Cytological Studies on Different Species of *Mentha* with Special Reference to the Occurrence of Chromosomal Biotypes

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Introduction

A number of species of the genus *Mentha* are cultivated widely in different parts of the world. This is mainly because of their use as mints meant for medical and other purposes. They provide interesting materials for cytological study as different polyploid series have been reported in a number of the species. In addition to this, individuals of the same species with different chromosome numbers are also on record.

In India too, several species of *Mentha* are grown in cultivation, where it is used both as spices as well as medicine. The importance of menthol is well known. Not only the various species cultivated here, but several wild forms have also been recently collected. Practically no cytological work has been carried out until now on the species of this genus growing wild and cultivated in India, whereas it is clear that ample possibilities for this study are existence. As in different species of this genus growing in different parts of the world, individuals with different chromosome numbers have been reported (Ruttle 1931, Heimans 1938, Glotov 1940, Nagao 1941, Löve and Löve 1942, Suzuka and Koriba 1949 and vide Darlington and Wylie 1955), It was thought highly desirable to investigate whether in the Indian forms too any such individuals with other chromosome numbers are in existence. Fortunately, as the text would reveal, a number of new polyploid and aneuploid types have been recorded and their behaviour has provided important clues as to their method of speciation and the lines of evolution within the genus.

Materials and methods

A. *Materials*

The following species of plants belonging to the genus *Mentha* were the source of materials for the present investigation.

1. *Mentha viridis* Linn. var. I.
2. *Mentha viridis* Linn. var. II.
3. *Mentha piperita* Linn. var. I.

4. *Mentha piperita* Linn. var. II.
5. *Mentha arvensis* Linn.

The genus *Mentha* is a native of North Temperate regions. *M. arvensis* is distributed in Europe, North West Asia to China. *M. piperita* is found in Europe, Asia and North America and *M. viridis* in Europe, Asia, North and South America. In India, most of the species are found in the temperate Western and Eastern Himalayas. *M. viridis* is found also in the plains. *M. viridis* var. II was collected from the suburbs of Calcutta. All the other species were collected from Rango (Darjeeling) at an altitude of 3,000–4,000 feet.

All of the species are shade-loving plants preferring damp soil, and were grown in shady humid nurseries. For root-tips, several runners were placed in suitable flat earthenware pots in a mixture of sand and soil. For flowering, one set of plants was grown in separate plots.

B. *Methods*

Fixation of somatic chromosomes presented much difficulty due to their high number in most cases. Various fixatives, involving both metallic and non-metallic constituents, were used with varying proportions of the constituents. Of them, a mixture of chromic-formalin, with the proportion of formalin high, 1 : 2, was effective in some species. Temporary squash preparations yielded good results in all species. Out of different trials of pre-treatment in various chemicals, para-dichlorobenzene produced best results (Sharma and Mookerjea 1955). Healthy root-tips were treated in a saturated aqueous solution of para-dichlorobenzene for $1\frac{1}{2}$ hours at 6°–8°C. The materials were then hydrolysed and stained in a mixture of 2 % Aceto-orcein and N/HCl (9 : 1) by heating over a flame for a few seconds. Smearing was done in 1 % Aceto-orcein solution and the slides were sealed properly.

For the fixation of meiotic chromosomes, Belling's modification of Navaschin's fluid A and B (1 : 1) was found most suitable. A pre-treatment in Carnoy's fluid for a few seconds followed by thorough washing in distilled water was necessary.

The peak period of mitotic and meiotic division was between 10 A.M. and 2 P.M.

Paraffin sections, 14 μ thick, were cut and staining was done following the usual schedule of Newton's crystal violet iodine technique.

The figures were drawn at a table magnification of approximately $\times 2{,}900$ using a Zeiss compensating eye-piece $\times 20$ and a 1.3 apochromatic objective with a condenser of 1.3 N.A.

In the drawings, chromosomes bearing secondary constrictions or satellites, have only been drawn in outline.

Observations

The records presented below would reveal at a first glance the role of

polyploidy in the different species. The normal somatic chromosome number has been found to vary from 2n=32 to 2n=90 in the different species. Nuclei with varying number of chromosomes are also observed. The somatic number occurring in the highest frequency is taken as the normal number for the species.

