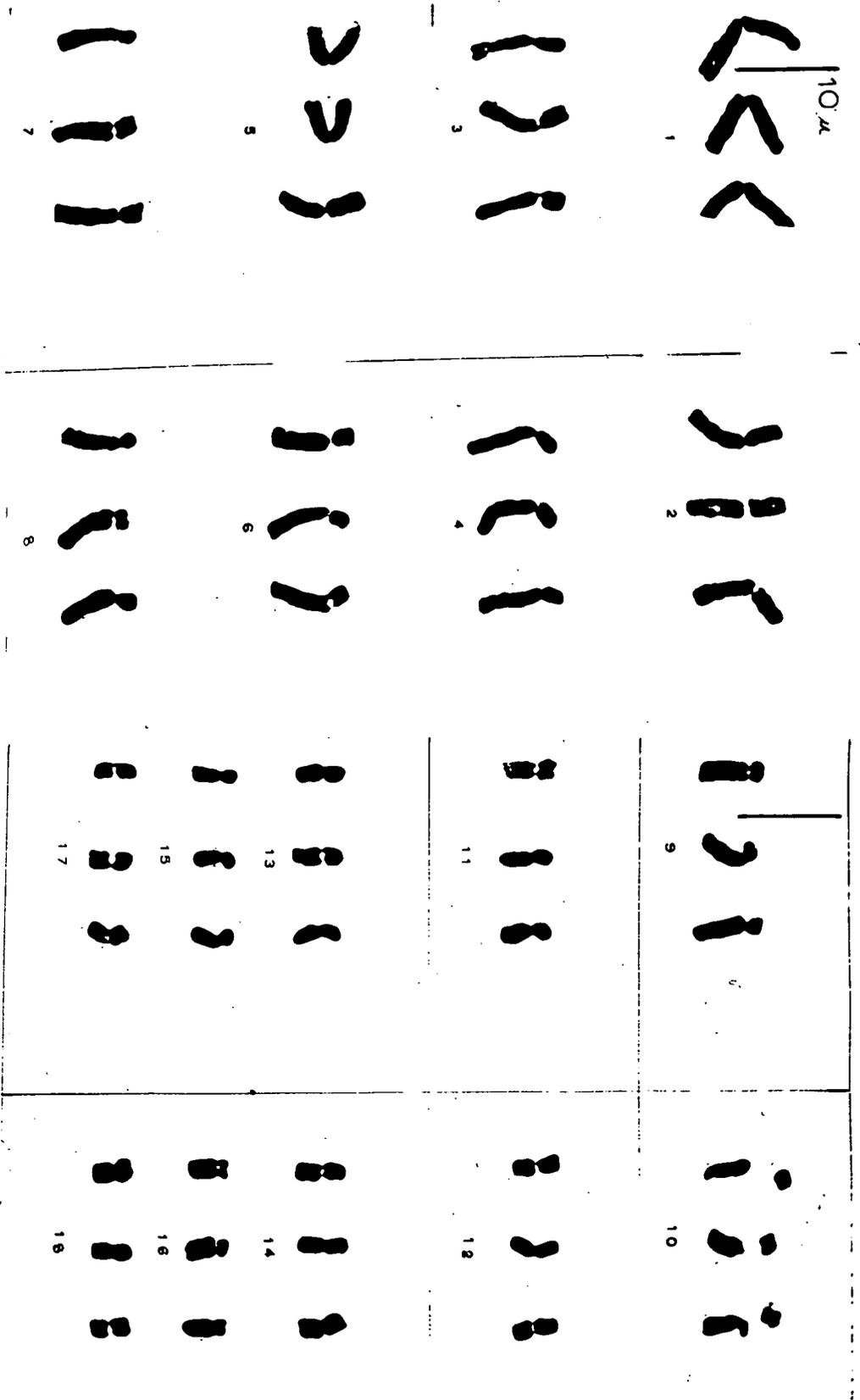


Fig. 14
14

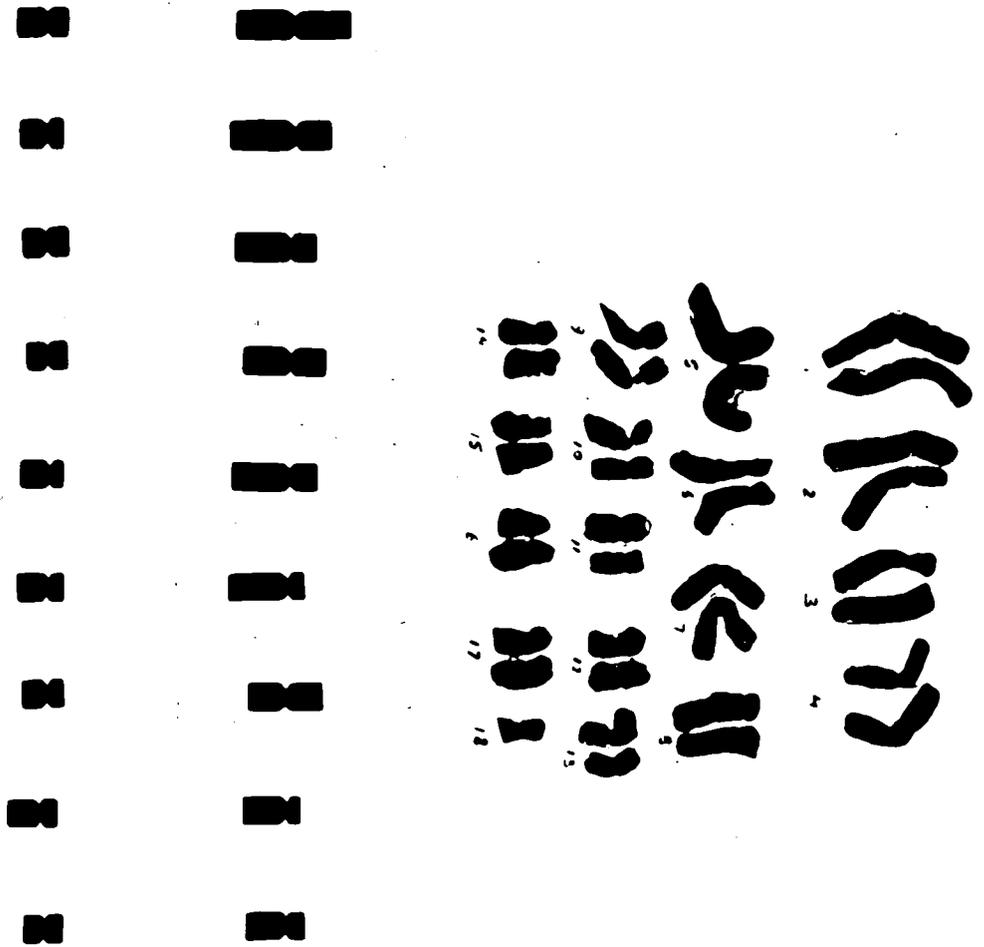


Fig. 15 : Somatic chromosomes and corresponding idiogram in M.dilatatum. This material has not been pretreated.

Fig. 16: Idiograms of Maianthemum, based on the means of chromosome pairs, trios or quartets.

16-a: Idiograms for M.bifolium $2n = 36$ from England, Poland, Denmark, and Czechoslovakia.

16-b: Idiograms for M.canadense var. interius (Minnesota) $2n = 72$, M.canadense (M4,1) Nova Scotia $2n = 54$, and M.canadense (M, 1,1) Quebec $2n = 54$. Open square represents satellited chromosome.

The chromosomes are arranged in order of length.

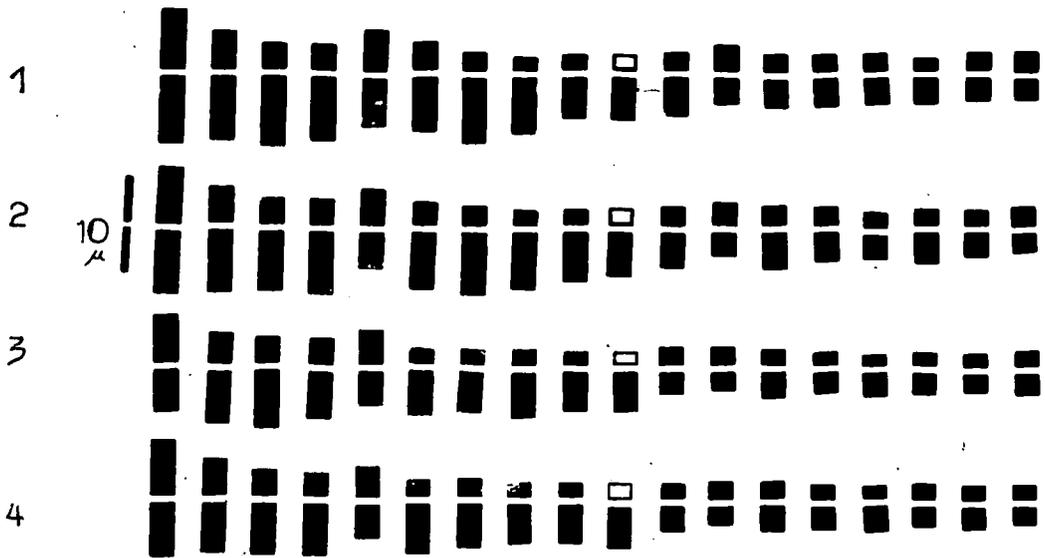


Fig. 16-a: Idiograms for the somatic karyome of British (1), Polish (2), Danish (3) and Czech (4) material of M. bifolium. ($2n=36$)

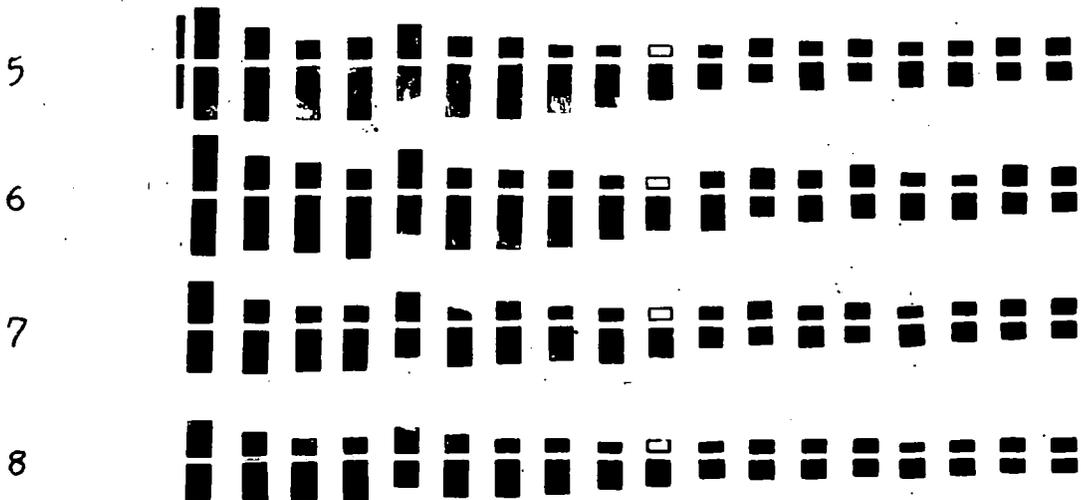
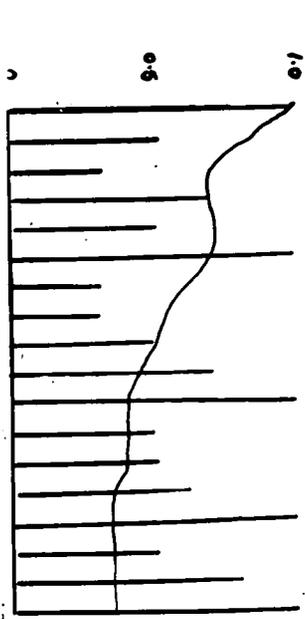


Fig. 16-b: Idiograms for the somatic karyome of American material; (5) var. interius ($2n=72$), (6) canadense, Nova Scotia ($2n=54$), and (7 & 8) canadense Quebec, ($2n=54$).

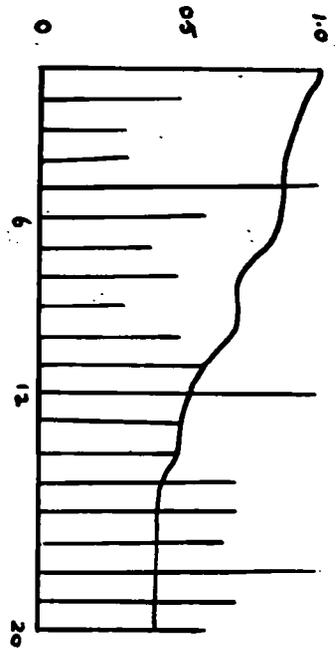
Fig. 17 Graphic representation of the karyotypes
 in Maianthemum.

- 17-1 For the hybrid canadense ($2n = 54$) x bifolium
 ($2n = 36$).
- 17-2 For M.dilatatum ($2n = 36$).
- 17-3 For M.canadense ($2n = 54$ - Quebec).
- 17-4 For M.canadense ($2n = 54$ - Quebec).
- 17-4 For M.canadense ($2n = 54$ - Nova Scotia).

The chromosomes of the diploids are treated in
pairs and those of the triploid as trios.
Continuous line indicates relative length.
Vertical lines indicate symmetry.

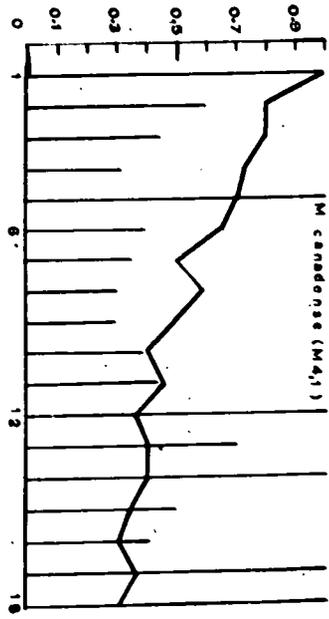


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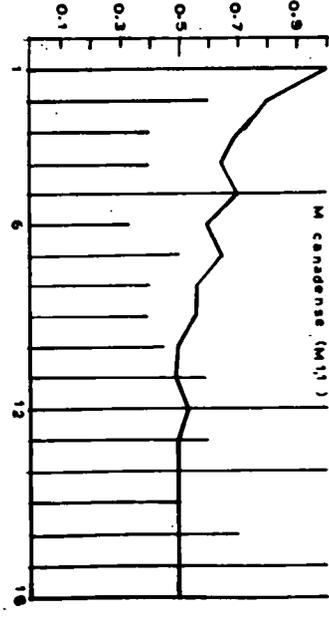


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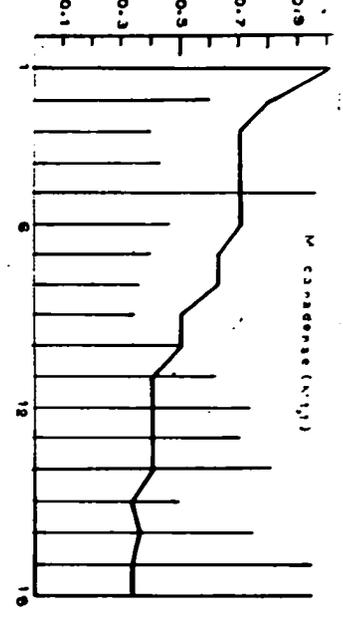
SYMMETRY AND LENGTH INDEX



(5)



(4)



(3)

Fig. 17

Fig. 18 Graphic representation of the
karyotypes of Maianthemum.

- 18-1 For M.bifolium, Scarborough, England.
- 18-2 For M.bifolium, Poland.
- 18-3 For M.bifolium, S. Germany.
- 18-4 For M.bifolium, Czechoslovakia.
- 18-5 For M.bifolium, Denmark.
- 18-6 For M.canadense var. interius (Minnesota)
($2n = 72$).

Legend as for Fig. 17.

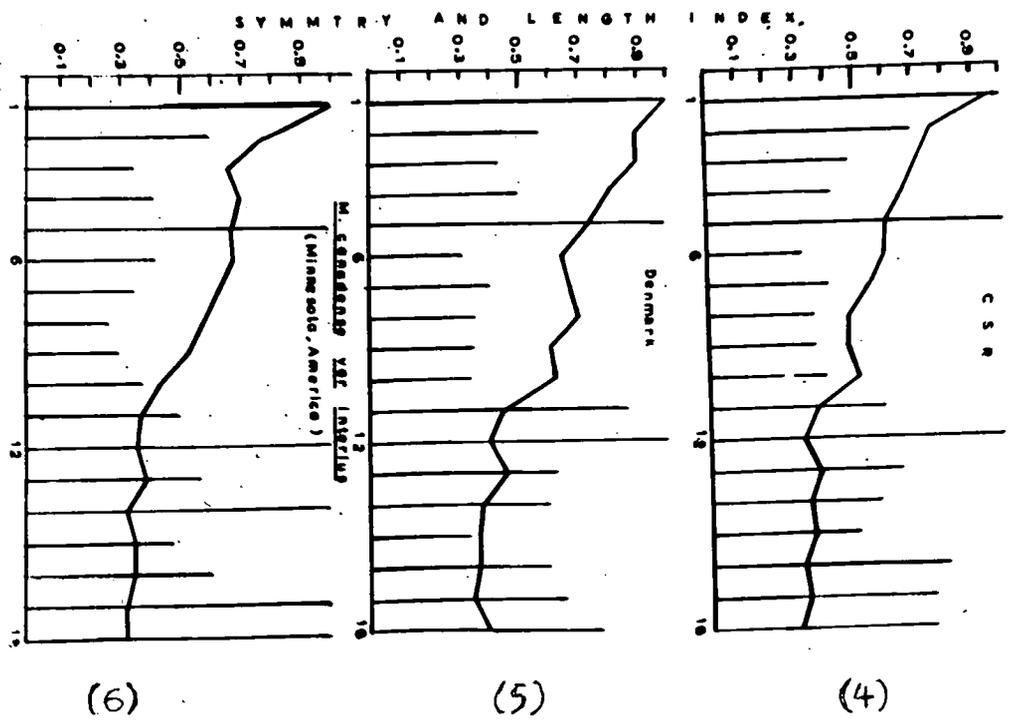
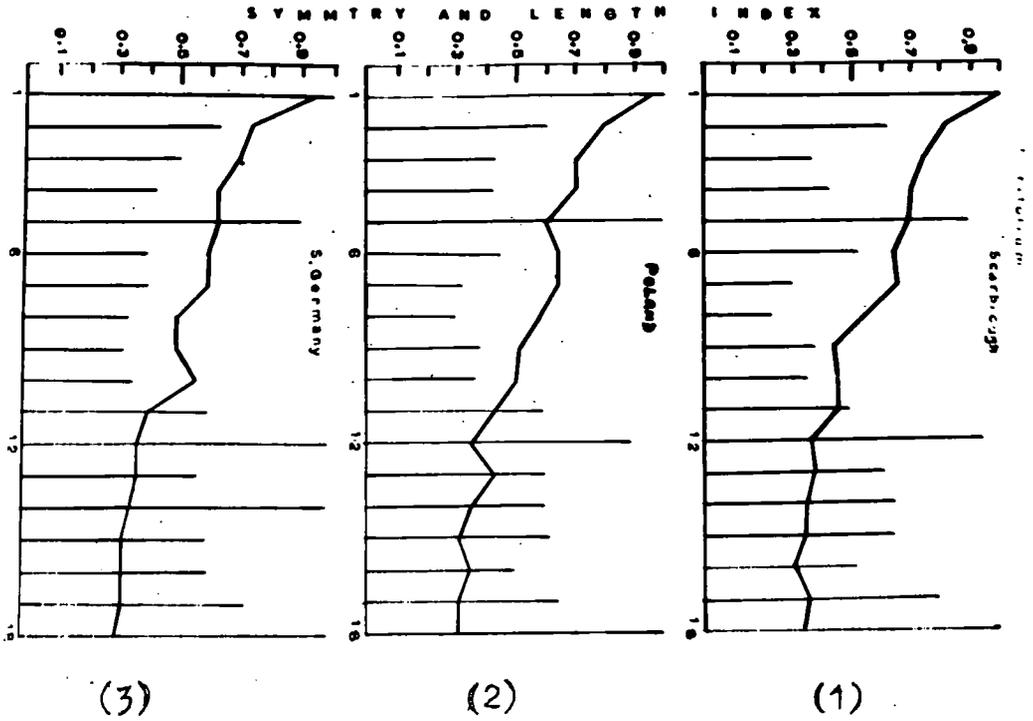


Fig. 18

Discussion of cytological results

1. Somatic Karyomes

The only other karyotype studies on Maianthemum are those of Matsuura and Suto (1935) on M.dilatatum Sato (1942) on M.bifolium and M.dilatatum, and very recently Kawano et al. (1967) on all species of Maianthemum.

Matsuura and Suto give the haploid number for dilatatum as $n = 16$ with the following comment "of the 16 bivalents, four are distinctly larger than the rest." This is not enough for us to make any comparison with other reports. We have already discussed this paper (p.42) and since we have not been able to examine meiosis in dilatatum, we can not even confirm this description.

Sato (1942) gives the counts of $2n = 54$ for all species of Maianthemum he worked on. Our results would lead us to expect seven trios of larger and eleven trios of small chromosomes, with one trio of satellited chromosomes. What Sato records for both species, is one quartet of large chromosomes and 50 small chromosomes, of which one quartet was satellited. We cannot at the present explain this difference; examination of more Japanese material both taxonomically and cytologically, is much to be desired. This fortunately, is done by Kawano et al. (1967). Their findings on the material examined and the detailed analyses of the chromosome complement of the species of Maianthemum "demonstrated

that all three possess an identical or at least basically very similar somatic karyome." On comparing their results with ours we find that the agreement is substantial indeed.

Their work is of great value as it covers a good deal of Japanese material not available to us, and thus a better understanding of the various taxa is possible.

We have, however, found it necessary to look into their data, and sometimes to analyse it in the manner we did ours in order to establish a basis for comparison.

The following points are obtained and/or calculated:

1. Ploidy level.
2. Absolute chromosome length.
3. Number and size of satellited chromosomes.
4. Ratio of total length of short arms to total length of long arms in the complement, i.e. symmetry.
5. Variation in chromosome length.
6. Karyotype formulae.
7. Geographical area.

Of these, 1, 2, 3, 6 and 7 are given in the original paper; whereas 4 and 5 have been calculated. Ploidy level and geographical area are mentioned where relevant.

Absolute chromosome length:

This is given in the following table (Table 22)

Table 22Absolute chromosome length in MaianthemumMeasurements in microns

Author	<u>bifolium</u>	<u>canadense</u>	<u>dilatatum</u>
Own	4.0 - 12.0	4.0 - 12.0	4.0 - 11.0
Kawano	2.1 - 8.0	3.6 - 12.0	2.0 - 9.0

The observed difference between our results and those of Kawano with regard to bifolium is apparently, significant; but we think of it as originating from differences in cytological technique and other technical factors such as pre-treatment and degree of contraction of the metaphase chromosomes at the time of fixation. The species, in our opinion, are very similar.

Number and size of the nucleolus chromosomes are given in Table 23.

Table 23Number and size of Satellited chromosomes

	<u>bifolium</u>	<u>canadense</u>	<u>dilatatum</u>
<u>Own</u>			
Number	2	3 & 4	2
size	6.6	5.3	4.2
<u>Kawano</u>			
Number	2	2	2
size	4.1	5.3	3.7

The difference in the chromosome size in bifolium in the two samples is possibly a result of treatment and of degree of contraction prior to fixation as explained above. The difference in number for M.canadense is due to the fact that in our material we have established a count of $2n = 54$ (triploid) and a count of $2n = 72$ (tetraploid), hence the corresponding increase in the number of satellited chromosomes. Kawano and associates have not reported any chromosome races in all the species, possibly because of the limitation of their samples, as it is apparent that their American clones have been collected from a single locality, i.e. Trout Lake Point, Vilas County, Wisconsin. Our material covers four different localities as shown in Appendix 3 and therefore possibly different in origin.

Symmetry

The ratio of the total length of short arms to that of long arms is given in Table 24.

Table 24

Ratio of short arms to long arms
in the somatic complement of the various taxa

	<u>bifolium</u>	<u>canadense</u>	<u>dilatatum</u>
<u>Own</u>	0.56	0.58	0.60
<u>Kawano</u>	0.57	0.56	0.52

The difference in symmetry in dilatatum complements from two different parts of its range is interesting. Our material is from California, that of Kawano is from Japan. There seems to be a reason for this as symmetry is a ratio and is not affected by degree of contraction or by technical factors. This however needs to be carefully investigated in larger samples and for the time not much weight should be laid on it pending further analysis.

Variation in chromosome length

We have established in our material seven large and eleven small pairs of chromosomes and we have looked into the table in the original paper of Kawano to see how far the individual chromosomes (as pairs) vary amongst themselves. The assembled data are given in Table 25.

Table 25

Variation in chromosome length.

Length expressed as an index whereby
the largest pair is taken as unity, and
the others as a fraction to the largest.

	<u>bifolium</u>	<u>canadense</u>	<u>dilatatum</u>
<u>Own</u>			
<u>Large</u>	7(0.65-1.0)	7(0.64-1.0)	7(0.62-1.0)
<u>Small</u>	11(0.34-0.54)	11(0.35-0.55)	11(0.23-0.55)
<u>Kawano</u>			
<u>Large</u>	7(0.66-1.0)	7(0.55-1.0)	7(0.6-1.0)
<u>Small</u>	11(0.26-0.51)	11(0.3-0.51)	11(0.23-0.5)

Thus it is evident that there is no marked difference and that the data of Kawano and co-workers are completely in line with ours.

