Review

Cytogenetics of the Genus *Mentha*

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Introduction

The genus *Mentha* of the family *Labiatae* consists of 15 species placed in two subgenera : *Pulegium* and *Menthustrum* (Table 3). Six of these species, *M. pulegium* L., *M. rotundifolia* (L.) HUDS., *M. viridis* L. (*M. spicata* L.), *M. longifolia* (L.) HUDS. (*M. spicata* L. var. *longifolia* L.), *M. arvensis* L. and *M. aquatica* L. are widely distributed throughout Europe and Asia, and also naturalized to America. *M. requinii* BENTH., a small creeping mint, is found in Corsica and Sardinia, and *M. tomentosa* D'URV. (*M. microphylla* C. KOCH) is found in Greece. The remaining seven species are indigenous to Australia and New Zealand. At a later date, *M. gattefossei* MAIRE was discovered in Northern Africa[6] and *M. japonica* MAKINO was found in Japan. Besides, *M. piperita* L. (peppermint) and *M. gentilis* L. (*M. cardiaca* Gerde ; Scotch spearmint) are believed to be interspecific hybrids. These two hybrid species, along with *M. spicata* including var. *crispata* (common spearmint) are cultivated in Europe and America, while *M. arvensis* var. *piperascens* (Japanese mint) is cultivated in Japan. All species are perennial.

Several cytological investigations have suggested that *Mentha* is a group of polyploids (table 1), with different chromosome numbers reported for each of the several species. This may be due to taxonomic confusion among the species or due to species complexes having cytotypes with different chromosome numbers. There are still many unsolved problems in the genus *Mentha*, e. g. different chromosome numbers within the same species, the nature of ploidy, especially auto- or alloploidy, basic chromosome number and the role of polyploidy in evolution. Since 1954, we have been studying the cytogenetics of *Mentha*, including a study of interspecific hybrids in the genus, and obtained new information on the role of polyploidy in evolution. The results are discussed briefly in this chapter.

1. Cytology of the Species

1.1. Somatic chromosomes

Available information on the cytology of several *Mentha* species is presented in Table 1. Based on two chromosome numbers ($2n=36$, 54), the genus *Mentha* was initially regarded to be a polyploid group produced through multiplication of a set of nine chromosomes[12),2)]. Later, a wide variation in chromosome number ($2n=18$, 20, 24, 36, 40, ca48, ca68, 72 and 96) was observed by Ruttle[1)], who proposed $x=12$ as the basic chromosome number in the subgenus *Menthustrum*[7)], examined the somatic chromosomes and found that many species of *Mentha* consisted of several cytotypes with

Table 1 Somatic and meiotic-pairing chromosome, and pollen and seed fertility of *Mentha* species used