Size difference in chromosomes amongst the different species is not marked, but a slow gradation in size, one group merging into other, is found within the complements. Chromosomes are characterized in having mostly median to submedian constrictions. The number of secondary constrictions varies from six to as many as fourteen. Chromosome size ranges from 1.0 μ to 3.1 μ and on its basis broadly two groups can be recognized, viz., medium and short.

In the detailed karyotype, the general chromosome types of the different species are described first on a comparative basis. Their finer details will be dealt with separately under karyotype description for each species. The main morphologically distinguishable types are as follows:

Type A — Represents medium-sized (nearly long) chromosomes, each with two constrictions, primary and secondary, one nearly median and the other placed at the distal end of the shorter arm.

Type A_1— Medium-sized chromosomes, each with a nearly median primary constriction and a satellite at the distal end of the longer arm.

Type A_2— Medium-sized chromosomes each with two constrictions, primary and secondary, one nearly median and the other nearly submedian placed at the distal end of the comparatively longer arm.

Type B — Medium-sized (nearly long in one pair) chromosomes, with two constrictions, primary and secondary, both in submedian positions at opposite ends of the chromosomes.

Type C — Medium-sized (nearly long) chromosomes with submedian primary constrictions.

Type D — Medium-sized chromosomes with nearly median primary constrictions.

Type E — Medium-sized chromosomes each having two constrictions, primary and secondary, both in submedian positions at opposite ends of the chromosome. It differs from the type B in being smaller in size.

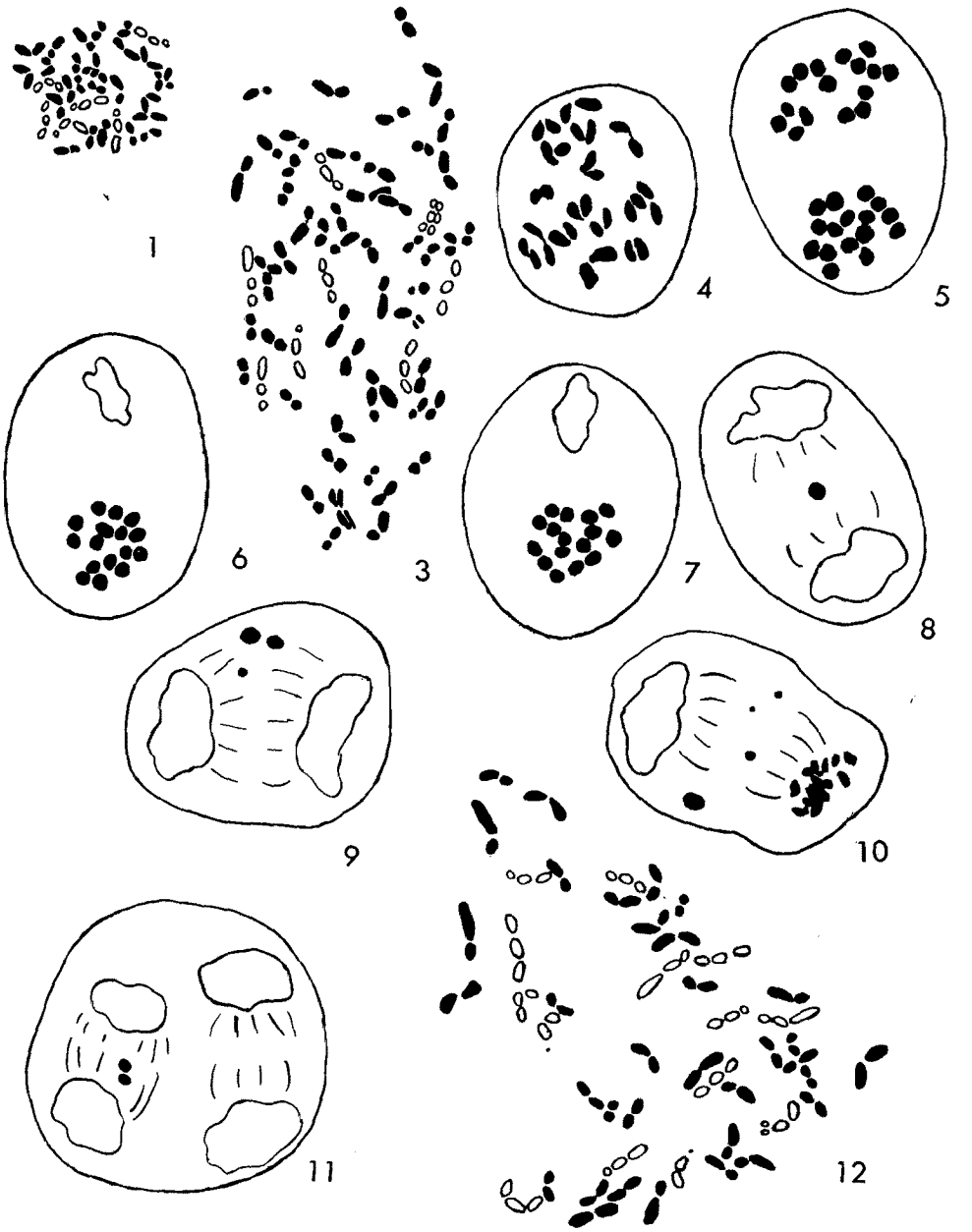
Type F — Medium-sized chromosomes smaller than type C with submedian primary constrictions.