Karyotype formulae

We have discussed in a great detail the karyotypes of the various taxa and their chromosome races and have come to the conclusion that they do not provide us with any information of taxonomic importance in distinguishing the species, (p.54). The various formulae are given in Table 21, and are here summarised giving only the most typical formulae, where:

M = metacentric Sm = Submedian St = Subterminal

M. bifolium (as pairs) $2n = 36 = (1M+1Sm+4St)+(4M+4Sm+4St)$

M. canadense (as trios or quartets)

$2n = 54$

$2n = 72 = (1M+1Sm+4St)+(4M+4Sm+4St)$

M. dilatatum (as pairs) $2n = 36 = (1M+1Sm+4St)+(4M+4Sm+4St)$

Thus the karyotype (in any of the figures 9-to-16) is composed of one large metacentric pair (No. 1) and one large submediocentric pair (No. 2) and then four pairs of J-shaped chromosomes all possibly subterminal. The remaining chromosomes are rather small in size and consist of four pairs of ^{sub-}metacentric chromosomes, one of which (No.10) is satellited, and eight pairs of J-shaped chromosomes.

This is almost identical to the general description of the karyotype of Maianthemum as reported by Kawano and co-workers (Kawano's figures are reproduced below for easy reference) which runs as follows:

"A close examination of the karyotype of this species (M.bifolium), reveals that of the eighteen pairs in the chromosome complement, two pairs, one large and one medium-sized, (Nos. 1 and 2), are meta-centric, eight pairs are J-shaped with the constriction submedian or subterminal (Nos. 3-10), one of which (No. 10), is nucleolar chromosomes. The remaining eight pairs are contrastingly small, of which four pairs (Nos. 13, 14, 15 and 16) are evidently metacentric but the remaining four pairs (Nos. 11, 12, 17 and 18) possess submedian or subterminal constriction."

"The karyotype of this species may roughly be expressed as follows:

$$K(2n)=36=4V(2v+2v)+16J+16V(\text{possibly } 8j+8v)"$$

The underlining is ours. It serves to point out the following:

1. That the second pair is not chosen in respect to size but to the position of the centromere. This same medium sized chromosome with its metacentric constriction is placed in the fifth position in all our figures and its place is taken by the second largest pair which is submediocentric.
2. That the agreement is evident in the case of the satellited chromosomes both in number and position in the karyotype.

The data presented by Kawano (1967) regarding chromosome length, briefly dealt with earlier, have been assembled with ours in Table 26. This variation in chromosome length within the complement is expressed as an index whereby the largest chromosome is considered as unity of which the shorter ones are expressed as fractions. The

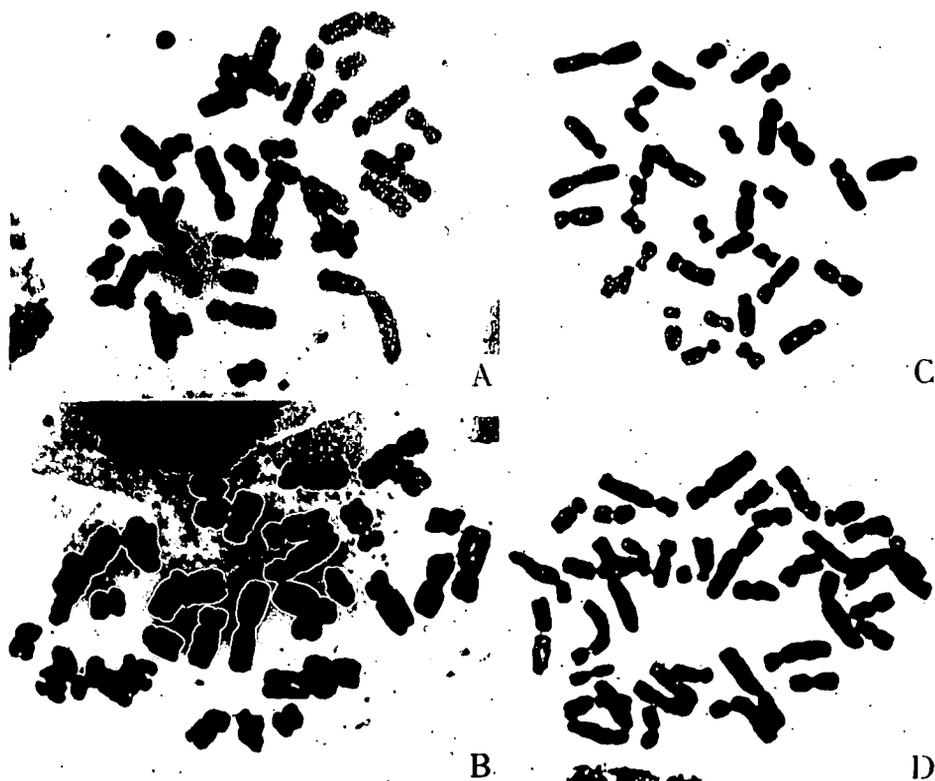


Fig. 1. Somatic chromosomes of *Maianthemum*. A, *M. bifolium* from Fuji-Aokigahara, Yamanashi ($2n=36$); B, *M. bifolium* from Fuji, Motosu, Yamanashi ($2n=36$); C, *M. dilatatum* from Nikko, Karikomi, Tochigi ($2n=36$); D, *M. canadense* from Trout Lake Point, Vilas County, Wisconsin.



Fig. 2. Somatic chromosomes of *M. bifolium* ($2n=36$, from Fuji-Aokigahara).

The cytological preparations were made by using modifications of the oxy-quinoline-aceto-orcein squash method (Kawano¹⁹). Voucher specimens are preserved in the Herbarium of the Department of Botany, University of Tokyo (TI) and in the Herbarium of the University of Wisconsin, Madison (WIS).

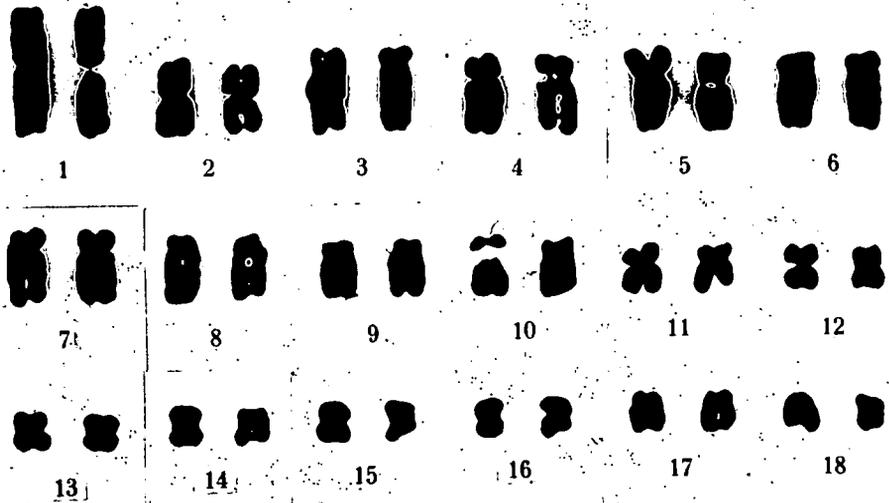


Fig. 3. Somatic chromosomes of *M. dilatatum* ($2n=36$, from Fuji-Aokigahara).

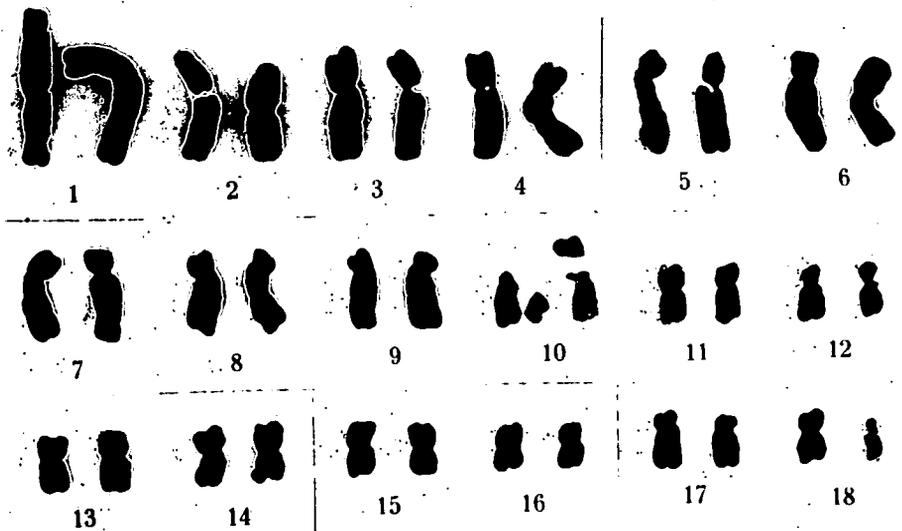


Fig. 4. Somatic chromosomes of *M. canadense* ($2n=36$, from Trout Lake Point, Vilas County, Wisconsin).

Table 26

Relative chromosome length in the somatic complement of *Maianthemum* (microns).

Kawano's material is all Japanese except *M. canadense*.

Pair No.	KAWANO			PRESENT RESULTS			
	<u>bifolium</u>	<u>canadense</u>	<u>dilatatum</u>	<u>bifolium</u>	<u>canadense</u>	<u>interius</u>	<u>dilatatum</u>
1	7.8	12.1	9.1	12.0	9.0	12.0	13.0
2	5.8	8.5	5.6	10.0	8.0	10.0	10.0
3	7.2	7.8	6.4	9.0	7.0	8.4	9.4
4	6.7	7.7	5.4	8.0	6.0	9.0	9.4
5	6.1	7.2	5.6	7.5	6.0	8.2	9.0
6	5.8	7.0	5.4	7.5	6.0	9.0	8.5
7	5.2	6.7	5.4	7.0	6.0	8.0	8.0
8	3.9	6.2	4.5	6.0	5.5	7.0	7.5
9	3.9	5.8	3.9	6.0	5.0	6.6	7.0
10	4.1	5.3	3.8	6.5	4.7	5.4	6.0
11	3.6	4.0	2.9	4.5	3.5	4.0	6.5
12	3.3	3.9	3.2	4.0	4.0	4.0	5.0
13	2.8	4.0	2.6	4.5	3.5	5.0	5.3
14	2.8	4.1	2.7	4.0	3.5	4.0	5.5
15	2.2	3.6	2.6	4.0	3.0	4.0	4.7
16	2.2	3.2	2.6	4.0	3.0	5.0	4.3
17	2.1	3.8	2.5	4.0	3.0	4.0	5.0
18	2.1	3.6	2.1	4.0	3.0	4.0	4.3

Kawano's results are presented by continuous lines in Fig. 19;

ours by dotted lines in the same figure.

graphs are plotted in the manner described earlier for Maianthemum (p.48) and are presented in Fig. 19.

It will be seen from the Figure that the pattern of variation exhibited by the Japanese material is identical to that of European and N. American material. The slight variation is attributed, possibly, to other factors such as the degree of contraction of the chromosomes and possibly in differences in the techniques. The major observed difference is the depression of the curve for the Japanese material at the point of the second chromosome pair. This has already been explained (p.61).

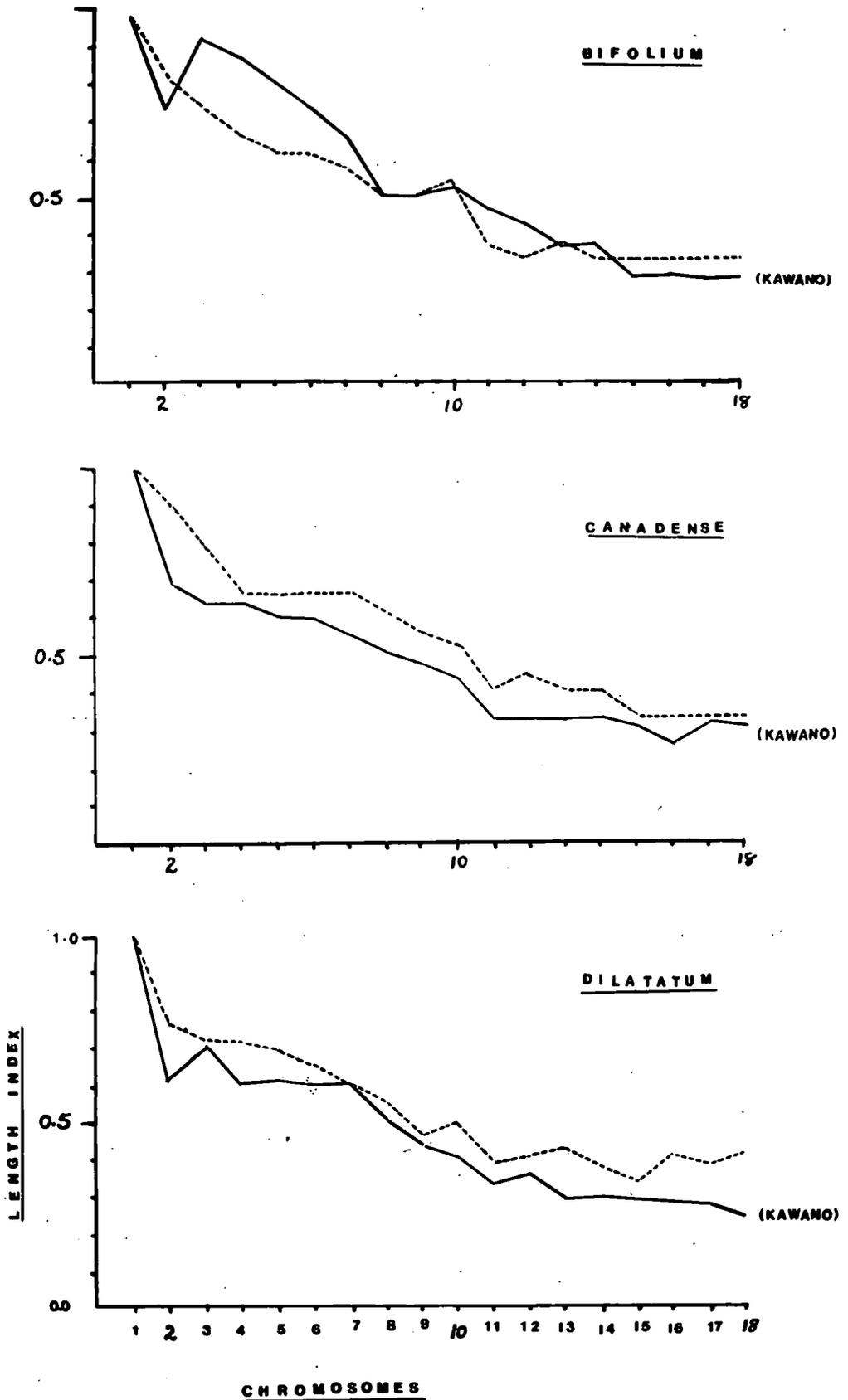


Fig. 19

CHAPTER SIX3. Studies of meiosis

As mentioned earlier, the species of Maianthemum do not always flower every year (p. 25). This has certainly limited our scope as regards the study of meiotic processes.

Whenever available, flower buds were collected and fixed in 1 : 3 acetic alcohol. A simple acetic-orcein or acetic-carmin technique was employed, and the slides, following examination, were made permanent by using dry ice and Euparal.

Meiosis in M.bifolium

Meiotic division has been studied in material originally obtained from several parts of the species range i.e. Britain, Germany, Poland and Czechoslovakia. We are now going to describe meiosis in clones from each of these areas.

(a) British material (code N4,2)

Metaphase I: most of the cells are found to have 18 bivalents, and some of these are illustrated in Fig. 20-1, 20-2, 20-3 and 20-4.

In Fig. 21 (top right) a group of four diakinesis cells have had their complements arranged in order of length, with an absolute length of 3 to 10 microns. One bivalent is seen closely associated with the nucleolus.

A count of $n = 18$ is not the sole one observed in this material. In some cells there are only 17 apparent bivalents. This could be due to the close association observed between some bivalents as in Fig. 20-7. On further examination, quadrivalents were observed and it is thought

that those complements possessing 17 bodies are likely to involve a quadrivalent. Fig. 20-5 and 20-6 show some such metaphase one cells including a quadrivalent in each case.

Metaphase I (side view): An exact analysis of the complements at this stage was not possible, but quadrivalents were occasionally observed. Some cells at this stage with 1 to 2 quadrivalents are shown in Fig. 21 (bottom left).

Anaphase I: In Fig. 21 (bottom right) 18 daughter chromosomes are seen at anaphase one. Anaphasic stages are generally very regular. There are, however, some observed abnormalities and these are here illustrated in Fig. 20-8, where in this group of PMC's we have an inversion bridge and a fragment in the top cell.

Second division: Second division in this material is mostly regular. Some very rare irregularities are shown in Figs. 20-9 and 20-10 where in the former we have a persistent bridge (possibly anaphase I bridge): and in the latter we have a lagging univalent. In Fig. 21-1 two lagging and dividing univalents are seen, one of these is satellited. The orientation of the chromatids of each suggest that this is possibly due to other factors such as squashing. In Fig. 21-2 micronuclei and a thick bridge are seen. The nature of the bridge is difficult to explain. In Fig. 21-3 a thin bridge connects two non-sister sets. Such bridges could be attributed to inversions incorporated in the chromosomes of the first anaphase polar sets.

German material (code N4, 1)

Diakinesis: Six selected cells are shown in Fig. 22 with their

complements arranged in order of length. The absolute length is 3 to 10 microns. Only one bivalent per cell is seen firmly attached to the nucleolus.

Metaphase I: This is very regular with the exception of the very rare occurrence of quadrivalents; one cell is shown in Fig. 22 (bottom centre).

Anaphase one: Bivalent members usually separate normally except in cases of difficulty due to chromosome stickiness where they lag. Fig. 22 (bottom left) is an example of such abnormality. Fig. 22 (bottom right) illustrates a bivalent and a fragment, possibly an inversion bridge.

Second division: Second division in this material is very regular.

Czechoslovakian Material (code N1, 1 and N5, 1)

Meiosis in N1, 1: Meiosis in this material is very regular; but the few disturbances observed are interesting. Fig. 23-1 shows a cell at a very early prophase exhibiting fragmentation and micronuclei formation: the reason for such behaviour is not known.

At diakinesis most of the chromosomes form bivalents, but it has been seen that some of the cells contain only 17 bodies. Further investigation revealed the presence of quadrivalents. The top cell in Fig. 23 illustrates a normal cell at diakinesis with $n = 18$. The middle row illustrates another with also 18 bivalents and two of these are closely associated with the nucleolus. The bottom row represents

one cell with only 17 bivalents and two nucleoli with their associated bivalents.

Metaphase I is generally normal, but we have seen quadrivalents at a very low frequency. Some such cells are shown in Fig. 23-2 and 23-3.

Anaphase I cells sometimes have only 17 bivalents at both polar sets. Such a cell is shown in Fig. 23-4. Figs. 23-5 and 23-6 are drawn to show the bridges and no exact analysis of the chromosomes is made. In Fig. 23-5 a bivalent bridge is seen; in Fig. 23-6 a bivalent bridge and a fragment are seen. Both bridges could be attributed to an inversion heterozygotes. Fig. 23-7 and 23-8 demonstrate further the occurrence of bridges. In Fig. 23-7 the bridge is attributed to an inversion crossing over; in Fig. 23-8 to difficulty of separation of a possible trivalent with a laggard univalent. Fig. 23-9 shows the occurrence of micronuclei.

A study was also made on a different plant from Czechoslovakia, code N5, 1. Four diakinesis cells from this plant are illustrated in Fig. 24. There are 18 bivalents in each, also one nucleolus and one associated bivalent, though rarely two nucleoli are seen (third row, Fig. 24).

Second division in this material is very normal.

Polish Material N5, 4.

Meiosis in this material is very regular; some of the few anomalies observed are illustrated here in Figs. 24-1, 24-2, 24-3 and 24-4, all at anaphase I.

It appears from the foregoing evidence that division in M.bifolium is fairly regular, resulting in the formation of bivalents in most of the cells except in certain cases where quadrivalents are formed. The most irregular material, comparatively speaking, is the British. M.bifolium in Britain is very rare and is found mainly at Scarborough. There are other scattered localities in the British Isles where the plant is probably introduced. It is quite possible that the population at Scarborough is also an introduction.

Fig. 20 Meiotic stages in *M.bifolium* (N4, 2)

2n = 36.

20-1, 20-2, 20-3 and 20-4: Metaphase I: plates with 18 bivalents each.

20-5 and 20-6: Metaphase I: plates with 16(ii) and 1(iv) each.

20-7 Metaphase I: plate with 18(ii), note the association of two bivalents (right hand corner).

20-8 First anaphase: bridge and a fragment, top cell.

20-9 Persistent bridge at telophase.

20-10 Fragment embedded in the wall at telophase.

20-11 A lagging univalent (top half) and a small group of laggards (bottom half) at telophase.

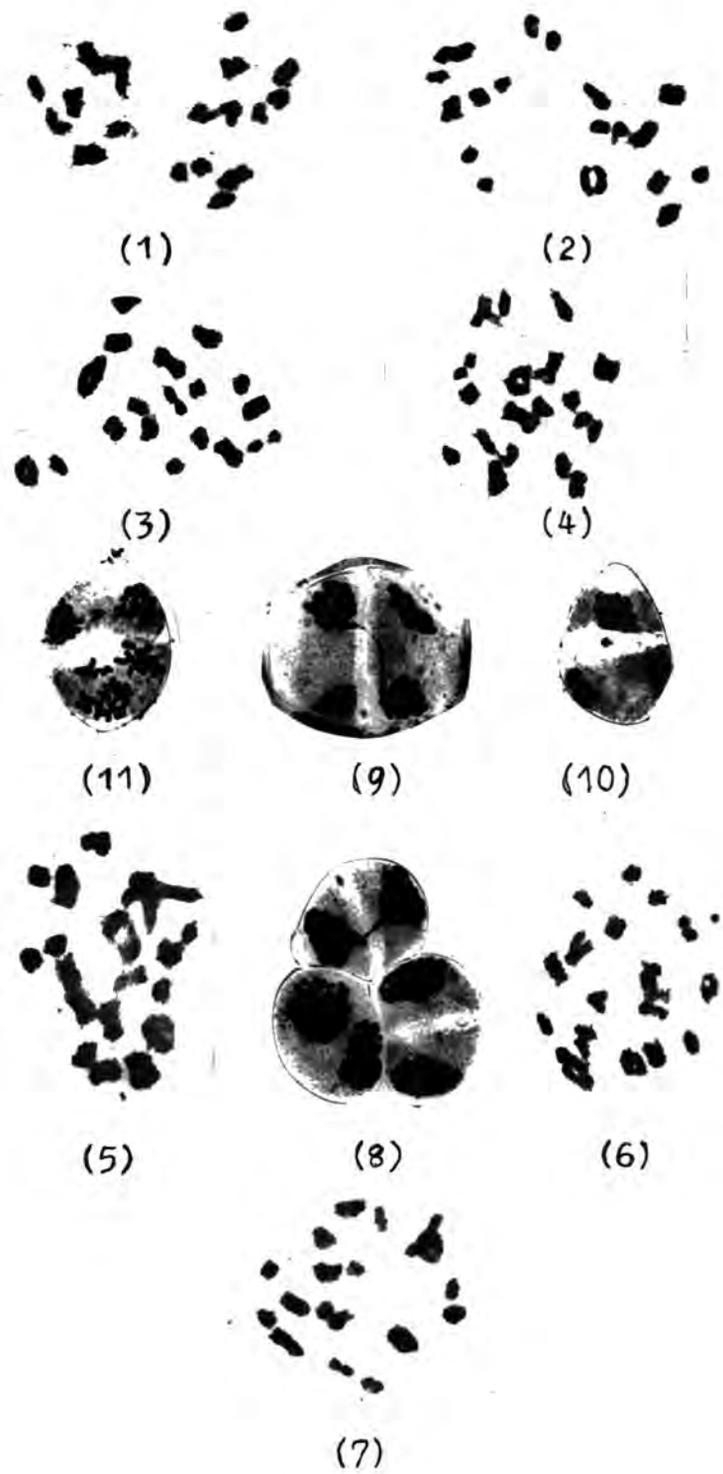


Fig. 20

Fig. 21 Meiotic stages in M.bifolium (N4, 2)

Top right: Four rows representing the complements of four complete nuclei at diakinesis and early metaphase I.

Note the nucleolus and the associated bivalent.

21-1 Late anaphase II with dividing univalents one of which satellited.

21-2 Double bridge and two micronuclei.

21-3 Single bridge connecting two polar sets at late anaphase II.

Bottom left: Side view of metaphase I illustrating some quadrivalents.

Bottom right: First Anaphase with 18 chromosomes.