Accession no.	Species studied	Present study: Pollen fertility (%)	Seed fertility (%)	$2n$	Meiotic figure	Previous work: $2n$ or n (author)
2	*M. requienii*	84.5	47.8	18	—	18,2(R)
4	*M. rotundifolia*	86.8	51.5	24	12_{II}	24,*12*(R),18(H),24(N)
9	*M. spicata* var. *longifolia*	65.5	60.8	24	12_{II}	*9*(L),24,*12*(R),48(N)
9-2	〃	0	52.5	24	—	
10	〃	0	0	36	$12_{II}+12_{I}$	
11	〃	92.6	76.0	48	24_{II}	
7	*M. spicata*	0	0	36	$12_{II}+12_{I}$	36,47,48,50~52,*27*(R)
8	〃	78.6	71.5	48	24_{II}	*18*(S),36,48,84(N)
12	var. *crispata*	0	0	36	$12_{II}+12_{I}$	48(R,N),*24*(Ts56)
13	〃	92.9	55.5	48	24_{II}	
14	var. *crispa*	85.8	55.7	48	$24_{II}^{a)}$	
15	〃	82.9	55.7	54	$26_{II}+2_{I}$,27_{II},$2_{III}+24_{II}$ $1_{III}+24_{II}+3_{I}$	
5	*M. tomentosa*	0	0	36	—	
6	〃	7.1	59.0	48	$24_{II}^{a)}$	
1	*M. pulegium*	100	72.5	48	$24_{II}^{a)}$	20,*10*;40,*20*(R),46(N)
3	*M. gattefossei*	94.6	69.8	48	$24_{II}^{a)}$	
16	*M. japonica*	91.6	81.8	48	24_{II}	49(N)
23	*M. aquatica*	67.0	22.0	60	$30_{II}^{a)}$	*18*(L),96,*48*(R)
24	〃	60.8	58.0	96	48_{II}	
17	*M. arvensis* var. *agrestis*	93.9	69.4	72	36_{II}	*36*(L),69~72,*36*(R)
17-2	〃	0	87.1	72	$36_{II}^{c)}$	
20	var. *piperascens*	0	0	72	$12_{II}+48_{I}$	*27*(W),*48*(R),64,90,92(N)
21	〃	0	0	72	$24_{II}+24_{I}$	96(Tu,Ts52)
22	〃	78.9	50.7	96	$48_{II}^{b)}$	
22-2	〃	0	43.0	96	—	
18	var. *canadensis*	28.2	13.5	84	$36_{II}+12_{I}$	
19	〃	97.7	94.5	96	$48_{II}^{b)}$	
25	*M. piperita*	0	0.3	72	$(20\sim24)_{II}+(32\sim24)_{I}$	*18*(W),65~69(R)
26	〃	0	0	72	$(17\sim23)_{II}+(38\sim26)_{I}$	36,64(G),68,72,84(N)
27	〃	0	0.3	72	$(23\sim24)_{II}+(26\sim24)_{I}$	
28	〃	0	3.4	96	$(0\sim1)_{IV}+(42\sim40)_{II}+(10\sim14)_{I}$	
29	〃	55.8	15.5	120	$48_{II}+24_{I}$	
30	*M. gentilis*	69.3	9.0	72	$(18\sim24)_{II}+(36\sim24)_{I}$	
31	〃	33.9	8.2	72	$(12\sim20)_{II}+(48\sim32)_{I}$	
32	〃	0	0	72	$(9\sim14)_{II}+(54\sim44)_{I}$	
33	〃	48.9	5.4	96	$(44\sim46)_{II}+(8\sim4)_{I}$	
34	〃	42.4	23.2	120	$(1\sim3)_{IV}+(54\sim48)_{II}+(8\sim12)_{I}$	

a) Race occurrence of 2 univalents.
b) Occasionally 2 or more univalents occurred.
c) Observed in megasporocytes.

different chromosome numbers. For instance, *M. spicata* had $2n$=36, 48 and 84 ; *M. piperita* had $2n$=68, 72 and 84, and *M. arvensis* had $2n$=64, 90 and 92. Based on these observations, it was concluded that polyploids might consist of genomes with basic sets of 12 chromosomes and another set of uncertain chromosome number.

Chromosome numbers from root-tip cells of 37 samples of 11 species determined by us[15], also varied from $2n$=24 to 120, expect for a $2n$=18 in *M. requienii* (Table 1). This indicated that *Mentha* has a number of species with cytotypes consisting of di-, tri-, tetra -, penta-, hexa-, hepta-, octo-, and decaploids with x=12 as the basic number. It is noteworthy that 6 of the 11 species each formed a complex with two or more different cytotypes. For example, 2x, 3x, 4x, and 4x+6 plants were found in *M. spicata*, and 6x, 8x and 10x plants were observed in *M. piperita* and *M. gentilis*.

1.2. Meiotic chromosome pairing

Tsuda conducted an extensive study of the meiotic chromosome associations in *M. rotundifolia* (L.) Huds. subsp. *rotundifolia* Briq. ($2n=24$) and *M. viridis* L. (= *M. spicata* L.) subsp. *crispata* Briq. ($2n=48$). In *M. rotundifolia*, 12 bivalents associated secondarily and resulted in six groups each with 2 bivalents, as much as 6_{IV} in an extreme case[13]. In *M. viridis* subsp. *crispata*, similar secondary associations were observed. These observations led to the conclution that these species were autoploids derived through multiplication of a set with six chromosomes.