Type G — Medium-sized (neary short) chromosomes each with a median primary constriction and a minute satellite at the distal end of one of the arms.

Type H — Nearly medium-sized to short chromosomes with nearly median to submedian primary constrictions.

Type I — Very short chromosomes with median primary constrictions.

Meiotic study could be made in only two species. Flowers of other species could not be collected. Further the plants grown in the nursery did not produce flowers. Meiotic irregularities are found in a considerable frequency. Tendency of association between the chromosomes in the second meiotic metaphase has also been noticed.



Figs. 1–11. *Mentha viridis* Linn. var. I. 1, normal somatic metaphase (2n=32). 3, variation somatic metaphase with 60 chromosomes. 4–11, meiotic stages including secondary association in metaphase II (For details, vide text, pp. 205). 12, *Mentha viridis* Linn. var. II. normal somatic metaphase (2n=48).

1. *Mentha viridis* Linn. var. I (2n=32=6M^s+6M+20S)

Strongly scented perennial herb growing wild in hilly areas; hairy, leaves sessile, comparatively longer and ovate in shape. Flowers few, axillary, comparatively large.

The somatic chromosome number in the normal nuclei is found to be thirty-two (Fig. 1). One variation plate with 2n=60 chromosomes has also been recorded (Fig. 3). Though size difference is not marked, the following groups can be seen.

i) Six pairs of medium-sized chromosomes.
ii) Ten pairs of short chromosomes

Of these, six chromosomes bear secondary constrictions. The size ranges from 1.1 μ to 2.1 μ. Table 1 shows the detailed karyotype (Fig. 2).

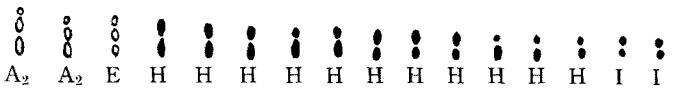


Fig. 2. *Mentha viridis* var. I (2n=32)

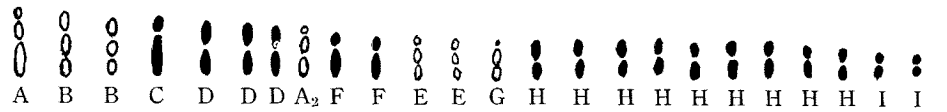


Fig. 13. *M. viridis* var. II (2n=48)

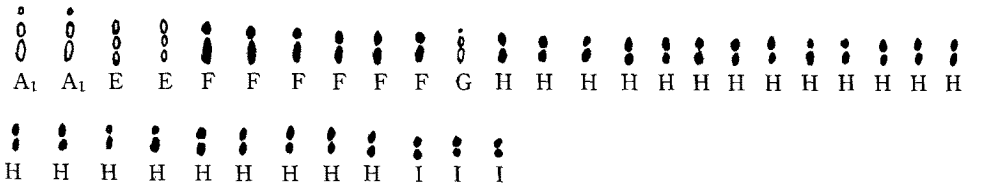


Fig. 17. *M. piperita* var. I (2n=72)