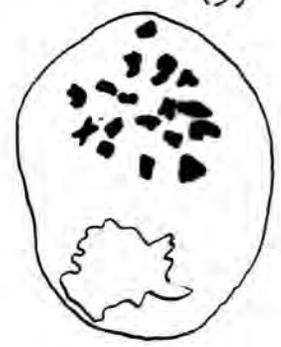
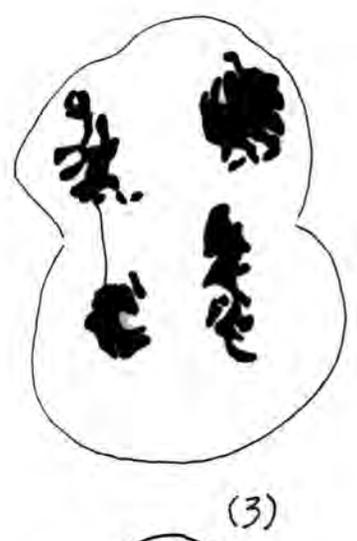
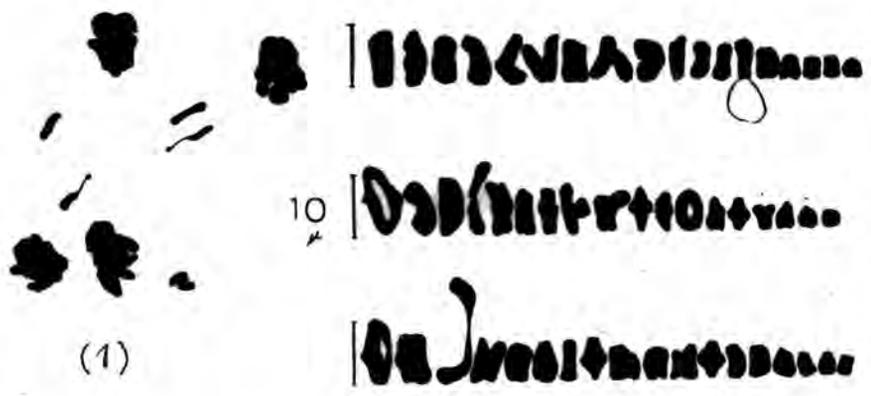


Fig. 21

Fig. 22 Meiotic stages in *M.bifolium* (N4, 1) $2n = 36$

Top: Six rows representing the complete bodies of six nuclei at diakinesis and early metaphase I. Note the nucleolus and associated bivalent.

Bottom left: Dividing lagging univalent and a bridge.

Bottom centre: Side view of metaphase I with two quadrivalents.

Bottom right: Univalent bridge and a fragment at first anaphase.

10. | **h v u n e d i a n d a n d e r s**

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| **h v u n e d i a n d a n d e r s**



Fig. 22

Fig. 23 Meiotic stages in *M.bifolium* (N1,1)2n=36

Top three rows: Complete bodies of three nuclei at diakinesis and early metaphase I, 18 bivalents in each.

- 23-1 Early prophase showing nucleolus, two micronuclei and three fragments. Unusual.
- 23-2 A quadrivalent at Metaphase I.
- 23-3 A figure of eight quadrivalent at first metaphase.
- 23-4 Anaphase I: Plate with only 17 bivalents.
- 23-5 Bivalent bridge at first anaphase.
- 23-6 Bivalent bridge and a fragment at first anaphase.
- 23-7 Double bridge at first anaphase.
- 23-8 Lagging trivalent and a univalent at first anaphase.
- 23-9 Two micro-nuclei at first anaphase.

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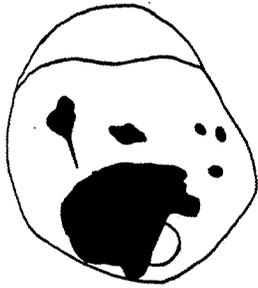
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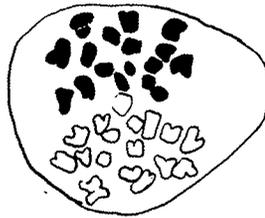
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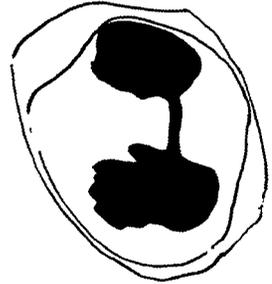
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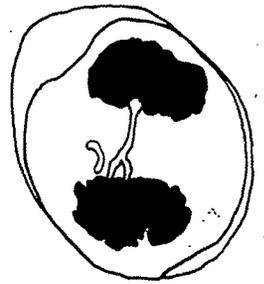
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(8)



(9)

Fig. 23

Fig. 24 Meiotic stages in M.bifolium

Top: Code N5,1. Four rows of complete four nuclei at diakinesis and early metaphase I. In the third cell there are two nucleolii and two associated bivalents - rare.

Bottom: Code N5, 4

- 24-1 Two lagging univalents at first anaphase; one already divided.
- 24-2 Bivalent bridge at first anaphase.
- 24-3 Two lagging trivalents.
- 24-4 Two lagging univalents at first anaphase.

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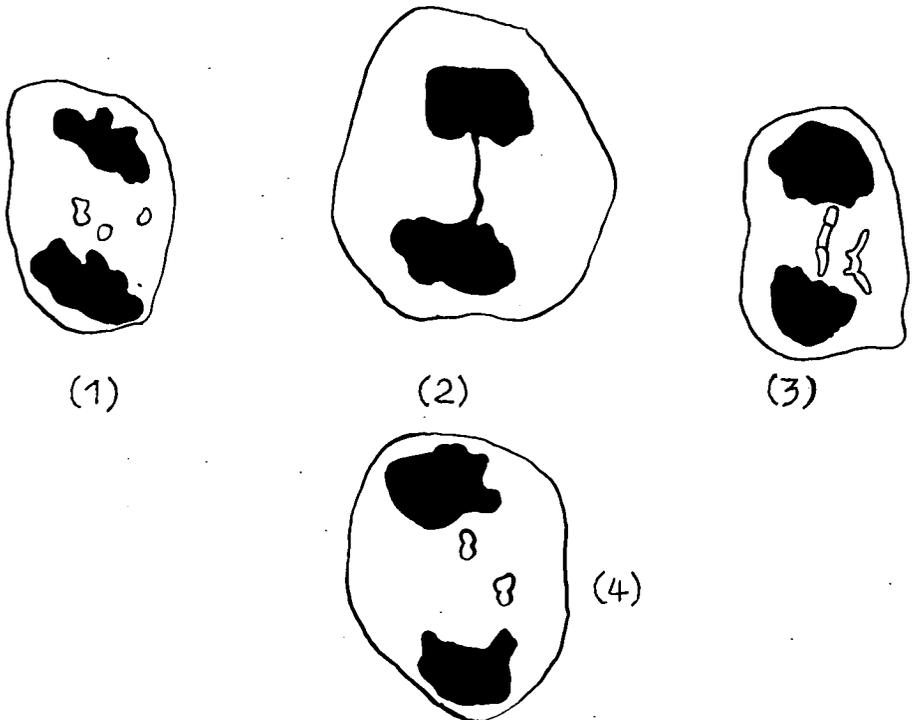


Fig. 24

Meiosis in diploid *M.canadense* (M6, 1)

The material on which this study is made was collected by Dr. T. Mosquin in the Gatineau Hills, P.Q., Hull, Canada. Meiosis has been found to be very regular at both the first and second divisions. No quadrivalents have been observed. Fig. 25 shows three late diakinesis cells each consisting of 18 bivalents of more or less the same absolute length as that seen in *M.bifolium*. There is also one nucleolus and one associated bivalent.

Meiosis in triploid *M.canadense* (M4, 1)

This material was collected by Dr. M.J. Harvey in Nova Scotia, Canada. Somatic chromosome counts reveal that it is triploid. Morphologically it is very similar to the diploid. There appear to be no differences in the vegetative features, apart from stomatal size (p. 22), but certain features of the flower and fruit are different. The pedicels are shorter and stouter, the flower buds attain a wider diameter before they undergo meiosis, the berry at maturity is of variable diameter but considerably larger than in the diploid and contains 2-3 seeds which appear to be similar but slightly larger than those of the diploid (p. 18).

Fig. 26-1 shows a cell at a very early prophase stage exhibiting the presence of paired and unpaired chromosomes. The unpaired one can be attributed to the presence of a univalent, which in turn could be part of a trivalent configuration. In Fig. 26-2



Fig. 25: Meiosis in diploid M. canadense. The 18 bivalents in each of the three metaphase I cells are arranged in order of length.

a possible inversion heterozygote is indicated by the characteristic loop. It could also be due to breakage reunion. In either situation the formation of bridges and fragments is possible.

Pollen-mother cells at metaphase I are difficult to analyse. Some few cells capable of analysis are illustrated in Fig. 27 and summarised in Table 27.

Table 27
Chromosome configurations at
metaphase I in triploid M.canadense

Cells examined	Univalents	Bivalents	Trivalents	Quadrivalents	2n
A	4	11	9	0	53
B	3	11	7	2	54
C	4	11	5	3	53
D	4	6	7	3	55
E	12	10	5	1	51
F	6	1	11	3	53
Mean	5	10	7	2	54

We will refer to this table later for discussion. At metaphase I (side view) it has been very difficult to study chromosome configurations and it has only been possible to count the univalents; the results are shown in Table 28.

Table 28

Distribution of visible univalents at
metaphase I (side view).

Univalents	0	1	2	3	4	5	6	7	8	9	10	11	12
Cells	42	-	2	32	40	57	38	28	20	19	9	5	3

Mean = 4.7 Univalents per cell.

At anaphse I many of the meiotic disturbances characteristic of triploids are observed; they will be briefly described here. It should be mentioned that in many of the observed pollen-mother-cells, the chromosome number is apparently variable, this is attributed to difficulty in getting well spread plates, as well as to the fact that some of the laggards do not reach the poles. The figures to be described below are drawn to illustrate the abnormalities rather than to give exact counts.

Fig. 28 shows a group of cells with varying number of laggards and bridges. Fig. 28-1, 28-2 and 28-4, are of individual cells with lagging univalents which have divided or are in the process of dividing, and rarely, bivalents. Fig. 28-1 shows a large lagging bivalent to the left, a bridge, and a univalent with its chromatids having their long arms stretched toward the poles. Fig. 28-2 shows two univalents, one already divided, and its chromatids approaching the poles, the other forming a univalent bridge. The nature of the fragment at the top right corner is not

known. Fig. 28-3 shows a lagging bivalent and three univalent laggards. Lagging bivalents, though infrequent, can be attributed to difficulty of separation.

Further anaphasic stages are illustrated in Fig. 29.

Fig. 29-1 shows two laggards, most likely to be bivalents suffering from difficulty of separation, possibly due to stickiness, as indicated by the presence of the bridges. Fig. 29-2 gives a possible segregation of 21 and 24. Fig. 29-3 gives a possible segregation of 19 plus 22 plus two lagging univalents and three fragments. Fig. 29-4 has an attenuated bivalent; Figs. 29-5, 29-6 and 29-7 show varying numbers of univalents some in the process of division, others divided, and in several of these dividing univalents, it is the long arm that seems to move first toward the pole. In Fig. 29-7 a bivalent bridge is seen. Fig. 29-8 shows a cell with unequal segregation of 27 and 22.

We have scored the number of univalents at this stage and the results are shown in Table 29 below.

Table 29

Lagging visible univalents at 1st anaphase

Univalents	1	2	3	4	5	6	7	8	9	10
No. cells	20	30	28	19	13	14	8	1	3	1

Mean = 3.5 univalents per cell.

It is clear from this table that most of the numerous laggards seen at metaphase I (Table 28) have divided and may have reached the poles at this stage.

Bridges at anaphase I are rather frequent and the results of scoring these are shown in Table 30.

Table 30

Frequency of bridges at anaphase I.

	Total	1 bridge	1 bridge fragment	2 bridges	3 bridges
No. of cells	100	39	4	13	3

The occurrence of bridges, whether of univalent, or bivalent origin, is attributed to possible difficulty in separation as stickiness is rather common. In certain cases some of these bridges can be explained as arising from structural changes in the chromosomes themselves such as inversions and breakage - reunion. Fragments are presumably lost in cells possessing only bridges.

Disturbances of meiosis are still seen in this material in the second division. Most of the anomalies encountered are bridges. Such bridges can be due to inversions incorporated in the polar members of bivalents at anaphase I. Univalents are still observed, rarely dividing, at the second anaphase. At telophase micronuclei are frequently seen. These vary in number and size, depending on whether they constitute single, or groups of, laggards undergoing cytogenesis. Some tetrads with varying number of micronuclei are illustrated in Figs. 30-1, 30-2 and 30-3.

Fig. 26 Meiotic stages in triploid

M.canadense (2n = 54)

- 26-1 Paired and unpaired chromosomes at
 diplotene.
- 26-2 Possible inversion heterozygote
 at diplotene.
- 26-3 Side view of first metaphase, variation
 in the number of visible univalents.

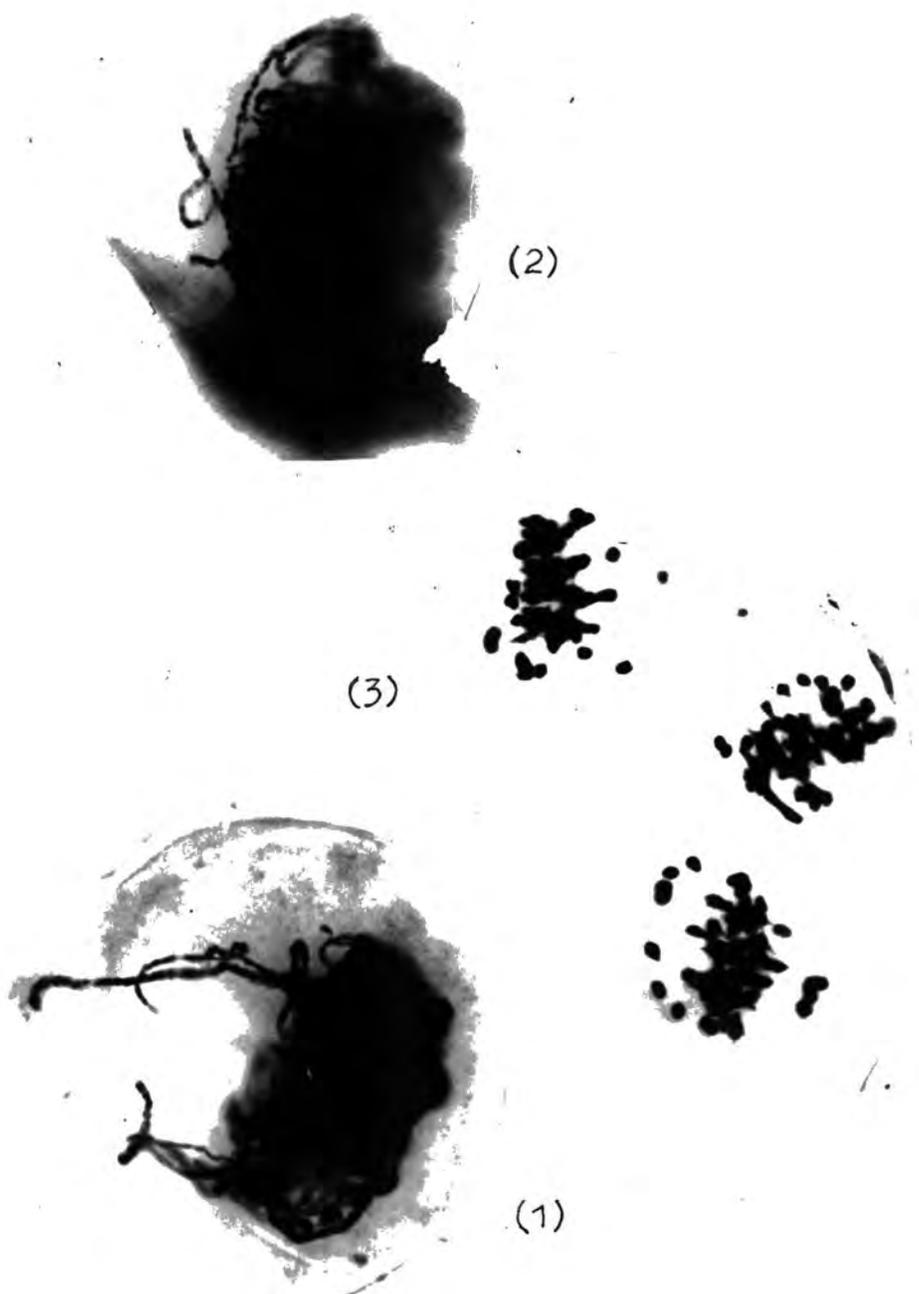


Fig. 26

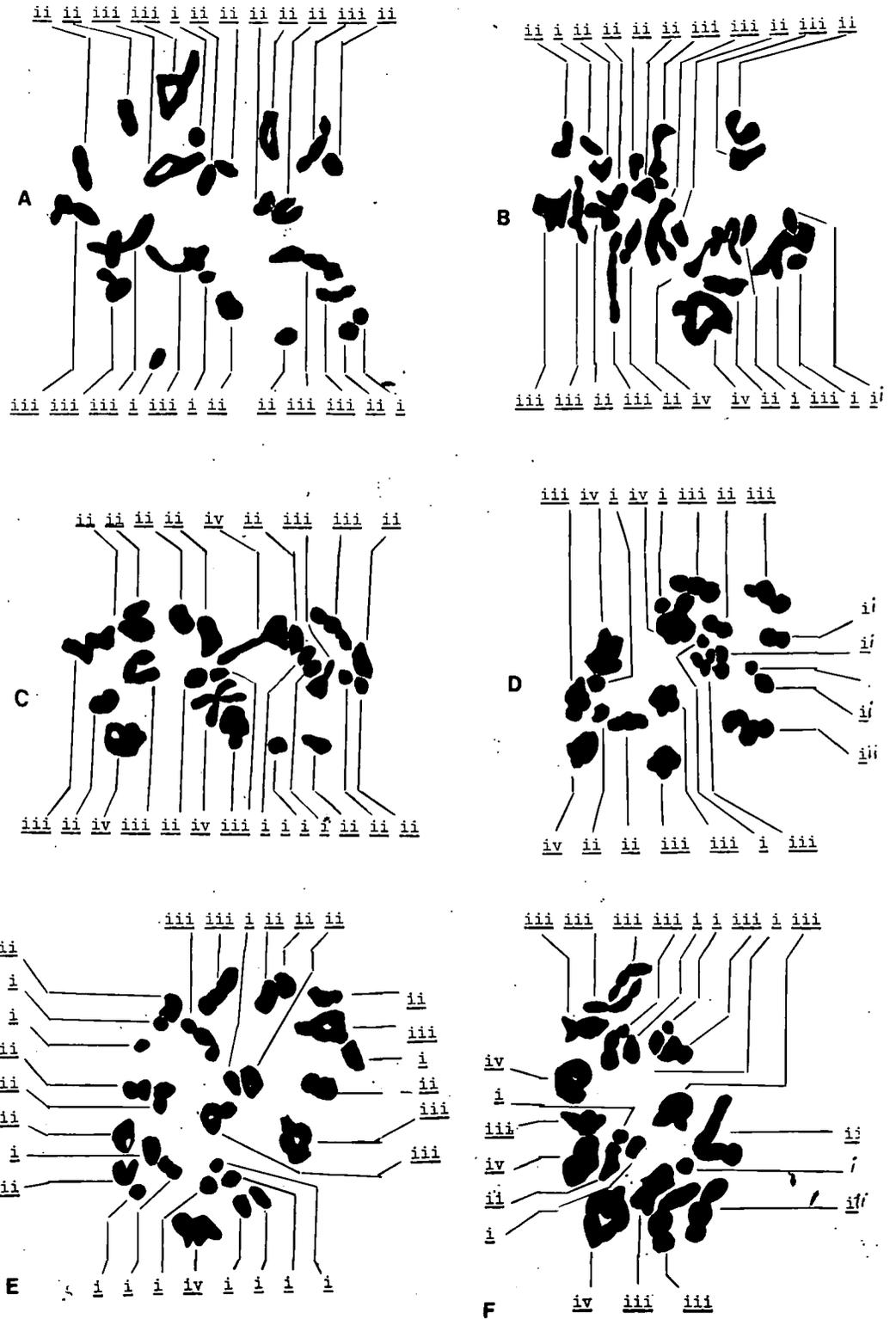


Fig. 27

Fig. 28 Meiotic stages in triploid

M.canadense $2n = 54$

- 28-1 Bivalent bridge, lagging bivalent
 (left hand corner)
 One univalent is exhibiting stretched arms.
 The other univalent is already divided.
- 28-2 First anaphase, univalent bridge;
 fragment; and an already divided
 univalent.
- 28-3 First anaphase; three dividing
 univalents and a lagging bivalent.
- 28-4 A group of P.M.C's with bridges and
 laggards.

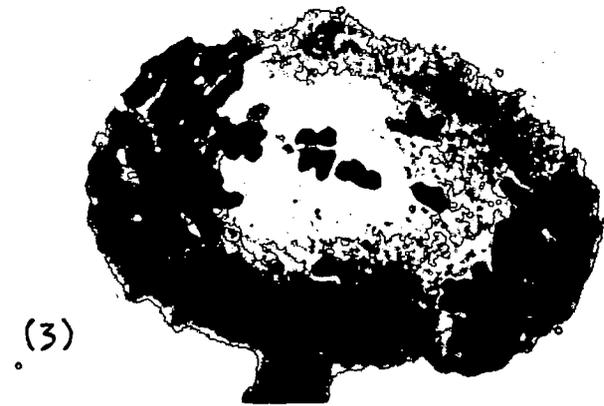
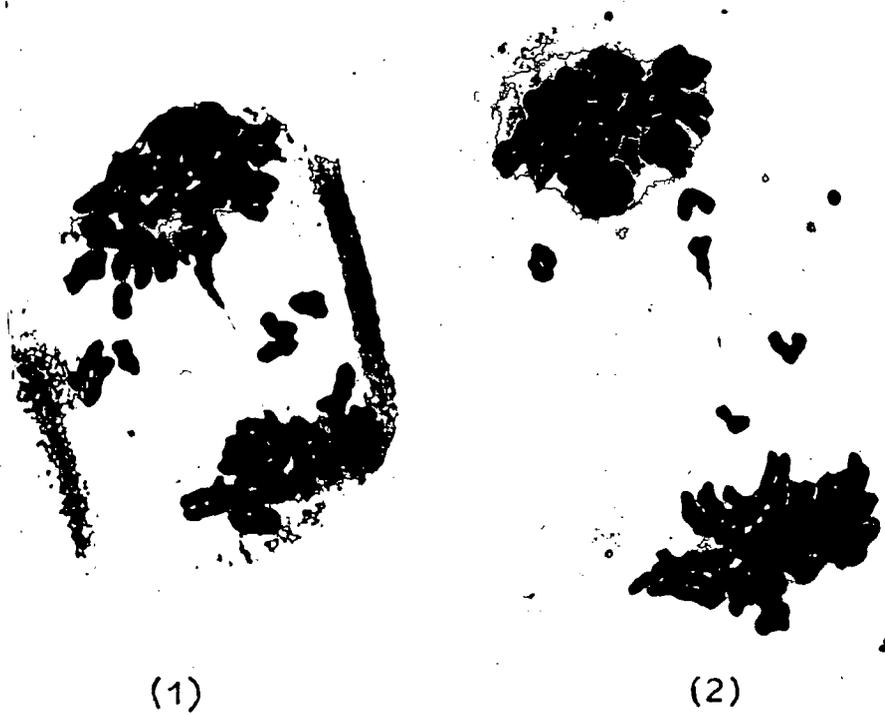
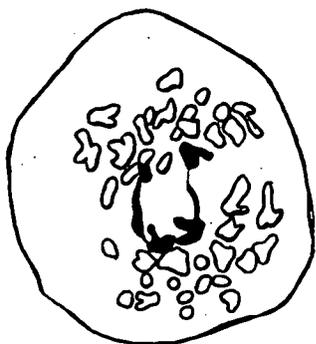
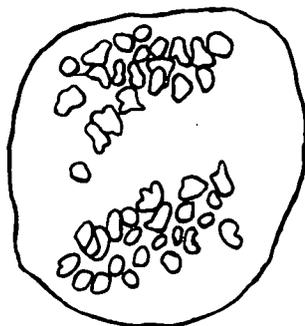


Fig. 28



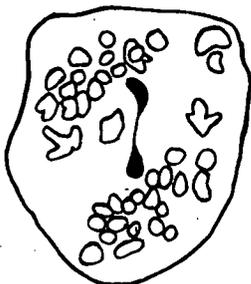
(1)



(2)



(3)



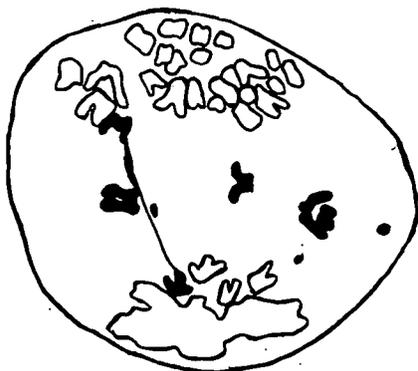
(4)



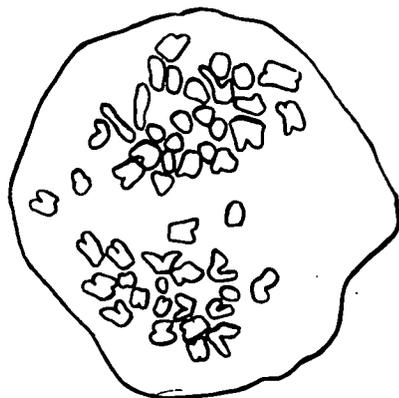
(5)



(6)



(7)



(8)

20 μ

Fig. 29

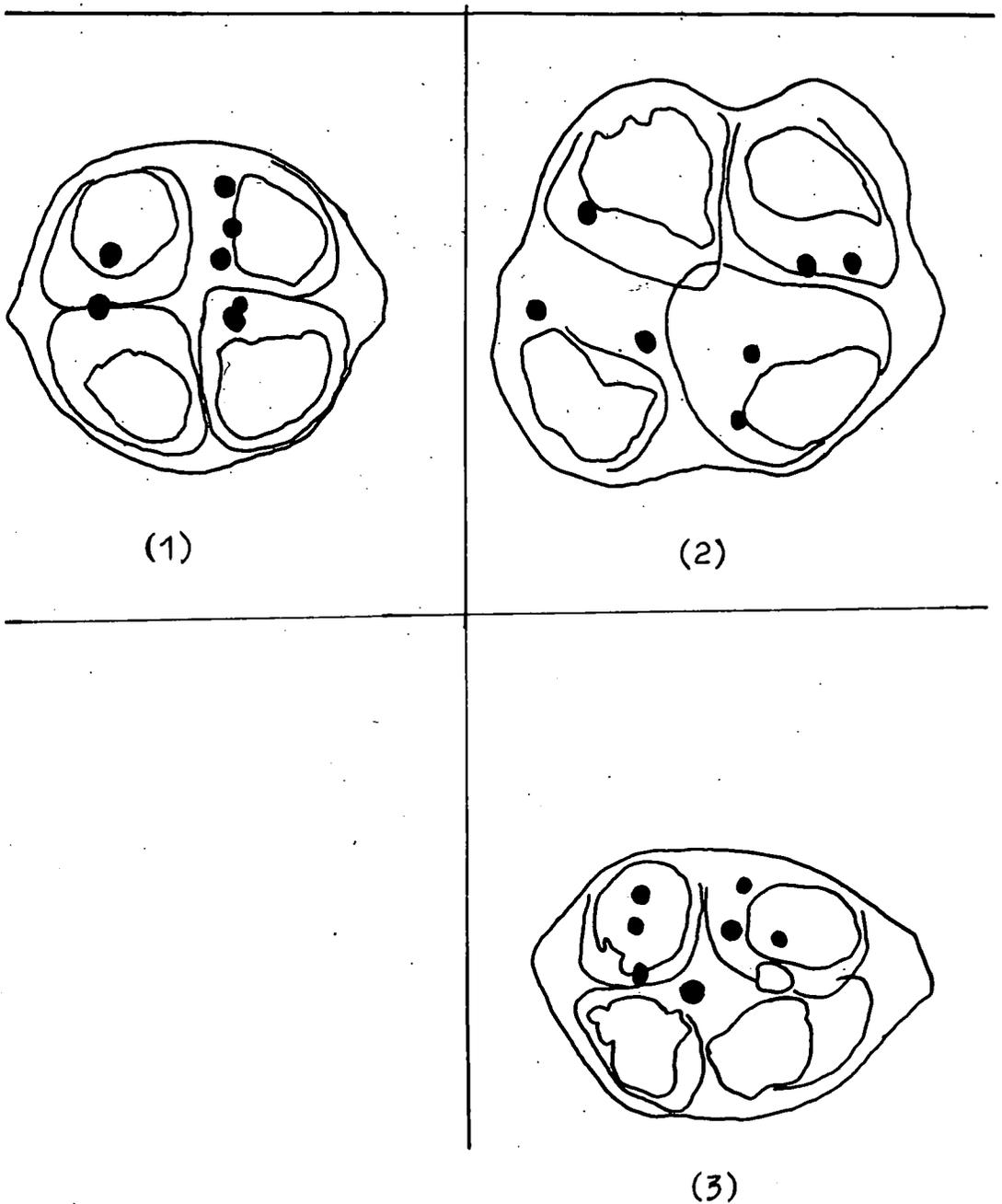


Fig. 30 : Tetrads in triploid
M. canadense with micronuclei.