The meiotic chromosome behaviour of several species and varieties was also examined by us and its relation with seed set open pollination was studied. As shown in Table 1, in many of the species and varieties, normal chromosome pairing was usually observed, though one or two pairs of chromosomes sometimes failed to pair, especially at higher ploidy levels. However, meiotic chromosome associations were complex and abnormal in tri-, hyper-tetra-, hexa-, hepta-, octo-, and decaploids. The triploids usually showed 12 uni- valents and 12 bivalents, as in *M. spicata* (10, 7, 12). Hexaploid *M. arvensis* var. *agrestis* (17, $2n=72$) showed normal 36_{II} but one clone of var. *piperascens* (20, $2n=72$) showed $12_{II}+48_{I}$ and the other (21, $2n=72$), $24_{II}+24_{I}$. Hexaploid *M. piperita* (25, 26, 27, $2n=72$) gave maximum pairing of $24_{II}+24_{I}$, but 1-7 pairs of chromosomes of ten failed to pair. Hexaploid *M. gentilis* (30, 31, 32, $2n=72$) gave more widely varied configurations $(9-24)_{II}+(54-24)_{I}$, that is, out of 24 bivalents, 1-15 chromosome pairs sometimes remained unpaired[9]. *M. arvensis* var. *canadensis* (18, $2n=84$) is the only heptaploid, showing $36_{II}+12_{I}$. The decaploid type of *M. piperita* (29, $2n=120$) gave $48_{II}+24_{I}$, while that of *M. gentilis* (34, $2n=120$) frequently showed $3_{IV}+48_{II}+12_{I}$ to $1_{IV}+54_{II}+8_{I}$, probably with a maximum of $4_{IV}+48_{II}+8_{I}$. In the latter, 8 genomes gave 48_{II} and the remaining 2 genomes showed a variable pairing which suggested the presence of 8 pairs of translocated chromosomes leading to 4_{IV}.

The chromosome configurations of hyper tetraploid *M. spicata* var. *crispa* (15, $2n=54$) at meiosis was usually $26_{II}+2_{I}$ or 27_{II} and occasionally $2_{III}+24_{II}$ or $1_{III}+24_{II}+3_{I}$. This means that the extra six chromosomes are so homologous that they can form 2_{III} with each other. The origin of the extra chromosomes is not clear. It was remarkable that *M. aquatica* (23) showed 30_{II} instead of the expected $24_{II}+12_{I}$, because it appeared to be a pentaploid with $2n=60$. It is thus possible that this forms a new ploidy series in the genus *Mentha* .

1.3. Fertility

Schurhoff (1927)[12] was the first to report partial sterility in *M. spicata*, and this was followed by a report of pollen degeneration in several species including *M. arvensis* and *M. spicata*[11),5)]. Later, male sterile plants were found in *M. japonica*. The data on pollen and seed fertility in several species/varieties in the genus are presented in Table 1 and it will be seen that seed fertility was 40-60 % (80 % in a few cases) which was associated with normal chromosome pairing at meiosis. This was, however, associated with reduced seed fertility, which was slightly lower than the pollen fertility. The fertility was often influenced by the age of plants and environmental conditions.

In some strains of *Mentha* species/varieties, e. g. *M. arvensis* var. *agrestis* (17-2, $2n=$

72), *M. arvensis* var. *piperascens* (22-2, $2n=96$) and *M. spicata* var. *longifolia* (9-2, $2n=24$), anther development was completely suppressed leading to male sterility, although seeds were set due to free pollination in the field . In these cases, degeneration of pollen grain usually occurred at the stage of archesporial tissue of the anther and meiosis could not be studied[15].

On the other hand, some species or varieties showing abnormal chromosome pairing were completely or highly sterile, e. g. *M. piperita, M. gentilis* and others, most of which were probably interspecific or ploidy hybrids (Table 1).