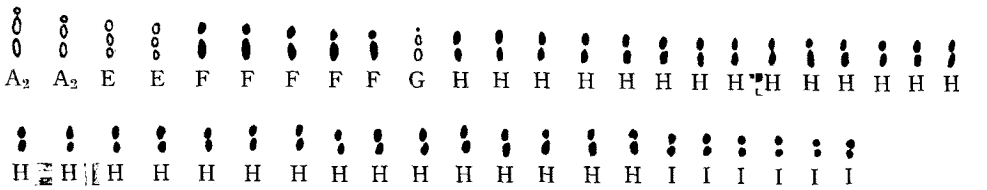


Fig. 25. *M. arvensis* (2n=90)

Idiogram table of the different species of *Mentha* so far investigated. Lettering of the chromosome types of the different species is prepared on a comparative basis.

Table 1. Karyotype analysis in *M. viridis* var. I

Type	Number	Special features
A_2	2 pairs	One pair slightly shorter than other A_2 types
E	1 pair	Common E type
H	11 pairs	Few pairs longer than others
I	2 pairs	Common I type

At diakinesis, sixteen clear bivalents have been observed (Fig. 4). The second meiotic metaphase showed clear sixteen chromosomes in polar view (Fig. 5). Meiotic irregularities, mainly lagging, have been observed in both the first and the second anaphase (Figs. 8–11). Many extra-nuclear bodies were also found at first anaphase (Fig. 10). Tendency of allo-polyploidy has been noticed in second meiotic metaphase. Various associations of the chromosomes noticed are revealed in figures (Figs. 5–7).

Figs. 14–25. *Mentha viridis* Linn. var. II. variation somatic metaphse with 38 and 81 chromosomes respectively. 16–20, *Mentha piperita* Linn. var. I. 16, normal somatic metaphase (2n=72). 18–20, variation somatic metaphase with 60, 70 and 132 chromosomes respectively.

2. *Mentha viridis* Linn. var. II ($2n=48=14M^s+18M+16S$)

Strongly aromatic perennial herb, glabrate, leaves ovate, shortly petioled, leaf-surface uneven. Cultivated as spearmint.

The normal complements of somatic cells are seen to contain forty-eight chromosomes (Fig. 12). Besides this, other variation nuclei with thirty-eight and eighty-one chromosomes are recorded (Figs. 14–15). Size difference is present and two groups can be recognised:

i) Sixteen pairs of medium-sized chromosomes, and

ii) Eight pairs of short to very short chromosomes.

Number of secondary constrictions is very high. Fourteen chromosomes bear secondary constrictions. The size ranges from 1.1 μ to 3.1 μ. The following table shows the detailed karyotype (Table 2, Fig. 13).

Table 2. Karyotype analysis in *M. viridis* var. II

Type	Number	Special features
A	1 pair	Normal A type
B	2 pairs	One pair slightly shorter than the other
C	1 pair	Normal C type
D	3 pairs	Common D type
A_2	1 pair	Common A_2 type
F	2 pairs	Longer than other F types
E	2 pairs	Common E type
G	1 pair	Longer than other G types
H	9 pairs	Common H type
I	2 pairs	Common I type

Table 3. Karyotype analysis in *M. piperita* var. I

Type	Number	Special features
A_1	2 pairs	Common A_1 types
E	2 pairs	Common E types
F	6 pairs	Few pairs shorter than common F types
G	1 pair	Common G type
H	22 pairs	Common H type
I	3 pairs	Common I type

3. *Mentha piperita* Linn. var. I ($2n=72=10M^s+12M+50S$)

Strongly scented perennial herb, glabrate, leaves thin in texture, petioled, lanceolate, serrate, flowers in axillary whorls. Cultivated as peppermint.

The normal somatic cells of the species show $2n=72$ chromosomes (Fig. 16). Abnormal cells with $2n=60$, 70 and 132 chromosomes are also seen (Figs. 18–20).

Size difference is not so marked and two groups can be distinguished.