Meiosis in *M. canadense* var. *interius*

The material on which the meiotic process has been studied was received, in flower, by air from Alberta, Canada, in 1965. Since then the plant has continued to reproduce vegetatively, until the spring of 1968 when two clones flowered.

One of the major features in this material is the clumping of the chromosomes around the nucleolus and the severe chromosome stickiness which has been observed up to late anaphase one (Figs. 34-1, 34-2 and 34-3). The matrix strands are so extensive as to connect chromosomes of one pole to each other as well as to those of the other pole (Fig. 34-1). Detailed analysis of pairing has thus been impossible. Other factors to this effect are: the crowded nucleus ($n = 36$), difficulty in obtaining well spread bodies and overlapping of chromosomes.

We have, however, attempted the analysis of some cells, and in these emphasis has been laid on the frequency of multivalents, expressed as a percentage, rather than a complete analysis of the various configurations.

Diakinesis cells, capable of analysis, are given in photograph Fig. 31, and the chromosome configurations, admittedly too limited for statistical analysis, is given in Table 31. The Table also includes some cells at early metaphase, such as the one illustrated in Fig. 32.

Table 31Frequency of visible multivalents in somePMC's in tetraploid canadense

Fig. no.	No. of bodies in the nucleus	No. of multivalents		Percentage
		iv	iii	
32	19 + two complex groups	7	3	51
	24 + three complex groups	7	4	56
31-1	7 + one large complex group	3	1	23
31-2	10 + four complex groups	4	1	27
31-3	17 + five complex groups	5	1	32
31-4	20 + one large complex group	4	2	30
31-5	9 + one large complex group	4	-	22
Mean/cell		5	2	36

It is admitted that the evidence revealed in this table is not conclusive and is inadequate. It could tentatively be assumed that autopolyploidy is possible. Further discussion on this subject will be resumed later.

Meiotic division in this material, on the whole, is regular and the multivalents appear to disjoin normally. There are, however, a

few anomalies, such as the rare occurrence of anaphase I bridges (Fig. 33-1) and some laggards in both divisions (Figs. 33-5 and 33-6).

Fig. 31 Meiosis in tetraploid *M. canadense*

var. Interius - $2n = 72$.

Diakinesis cells:

Only the visible configurations
are drawn and labelled.

- 1 Top left: $4(iv)+1(iii)+\text{complex groups.}$
- 2 Top right: $4(iv)+1(iii)+\text{complex groups.}$
- 3 Centre: $5(iv)+1(iii)+11(ii) +\text{complex groups.}$
- 4 Bottom left: $4(iv)+1(iii)+16(ii)+\text{complex groups.}$
- 5 Bottom right: $4(iv)+5(ii)+\text{complex groups.}$

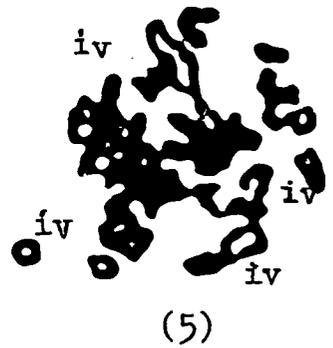
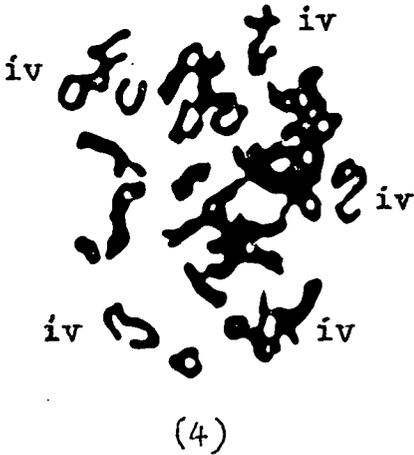
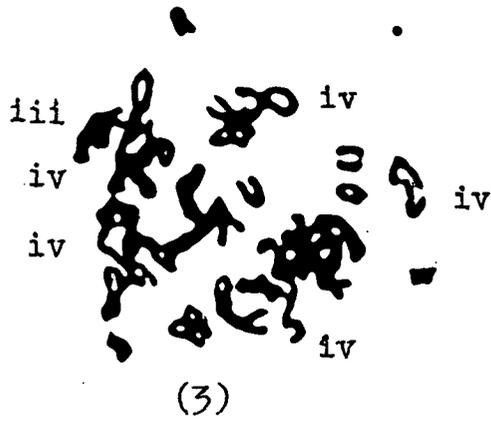
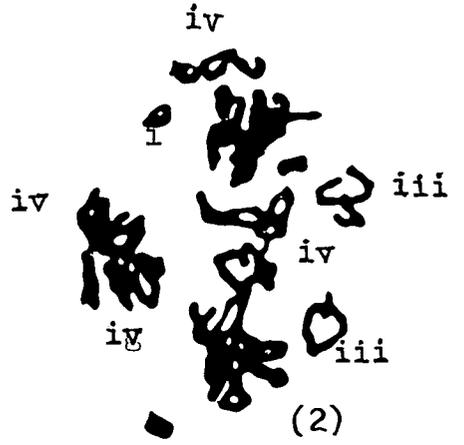
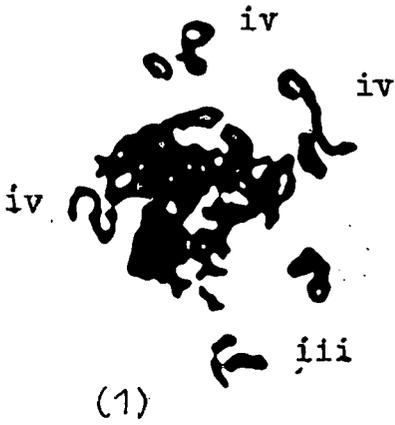


Fig. 31

Fig. 32 Meiosis in tetraploid M.canadense var.Interius

2n = 72

Metaphase I (actual cell to the right). Only the visible configurations are labelled.

Complex parts are shown as spotted areas.

The possible configuration in this cell is
7(iv)+3(iii)+9(ii)+complex group.

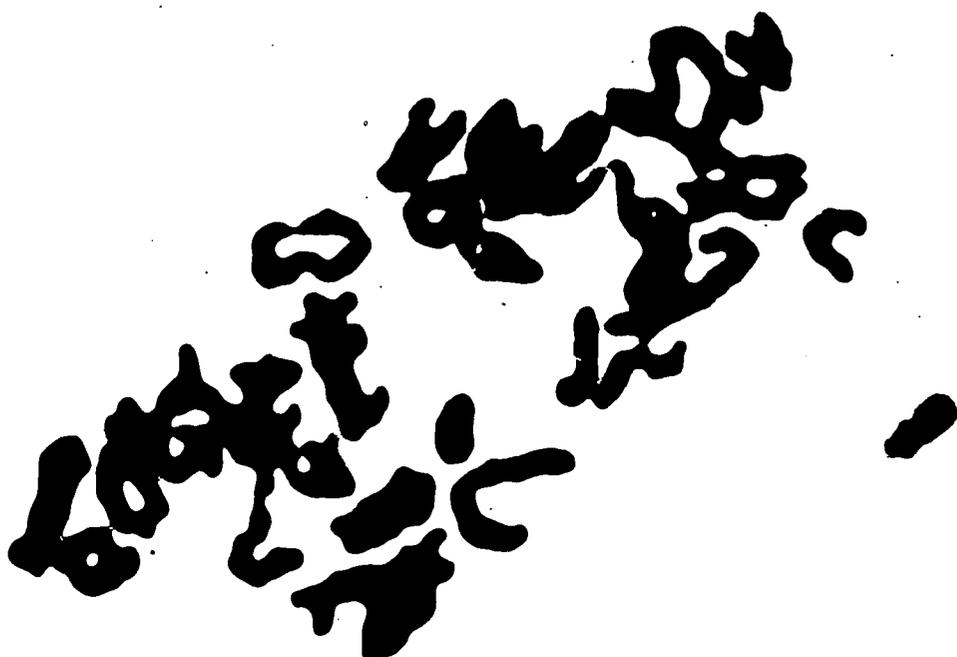
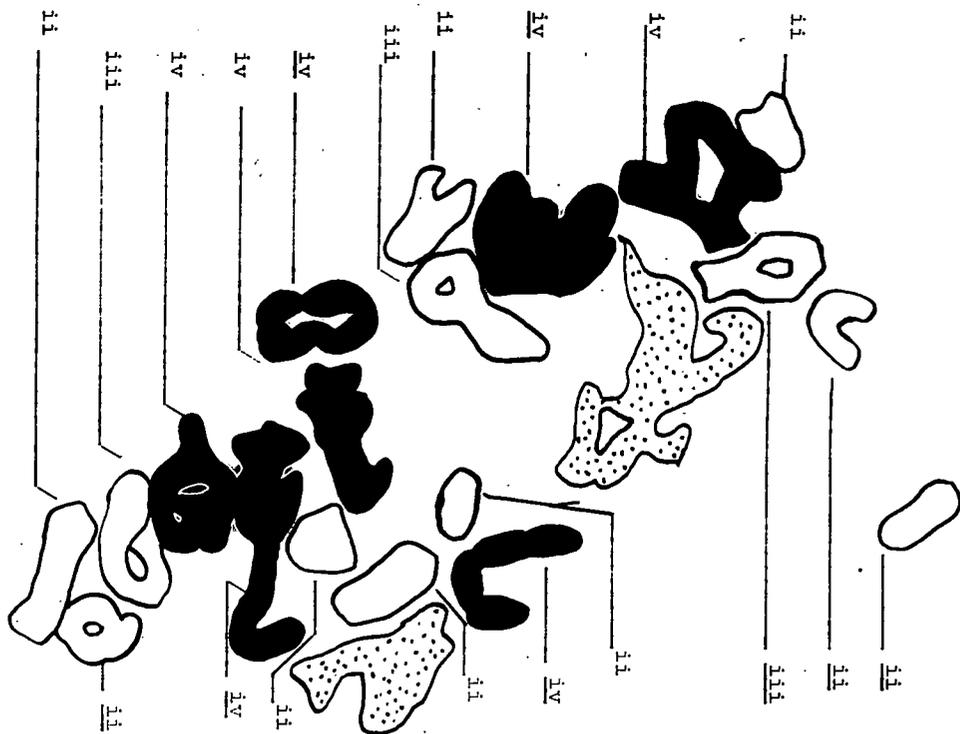
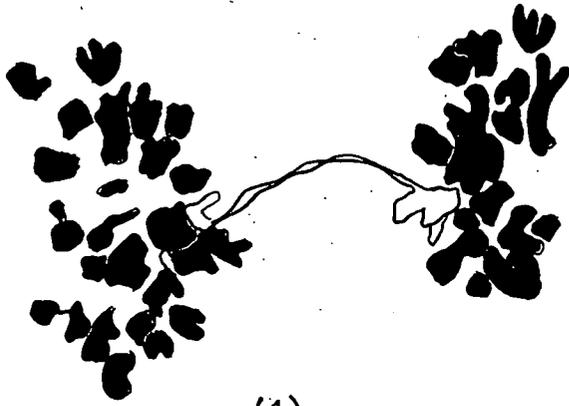


Fig. 32

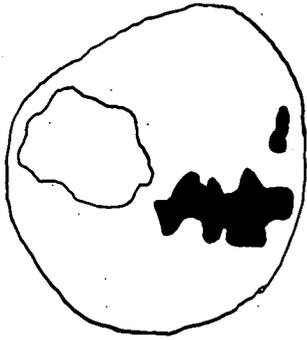
Fig. 33 Meiotic stages in tetraploid *M. canadense*

var. *interius*. ($2n = 72$)

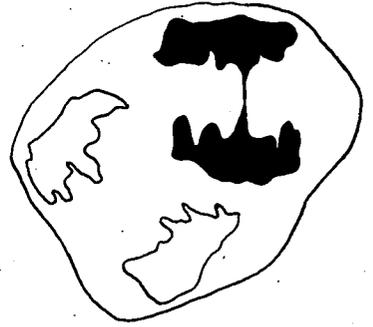
- 33-1 Anaphase I, bivalent bridge.
- 33-2 Metaphase two, lagging univalent.
- 33-3 Bivalent bridge
- 33-4 Metaphase one, two quadrivalents of
 the ring and figure of eight type.
- 33-5 Late second anaphase, two bridges and
 two laggards.
- 33-6 Late second anaphase, lagging univalents.



(1)



(2)



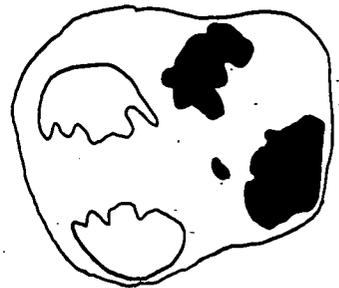
(3)



(4)

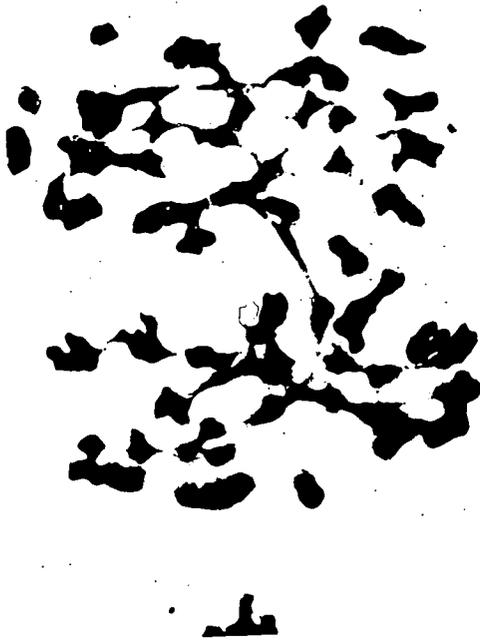


(5)

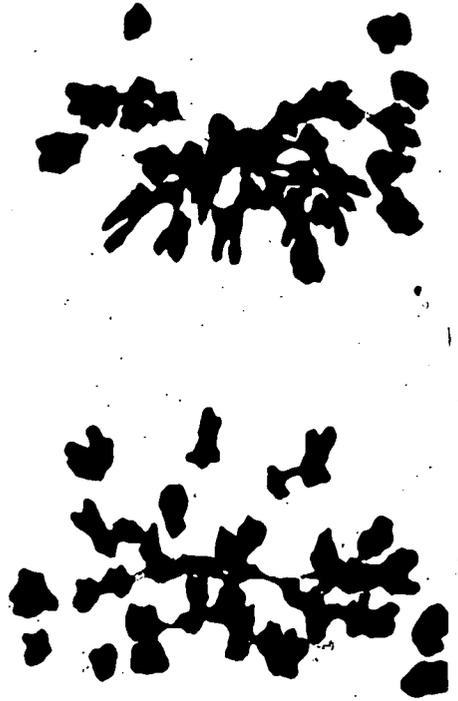


(6)

Fig. 33



(1)



(2)



(3)

Fig. 34: Anaphase I : Chromosome stickiness
in tetraploid interius ($n = 36$).

DISCUSSION OF CYTOLOGICAL RESULTS

We have seen above that the karyotypes of several different clones of Maianthemum are basically similar (p.54). Stebbins (p.442) says that "chromosomes that resemble each other in outward appearance are not necessarily alike in genic content or in hereditary potentiality". It is therefore necessary to analyse meiotic behaviour of the chromosomes if any reasonable conclusions regarding matters related to evolution and relationship are to be reached.

Meiosis in almost all the clones has been described above. We will look into the relationship between the evidence collected from meiosis and mitosis; also between these two and fertility, and, finally, into the origin of the various taxa in relation to polyploidy.

The various taxa have a uniform karyotype that is not accompanied by any major structural changes in the chromosomes, as the evidence from meiosis reveals. This indicates that the whole group is morphologically slightly variable and cytologically fairly stable, despite the disjunct distribution and the presumed long period of isolation.

It is of interest to note that all the clones in cultivation have shown a tendency towards self-incompatibility (p.28)but are readily crossable with each other at all levels of ploidy. On pollen-stainability basis, most of the clones are fertile, but there seems to be a correlation between meiotic pairing and fertility as shown in Table 32.

Table 32
Meiotic pairing and fertility
in *Maianthemum*

Material	Origin	Configuration at Metaphase I	Fertility Percentage
<u>bifolium</u> 2n = 36	Germany	18(ii)	96
	Poland	18(ii) or 16(ii) + 1(iv) rare	85
	Czechoslovakia	18(ii) or 16(ii) + 1(iv) rare	80
	Britain	18(ii) or 16(ii) + 1(iv) occasional	65
<u>canadense</u> (2n = 54)	Canada (2n = 36)	18(ii)	100
	Canada (2n = 54)	5(i) + 10(ii) + 7(iii) + 2(iv)	46-50
	Canada	5(iv) + 2(iii) + 23(ii) and a possible maximum of 7(iv)	100
<u>interius</u> 2n = 72			
<u>dilatatum</u>	California	18(ii) Therman	-

It is noted that in the 36-chromosomed clones, fertility is slightly affected by the rare occurrence of multivalents, but when these become occasional, fertility is affected to a much greater degree. It appears that these multivalents together with the observed, but less frequent, disturbances in the meiotic course, contribute to the formation of unbalanced gametes. It could also be argued that structural hybridity to a limited degree occurs in these clones, which could explain their polyploid origin as will be

seen below.

In the 72-chromosomed race fertility is not affected by the frequent association of the chromosomes into higher multivalents. This means that they disjoin normally and form balanced gametes. Higher associations can indicate homology rather than structural differences as will be seen below.

In the triploid race, as in all organisms of this ploidy level, meiosis is irregular and many of the gametes are abortive. It is surprising that despite the various anomalies seen in the meiotic processes in this race, there are still some viable gametes formed and that the triploid is not only partially fertile but also productive of good berries and good seeds on selfing. It is unlikely that it is of hybrid origin, but we will discuss this later.

The question of whether a species is diploid or polyploid can in uncertain cases be solved on the basis of the shape of the chromosomes (Soumalainen, 1947). If polyploidy is assumed in Maianthemum, then the base number is $x = 9$. It is necessary to examine this by looking at the evidence both from the karyotype and from meiosis.

In the tribe Polygonatae, Therman (1956) gives the following base numbers:

<u>Polygonatum</u> group <u>Alternifolia</u>	$x = 9, 10$
<u>Polygonatum</u> group <u>Verticillata</u>	$x = 13, 14$
<u>Reineckia</u>	$x = 19$
<u>Convallaria</u>	$x = 19$
<u>Smilacina</u>	$x = 18$

<u>Maianthemum</u>	x = 18
<u>Streptopus</u>	x = 8
<u>Disporum</u>	x = 8, 9
<u>Clintonia</u>	x = 14, 16

According to Therman the base number for the tribe is $x = 9$ and for Maianthemum is $x = 18$. Therman rules out autopolyploidy and suggests a possible allopolyploid origin for Maianthemum.

If we follow the argument that the base number for Maianthemum is possibly $x = 9$, as in its nearest ally, Polygonatum, then we expect in the 36-chromosomed strains to find the chromosomes in sets of four. But this is not the case. We have two main types of chromosomes, 7 large and 11 small pairs. Within each of these two series we have only two identical chromosomes of any one kind. Thus, the argument whether these polyploids are allo- or auto- in origin is partly settled on the basis of karyotype analysis, which indicates allopolyploidy; also from the meiotic pairing, although this is very limited, as we have seen earlier (p. 64). Autopolyploidy can only be postulated on the basis of chromosome differentiation following polyploidization, in Stebbins' sense, and this does not seem very likely.

The data for the karyotype (p. 75) strongly suggests, cytologically, at least, that the $2n = 72$ forms of M. canadense are autopolyploids. The meiotic behaviour of the chromosomes is also indicative of autopolyploidy. As has already been indicated, it is not possible to analyse all the configurations in any single PMC, but the information we have (Table 29) indicates that the most

probable configuration is 5(iv) + 2(iii) + 23(ii), with a maximum observed number of 7(iv). Thus about 36 - 55 per cent of the chromosomes are involved in higher configurations. This is within the figure calculated for the Gramineae by Morrison and Rajhathy (1960). In the Gramineae, an average of 57 per cent of the chromosomes form multivalents in known autopolyploid forms. Manton (1937) produces similar figures on autotetraploid Biscutella.

The triploid form of M.canadense can be looked at from two different angles. The first is, whether it is cytologically autopolyploid. The karyotype evidence, showing that the chromosomes can be arranged in trios, is in favour of this hypothesis, as is also the evidence from meiosis. The most typical configuration (Table 25) is possibly 5(i) + 10(ii) + 7(iii) + 2(iv) (with a maximum of 11 trivalents). Here the mean percentage of chromosomes involved in higher associations is 68 per cent, which is within the range calculated by Morrison and Rajhathy (1960). The second question about the triploid is whether it arises from a diploid-tetraploid hybrid, or directly from a diploid. The cytological evidence could fit either hypothesis; and we have insufficient data to judge on the basis of morphological evidence. If all triploid canadense turned out to be like the diploid, and if all the plants of var. interius turned out to be tetraploid, then a conclusion could be reached. But at present not enough populations have been cytologically examined.

Therman, 1956, dealing with the tribe Polygonatae, says that

"clear polyploidy has been found in the North American Polygonatum species representing the group Alternifolia, within the species representing the group Verticillatum and in the genus Streptopus". This can be extended by the addition of the North American Smilacina (Kawano and Itlis 1963) and the North American representatives of Maianthemum (present report).

CHAPTER SEVENDISCUSSION

In the present work we have investigated the small genus Maianthemum. The results can be looked at from at least three different points of view, viz., taxonomic, evolutionary and phytogeographical.

In our investigation on the morphology of the various taxa, evidence has been collected from dried and living material. It has been found difficult to use the flower for the reasons given earlier (p. 19), and p. 20) and the limited work done on these (p.20) showed that the flowers in all the various taxa are basically similar except for minute, but sometimes obvious differences. Such differences as exposed by width of ovary and perianth; and thickness of pedicel and style, have been incorporated in our main discussion that will follow.

In the fresh material the anatomy of the rhizome has proved of little taxonomic importance (p.24); and characters such as stomatal length and width, and size of the nuclei of the subsidiary cells, although revealing striking differences (p.22) have been found to be correlated with polyploidy.

It would certainly be profitable if further analysis could be made on the dried material with these characters in mind. This would add to our knowledge of the prevalence of these chromosome races in nature. It should be mentioned here that both the tetraploid and

the triploid material have been sent to us from widely separated localities (Appendix 3), and this could indicate that they are quite widely distributed; but their complete distribution is still unknown; and we cannot even be sure that there is any correlation between chromosome number and morphology, apart from the cell-size characters. Hence the discussion which follows, in the relationship and status of these taxa, will be based primarily on the herbarium material.