2. Interspecific Interploidy and Interspecific Hybrids

2.1. Occurrence and production of hybrids

Natural as well as artificial hybrids were available for study in the genus *Mentha* . A natural hybrid, *M. arvensis* × *M. aquatica* was reported by Moews (1983)[7] and two artificial hybrids, *M. viridis* × *M. aquatica and M. aquatica* × *M. rotundifolia*, were studied respectively[12),8)]. The latter was sterile but recovered fertility when its chromosome number was doubled. Tsuda[14)] reported secondary association of bivalents not only in the parents but also in the F_1 hybrid, *M. viridis* subsp. *crispata* ($2n=48$)× *M. rotundifolia* ($2n=24$), and concluded that the basic chromosome number in *Mentha* may be $x=6$ (see also section 2.2). Inter subgenetic crosses between species of subgenera *Menthustrum* and *Pulegium* were not successful, but hybrids between *M. arvensis* var. *agrestis* and *M. pulegium* could be obtained with embryo culture[10)]. In subgenus *Menthustrum*, using 14 different strains with almost normal meiotic chromosome behavior, interspecific hybrids could be produced through reciprocal crosses, which were successful irrespective of the chromosome number of the parental species with some exceptions (Table 2). In interploidy crosses, a cross involving higher chromosome number (♀)× lower chromosome number (♂) gave better seed setting and better germination of F_1 seeds than in the reciprocal cross.

2.2. Fertility of hybrids

The data on seed set recorded on open-pollination in interspecific and intervarietal F_1 hybrids are shown in Table 2. All hybrids with regular meiotic pairing of chromosomes showed normal seed set, with maximum fertility of 49-87 %, but a reduced seed set and fertility were observed in hybrids between parents with different ploidy levels. Occasional high sterile hybrids were also obtained in some cases involving parents with same ploidy level and this was attributed to abnormal meiotic pairing of chromosomes.

2.3. Meiosis in hybrids

The meiotic chromosme pairing in F_1 hybrids was variable (Table 2). Diploid *M. spicata* var. *longifolia* (9, $2n=24$)× *M. rotundifolia* (4, $2n=24$) and tetraploid *M. tomentosa* (6, $2n=48$)×tetraploid *M. spicata* var. *longifolia* (11, $2n=48$) showed normal 12_{II} and 24_{II}, respectvely. The interspecific tetraploid hybrid *M. spicata* var. *longifolia* (11, $2n=48$)× *M. spicata* (13, $2n=48$) gave quite a similar result of 24_{II}, whereas another interspecific tetraploid hybrid of *M. spicata* (11×14, each with $2n=48$) showed a variable chromosome pairing of $(12\text{-}23)_{II}+(24\text{-}2)_{I}$. In some interspecific triploid hybrids, e. g. *M. spicata* var. *crispa* (14, $2n=48$)× *M. rotundifolia* (4, $2n=24$), *M. rotundifolia* (4, $2n=24$)× *M. spicata* (8, $2n=48$) etc. $12_{II}+12_{I}$ were frequently observed, though occasionally some univalents

Table 2 Somatic and meiotic-pairing chromosome, and seed fertility in F_1 hybrids, and their parents