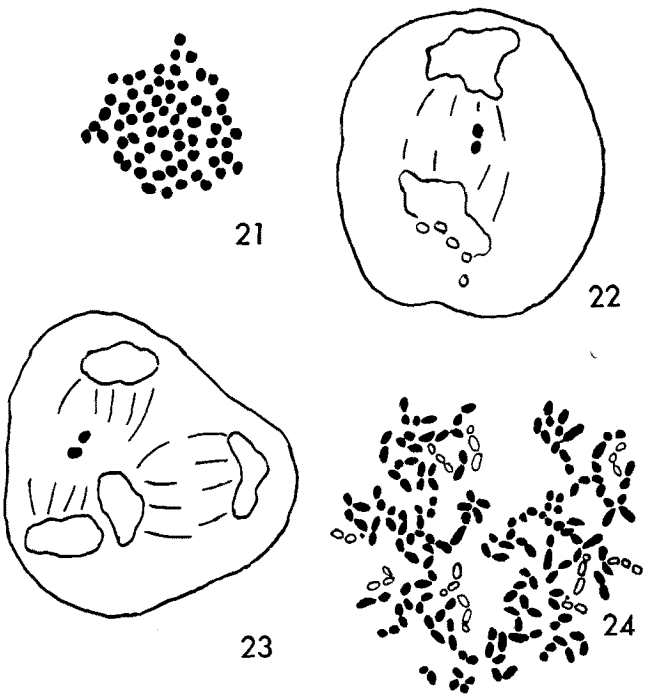
i) Eleven pairs of medium-sized chromosomes, and

ii) Twenty-five pairs of short to very short chromosomes.

Ten chromosomes bear secondary constrictions. The size ranges from 1.0 μ to 2.4 μ. The detailed karyotype is as follows (Table 3, Fig. 17):—

4. *Mentha piperita* Linn. var. II (n=66)

External morphology almost similar to the variety I. But leaves are comparatively thick and large. Flowers small, in axillary and terminal clusters.



Figs. 21-23. *Mentha piperita* Linn. Var. II. meiotic stages (for details, vide text, p. 209). 24, *Mentha arvensis* Linn. normal somatic metaphase (2n=90).

The somatic chromosomes of this species could not be examined as the materials brought from Rango (Darjeeling) did not survive. Meiotic studies revealed sixty-six clear bivalents at Metaphase I (Fig. 21). Several extranuclear bodies were observed at prophase. Meiotic irregularities, mainly lagging, were noticed at anaphase I and II (Figs. 22-23).

5. *Mentha arvensis* Linn. ($2n=90=10M^s+10M+70S$)

Strongly scented perennial herb; glabrate, leaves shortly petioled, oblong-ovate, serrate.

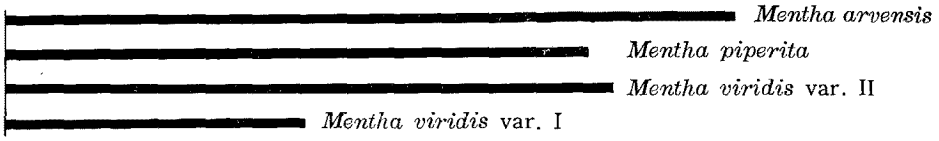
The normal complement of somatic cells is seen to contain ninety chromosomes (Fig. 24). Size difference is not well marked and two groups can be recognised.

i) Ten pairs of medium-sized chromosomes, and
ii) Thirty-five pairs of short to very short chromosomes.

Ten chromosomes bear secondary constrictions. The size ranges from 1.0 μ to 2.1 μ. The detailed karyotype is revealed from Table 4 (Fig. 25).

Table 4. Karyotype analysis in *M. arvensis*

Type	Number	Special features
A_2	2 pairs	One pair longer than the other
E	2 pairs	Common E types
F	5 pairs	Few pairs shorter than common F type
G	1 pair	Common G type
H	29 pairs	Common H type
I	6 pairs	Common I type



Histogram showing the total length of chromatin matter in the haploid complement of the different species of *Mentha* so far investigated.

Discussion

1. *A correlation between the principal findings of the previous and the present records*

Previous records show that in different species of *Mentha*, chromosome numbers as multiples of six, nine and ten have been found (vide table 5).