In our earlier attempts (p.14) we considered the taxa on a regional basis. Thus M.dilatatum was closely examined for variation in four different parts of its range, i.e. North American Pacific coast; Alaska and the Aleutian Islands; Asiatic Pacific coast; and Japan. It was found that the N.American Pacific plants are similar to those of Alaska and Aleutian Islands; but they both are different from the Asiatic and Japanese ones. Thus it was possible to group the dilatatum plants into two main sections; one along the Pacific coast of North America, including Alaska; the other along the Pacific coast of Asia, including Japan. For M.bifolium the European and Asiatic samples were found to be very similar and hence considered as one group, Eurasiatic.

We have summarised our data first, into the form of resemblances and differences (Table 33), and then in the form of a similarity index (Table 34) which is the number of characters shared by any two taxa, expressed as a percentage of the total number of characters considered.

Table 33

The taxa and their resemblances and differences.All measurements in mms. Means and standard error of means.Herbarium data.Key for the taxa:-

- A: M.dilatatum, American
 B: M.dilatatum, Asiatic
 C: M.bifolium, Eurasiatic
 D: M.canadense var. interius
C.N.America
 E: M.canadense, E.N. America

Character	A	B	C	D	E
Stem length	260 \pm 9.3	141 \pm 8.5	170 \pm 6.4	152 \pm 24.3	130 \pm 3.6
Petiole length	46 \pm 3.4	24 \pm 1.1	19 \pm 1.2	5 \pm 0.9	2 \pm 0.2
Sinus depth	16.5 \pm 1.0	12 \pm 0.7	8 \pm 0.4	2 \pm 0.4	1.7 \pm 0.1
Lamina width	62 \pm 2.4	37 \pm 1.8	35 \pm 0.4	30 \pm 1.0	20 \pm 0.6
Midrib length	66 \pm 2.4	39 \pm 1.4	49 \pm 1.7	40 \pm 3.5	25 \pm 2.3
No. of fls/ raceme	36 \pm 2.4	17 \pm 1.4	25 \pm 1	28 \pm 1.1	21 \pm 0.5
Ovary width	0.9	1.0	1.0	1.35	1.0
Perianth width	1.8	1.4	1.2	1.6	1.2
Pedicel thickness	thick	thick	slender	slender	slender
Style thickness	thick	thick	slender	slender	slender
Rhizome thickness	thick	thick	slender	slender	slender
Endowment	glabrous	glabrous	pilose	pilose	glabrous

The similarity index is shown diagrammatically in Fig. 35. In this case the thickness of the line connecting any two taxa is an expression of their degree of similarity; the thinner the line the more different they are.

Table 33 shows that M.dialtatum (A) is the most distinct taxon in the group; consequently, it is compared to the remaining taxa in the manner presented in Table 34. In this Table the comparison is made along the following lines: In Column 1, taxon A is compared to B, C, D, and E. This shows that taxon A has more characters in common with B than with any of the remaining taxa. In the same way, in column 2, taxon B is compared to C, D and E; in column 3, taxon C is compared to D, and E; and in column 4, D and E are compared. Similarity, in all cases, is expressed in number of characters shared, and as a percentage.

Table 34

The species and their similarity index. Based on data in Table 31.

(For index calculation see p. 84)

Key for the taxa as in Table 31

0 = character not significant.

+ = character significantly different.

Character	(1)				(2)			(3)		(4)
	A				B			C		D
	B	C	D	E	C	D	E	D	E	E
Stem length	+	+	+	+	+	0	+	0	+	0
Petiole length	+	+	+	+	+	+	+	+	+	+
Sinus depth	+	+	+	+	+	+	+	+	+	0
Lamina width	+	+	+	+	0	+	+	+	+	+
Midrib length	+	+	+	+	+	0	+	0	+	+
No. of fls/ raceme	+	+	+	+	+	+	+	0	0	+
Ovary width	0	0	+	0	0	+	0	+	0	+
Perianth width	+	+	+	+	0	+	+	+	0	+
Pedical thickness:										
thick	0	+	+	+	+	+	+	0	0	0
Style thickness:										
thick	0	+	+	+	+	+	+	0	0	0
Rhizome thickness:										
thick	0	+	+	+	+	+	+	0	0	0
Indumentum, glabrous	0	+	+	0	+	+	0	0	+	+
<u>NO OF CHARACTERS SHARED (OUT OF 12)</u>	5	1	0	2	3	2	2	7	6	5
<u>PERCENTAGE</u>	42	8	0	17	25	17	17	58	50	42

Fig. 35 summarizes these results. It is evident that canadense, interius, and bifolium resemble one another fairly closely and are all rather distinct from dilatatum. It is also clear that N.American dilatatum is linked with Eurasiatic bifolium through E. Asiatic dilatatum. The leaf of the latter retains the general shape and texture of dilatatum, but approaches bifolium in size; its flowers have the general characteristics of dilatatum, but the number of flowers per spike is reduced in the direction of bifolium. The evidence thus suggests that E. Asiatic dilatatum may have been modified by contact with bifolium, and the occurrence of hybridization and introgression. As noted earlier (p. 8) Kawano has reported that the species occur together in several places in Japan and that intermediates, presumably of hybrid origin, are found. Hybridization may have been extensive in the past and have led to introgression of the kind suggested. We know nothing of the fertility of the hybrids and unfortunately a synthesis has not been possible; but it seems quite likely that the hybrids are at least partially fertile.

The general questions of speciation on the genus will be discussed in the final chapter.

As regards the taxonomy of the genus, our results lead us to suggest the following scheme.

M. dilatatum (Wood) Nelson and Macbride

var. dilatatum (American Pacific coast).

var. kamtschaticum (Asiatic Pacific coast).

f. vegetior Sokolovskaya (Far East).

M.bifolium (L)Schmidtsubsp. bifolium (Eurasia)subsp. canadense (E.N.America)subsp. interius (C.N.America)

It may be that further investigation may justify the separation of var. carolinianum (p. 5) as a subspecies under M.bifolium.

A new species is reported in the genus in the Far East, i.e. M.intermedium Worosch, described by Voroshilov (1960). It has not been possible for us to obtain this particular paper. Sokolonvskaya, however, is of the opinion that this species is a caryological form similar to the higher polyploid ($2n = 64 - 70$) of bifolium discovered by her (p.42).

As has been shown (p.54) evidence from the karyotype does not help in drawing taxonomic conclusions. Uniformity of the karyotypes of all species suggests that these long-lived perennials have had a very slow rate of evolution. Again, it has been shown that at least three of the taxa have developed polyploid races especially in N.America; but it is not yet clear whether these are local and rare, or widespread and abundant, so that their taxonomic significance cannot be assessed. It is interesting that Kawano and co-workers (1967) did not report any polyploid races in Maianthemum (p.58) Subsp. canadense certainly exists in both diploid and triploid (possibly also in a tetraploid) forms, and subspecies interius as both diploid and tetraploid.

Evidence from both wild populations and experiments indicate that hybridization can occur in the genus, and that, in particular, triploid canadense can be crossed with diploid bifolium, but nothing is known yet of the fertility of the hybrids, and the criteria of ability to exchange genes cannot be applied to the taxonomic problem.

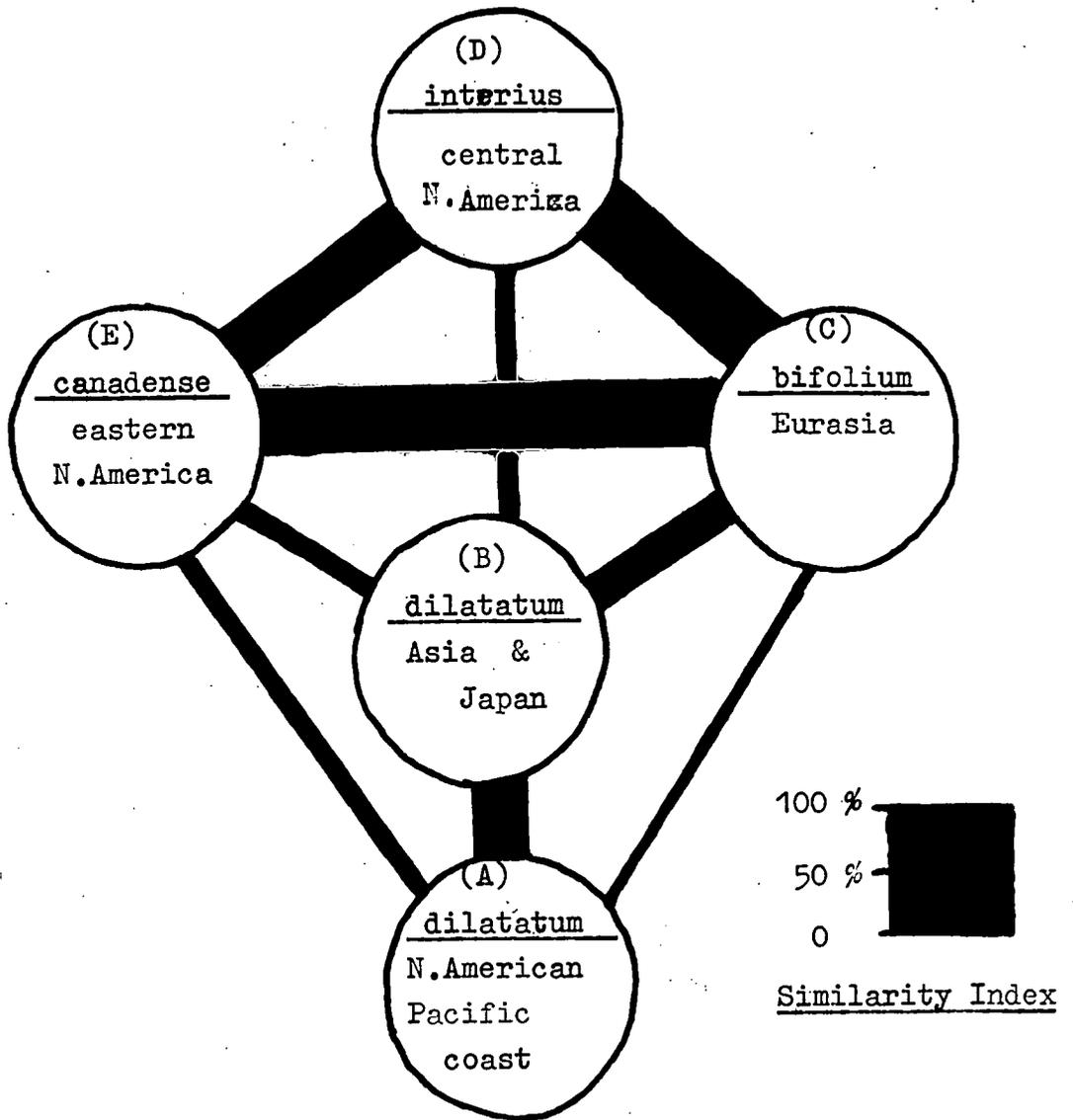


Fig. 35: Morphological affinities between the various taxa of Maianthemum.

PART TWO

OXALIS

SECTION ACETOSELLAE

INTRODUCTION

In this part we will be investigating the relationship between O.acetosella of Europe and Asia, and O.montana of N. America. Fernald (1918, 1920, 1929) believes that the American plant is distinct from the European. Hara (1952, 1962, 1966) considers the American plant to be only a part of the European. Hultén (1958) lists the characters of the American plant (as pointed out by Fernald without actually mentioning Fernald) and accordingly degrades the plant to a subspecies level i.e. O.acetosella subsp. montana (Raf.) Hultén, giving no further details. Love (1968 and personal communication) says that she has verified the subspecific status which Hultén failed to establish. Details of her work are not available to us.

These two taxa are obviously like one another and it is not clear what, if any, the differences are.

The relationship between these two taxa and a third taxon, namely O.oregana of north-west America, is also investigated. Our evidence suggests that the last named taxon is different from the other two taxa and should be maintained as a separate species. Love (1968, and personal communication) has recently published this taxon as a subspecies under O.acetosella giving no details, a treatment we cannot confirm.

There are, however, other species in the section Acetosellae; Knuth (1930) divides them into two series. In the Southern hemisphere

there are O. magellanica Forst (including O. lactea Hook), O. Comberi R. Knuth, O. Berningeri R. Knuth, O. Pachyrrhiza Wedd. Chlor., O. Schickendantzii R. Knuth; of these we have made a few investigations on O. magellanica and O. lactea and these are described on page 128.

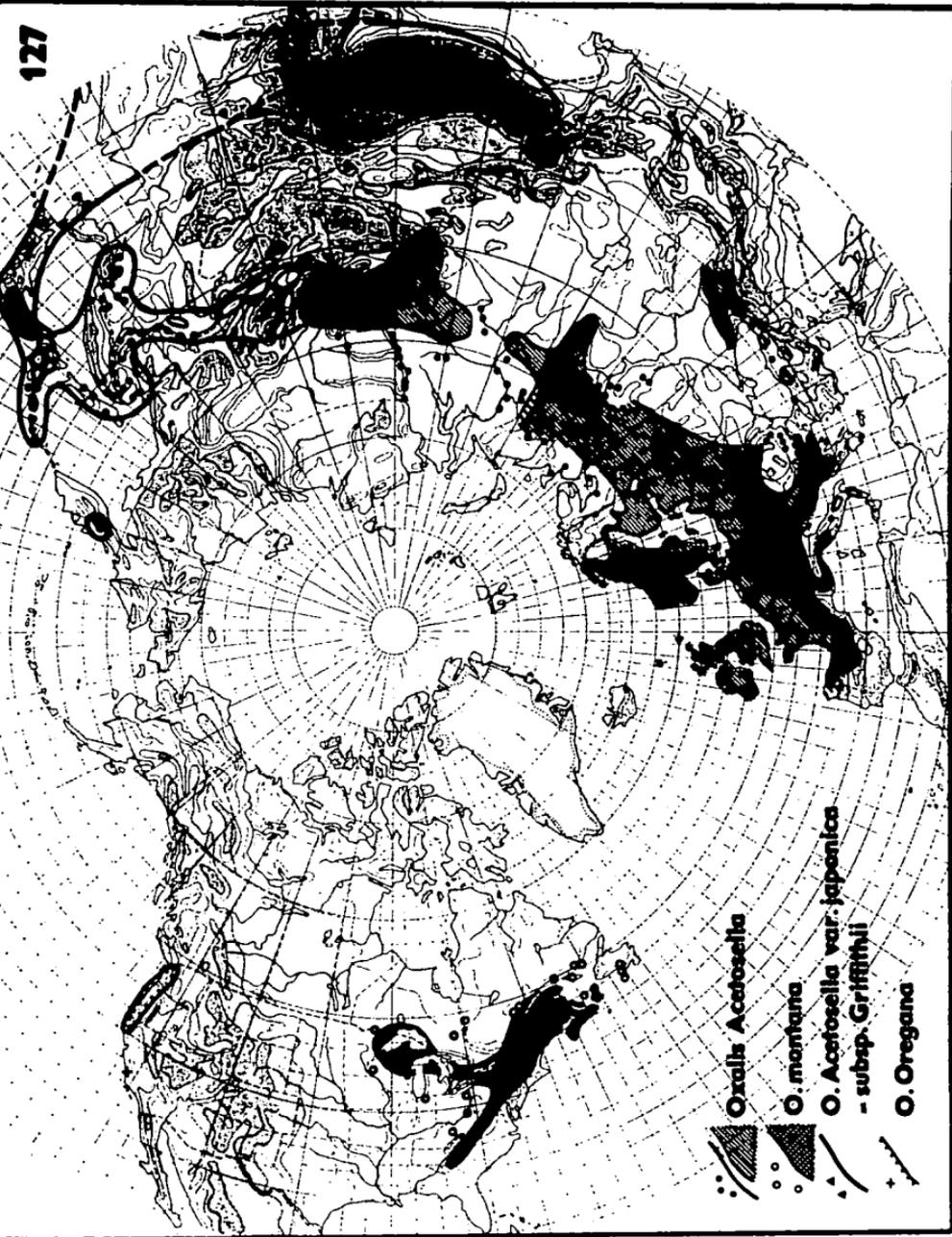
In the Northern hemisphere, Knuth records the following species: O. oregana Nutt, (including O. Smallii) O. obtriangulata Maxim, O. griffithii Edgew, and Hook O. hupehensis R. Knuth, and O. acetosella L. (including O. montana Raf.).

The geographical distribution of the section Acetosellae is given in the map Fig. 36. Some species are very localized. O. magellanica: Bolivia, Chile, Argentine, S. Australia, Tasmania and New Zealand. O. Schickendantzii: Argentine. O. Comberi: Argentine. O. Berningeri: Chile. O. Pachyrrhiza: Peru and Argentine. O. acetosella: Eurasia and N. America. O. oregana: American Pacific coast. O. obtriangulata: Korea, Manchuria, China, Japan. O. griffithii Himalaya, China, Japan, Formosa. O. hupehensis: China.

The geographical distribution of the species of the Northern hemisphere is given in the map, Fig. 37, reproduced from Hultén (1958).



Fig. 36: Distribution of Oxalis section Acetosellae.



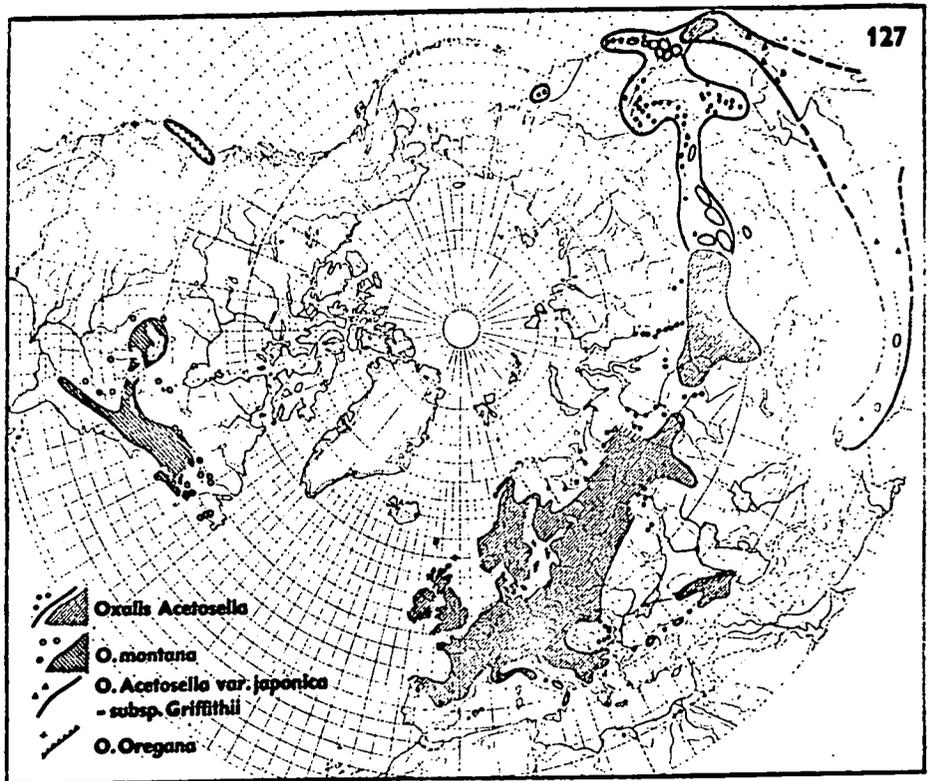


Fig. 37: Distribution of the northern hemisphere species of Oxalis section Acetosellae (from Hulten, 1958).

CHAPTER EIGHT
COMPARATIVE MORPHOLOGY

The species investigated are keyed as follows:-

Key to the taxa investigated in the present work

1. Petals white, without coloured veins or yellow base

O. magellanica

O. lactea

1. Petals with coloured veins and yellow base

2. Plant with slender rhizomes; petals 9-12mm long

3. Capsules 3-7mm long, with acute apex O. acetosella

3. Capsules 3-4mm long, with rounded apex O. montana

2. Plants with thick rhizomes; petals 15-18mm long

4. Leaflets obtriangular, with acute angles;

chasmogamous capsules cylindrical, 20mm long

O. obtriangulata

4. Leaflets obcordate-to-depressed obtriangular, with rounded margins

5. Plant with no cleistogamous flowers; capsules ovoid, 8-10mm long

O. oregana

5. Plant with cleistogamous flowers, chasmogamous capsules oblong, 10-12mm long

O. griffithii

Comparative study on O. acetosella and O. montana. Herbarium studies

In our investigation of herbarium material a number of quantitative characters were looked at to see to what extent these differed in the European and N. American materials.

The characters selected for the investigation and the results obtained are shown in Table 35, and Fig. 38. The measurements are based on a considerable number of samples from a wide range of localities. For each locality, the largest specimen on the sheet was selected for measurement; it is realised that the results must be treated with caution, as the plants are plastic, and their size is affected by the environment.

Bearing this in mind, a number of conclusions can tentatively be drawn. O. acetosella differs from montana in a number of characters and some of the differences are significant. This is true, for example, of the shape of leaflet, and the length of the capsule. Cultivation experiments involving these two taxa have shown that the differences between them are genotypic.

The differences between the two taxa are presented diagrammatically in polygon Fig. 38, where it is evident that they are very similar.

Table 35

Morphological differences and similaritiesBetween the American *O. montana* and the European *O. acetosella*.All measurements in mms. Means and standard errors of means.Herbarium data.

Character	<u><i>O. montana</i></u>	<u><i>O. acetosella</i></u>
Width of lamina at apex	21.6 \pm 0.56	16 \pm 0.5
Width of lamina at midpoint	16 \pm 0.8	18.6 \pm 0.58
Midrib length	13.9 \pm 0.46	13 \pm 0.45
Petiole length	75 \pm 1.4	79 \pm 3.6
Pedicel length	79 \pm 4	92 \pm 2
Flower length	12 \pm 0.27	12 \pm 0.25
Petal width	4 \pm 0.1	4 \pm 0.2
Fruit length	3 \pm 0.2	4.9 \pm 0.1
Fruit width	3 \pm 0.2	3.2 \pm 0.2

Fernald (1918) reviewed the literature on O. montana and summed up his views on the characters which, in his opinion, justified its separation as a species distinct from O. acetosella. These are in Table 36.

Table 36

Differences between O. acetosella and O. montana

as pointed out by Fernald (1918)

Character	O. montana	O. acetosella
Habitat	Cool mossy woods	Open, dry habitats
Flowering season	Mid-June to August	April-May
Rhizome	Persistent petiole bases conspicuous	Persistent petiole bases less conspicuous
Sepals	Hairs spreading	Hairs appressed
Petals	Oblong, deeply emarginate	Normally obovate, slightly emarginate
Capsule	Oblate, broader than long	Ovoid, longer than broad
Seeds	Smooth or obscurely ridged	Conspicuously ridged

We will now re-examine all these characters in the light of (a) herbarium material, and (b) fresh material.

Habitat

It is very difficult to confirm this difference as both plants

are found in woodlands, under varying degrees of cool humid surroundings.

Flowering time

This is confirmed by an examination of the records on the herbarium sheets, which are summarized in Table 37.

Table 37

Flowering time in populations of European and American plants from herbarium records.

Taxon	April	May	June	July
	%	%	%	%
<u>O. montana</u> (35 localities)	0	8.5	28.5	43
<u>O. acetosella</u> (61 localities)	33	28	11	3

A difference has also been observed in the plants in cultivation in the greenhouse, though there is sufficient overlap to make hybridization experiments possible.

Rhizome

It has not been possible to confirm the difference in the rhizome character listed by Fernald; indeed Fernald himself, in his latest paper (1929), does not lay emphasis upon it.

Sepals

Observations on the indumentum of the sepals, which is Fernald's fourth character, have shown that the hairs in both

species can be either spreading or appressed; but there does appear to be a difference not noted by Fernald, in that, in *O. acetosella* the sepals are sometimes notched (50 per cent of samples) at the apex, while in *O. montana* they are always entire. Characters of hairs and sepal apex are illustrated in Fig. 39 (not to scale), also in Fig. 40.

Petals

As regards the shape and emargination of the petals, it has not been possible to obtain enough material of *montana* to make adequate petal measurements, so as to investigate the shape; dried material showed that emargination is very consistent. Flowers of *O. acetosella* on the other hand, with emarginate petals are occasionally seen, so that the character is of limited diagnostic value (Fig. 40).

Capsule

The capsule characters given by Fernald do seem to be good. The difference in capsule length shown by the herbarium specimens are retained in cultivation, and there is also a difference in shape; the capsule of *montana* tends to be rounded at the apex, that of *acetosella* acute (Fig. 40).

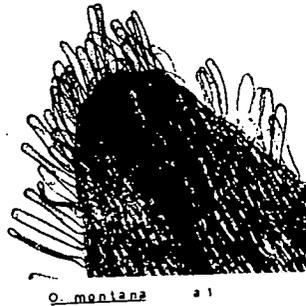
Seeds

Seeds of both species have been examined and no significant difference in size or ridging has been found; in this case, Fernald's diagnosis is not confirmed.

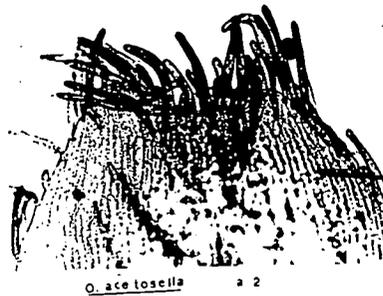
We may sum up these results by saying that N.American *O. montana*

differs from European acetosella in flowering time, in characters of the apex of sepal, and petal, and in capsule shape, though none of these characters is absolutely diagnostic by itself. The best taxonomic treatment for these taxa is probably to treat them as ~~by~~ subspecies of a single species. This was also suggested by Hultén (1958) and Löve (personal communication) has reached the same conclusion.