Ploidy	Cross	F1		
		Range of seed Fertility %	2n	Meiotic figure
	Crosses bet. homologous genome type			
2x×2x	*M. spicata* v. *longifolia* (9)×*M. rotundifolia* (4)	42~49	24	12_{II}
4x×4x	*M. spicata* v. *longifolia* (11) ×*M. spicata* v. *crispata* (13)	44~74	48	24_{II}
〃	*M. tomentosa* (6)×*M. spicata* v. *longifolia* (11)	75~87	48	24_{II}
8x×8x	*M. arvensis* v. *canadensis* (19) ×*M. arvensis* v. *piperascens* (22)	41~51	96	48_{II}
	Crosses bet. non-homologous genome type			
4x×2x	*M. spicata* v. *longifolia* (11) ×*M. spicata* v. *longifolia* (9)	0~1	36	$12_{II}+12_{I}$
〃	*M. tomentosa* (6)×*M. rotundifolia* (4)	1~4	36	$12_{II}+12_{I}$
〃	*M. spicata* v. *cripata* (13)×*M. rotundifolia* (4)	6	36	$12_{II}+12_{I}$ rarely $1\sim2_{III}$
〃	*M. spicata* v. *crispa* (14)×*M. rotundifolia* (4)	0~6	36	$12_{II}+12_{I}$ rarely $1\sim2_{III}$
2x×4x	*M. rotundifolia* (4)×*M. spicata* (8)	3~30	36	$12_{II}+12_{I}$ rarely $1\sim6_{III}$
4x×2x	*M. japonica* (16)×*M. rotundifolia* (4)	0	36	$(4\sim12)_{II}+(28\sim12)_{I}$
4x×4x	*M. spicata* v. *longifolia* (11) ×*M. spicata* v. *crispa* (14)	15~38	48	$(12\sim23)_{II}+(24\sim2)_{I}$
〃	*M. japonica* (16)×*M. spicata* v. *longifolia* (11)	0	48	$(4\sim11)_{II}+(40\sim26)_{I}$
4x[a]×2x	*M. spicata* v. *crispa* (15)×*M. rotundifolia* (4)	3~12	39	$(12\sim13)_{II}+(15\sim13)_{I}$
4x×4x[a]	*M. spicata* (8)×*M. spicata* v. *crispa* (15)	57~68	51	$(22\sim25)_{II}+(7\sim1)_{I}$
4x[a]×4x	*M. spicata* v. *crispa* (15) ×*M. spicata* v. *crispa* (14)	7~56	51	$(24\sim25)_{II}+(3\sim1)_{I}$ rarely $1_{III}+24_{II}$
5x[b]×2x	*M. aquatica* (23)×*M. rotundifolia* (4)	0	42	$(6\sim12)_{II}+(30\sim18)_{I}$
5x[b]×4x	*M. aquatica* (23)×*M. spicata* v. *longifolia* (11)	0~1	54	$(14\sim17)_{II}+(26\sim20)_{I}$
6x×5x[b]	*M. arvensis* v. *agrestis* (17)×*M. aquatica* (23)	3	66	$(24\sim30)_{II}+(18\sim6)_{I}$
6x×4x	*M. arvensis* v. *agrestis* (17)×*M. japonica* (16)	1~3	60	$24_{II}+12_{I}$
〃	*M. arvensis* v. *agrestis* (17) ×*M. spicata* v. *longifolia* (11)	0~5	60	$(13\sim24)_{II}+(34\sim12)_{I}$
8x×4x	*M. aquatica* (24)×*M. spicata* v. *longifolia* (11)	0	72	$(17\sim24)_{II}+(38\sim24)_{I}$
〃	*M. arvensis* v. *piperascens* (22) ×*M. spicata* v. *longifolia* (11)	0~1	72	$(12\sim21)_{II}+(48\sim30)_{I}$
〃	*M. arvensis* v. *piperascens* (22) ×*M. spicata* v. *crispa* (11)	0	72	$(3\sim17)_{II}+(66\sim38)_{I}$
8x×6x	*M. arvensis* v. *piperascens* (22) ×*M. arvensis* v. *agrestis* (17)	3~9	84	$36_{II}+12_{I}$
6x×8x	*M. arvensis* v. *agrestis* (17)×*M. aquatica* (24)	0~1	84	$36_{II}+12_{I}$
8x×8x	*M. aquatica* (24)×*M. arvensis* v. *piperascens* (22)	30~48	96	$(36\sim48)_{II}+(24\sim0)_{I}$

a) 4x+ : hyper-tetraploid.
b) 5x : See *M. aquatica* (23) in Section 4.
c) Number in the Parentheses is acc. in Table 1.