Table 5. Previous and present records of chromosome number

Species	Previous records		Present record (2n No.)
	(2n No.)	Author	
Mentha piperita	36, 64	Glotov (1940)	var. I-72
	66, 68, 70	Ruttle (1931)	var. II-132 (from n=66)
M. arvensis	12, 60, 72	Löve and Löve (1942)	90
	54	Wolf (1929)	
	72	Ruttle (1931)	
	64, 92	Nagao (1941)	
M. viridis	36, 48	Löve and Löve (1942)	var. I-32 var. II 48
	36	Schurhoff (1929)	
	36	Nagao (1941)	
	48	〃 〃	
	84	〃 〃	
M. longifolia	18	Heimans (1938)	
	24	Ruttle (1931)	
	48	Suzuka and Koriba (1949)	
M. rotundifolia	18	Heimans (1938)	
	24	Nagao (1941)	
	54	Schurhoff (1929)	
M. niliaca	24, 56	Ruttle (1931)	
M. aquatica	36	Schurhoff (1929)	
	96	Ruttle (1931)	
M. pulegium	20, 40	Ruttle (1931)	

The most interesting feature recorded in practically all of them is the difference in chromosome number between different individuals of the same species. In a number of cases, they seem to represent a polyploid set whereas in others they are not necessarily multiples of the basic number. It is quite interesting that investigations so far carried out do not reveal any significant difference between the ecological tolerance of such chromosomal biotypes, at least in a number of species. The term chromosomal biotype can obviously be applied here as the individuals of the same species having difference in

the chromosome number also differ in minute details of the external morphology of the plants. In the present investigation too, difference in chromosome number in different individuals collected from the same locality has been noted. As viable seed setting in the tropical countries, even following selfing is very rare, it is very difficult to investigate whether these different biotypes show cross compatibility. In any case, there is no doubt that the stability of these individuals is maintained as it is, as they are propagated through vegetative means and so have no chances of merging with one another through natural crossing.

The five different species and varieties of *Mentha* reported in the present paper reveal also certain interesting details in their cytology. The different individuals of these species have been investigated by previous authors too on materials collected from different geographical zones, viz., Egypt, Europe, North Africa and North Asia. No record is available of the Indian species whether growing as wild or cultivated in this subcontinent. If the records of chromosome number of the different individual species including those investigated here are reviewed, the situation stands as thus:

In *M. piperita*, the common cultivated peppermint, individuals with 2n =36 and 64 chromosomes are reported by Glotov (1940), and 2n=66, 68 and 70 chromosome by Ruttle (1931). The materials were collected from Europe, several areas of Asia and Northern Africa. The cultivated species of *M. piperita* growing in India, so far investigated here, on the other hand, are constituted of individuals with 2n=72 and n=66 chromosomes respectively. In this cultivated species, therefore, it is quite evident that various chromosomal biotypes are present, which might have arisen through cultivation. The two individuals, one with n=66 and the other with 2n=72 chromosomes, reported here, though were collected from the same locality of the temperate Himalayas, had slight difference in the texture of the leaves, one being thick (n=66 form) and the other comparatively thinner (2n=72 form).

Similarly in *M. arvensis*, individuals with 2n=12, 60 and 72 chromosomes have been reported by Löve and Löve (1942), 2n=54 by Wolf (1929), 2n=72 by Ruttle (1931) and 2n=64 and 92 chromosomes by Nagao (1941) respectively, collection being made from different parts of Europe and Northern Asia. The cultivated species of *M. arvensis* growing in the temperate Himalayas, as reported here, show 2n=90 chromosomes. Here also the presence of individuals with varying chromosome numbers in the same species can be noted. Though a number of individuals of this species has been studied here in course of the present investigation, all of them show a constant chromosome number of 2n=90.

Of *M. viridis*, two different varieties have been investigated here. One of them, *M. viridis* var. II which grows wild as well as in cultivation in the plains as mint, shows 2n=48 chromosomes in both the wild and the cultivated individuals.