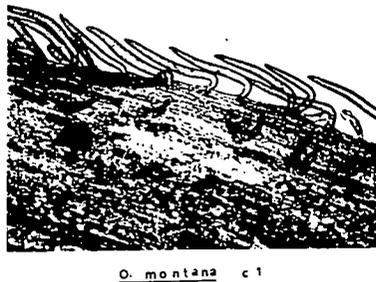
Sepal apex



Sepal apex



Sepal margin



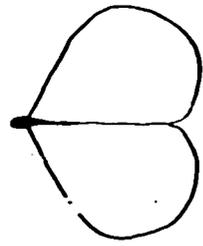
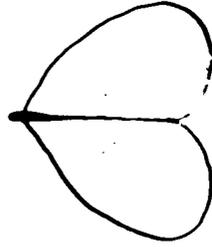
Sepal margin



Fig. 39: Ciliation of sepals and sepal apex. (Not to scale).

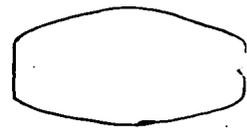
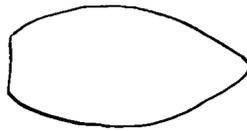
Leaflet

| = 5 mm.



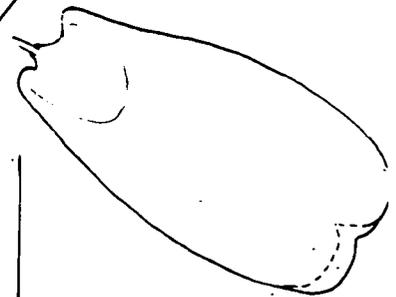
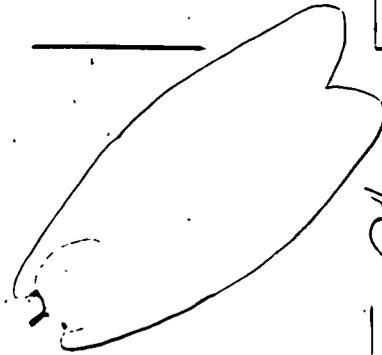
Sepal

| = 1 mm.



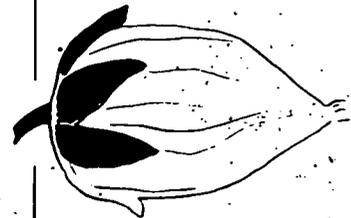
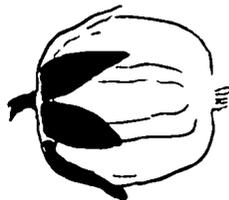
Petal

| = 2 mm.



Capsule

| = 1 mm.



(B)

(A)

Fig. 40 Characters of leaflet, sepal, petal, and capsule in O. acetosella (A), and O. montana (B).

Morphological comparison between *O.acetosella* agg. and *O.oregana*

Our observations on *O.oregana* were made on herbarium material and on three cultures, two directly from California, the third from Wisley, England.

The evidence strongly suggests that from the geographical and morphological points of view, *O.oregana* is distinct from *O.acetosella*. The plant is larger in nearly all its parts; the leaflets attain on the average a larger size (31 x 22 mm), with longer petioles. The flowers are larger (18 x 6mm), with longer pedicels. The capsules are larger (9 x 5mm) with larger seeds (3 x 2mm). All these are maintained in cultivation. The plant is, moreover, densely pubescent and has thicker rhizomes, and does not produce cleistogamous flowers.

In subsequent chapters, other lines of evidence will be utilised to discover as much as possible about the relationship between *O.acetosella* and *O.montana* on the one hand, and these and *O.oregana* on the other.



Variability of *O. acetosella*

It had been noticed that some collections of material from montane habitats had small leaves, and it was of interest to see to what extent this kind of difference was maintained in cultivation.

26 stocks of *O. acetosella* were selected from those available. Their provenance is given in Table 38. They had been in cultivation for various periods from a few months to three or four years. Each stock was cloned and divided into four portions; and each portion was potted in a 4" pot, in a well-mixed batch of compost. Each set of 26 pots, made up in the autumn, was then allowed to overwinter in four different environmental conditions, viz. (a) in shade under a bench in a warm greenhouse, (b) sunk in the ground out of doors, (c) out of doors in a cold frame, and (d) in a warm greenhouse on a bench which, in addition to daylight, received eight hours of artificial light per day. The plants were examined from time to time, and detailed notes were made in mid-winter and in spring (April).

The survival rates of the plants under the four treatments are shown in Table 39. Shade conditions in the greenhouse were very unfavourable, but well-lighted conditions in the greenhouse were favourable, and the plants did best here. There was quite good survival out of doors, but the plants in the cold frame did distinctly better than those without any protection at all.

Table 38

Localities for plants of *O. acetosella*
used in cultivation experiment.

Code No.	Country	Location	Altitude	Habitat
A4, 5	England	Co. Durham	Lowland	-
A4, 6	"	Near Middleton in Teesdale	800 ft.	Alder woods
A4, 8	"	Shincliffe, Co. Durham	200 ft.	Woodland
A4, 11	"	Co. Durham	Lowland	-
A5, 9	"	Durham	200 ft.	Woodland
A5, 11	"	Shincliffe, Durham	200 ft.	Woodland
A4, 2	"	Farley Hill, Berks.	Lowland	Woodland
A4, 3	"	Bucklebury, Berks.	Lowland	Woodland
A5, 8	"	Near Scarborough	Lowland	"
A4, 1	"	Sunbiggin, Westmorland	c.1500 ft.	Limestone rocks
A5, 13	"	"	c.1500 ft.	"
A1, 1	"	Great Dun Fell, Yorkshire	2400 ft.	Rock crevices
A4, 9	"	Cleveland Hills	1000 ft.	Grassland, under bracken
A4, 4	Scotland	Ben Lawers	c.2000 ft.	Rock crevices
A5, 2	"	Perthshire	2300 ft.	Cliffs
A5, 3	"	Skye	1850 ft.	"
A5, 4	"	Ben Lawers	3250 ft.	"
A5, 5	"	Caenlochan, Glen Angus	2850 ft.	"
A5, 6	"	Glen Doll, Angus	2350 ft.	"
A5, 12	"	Glen Fell, Angus	2700 ft.	-
A5, 10	"	Loch Sunart Argyll	-	Oak-birch Woodland

Table 38
(continued)

Code No.	Country	Location	Altitude	Habitat
A4, 10	<u>European</u> Denmark	Zealand	100ft.	-
A5, 14	"	-	100ft.	-
A3, 1	S. Finland	Tammisaari	Sea level	-
A2, 1	Switzerland	S. Gemmi	-	-
A4, 7	Germany	Murnau, Bavaria	-	-
A5, 1	Czechoslovakia	Bratislava	100ft.	-
A5, 7	Poland	Szczecin	-	-

Table 39

Response of 26 clones of O. acetosella
to four different winter environments

Environment	No. of plants surviving	Mean No. of leaves per plant, April	Mean No. of flower buds per plant, April
A) Warm greenhouse, in shade under bench.	6	6	0
B) Sunk in ground out of doors.	16	16	7
C) Cold frame	23	29	7
D) Warm greenhouse, 8 hours artificial light per day.	23	66	14

The leaflets of selected plants were measured on two occasions, in early spring and early winter; the results for the best grown plants, measured in early spring, are given in Table 40. This shows that many of the plants from high altitudes in England and Scotland have leaflets significantly smaller than those from low altitudes, though there is some overlap. This difference is not found in herbarium material from E.Asia, including Japan. Asian material (Table 41) appear to be always alpine in habitat, and of an altitudinal range of 7000-13500 ft. (about 2-4 times the highest peak in Britain). The plants collected from such high altitudes do not show any reduction in size and do not differ from the lowland populations of Europe and Britain. It appears that in its extreme north-western parts of the range, the species, besides occupying the lowlands, also ascends to well above the tree-line in some scattered isolated peaks; whereas in the extreme south-eastern parts of its range (Asia), the species becomes extremely rare in the lowlands and occurs only in the mountains. Its place in the lowlands is taken by another distinct taxon, viz., O.griffithii.

Table 40

Potted plants grown over winter
in warm greenhouse with artificial
light. Leaflets measured in April, in mms.

Code	Locality	Altitude	Mean mid-rib length	Mean lamina width
A5, 7	Poland	Lowland	17.0	25.0
A4, 7	Germany	"	10.5	16.0
A5, 1	Czechoslovakia	100ft.	10.0	15.0
A5, 9	Durham	200ft.	10.0	14.0
A5, 11	Durham	200ft.	9.0	14.0
A5, 6	Scotland	2350ft.	9.0	14.0
A5, 2	"	2300ft.	8.0	12.0
A5, 3	"	1850ft.	8.0	12.0
A5, 4	"	3250ft.	7.0	10.0
A5, 5	"	2850ft.	5.5	8.0
A4, 1	Westmorland	1500ft.	5.0	7.5
A1, 1	Yorkshire	2400ft.	4.0	6.0

Table 41

Asiatic high altitude populations
of O. acetosella. Herbarium data.

Means. Measured in mms.

Locality	Altitude	Mean midrib length	Mean lamina width
Sikkim	9500 ft.	8.0	8.0
Japan, Central mountains	2-7000ft.	8.0	10.0
Kanchiang range, China	11000 ft.	10.0	19.0
Kanchiang range	10-12000 ft.	11.0	16.0
Kashmir	7-9000 ft.	10.0	12.0
Burma-Tibet frontier	12000 ft.	11.0	9.0
Tibet frontier	9-13500 ft.	11.0	15.0
Yunnan	13500 ft.	12.0	16.0
W. Nepal	10000 ft.	13.0	17.0
India	8000 ft.	14.0	16.0
Japan, Nikko mt.		12.0	17.0

CHAPTER NINECytological comparisons

The chromosomes of the three *Oxalis* species with which we are concerned have been examined by several authors. Their results are summarised in Table 42.

Table 42Chromosome counts made byother authors

<u>Taxon</u>	<u>Place of origin</u>	<u>Somatic count</u>	<u>Meiotic count</u>	<u>Author</u>
<u>O. acetosella</u>	-		11-12(?)	Heitz (1927) ^a
	Lund, Sweden	22		Love & Love (1944)
	Iceland	22		Love & Love (1956 ^b)
	Japan	22		Hara (1966)
	Japan	22		Nakajima (1936)
	Calcutta, India	30		Sharma (1960)
	Eastern Himalaya	22		Hara (1966)
<u>O. acetosella</u> ssp	Japan	22		Hara (1952)
<u>griffithii</u>	Eastern Nepal	22		Hara (1966)
<u>O. montana</u>	Canada	22		Love & Love (1956)
<u>O. oregana</u>	Bot. garden, Wisley	22		Marks (1964)
<u>O. oregana</u>	" " "	33		Marks (1964)

The count of $2n=30$ can be eliminated straight away from this list. The plant is described by Sharma as having large, broad, membranous stipules, and this can not be O.acetosella. It has not been possible to consult the original paper of Heitz, and the reason for the variation in number which is given (in published lists) is not known. All the remaining counts are in agreement; none of them gives any details of chromosome morphology, though Nakajima states that the chromosomes are rod-shaped and of similar size.

Our own counts are given in Table 43. The technique used is similar to that described (p.46) for Maianthemum. Divisions in root-tips are difficult to find.

Table 43

Our own chromosome counts

Taxon	Place of origin	Somatic number (root-tip)	Meiotic count	No. of plants examined
<u>O.acetosella</u>	Durham		11	1
	Scarborough	22	11	1
	Poland	22	-	1
<u>O.montana</u>	Nova Scotia	22	-	1
<u>O.oregana</u>	California	22	11	1

The chromosomes are small, but there is a marked variation in size. A karyotype analysis has been carried out in the same way as for Maianthemum (p.47). Fig.41 shows the chromosomes, photographed after they have been cut and arranged on a card. The pairs are arranged in order of size and measured. The largest is taken as unity

and the length of each of the others expressed as a fraction of the largest. A symmetry index, of the ratio of the short arm to the long arm, is also worked out. The data obtained, and the graphs plotted from them, are shown in Figs. 41 and 42 and summarised in Table 44.

It will be seen that the size ranges in O.acetosella and O.montana are very similar; so far as can be judged, the absolute sizes are about the same (4-8 microns). The karyotype formulae are:-

O.acetosella: 4 median + 5 submedian + 2 subterminal

O.montana: 4 median + 4 submedian + 3 subterminal

The correspondence in size and symmetry in the two species is not exact, but on the basis of the small sample examined, it is not possible to say that there is a significant difference.

The karyotype formula of O.oregana is 4 median + 5 submedian + 2 subterminal, but here there is rather less difference in size between the smallest and the largest chromosomes, and this might be a significant difference, if it could be established in a larger sample. But certainly the data, so far as they go, support the morphological evidence, in indicating close similarity between O.acetosella and O.montana.

Table 44

Karyotype analysis of somatic chromosomes
of O.acetosella, O.montana and O.oregana

Chromosome pair number	Short arm	Long arm	Symmetry index	Total length	Length index	Centromere position
<u>O.acetosella</u>						
1	4.0	4.0	1.0	8.0	1.0	Median
2	2.2	3.3	0.6	5.5	0.7	Submedian
3	3.0	3.5	0.9	6.5	0.8	Submedian
4	2.7	3.8	0.7	6.5	0.8	Submedian
5	2.2	3.9	0.6	6.1	0.75	Submedian
6	2.7	2.7	1.0	5.4	0.7	Median
7	2.7	2.7	1.0	5.4	0.7	Median
8	1.4	3.6	0.4	5.0	0.62	Subterminal
9	1.4	3.6	0.4	5.0	0.62	Subterminal
10	2.5	2.5	1.0	5.0	0.62	Subterminal
11	1.4	2.6	0.5	4.0	0.5	Submedian
<u>O.montana</u>						
1	4.0	4.0	1.0	8.0	1.0	Median
2	2.7	4.8	0.6	7.6	0.95	Submedian
3	3.4	3.4	1.0	6.8	0.85	Median
4	3.3	3.3	1.0	6.6	0.8	Median
5	2.2	3.8	0.6	6.0	0.75	Submedian
6	2.2	3.8	0.6	6.0	0.75	Submedian
7	2.0	3.4	0.6	5.4	0.7	Submedian
8	1.6	3.8	0.4	5.4	0.7	Subterminal
9	1.4	3.6	0.4	5.0	0.62	Subterminal
10	1.4	3.6	0.4	5.0	0.62	Subterminal
11	2.0	2.0	1.0	4.0	0.5	Median
<u>O.oregana</u>						
1	3.5	3.5	1.0	7.0	1.0	Median
2	3.1	3.1	1.0	6.2	0.9	Median
3	2.7	2.7	1.0	5.4	0.8	Median
4	2.3	3.1	0.8	5.4	0.8	Submedian
5	2.7	2.7	1.0	5.4	0.8	Median
6	1.7	3.4	0.5	5.1	0.7	Submedian
7	2.0	3.0	0.6	5.0	0.7	Submedian
8	1.4	3.6	0.4	5.0	0.7	Subterminal
9	1.4	3.6	0.4	5.0	0.7	Subterminal
10	2.3	2.7	0.9	5.0	0.7	Submedian
11	1.4	2.9	0.5	4.3	0.6	Submedian

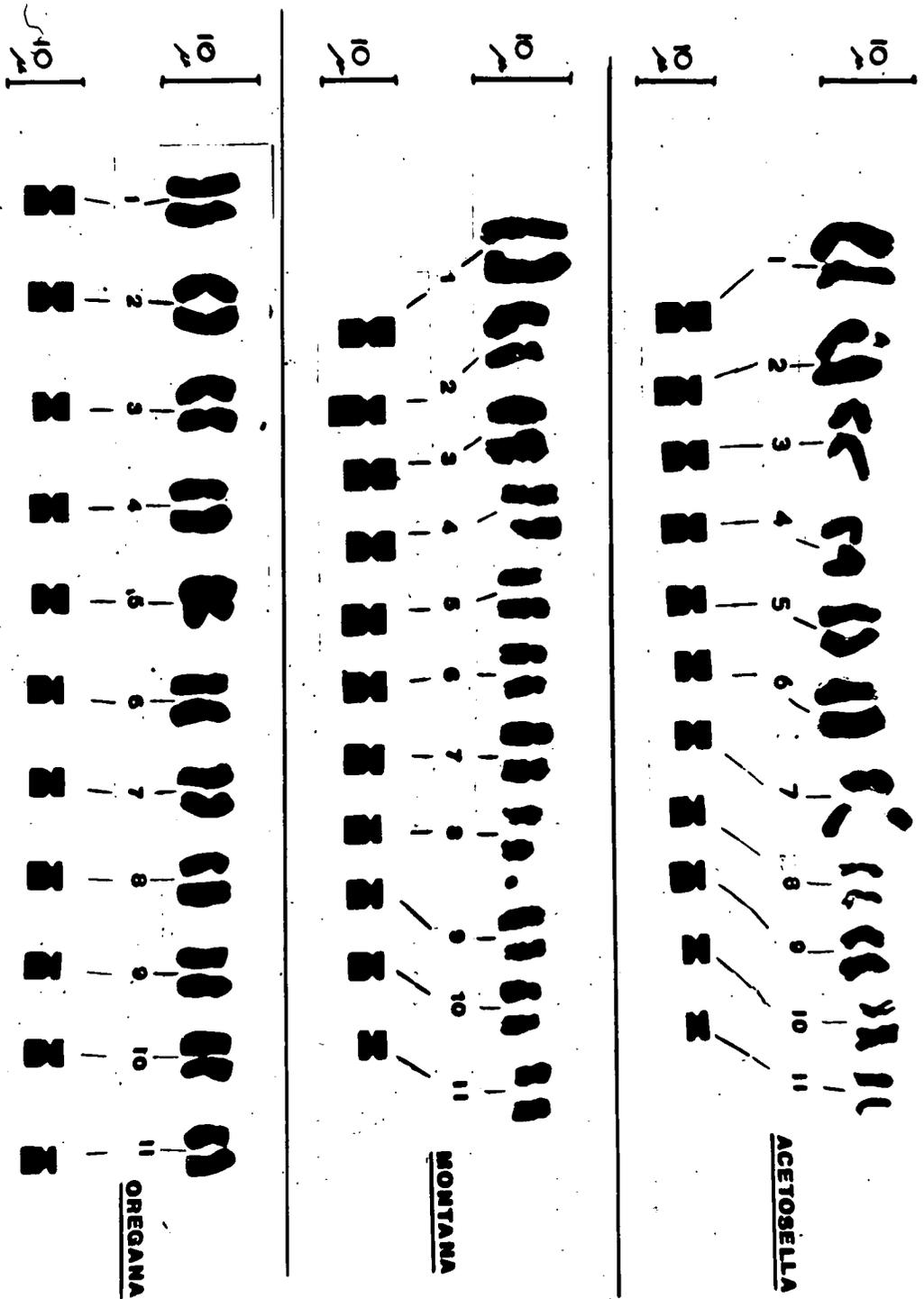


Fig. 41: Somatic karyotype and idiograms for O. acetosella, O. montana, and O. oregana.
 ($2n=22$ arranged in pairs in order of length).

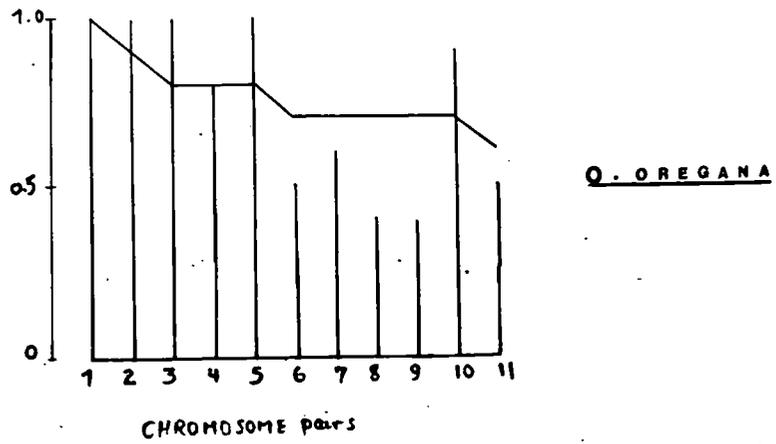
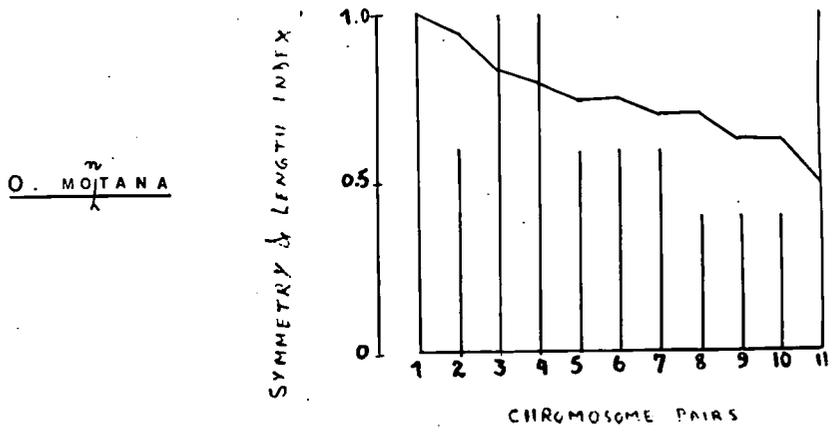
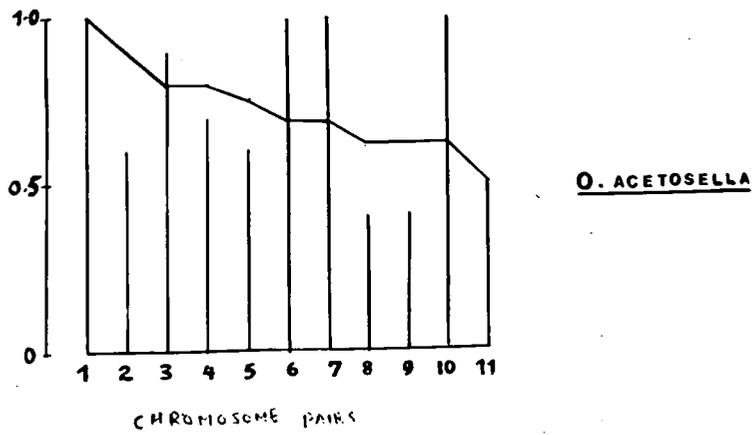


Fig. 42: Graphic representation of the karyotypes of O. acetosella, O. montana, and O. oregana.

Meiosis in O.acetosella

It has not been possible to obtain meiotic stages in O.montana, partly because we had so few plants in cultivation, but it has been studied in plants of O.acetosella from Durham, Scarborough and Poland. This could only be done by taking many samples to obtain buds at the right stage (often rather early in the year, in February and March). The squash technique used was similar to that described already (p.46) for Maianthemum.

Diakinesis is difficult to find and analyse, but it was seen in stocks from Durham (Fig. 43-1) and Scarborough (Fig. 43-2). There is a marked variation in the length of the bivalents at this stage.

Metaphase I plates have been seen in side view in the Scarborough and the Polish materials (Figs. 43-3 and 43-4 respectively); the bivalents are of almost the same size.

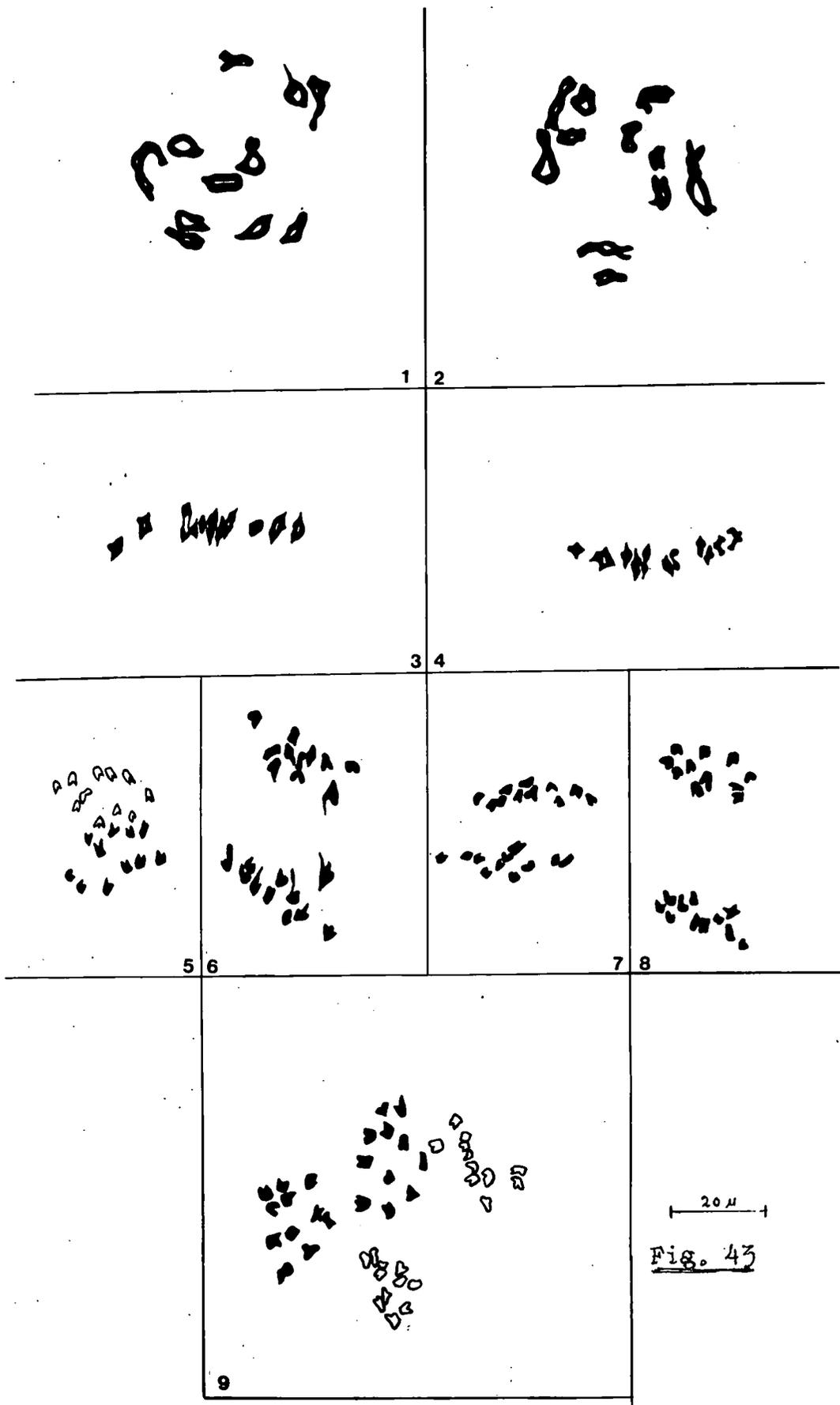
Anaphase I stages were seen in all three stocks; the English material (Figs. 43-5, 43-7 and 43-8) and the Polish material (Fig. 3-6) are illustrated. Eleven daughter chromosomes are countable at each pole, all of the same size.