were able to form trivalents. While, in another similar triploid cross, *M. japonica* (16, $2n=48$)×*M. rotundifolia* (4, $2n=24$), number of univalents was higher. $12_{II}+12_{I}$ were also frequently observed in an intraspecific but interploidy triploid hybrid, *M. spicata* var. *longifolia* (11, $2n=48$)×*M. spicata* var. *longifolia* (9, $2n=24$). A pentaploid hybrid *M. arvensis* (17, $2n=72$)×*M. japonica* (16, $2n=48$) exhibited $24_{II}+12_{I}$, although the remaining pentaploid hybrid showed variable combination of bivalents and univalents. Similarly, in the interspecific heptaploid hybrid *M. arvensis* var. *agrestis* (17, $2n=72$)$\times$*M. aquatica* (24, $2n=96$) and in the interspecific but interploidy heptaploid of *M. arvensis* (22, $2n=96\times17$, $2n=72$), $36_{II}+12_{I}$ were frequently observed. The interspecific octoploid hybrid *M. arvensis* var. *canadensis* (19, $2n=96$)$\times$*M. arvensis* var. *piperesces* (22, $2n=96$) gave 48_{II}. In the remaining interspecific highploidy crosses (8x$\times$4x and 8x$\times$8x), very unstable chromosome pairing was observed.

M. spicata var. *crispa* (15, $2n=54$, 27_{II}, $2_{III}+24_{II}$ in maximun) was separately crossed with *M. spicata* (8, $2n=48$) and *M. spicata* var. *crispa* (14, $2n=48$). The F_1 hybrids frequently exhibited $2n=51$ and $25_{II}+1_{I}$ at metaphase I respectively, although sometimes, 1-3 pairs of chromosomes failed to pair. Similarly, *M. spicata* var. *crispa* (15, $2n=54$)$\times$*M. rotundifolia* (4, $2n=24$) gave hybrids with $2n=39$, which formed $(12\text{-}13)_{II}+(15\text{-}13)_{I}$ at MI.

M. aquatica (23, $2n=60$) was crossed separately with *M. rotundifolia*(4, $2n=24$), *M. spicata* var. *longifolia* (11, $2n=48$) and with *M. arvensis* var. *agrestis* (17, $2n=72$), to get three hybrids ($2n=42$,54 and 66, respectively) which showed unstable pairings with maximum of 12_{II}, 17_{II} and 30_{II} (30_{II} was in most cases), respectively, the remaining chromosomes forming univalents.

2.4. Origin of species through natural hybridization

Based on morphological characteristics, *M. piterita* is considered to have originated from the cross between *M. aquatica* and *M. spicata*. Similarly, *M. gentilis* is believed to have originated from the cross between *M. arvensis* and *M. spicata*.

This is also supported by the cytological data in our own study[14]. The meiotic pairing data for *M. piperita* (25, 26, 27, $2n=72$) and *M. gentilis* (30, 31 or 32, $2n=72$) as presented in Table 1, show remarkable similarity with those of F_1 hybrid, *M. aquatica* (24, $2n=96$)$\times$ *M. spicata* var. *longifolia* (11, $2n=48$) and F_1 hybrids, *M. arvensis* var. *piperascns* (22, $2n=96$)$\times$*M. spicata* var. *longifolia* (11, $2n=48$) or *M. spicata* var. *crispa* (14, $2n=48$), with a variation of bivalents from 12_{II} to 24_{II} from under 12_{II} to 24_{II} in each case (Table 2). *M. piperita* and *M. gentilis* with $2n=96$ and $2n=120$ may have been produced by some secondary variation in chromosome number, respectively from *M. piperita* and *M. gentilis* each with $2n=72$.

3. Genome analysis

Based on the data of meiotic chromosome pairing in hybrids (Table 2), an attempt was made to establish genome relationships among different species in the genus *Mentha* . Figure 1 shows the genome affinity in hybrids between diploid and tetraploid species including hyper-tetraploid. *M. spicata* var. *longifolia* (4, $2n=24$) is tentatively assumed as the basic diploid species with two sets of the basic chromosome number $x=12$, for which genome symbol RR is assigned. Triploid hybrids of each of these diploids with each of the five tetraploid varieties (including hypertetraploid) of *M. spicata* and *M. tomentosa* ($2n=48$), showed $12_{II}+12_{I}$ or its modification. This suggests that these species have the R genome in common, resulting in 12_{II}. Tetraploid hybrids, *M. spicata* var. *longifoilia* (11)$\times$ *M. spicata* var. *crispata* (13) and *M. spicata* var. *longifolia* (11)$\times$*M. tomentosa* (6), each showed 24_{II}. They may have the same genome constitution RRSS. In similar hybrid, *M. spicata* var. *longifolia* (11)$\times$*M. spicata* var. *crispa* (14), variable chromosome pairings (12