Löve and Löve (1942) reported plants with 2n=36 and 48 chromosomes in the same species, *M. viridis*. Other records include individuals with 2n=36 chromosomes recorded by Schurhoff (1929), 2n=36 in another variety by Nagao (1941) and 2n=48 and 84 chromosomes in Italian Black and American Black spearmint respectively by the same author. Italian Black spearmint is a different type than the commonly used Indian mint. It will be interesting to compare the karyotype of the Indian mint with 2n=48 chromosomes with that of Northern Asian mint with 2n=36 and 48 chromosomes reported by Löve and Löve (*l.c.*)

Another wild variety of *Mentha*, namely *M. viridis* var. I (hill variety), has been reported here, which shows some difference in the external morphology of leaf character and in the growth of the plant. This particular variety shows chromosome number as 2n=32. This number has not so far been found in any of the other individuals of *M. viridis* or in any other species of *Mentha*. Therefore, like other species of this genus, *M. viridis* is also characterized by a number of chromosomal biotypes.

It seems apparent that the occurrence of individuals with different chromosome numbers in the same species growing in the same area indicates that, possibly so far as the genus *Mentha* is concerned, the survival of these biotypes is not governed by any detectable cyto-ecological principle. Recently a number of plant species have been recorded where the chromosome numbers have been found to be different in plants growing in different ecological conditions (Löve and Löve 1943, Lovkvist 1947, Banach 1950, Leoncini 1951, Haskel 1954, Janaki Ammal 1954). The existence of such ecotypes with different genomic constitutions have been brought forth by a number of authors (Sokolovskaja and Strelkova 1940 and 1941, Soo 1947, Löve and Löve 1949). In *Mentha*, a glance in the previous record does not reveal any such correlation between chromosome number and the ecology. Even then, one cannot ignore the influence of micro-climate in the growth of the different individuals of the same species. More precisely it may be said that in an apparent common area, the different zones may represent various micro-climatic conditions, mainly the soil factor. It is worth while to investigate, whether these different chromosomal forms in the same species show any correlation with the micro-climate to which they are exposed during their growth. An analysis of the soil and other allied factors necessary for the growth of these different chromosomal forms may reveal facts fundamental from a cyto-ecological standpoint.

2. *Role of polyploidy and possibility of six being the basic number of chromosomes*

The most important role in the speciation of the species of *Mentha* is possibly played by polyploidy. A glance at the table (Table 5) reveals that in every species, a polyploid series is clear and in practically most of them in addition to distinct polyploid numbers, other aneuploid numbers too have

been found. In absence of any observation on the detailed meiotic studies in all the species of *Mentha*, so far cytologically examined, it is difficult to find out the role of auto- and allo-polyploidy in the evolution of the different species. The chromosome numbers, so far noted, are either multiples of six, nine or ten. Further, in species such as *M. piperita*, having individuals with 2n=36, 64, 68 and 72 chromosomes, multiples of different basic numbers can be seen. The occurrence of such varying numbers in the same species obviously indicates that they have all originated from a single basic set, though apparently they denote multiples of different basic series.

If one takes into consideration all the species of *Mentha* cytologically known so far, the problem is posed at to whether they represent a single evolutionary series or are characterized by different lines of evolution which may or may not have a remote common ancestry. Regarding this issue, one cannot deny that multiples of different basic sets are present in different species. But if all the species are considered collectively, it will be seen that all of them have at least some individuals which show multiples of chromosome sets common to all of them. In every species, the chromosome numbers of most of the individuals are multiples of six. This number seems to be common to all of them inspite of the presence of other individuals whose chromosome numbers may be interpreted as multiples of nine and ten. In view of this fact, it appears clear that six is possibly the basic set from which different euploid and aneuploid series have evolved which have been responsible for the evolution of the different biotypes or different species. These observations reveal, so far as the genus *Mentha* is concerned, that its species represent quite a homogeneous assemblage, all being derived directly or indirectly from a common basic set.