Anaphase II has been observed in only one stock, Durham, Fig. 43-9, where 11 chromosomes are countable at each pole.

Meiosis in O.oregana

This was studied in both the Wisley and Californian diploids; the first is white flowered, the second pink-flowered. The only countable preparation obtained was at early metaphase and showed 11 bivalents.

- Fig. 43 Meiotic stages in O.acetosella
- 43-1 Diakinesis in Durham material, 11 bivalents.
- 43-2 Diakinesis in Scarborough material,
 11 bivalents.
- 43-3 Metaphase I in Polish material, 11 bivalents.
- 43-4 Metaphase I in Durham material, 11 bivalents.
- 43-5 Anaphase I in Durham material, 11
 chromosomes at each pole.
- 43-6 Anaphase I in Polish material,
 11 chromosomes at each pole.
- 43-7 Anaphase I in Scarborough material,
 11 chromosomes at each pole.
- 43-8 Anaphase I in Durham material,
 11 chromosomes at each pole.
- 43-9 Late anaphase II in Durham material,
 11 chromosomes in each polar set.



The triploid O.oregana (white flowered) obtained from Wisley was also examined, but only tetrad stages were found, and these were irregular with many micronuclei. This plant produced capsules when pollinated by a diploid, but the capsules were empty at maturity; it appears to be highly sterile. Nothing is known about its origin.

CHAPTER 10Hybridization Experiments

The morphology of the flower in the three species of Oxalis selected for this study is fundamentally similar, and the plants are, with one exception, self-compatible. The styles in all these plants project beyond the outer larger ring of stamens, but occasionally some variation in length is observed where they become equal to the stamens, or slightly shorter. It was only in one case, a plant from Poland, that the number of stamens was found to be five instead of the normal 10, (A5, 7). In other respects, including chromosome number, this plant was normal. Meristic variation in number of floral parts was found in a plant from Scarborough which was grown in the greenhouse, but the wild population appeared to be perfectly normal.

As has already been pointed out, (p. 98) there is a considerable period of overlap in the flowering season of all three taxa, a fact that made hybridization possible. In the following pages our hybridization experiments will be described and discussed. The method used is to emasculate the flowers before the stamens reach maturity, and it is sometimes necessary to dissect out one petal in order to reach the stamens. The flowers tend to fold in and close when the weather becomes dull and cold. Dusting of pollen is carried out by brushing a whole anther against the stigmas and

repeating this when necessary. All the experiments were carried out in an insect-proof greenhouse.

Selfing and intraspecific crosses

Before describing the hybridization experiments, a word should be said about seed-set in the species themselves. Many self-pollinations have been made, on various stocks of O.acetosella and these regularly produce seeds in about 30 days, the number of seeds per capsule being 6-15, with a mean of 9. In the summer, cleistogamous flowers regularly follow, and these too produce seeds at about the same time and in the same numbers. Selfing of open flowers of O.montana has not been done, but the cleistogamous capsules and their productivity are both very similar to those of O.acetosella. The seeds in both are longitudinally ridged and measure on the average 2 x 1.5 mm. Mechanism of seed dispersal in all three northern hemisphere species is mechanical; the capsules dehisce by slits along the sides and the seeds are shot out laterally. This is largely brought about by the drying up of the mucilagenous layer that surrounds the individual seeds, (Ridley 1930; V.Royale 1918). Successful crosses have been protected against loss of seeds by simply covering the developing ovary by a light bag of well-ventilated paper with the weight of the bag suspended on a bridge of hard paper.

O.oregana does not produce cleistogamous flowers, and early experiments seemed to indicate that it was self-incompatible. The most recent experiments are summarised in Table 45.

Table 45

Pollination of *O.oregana*,
stock (D5,3) California, April-May 1967

Procedure	No. of flowers treated	Result	No. of seeds produced
Flowers left intact	15	Ovaries not stimulated	No seed-set
Flowers selfed one dusting of pollen	12	Ovaries not stimulated	No seed-set
Flowers selfed, several dustings	2	Seed set	3
Flowers pollinated from another flower on same plant	1	Seed set	3
Flowers pollinated from another plant of same stock	1	Seed set	5

These experiments are obviously not conclusive and further investigation is needed. There is obviously a tendency to self-incompatibility, though this can apparently be overcome by repeated pollination. We thought that the plant might be heterostyled and that failure of selfing might be to this phenomenon, but Professor H.G. Baker (in correspondence) says that it is not. It is however, of interest to note that the two cultures of *O.oregana* received recently from Professor Baker show substantial difference in style, size and shape from the material on

which we have carried out our present experiments. In one strain the styles are longer than the stamens, in the other they are considerably short and are well below the stamens.

Inter-specific crosses:

(a) O.acetosella and O.montana

Difficulties were met with in making this cross, partly because of the non-coincidence of flowering time and partly because of the not very abundant flowering of the two montana stocks available. The results are shown in Table 46.

Table 46

Crosses between O.acetosella and O.montana

Seed parent	Pollen parent	No. of flowers pollinated	No. of capsules	No. of seeds	Time for capsule to mature
<u>acetosella</u>	<u>montana</u>				
A4, 8	B1, 1	4	0/4	0	7.4-11.5.65
A5, 7	B1, 1	3	3/3	3,1,3	17.3-14.5.66*
<u>montana</u>	<u>acetosella</u>				
B4, 2	A5, 1	3	1/3	3	12.4-19.5.65
B4, 2	A5, 15	4	1/4	0	19.6-12.7.66@

* development of ovary to maturity took 8 weeks instead of the usual 4 weeks.

@ the capsule developed normally and then dried up.

Table 46 shows that the yield of seed was very poor, averaging less than one seed per capsule; but in view of the small number of experiments that was possible, too much weight must not be put on this result.

(b) O.acetosella and O.oregana

These species flowered at about the same time, and it was fairly easy to make crosses. The results are given in Table 47.

Taking first the crosses in which O.acetosella was the seed parent, it can be seen that the 30 pollinations made in 1965 produced only two capsules, each of which contained one or more seeds. None of these germinated, and it is not possible to assess

Table 47

Crosses between O. acetosella and O. oregana

Seed parent	Pollen parent	No. of flowers poll.	No. of capsules	Time for capsule to mature	Remarks
<u>1965</u>					
<u>acetosella</u>	<u>oregana</u>				
A5, 1	D5, 3	30	2	16.4-13.5	One capsule with 3 seeds the other with one seed.
<u>1966</u>					
<u>acetosella</u>	<u>oregana</u>				
A2, 1	D5, 3	1	1	25.4-25.5	Capsule developing normally, but seeds imperfect.
A4, 2	D5, 3a	1	1	27.4-22.5	"
A5, 4a	D5, 3f	2	2	28.4-15.5	"
A4, 5b	D5, 3e	1	1	25.4-24.5	"
A4, 5a	D5, 3e	4	4	20.4-24.5	"
A4, 5b	D5, 3d	3	3	25.4-23.5	"
A4, 9a	D5, 3e	1	1	24.4-13.5	"
A4, 10a	D5, 3	2	2	11.4-14.5	"
A4, 11	D5, 3	1	1	7.5-22.5	"
A5, 1b	D5, 3b	2	2	21.3-12.5	"
A5, 7c	D5, 3	1	1	28.3-13.5	"
A5, 7b	D5, 3	1	1	20.4- 5.5	"
A5, 9	D5, 3e	1	1	25.4-22.5	"
A5, 10a	D5, 3e	1	1	27.4-27.5	"
A5, 15	D5, 3b	3	3	28.4-17.5	"
A6, 3	D5, 3f	2	2	28.4-24.5	"
D5, 3a	A5, 1b	1	failed	25.4-14.5	
D5, 3a	A5, 7	4	"	20.4-14.5	
D5, 3d	A5, 7a	4	"	20.4-12.5	
D5, 3f	A5, 15	2	"	20.4-12.5	
D5, 3f	A5, 11	1	"	22.4-12.5	
D5, 3a	A4, 5	3	"	3.4-14.5	

the significance of this result, though it is possible that the seeds were accidental selfs. This is made more likely by the numerous results of 1966. Here, all the pollinations, on a variety of seed parents, produced capsules, which matured in the normal four-week period, but the seeds were always imperfect. The seed-coat was well developed, but only a small amount of endosperm was produced. In Fig. 44, squash preparations with developing endosperm (four weeks old) are shown. The method followed for this preparation is that of Maxwell and Valentine (1966).

The reciprocal cross, O.oregana x O.acetosella failed completely; the ovary is not stimulated to develop by pollination, and there is no evidence that fertilization takes place. It is noteworthy that the style of O.oregana used in this cross is never less than 10 mm long, while that of O.acetosella is never more than 7 mm. It is thus possible that the pollen of O.acetosella is unable to grow strongly enough in the style of O.oregana to reach the ovules.

This cross provides an excellent example of seed incompatibility. The difference in the reciprocal crosses is characteristic, as is also the incomplete development of the seed in the O.acetosella x oregana cross. The result can be taken to demonstrate positive genetic affinity between the species; in that they are crossable to produce at least hybrid endosperm; on the other hand, the failure to produce viable seed or hybrids may be taken, with due caution, to indicate the not very close relationship between the species. It is unfortunate that from the other cross, acetosella x montana, no

really firm conclusions can be drawn, because of inadequate data.

The seeds produced in the crosses did not germinate (for germination difficulties see Chapter 11).

Fig. 44: Dissected ovules of the cross

(a) O.oregana ♀ x O.acetosella ♂

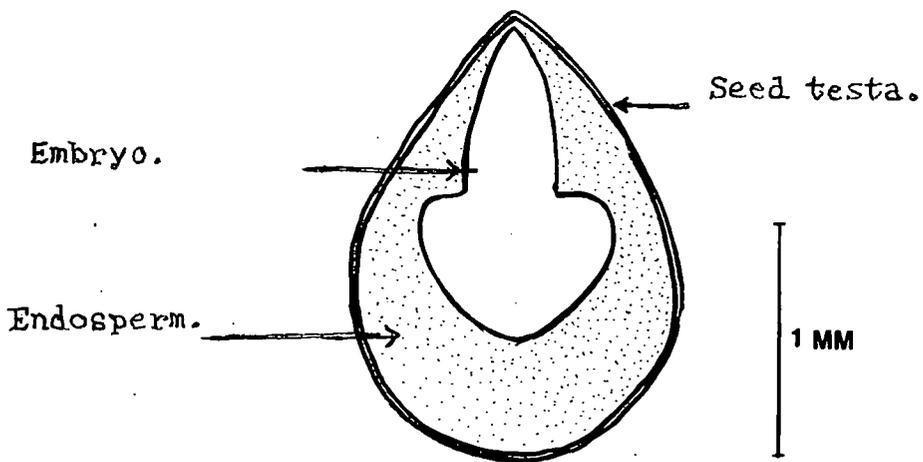
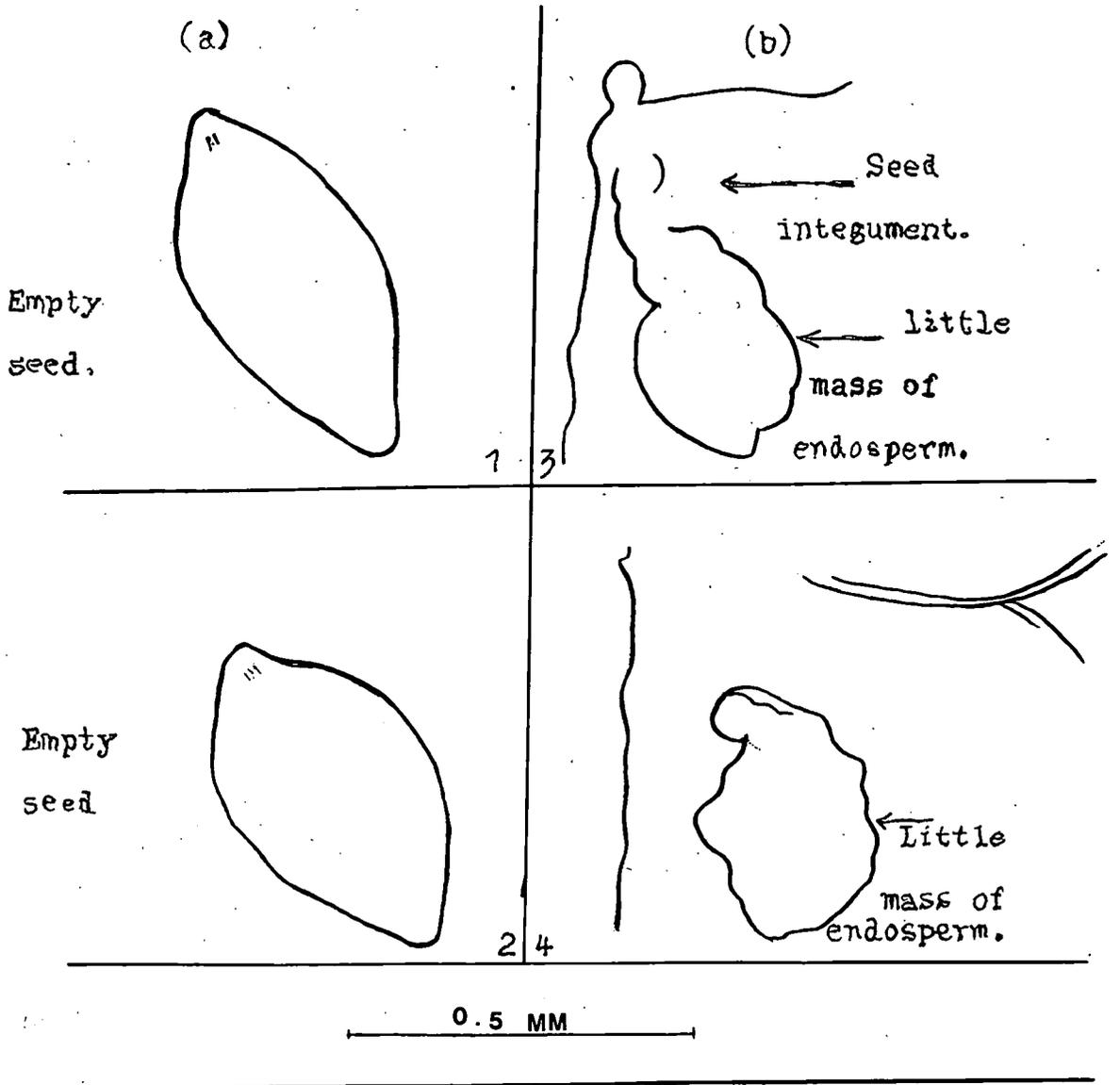
In both Fig. 44-1 and 44-2, no stimulation of ovary is observed, and no sign of fertilization is seen.

(b) O.oregana ♂ x O.acetosella ♀

In both Fig. 44-3 and 44-4 the ovary is stimulated following pollination, but the endosperm is poorly developed, and the ovule contains a small mass of it.

(c) Longitudinal section of the mature seed of a normal cross in O.acetosella.

N.B. All are of the same age of four weeks following pollination.



(c)

Fig. 44

CHAPTER ELEVENGermination Experiments

An unexpected difficulty in this study is that the seeds of *Oxalis* species have germinated very badly. As already mentioned, this has severely hampered experiments on ecotypic differences, and also made it impossible to multiply rapidly stocks of overseas material, especially *O. montana*.

The seeds of *O. acetosella* and *O. montana* have been described above. They weigh about 1.5mg and are endospermic with straight embryos. Those of *O. oregana* are larger in size, 3.3 x 2.4mm. and the ribs on their surface are irregular, forming a network.

The seeds were normally sown in pots on John Innes compost in autumn, and exposed to winter temperatures in a cold greenhouse. The results for seeds sown in the autumn of 1964 and the spring of 1965 are given in Table 48. All seeds are from cleistogamous or selfed flowers.

It is evident from Table 48 that a period of two years is necessary for the initiation of germination. The seeds seem to require at least two winters before germinating and even then a very low percentage is obtained. The experiment was repeated in the season 1965-66 and the results are shown in Table 49.

Table 48

Germination of Oxalis seeds, season 1964-65

Code	Taxon	Date of sowing	No. of seeds	Germination	
				1965	1966
A4, 11	<u>O.acetosella</u>	Sep. '64	15	0	1
A4, 7	"	"	15	0	6
A4, 5	"(pink)	"	15	0	6
A4, 8	"	"	15	0	0
A3, 1	"	"	15	0	1
	"	"	15	0	2
	"	"	15	0	0
	"	May '65	15	0	4
	"	"	15	0	0
	"	Sept. '64	15	0	0
	"	Feb. '65	15	0	2
Total Percentage germination			165	0	22 13%
B1, 1	<u>O.montana</u>	Sep. 64	15	1	3
Percentage germination				7%	20%
B5, 3	<u>O.oregana</u>	Mar. '65	10	0	0
	"	"	10	0	0

Table 49Germination of Oxalis seeds.All seeds sown on November 1st 1965.Seeds collected from selfed capsules June-August, 1965.

Code	Taxon	No. of seeds sown	No. germinating	
			1966	1967
A1, 1	<u>O.acetosella</u>	90	0	0
A2, 1	"	30	0	0
A2, 2	"	60	0	0
A3, 1	"	60	0	0
A3, 2	"	60	0	0
A4, 3	"	60	1	5
A4, 4	"	10	0	0
A4, 5	"	30	1	1
A4, 6	"	15	5	4
A4, 7	"	60	18	5
A4, 9	"	15	4	4
A5, 1	"	10	0	0
A5, 9	"	90	11	4
A5, 10	"	40	4	2
A5, 11	"	90	6	2
A5, 15	"	90	4	6
	Total	810	54	33
	Percentage germination			
	1966	7		
	1967	10.7		

The results for germination for O.acetosella show a germination of 0 per cent in 1964-65, 6.7 per cent in 1966, and 10.7 per cent in 1967 (Table 49). It is noteworthy that half of this germination came from two samples, A4, 7 (S.Germany) and A5, 9 (Durham). In both cases, replicate samples showed little or no germination, and it is not easy to find an explanation of this.

It was thought worth while to carry out a few germination experiments, and a brief account of them will be given here.

For the first experiment, 2000 seeds were collected, all from O.acetosella in one locality. These were then given a series of eight treatments, in which light and dark, presence and absence of 2000 p.p.m. of gibberellic acid, and high and low temperature (23°C. and 5°C.) were combined in all possible ways. The experiment was further elaborated by moving batches of seed from light to dark and from warm to cold, and back, at 24 hour intervals. None of these treatments produced any germination during the period December 1965 to July 1966, except one, and this was that in which the seeds were kept under cold, dark conditions. The relevant data are given in Table 50.

Table 50

Germination of treated seeds of
Oxalis acetosella

Code	No. of seeds	Percentage germination	Prevailing conditions Dec. '65 - July 1966	Previous treatment Nov.-Dec. '65
<u>1) With gibberellic acids:</u>				
A, 14	60	93	cold, dark	warm dark, cold dark.
A, 18	60	100	cold, dark	cold dark, cold light
A, 21	60	93	cold, dark	warm light, cold dark.
A, 5	20	100	(stationary under cold dark)	
<u>2) Without gibberellic acids:</u>				
A, 15	60	93	cold, dark	warm dark, cold light.
A, 25	60	70	cold, dark	cold light, cold dark.
A, 29	60	56	cold, dark	warm light, cold dark.
A, 7	20	65	cold, dark (stationary)	

It is clear from Table 50 that gibberellic acid had little or no effect on germination. It is perhaps, surprising, in view of the success of the cold, dark treatment, that the vernalisation of the seeds in soil, which was the treatment given in the winters of

1964-65 (Table 48) and 1965-66 (Table 49) provided so little result. It is possible that in a mild winter, the exposure to cold would be of too short duration, and that a second winter is needed to complete the treatment.

In a supplementary experiment, carried out at the same time, exposure to about zero temperature for a month, followed by a period of 10°C. in the light, produced no germination. Attempts to produce germination by softening the testa by treatment with sulphuric acid also produced no result.

A Note on O.lactea and O.magellanica

O.lactea Hook is a species found in Tasmania, Australia and New Zealand which appears to be rightly placed in the Acetosellae. It differs from O.acetosella in its pure white flowers, which do not have coloured veins or a yellow base, and in its generally smaller size. O.magellanica Forst is a taxon found in S.America, which has sometimes been included in O.lactea.

Material of O.lactea was received from New Zealand and of a plant said to be O.magellanica from the garden of Dr. D.P. Young, but the plants were difficult to cultivate and few significant observations were made.

As regards germination, ten seeds of O.lactea (E5, 1) sown in August 1965 produced 0 percent germination in 1966 and 50 percent germination in 1967. The seeds are very small (0.8 x 1.0 mm). Seeds of O.magellanica (C4, 1) sown in November 1965 produced no germination in 1966 and 1967. Thus these two taxa seem to require at least two years, i.e. two winters, before any germination is induced, in this they are similar to species of the Northern hemisphere.

It has been possible to do some hybridization experiments between O.magellanica and O.acetosella. All crosses, including the reciprocals, completely failed and no stimulation on the development of the ovary is observed. About 35 crosses were done, in 1965.

CHAPTER TWELVEDiscussion of Morphological findings

We have discussed earlier in this part of the findings and the opinions of the American botanist, Fernald, in connection with the American taxon O. montana and its relative the European O. acetosella, and we have come to the conclusion that the American plant is best treated as a subspecies of the European. It is important now to review the opinions of other workers on this section of the genus Oxalis.

In dealing with O. acetosella, Hultén (1958), says "the American plant, at first taken to be identical with the European and very similar in general appearance, differs in having narrower emarginate petals, a more depressed, globose capsule, more obsoletely ribbed seeds, and in the different ciliation of the sepals" and that the plant should be taken as a subspecies of O. acetosella. Dr. D. Löve (personal communication) says that "As Hulten failed to validate his transfer of O. montana Raf. to subspecific status, this has been done for him by D. Löve (1968)". Our evidence supports both Hulten and Löve.

Hara (1952) expressed the opinion that the American plant O. montana was not specifically distinct from O. acetosella. He states that, "the specific distinction between the European and

the American plants, pointed out by Fernald, seems to me to be untenable, and some Japanese specimens have oblong, emarginate petals, distinctly ciliate sepals, and depressed capsules as in the American plants. They have slender rhizomes and broad obovate leaves with round lobes. Hara thus considers the two taxa as part of one and gives the geographical distribution for the European O. acetosella as extending into N. America. He also stated that O. acetosella was common in cool, coniferous woods on higher mountains in Japan; and later (1966), he pointed out its occurrence at high altitudes in the Himalaya, e.g. in Sikkim at 3000m. in Rhododendron scrub. We have seen several herbarium specimens in the British Museum from high mountains in Asia and Japan (Table 41), which are identifiable as O. acetosella. We have been unable to confirm the reported plants with depressed capsules and emarginate petals in all the herbarium specimens of Asian and Japanese origin. These characters are seen only in the American plants and are in our opinion enough to distinguish the N. American plants from the European and Asiatic material.

Hara also referred to another taxon, first as subspecies *japonica* (Franch and Sav.) Hara and later as subspecies griffithii (Edgew and Hook f.) Hara, (Plate 1). This taxon occurs at lower altitudes in both Japan and Sikkim (Appendix). It differs from typical O. acetosella in having thicker rhizomes, that are also different in being densely covered by petiole bases (Plate 1) and in having larger and more obtriangular leaflets, with more divaricate

obtuse lobes, larger capsules and larger seeds. It appears to be distinct from subspecies acetosella in Sikkim, but according to Hara intermediates between the two subspecies occur in Honshu province of Japan. Like subspecies acetosella, subspecies griffithii has $2n = 22$.

Knuth (1930) describes the plant as a species O. griffithii Edgew and Hook, and cites several high altitude localities for it in the Himalaya. No reference is made to the altitude factor in China and Japan, but in Formosa the plant is found at 2600 m. Dr. Love (in correspondence) is of the opinion that this taxon should be considered as a separate species until further cytological information is obtained. Our evidence clearly reveals that this taxon is larger than subspecies acetosella, and has several other characters, such as shape of leaflets and capsule, that mark it off even from O. oregana the largest of the Acetosellae group studied in this thesis.