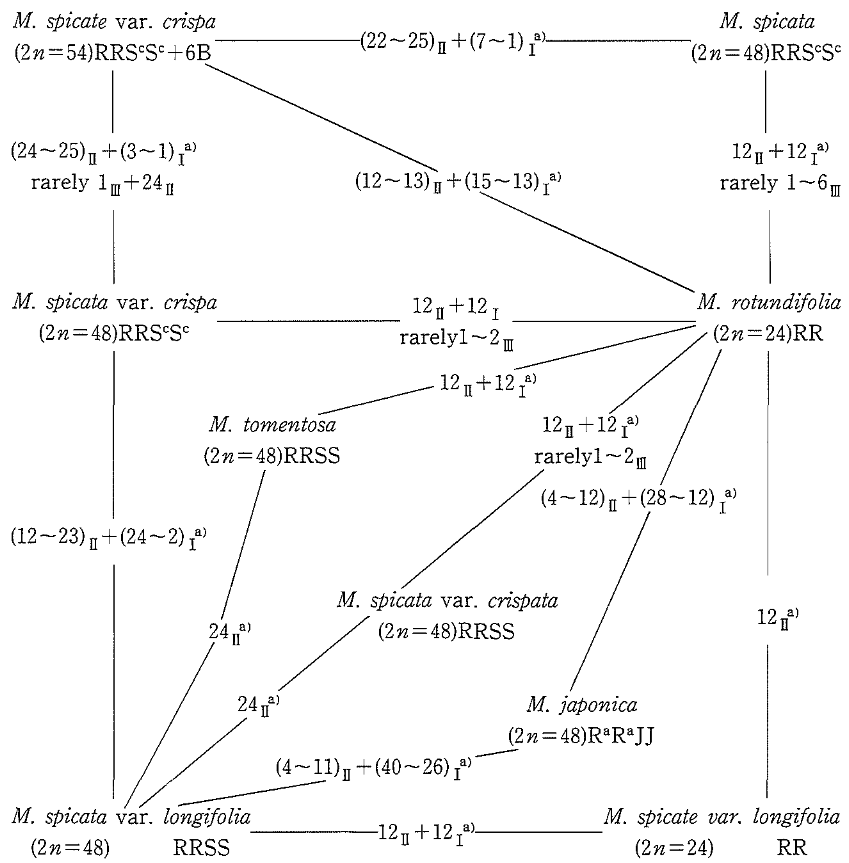

Fig. 1 Diagramatic illustration of meiotic chromosome pairing (marked with[a)]) in F_1 hybrids, 2x×2x, 2x×4x and 4x×4x (including hyper-4x). Some hybrids gave some trivalents in a few cases.

$-13)_{II}+(24-2)_{I}$ were observed. This suggests that the second genome (S^c) of the latter is partially homologous with that of the former (S). On the other hand, hyper tetraploid *M. spicata* var. *crispa* (15) was crossed separately with tetraploid *M. spicata* var. *crispa* (14) and *M. spicata* (8). Chromosome association of $22\text{-}25_{II}$, 24_{II} in most cases, was observed in meiosis of each F_1 hybrid. The meiotic figure seems to be due to 24_{II}, formed from chromosomes of both parents and 1_{II}, formed from extra chromosomes of the hyper tetraploid (15). From these facts, these tetraploid species may have the same genome constitution, RRS^cS^c, and the hyper tetrploid species seems to possess RRS^cS^c, and six extra chromosomes. As mentioned above, the extra chromosomes are able to form 3_{II} or 2_{III}, i. e. they are homologous with each other. Their origin is not yet known, but these could be B-chromosomes. From the unstable chromosome pairing, *M. japonica* (16, $2n=48$) appears to have one partially homologous genome R^a and another distinct non-homologous genome[4)].