3. *Structural differences between chromosome complements of different species*

In addition to numerical difference, these species also show structural

Table 6. Difference in chromosome morphology, variations and length of chromatin matter

Species	Normal somatic number (2n)	Size difference in diploid complement (2n)	Variations in somatic number (2n)	Total length of chromatin matter in haploid complement
Mentha viridis var. I	32	*6M^s+6M+20S	60	22.6 μ
M. viridis var. II	48	14M^s+18M+16S	38 & 81	45.6 μ
M. piperita var. I	72	10M^s+12M+50S	60, 70 & 132	47.1 μ
M. arvensis	90	10M^s+10M+70S	—	54.5 μ

*M—Medium-sized chromosome
S —Short chromosome
M^s—Medium-sized chromosome with secondary constrictions

difference in the chromosome complement. Though characterised by a gross homogeneity in the chromosome morphology in different species, each and every one of them has a distinct karyotype of its own. In general, the chromosomes are mostly medium to short in size with primary constrictions mainly varying from median to submedian position. The position of the primary and secondary constrictions varies in their minute details between different species. This fact immediately emphasizes the role of structural alteration of chromosomes in the evolution of the species of *Mentha*. The role of this process in speciation is well known in a number of other plant genera (Kihara 1940, Babcock 1947, Stewart 1948 and vide Stebbins 1951).

4. *Origin of chromosomal biotypes or individuals with different chromosome numbers*

In the present as well as the previous records, the presence of different individuals with different chromosome numbers has been emphasized. It has already been pointed out that the chromosome numbers in them may not necessarily be multiples of the basic number but rather a number of aneuploid forms in a number of individuals have been found. The problem naturally arises as to how these forms or chromosomal biotypes originate.

In this connection it is worth noting that in all of these species excepting *M. arvensis*, investigated here, considerable variations in chromosome numbers within the same somatic tissue of the individual have been found (Table 6). Such variations in the somatic tissue are constituted both of polyploid and aneuploid numbers. Recently, this behaviour has been noted in a number of species where sexual reproduction is obsolete, and the propagation is mainly carried out through vegetative means. It has been pointed out in a series of publications that such altered nuclei participate in the formation of young daughter shoots and thus shoots with different genomic constitutions arise (Sharma and Das 1954, Mookerjea 1955, Sharma 1956, Sharma and Bal 1956, Sharma and Sharma 1956, Sharma and Bhattacharyya 1956, Sharma and Mazumdar 1956). Being detached from the mother plant, they behave as independent new individuals. In absence of any regular method of sexual reproduction, this seems to be the only way through which new genotypes arise in a number of plants, specially those grown in cultivation.

So far as the genus *Mentha* is concerned, a number of its species are cultivated as mints used as spices as well as in medicines in India. They are all propagated through cuttings of runners. Seeds are no doubt formed in a number of species, but this vegetative means is the main method of reproduction of its members. The regular occurrence of variations in the somatic tissue seems to indicate that in species of *Mentha* too, speciation is affected through their participation in the formation of new shoots as emphasized in a number of other vegetatively reproducing plant species.

Summary

1. A cytological investigation of five different species and varieties of the genus *Mentha* have been carried out. Their chromosome numbers noted here are as follows :

i)	*Mentha viridis* Linn. var. I	2n=32
ii)	*Mentha viridis* Linn. var. II	2n=48
iii)	*M. piperita* Linn. var. I	2n=72
iv)	*M. piperita* Linn. var. II	n=66
v)	*M. arvensis* Linn.	2n=90

2. Karyotype analysis in detail has been performed in four species and varieties. Meiosis has been worked out in *M. viridis* var. I and *M. piperita* var. II.

3. It has been shown that each species and variety has got a karyotype, characteristic of its own. This fact indicates that evolution has been associated with a considerable degree of structural changes of chromosomes in them.

4. Role of polyploidy in speciation in this genus has been emphasized in view of the present and the previous data available on this aspect in the different species. Six has been considered as the possible basic number of chromosomes, from which all the euploid and aneuploid series have evolved.

5. Early literature as well as the present records show that in species of this genus, a large number of chromosomal biotypes occur. The importance of analysis of these biotypes from a cyto-ecological standpoint has been emphasized.

6. Variation in chromosome number in the same somatic tissue and the ineffective method of sexual reproduction have been pointed out as responsible for the origin of chromosomal biotypes or individuals with different chromosome numbers. Their role in speciation as an additional means is obvious.

In conclusion, the authors wish to express their sincere thanks to Dr. K. P. Biswas, Director in charge, Medicinal Plants Scheme, Government of West Bengal, for giving us all facilities to collect different wild and cultivated species of *Mentha* at Rongo, Darjeeling.

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