The Asiatic taxa presented in the map Fig. 36 and in the key (p. 94) need further investigation. It would be of interest if the relationship between O. obtriangulata, O. griffithii, and O. hupehensis, is closely examined. They have a considerable area of overlap and hybridization is possible. It appears that the taxon O. leucelepis R. Knuth, is a possible population of intergrading forms between O. griffithii and O. hupehensis. Knuth describes it as a synonym for either. Further investigation of these taxa is obviously needed.

To sum up our findings, the data are presented in Table 51 and in Fig. 45. The Table shows that:

- (1) O.oregana and O.griffithii are larger in size than O.acetosella and O.montana.
- (2) the most closely similar taxa are O.montana and O.acetosella.

In addition there are other important differences marking off O.oregana from the rest; these are given on page 101. Little is known of the Japanese and Asiatic O.griffithii and it would be of great interest to grow the Japanese taxa alongside the American and the European and in particular to look for physiological and genetical differences such as flowering time cleistogamy, compatibility, and ability to cross with other related taxa. Apparently it is only in Asia that O.acetosella has been able to invade the high mountains. It may well be that the ancestral circumpolar species in this group resembled morphologically subspecies acetosella; and that in N.America this gave rise to subspecies montana.

The attempts to grow and cross O.lactea with other species (p.128) indicate that the southern hemisphere species may not be able to exchange genes with those of the northern hemisphere. Little is known of the biosystematics of groups which have representatives in both northern and southern hemispheres; and it is hoped that further experiments can be made. We will deal in the general discussion with other points of phytogeographical interest.

Table 51

The taxa and their similarities and differencesAll herbarium data except No. 18

Character	<u>oregana</u>	<u>griffithii</u>	<u>acetosella</u>	<u>montana</u>
1 Leaflet ^d obcorate	+	-	+	+
2 Leaflet lateral lobes rounded	+	-	+	+
3 Leaflets pilose on both sides	+	-	+	+
4 Lamina > 25mm wide	+	+	-	-
5 Midrib > 15mm long	+	+	-	-
6 Petiole > 90mm	+	+	-	-
7 Pedicel > 80mm	+	+	-	-
8 Flower > 15mm long	+	+	-	-
9 Petal > 5mm wide	+	+	-	-
10 Petal undulate emarginate at apex	-	-	+	+
11 Sepal emarginate	-	-	+	-
12 Capsule > 8mm long	+	+	-	-
13 Capsule > 4mm wide	+	+	-	-
14 Capsule pointed at apex	+	+	+	-
15 with cleistogamous flowers	-	+	+	+
16 Rhizome slender sparsely pubescent	-	-	+	+
17 Rhizome thick densely pubescent	+	+	-	-
18 Plant sets seeds freely on selfing	-		+	+

Fig. 45 Morphology of Oxalis species

Polygon presentation.

The characters plotted along the radii,
in the following manner: Herbarium data.

- A. Lamina width at apex.
- B. Width of lamina at midpoint.
- C. Midrib length.
- D. Petiole length.
- E. Pedicel length.
- F. Flower length.
- G. Petal width.
- H. Fruit length.

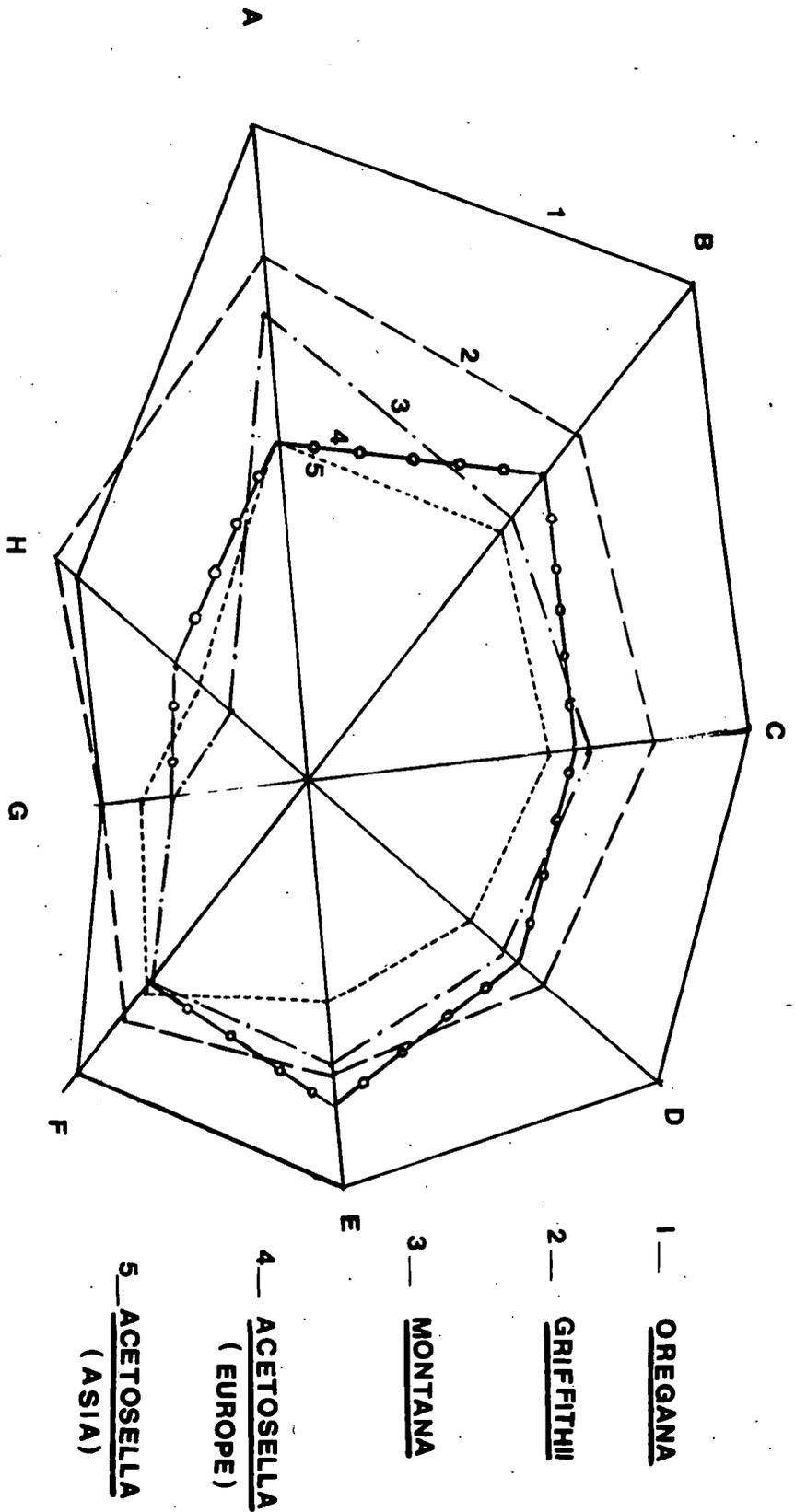


Fig. 45

CHAPTER THIRTEENGeneral Discussion

In this final discussion, we shall deal with some of the broad points of resemblance and difference between Oxalis and Maianthemum from the point of view of evolution and geographical distribution. If we leave out of account the S.hemisphere representatives of the section Acetosellae, then the distribution of the two groups we have dealt with shows a striking similarity, as well as some points of resemblance in the pattern of variation, thus:-

Geographical area	Genus <u>Maianthemum</u>	Genus <u>Oxalis</u> section <u>Acetosellae</u>
Europe	<u>M.bifolium</u> subspecies <u>bifolium</u>	<u>O.acetosella</u> subspecies <u>acetosella</u>
Asia (including Japan)	<u>M.bifolium</u> subspecies <u>bifolium</u> <u>M.dilatatum</u> var. <u>kamtschatichum</u> var. <u>vegetior</u>	<u>O.acetosella</u> subspecies <u>acetosella</u> <u>O.griffithii</u> <u>O.obtriangulata</u>
C. & E. North America	<u>M.bifolium</u> subspecies <u>canadense</u> subspecies <u>interius</u>	<u>O.acetosella</u> subspecies <u>montana</u>
Pacific north-west	<u>M.dilatatum</u> var. <u>dilatatum</u>	<u>O.oregana</u>

Stebbins (1950 p.531) says "The contemporary distribution patterns, like the external appearance and genetic constitutions of the organisms themselves, are the end results of the interaction of various evolutionary processes and of changes in the earth surface and climate over long periods of time. If, therefore, two or more unrelated groups of organisms have identical or similar modern pattern of distribution, we can logically infer that their evolutionary history have been similar, at least in certain respects and during the more recent periods of geological times."

The variational pattern of our two groups is very similar, and in both cases the European, Asiatic, C & E.N. American vicarious taxa are different, though most of the differences are estimated to be only at the subspecific level; in both cases the Pacific northwest taxa are distinct from the European and C. & E.N. American. In Maianthemum C. & E.N. America shows a maximum degree of variation, whereas the maximum centre of variation for northern hemisphere species of the section Acetosellae is possibly in eastern Asia and Japan. The European taxa of both groups are very stable. It is also of interest to note that the two most distinct taxa in both groups of plants are found on the forest floor of the Redwoods of Pacific North America. The Redwoods have had an interesting history (Chaney, 1948) and the Pacific northwest apparently had a different climatic history which could have resulted in the localization of these and other species (Japson 1951).

Vicariism in most of the taxa investigated is mostly horizontal, the taxa replacing each other geographically in a circumpolar manner. In eastern Asia, in the case of O.acetosella, vicariism appears to be altitudinal, in that typical acetosella, absent from the lowlands, is found in the mountains, partly mingling with another taxon, O.griffithii, which is mostly of lowland distribution.

Probably the most important historical events which have influenced the distribution of plants of the north temperate flora are the glaciations. According to Fernald (1929) and Cain (1944), these have had almost the same sequence in Europe and N.America, with one major difference. The main mountain series in Europe runs from east to west, that in N.America from north to south. This has affected the extent of plant migration, and has probably led to the extinction in Europe of many species which have been able to survive in N. America. Hara (1962) writes "Most temperate genera which constitute the present flora of Japan had already been differentiated by the middle Tertiary, and many temperate plants of ancient origin, including the so-called Arcto-Tertiary elements were later greatly disturbed in Europe and western N.America, but have survived in Japan and eastern N.America, mainly because of the mild climate during the Ice Age and the present temperate climate of well distributed rainfall. The glacial periods, however, have played an important role in the differentiation of the Japanese plants which have been the same as the N.American ones in the Tertiary period." Such affinities

between eastern Asia and eastern N.America have long been observed (Gray 1846). Cain (1944) remarks that many vicarious pairs of species in eastern N.America and eastern Asia are exceedingly close to each other. Hara (1952, 1956) lists several Japanese plants that are conspecific with or vicarious for the European and N.American ones. In our species, however, this particular phenomenon is not found.

Corresponding taxa on both sides of the Atlantic have been studied by several authors. Fernald (1929), Love (1954), and Marie-Victorin (1938) have listed many genera, including Maianthemum and Oxalis, with pairs of taxa on either side of the Atlantic which show varying degrees of resemblance. Some of these authors consider the American member of any such corresponding pair as equally ancient as, and perfectly distinct from, its relative on the other side. Fernald (1929) says that "species which in America are confined chiefly to the Alleghanian region will be found to differ in very fundamental characters from their nearest allies of Continental Europe." To establish this further, Fernald draws an exact parallel to the glaciation sequence in both the N.American and European continents; and is inclined to explain the ancient origin as a result of the disappearance of a North Atlantic Continent, which formerly linked America and Europe. Marie-Victorin (1938) holds the same point of view; that the corresponding taxa "up to the present remarkably similar due to continuity of land and

similarity of habitat and climate", are examples of divergent evolution following the gradual disappearance of the former north-Atlantic bridge. Hultén (1963), however, finds little phytogeographical evidence to support the land-bridge connection. He finds that in many cases most of the species are absent from Greenland, Iceland, and other North Atlantic Islands. In this connection it may be noted that there is no reference to the occurrence of Maianthemum in any of the North Atlantic islands; as for O. acetosella, it has been reported in Iceland and the Faeroes (Ostenfeld and Grøntved, 1934) and as having its refuge area in Iceland during the Ice Age (Steindorsson 1963).

Hultén (1937) assumed that Beringia and parts of N.E. Asia were relatively free from ice during the glacial periods, and had constituted areas from which species were able to radiate W. E. or S. when conditions were favourable. Recent evidence (Jonker 1968) has indicated that in Miocene times the forests of Siberia and Alaska were continuous, becoming disjunct in the late Miocene. Colinvaus (1967) has shown that the land bridge between America and Asia supported only tundra vegetation during the Pleistocene and that the forests did not merge again. Nevertheless, Hultén's ideas for the centres of distribution in the Bering region are still valid, at least as a basis for discussion. He lists several radiation centres, as follows:

1. N.E. Siberia and Amur-Manchuria region.
2. The Altai-Sajam region.
3. North Japan.
4. The Northern part of the Bering Sea.
5. From the Yukon Valley along the Arctic coast.
6. The Arctic Archipelago.
7. From the State of Washington along the American coast,
or the Rocky Mountains to Alaska.

According to Hulten M.bifolium has its centre in eastern Asia, dilatatum in the Bering Sea area. It is possible to assume that the American populations are migrants from the Eurasiatic centre. The same treatment is possible for O.acetosella agg., in that the main centre is eastern Asia. As for O.oregana, which in California is restricted to the belt of Redwood transition (Jepson 1951), it seems to have radiated from refuge areas in the State of Washington, into California in the south, and to Oregon in the north.

The situation for the maritime and continental Maianthemum of N.America has been interpreted differently by other workers. Butters (1927) is of the opinion that there were originally two distinct entities, one glabrous and north eastern (canadense) the other pubescent and western (interius). These could have developed into two perfect species had there been any strong barrier between them. The two taxa, he believes, were disturbed by the glaciations and on migration north, east, and westwards, they met each other in the

northern part and are now in the process of reuniting. Love (1959) thinks of the pubescent form (interius) as a "western" element in the process of restocking of the flora of Manitoba, implying that it found refuge in the foothills of the Rocky Mountains. In our opinion, subspecies interius is morphologically and geographically, possible also ecologically (Love and Love 1954 - also p. 5) rather distinct from canadense and is possibly on the way to becoming a distinct species.

Our own results do not lead to any startling phytogeographical conclusions, though they have been helpful in clarifying the taxonomy of the species. We had hoped to find structural differences between the chromosomes of the different taxa, and also by hybridization to investigate their genetical relationships. Structural differences in the chromosomes apparently do not exist, and it has not yet been possible to obtain mature hybrids. As regards polyploidy, the results have been interesting, but inconclusive from the phytogeographical point of view. In Maianthemum, it would appear that there has been gradual evolution at the $2n = 36$ level in Valentine's sense (Valentine, 1949 and 1963), and that polyploids have arisen here and there, and apparently quite independently, in different parts of the area of the genus; and except possibly in M. canadense, polyploidy has had little effect on the formation of new taxa.

It is clearly not possible from our results to come to any definite decisions about the relationship between subspp. acetosella and montana of O. acetosella. They are apparently closely related;

and it is possible that they had a common origin in E.Asia, but that is all that can be said. O.oregana is very distinct and probably originated in Western North America at an early date.

As regards Maianthemum, aⁿ eastern Asiatic origin also seems the most probable; here subsp. bifolium would correspond to subsp. acetosella, and subsp. canadense to subsp. montana. The position of M.dilatatum is not so clear; the fact that, like O.oregana it is part of the redwood flora of California (Japson 1951) may indicate that it split off early from the main stock of the genus. Its hybridization with M.bifolium, and the possible introgression that has been recorded, may be a later occurrence.

Sources of the herbarium specimens:

In the following Appendix, the localities examined are enumerated with reference to the region or area. The plants for both Maianthemum and Oxalis are borrowed from the following herbaria:

1. Cambridge University
2. Durham University
3. Dublin University
4. Harvard University
5. Munich University
6. Manchester University
7. The New York Botanical Garden

A good many herbarium sheets have also been examined at the British Museum (Natural History).

In preparation of the map Fig. 2 the exact location of some of the localities has been obtained from the "Index Gazeteer to the TIMES Survey Atlas of the World" - 1922.

APPENDIX 1Localities for Maianthemum and Oxalisa) Maianthemum

Taxon	Country or State	No. of localities examined	Country or State	No. of localities examined
<u>bifolium</u>	Norway	8	Sweden	6
	Germany	5	Poland	4
	Denmark	3	Yugoslavia	3
	Britain	3	Netherlands	2
	France	2	Austria	2
	Czechoslovakia	1	Finland	1
	China	5	Japan	5
	N. Russia	3	Korea	1
<u>Total number of localities = 54</u>				
<u>dilatatum</u>	Alaska	6	California	5
	Oregon	3	Washington	5
	Vancouver Island	6	British Columbia	1
	Russia	3	Kamchatka	2
	Korea	1	Saghalin	1
	China	2	Japan	12
	<u>Total number of localities = 47</u>			

APPENDIX I (continued)Localities for Maianthemum and Oxalis.a) Maianthemum (continued)

Taxon	Country or State	No. of localities examined	Country or State	No. of localities examined
<u>canadense</u>	Pennsylvania	2	New Jersey	2
	Minnesota	4	S.W. Virginia	1
	Gaspé Penn.	1	Manitoba	1
	Tennessee	1	Mass.	2
	N. Dakota	1	Connecticut	1
	Labrador	2	Ontario	4
	Maine	2	Indiana	2
	Prince Edward Island	1	Quebec	3
	Michigan	2	Ottawa	1
	N. Carolina	1	Newfoundland	4
	Magdalen Islands	1	Vermont	1
	Philadelphia	1	New York	4
	<u>Total number of localities = 45</u>			
<u>interius</u>	Minnesota	1	Manitoba	1
	N. Dakota	2	Ontario	1
	Indiana	1	Cumberland	1
	New York	1	Rochester	1
	Alberta	3	Mackenzie District	1
	Wisconsin	1	S. Dakota	1
<u>Total number of localities = 15</u>				

APPENDIX 1 (continued)Localities for Maianthemum and Oxalis.b) Oxalis

Taxon	Country or State	No. of localities examined	Country or State	No. of localities examined
<u>acetosella</u>	<u>Europe</u>	20	Britain	68
	<u>Asia</u>			
	India	8	Kashmir	2
	China	6	Japan	5
	<u>Total number of localities = 109</u>			
<u>griffithii</u>	China	4	<u>India</u>	7
	Kashmir	1	Japan	
	<u>Total number of localities = 14</u>			
<u>montana</u>	Quebec	9	Nova Scotia	6
	New York	6	Pennsylvania	3
	Virginia	3	Massachusetts	2
	Vermont	4	Prince Edward Island	2
	Maine	1	S. Appalachian Region	1
	New Brunswick	2	Connecticut	1
	N. Carolina	2	New Hampshire	2
	<u>Total number of localities examined = 44</u>			
<u>oregana</u>	California	6	Washington	3
	Oregon	4		
	<u>Total number of localities examined = 13</u>			

APPENDIX 2Maianthemum Seed Collections

<u>Locality reference as in Tables</u>	<u>Taxon, locality, name of collector and date</u>
(1)	<u>M.bifolium</u> , Finland, Wusemma prov., Tuusula, Maantickyla, fresh spruce forest, col. Raya-Leena, Hamet-Ahti 26.9.1964
(2)	<u>M.bifolium</u> , Finland, prov. Wusemaa, Provoo rural commune, Suomenkyla, North of Lake Papinjaru, co. Leena Saaricalo 1964.
(3)	<u>M.bifolium</u> , Finland, Wusemma prov., Zoo. Station, Taurmine, col. H.Luther 4.10.64
(4) & (14)	<u>M.bifolium</u> , Finland, Porv. Wusemma, Espoo, Royla Dasbacka, col. Marjatta Jsovuta 1964.
(5) & (18)	<u>M.bifolium</u> , Sweden, Uppland, Uppsalla, col. O.Hedberg, 1964.
(6)	<u>M.bifolium</u> , Sweden, Graso parish, Uppland, 1964.
(7) & (12)	<u>M.bifolium</u> , Sweden, Morkarla parish, Uppland, 0.5 km south of "Vatvik", col. B.Jonsell and L. Junell, 27.9.1964.
(8) & (11)	<u>M.bifolium</u> , Sweden, Almungo parish, Uppland 1964.
(9)	<u>M.canadense</u> , (Canada), N.Scotia, col. M.J.Harvey 1964
(10)	<u>M.dilatatum</u> , California, University of British Columbia.
(13) & (31)	<u>M.canadense</u> , Lac Philippe, Gatineau Park, Ottawa, Canada. col. W.J. Cody, August 8th, 1963.

Locality reference as
in Tables

Taxon, locality, name of collector and date

- (15) M.bifolium, Hortus Botanicus Universitalis
Uppsala, Suecia.
- (16) M.bifolium, Bot. Garden, Lund, Sweden.
- (17) M.bifolium, Institute de Botanique,
Strasbourg.
- (19) M.bifolium, Oulu, Finland.
- (20) M.dilatatum, USSR.
- (21) M.bifolium, Pruhinice, Czechoslovakia.
- (22) M.bifolium, Oulu, Finland, Kiiminki.
- (23) M.bifolium, Oulu, Finland, Liminka.
- (24) M.bifolium, Oulu, Finland, Kempele.
- (25) M.bifolium, Botanic Garden, Lund, Sweden.
- (26) M.bifolium, Institut fur Spezielle Botanik
und Arboretum, Berlin University.
- (27) M.bifolium, Belfause, 600 m (Fribourg)
Nature.
- (28) M.bifolium, Rheinsberg, Mark
- (29) M.bifolium, Jardin Botanique de Dijon 1965.
- (30) M.bifolium var. Kantschaticum, University
of British Columbia, Vancouver, Canada.
- (32) M.bifolium, Finland, Porv. of Uusimaa,
Espoo, Royla, Dalsbacka, Spruce forest
of Myrtillus type. col. Marjatta Jsoviita 1964

APPENDIX 3List of collection of living Maianthemum plants

<u>Country and code</u>	<u>Habitat and altitude</u>	<u>Collector</u>
<u>M. bifolium</u>		
Czechoslovakia (N1, 1)		D.H. Valentine 1957
Switzerland (N2, 1)	S. Gemmi	T.T. Elkington 1960
Switzerland (N2, 2)		M.E. Bradshaw 1960
Denmark (N2, 3a, N2, 3b, N2, 3c)	Seeland	D.H. Valentine 1960
England, Scarborough (N4, 2)	Wrench Green	M. Wilson 1963
Germany (N4, 1)	Murnau, Bavaria	D.J. Bellamy 1964
Denmark (N3, 1)		D.H. Valentine 1964
Czechoslovakia (N5, 1)	Zvolen	D.H. Valentine 1965
Denmark (N5, 2)	Sieleborg	D.H. Valentine 1965
Denmark (N5, 3)	Kas	D.H. Valentine 1965
Poland (N5, 4)	Szczecin	M. Jasnowski 1965
<u>M. canadense</u>		
Canada (M1, 1)	Morgan arboretum PQ	D.H. Valentine 1959
Canada (M4, 1)	Nova Scotia	Harvey 1964

APPENDIX 3 (continued)

<u>Country and code</u>	<u>Habitat and altitude</u>	<u>Collector</u>
<u>M. canadense</u>		
Canada (M6, 2)	Ile Penot, Vaudrevil Co. 30 miles west of Montreal Quebec	W.F. Grant 1966
Canada (M6, 4)	Gatineau Hills, PQ, Hull	T. Mosquin 1966
Canada (M6, 3)	Gatineau Park, Hull	C. Frankton 1966
Canada (M6, 4)	Harriston, Co. Wellington, Ontario	1966
Canada (M6, 5)	Co. Gaspe, Quebec	1966
<u>M. canadense var. interius</u>		
U.S.A. (Mv4, 1)	Anoke Co. Minnesota	G.B. Ownbey 1964
Canada (Mv6, 1)	Alberta, Near Edmonton	J.G. Packer 1966
<u>M. dilatatum</u>		
California (L5, 1)	South of Big Lagoon Co., Humboldt, California, on high wooded ground near the sea	H M. & R.S. Beard 1959

APPENDIX 4List of collections of living Oxalis plants.

Virtually all the *O. acetosella* collections are listed in Table in the text.

Oxalis montana

- B1, 1 Maple woodland, Napierville, PQ, Canada
(coll. D.H. Valentine 1959)
- B4, 2 Nova Scotia, Canada (coll. M.J. Harvey, 19.9.64)
- B5, 1 Rouge River, near Harrington, Quebec
(W.F. Grant, 1966)
- B6, 1 Harrietsfield, near Halifax, Nova Scotia
(coll. M.J. Harvey 1967)

O. oregana

- D4, 1 From garden of John Innes Institute,
Hertford, Herts., (sent by G. E. Marks 1964)
- D4, 2 From garden of John Innes Institute,
Hertford, Herts. (G.E. Marks 1964)
- D5, 3 Oregon Canyon (near Forestville), Sonoma Co.,
California. (coll. Dr. H.G. Baker, 4.1.65)
- D7, 1 - a & b California (coll. Prof. H.G. Baker 1967)

O. lactea

- E5, 1 New Zealand (coll. N.W. Nelson 20.4.65)

O. magellanica

- C4, 1 Garden material, Dr. D.P. Young 12.9.64

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Plate I



DR. AUG. HENRY'S COLLECTIONS FROM
CENTRAL CHINA, 1885-88.

NO. 528'S B

Prov. HUPEN. *Trifolium*