Using tetraploids with known genome constitutions, the genome of *M. arvensis* var. *agrestis* (17, $2n=72$) was analysed (Fig. 2). This *M. arvensis* shares two common genomes (R^a, J) with *M. japonica* (16, $2n=48$), resulting in $24_{II}+12_{I}$ in their hybrids. It also shares one homologous genome and another partially homologous genome with *M. spicata* var.

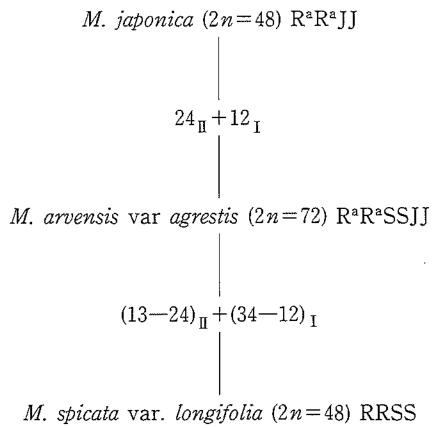

Fig. 2 Diagrammatic illustration of meiotic chromosome pairing in F_1 hybrids, (4x×6x).

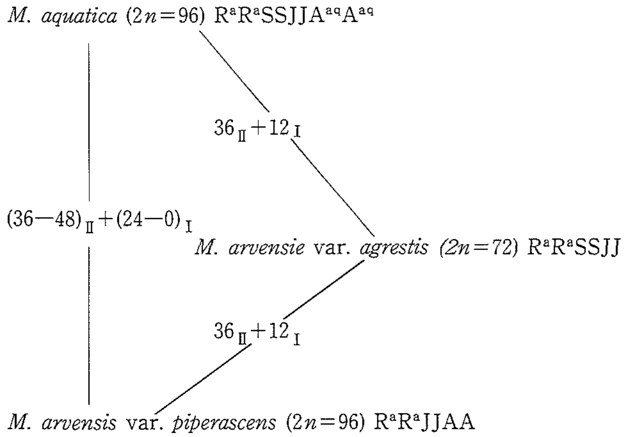

Fig. 3 Diagrammatic illustration of meiotic chromosome pairing in F_1 hybrids, (6x×8x and 8x×8x).

longifolia (11, $2n=48$, RRSS), resulting in $(13\text{-}24)_{II}+(34\text{-}12)_I$ at meiosis of their hybrid. Since R^a of this *M. arvensis* is partially homologous to R of *M. spicata* var. *longifolia*, the third genome of this *M. arvensis* must be S found in *M. spicata* var. *longifolia.*

By the same method, *M. arvensis* var. *piperascens* (22, $2n=96$) and *M. aquatica* (24, $2n=96$) were found to have $R^aR^aSSJJAA$ and $R^aR^aSSJJA^{aq}A^{aq}$, respectively (Fig. 3). Here, A and A^{aq} are partially homologous genomes. Figure 4 presents genome analysis for *M. aquatica* (23, $2n=60$, 30_{II}) which should be called a pentaploid or hyper tetraploid. This *M. aquatica* formed 30_{II} of which 24_{II} came from R^aR^aSS and 6_{II} from the remaining 12 chromosomes. That is, the 12 chromosomes are not the whole set of the genome but a half of the J genome. While in most polyploid plants such an aneuploid would be lethal, this *M. aquatica* grows vigorously and shows 22 % seed fertility in open-pollination. We will tentatively name this set of six chromosomes J/2 and consequently use the genome formula $R^aR^aSS+J/2\,J/2$ for this *M. aquatica.*

It is interesting that aneuploid *M. aquatica* ($R^aR^aSS+J/2\,J/2$) and hexaploid *M.*

M. arvensis var. *agrestis* ($2n=72$) R^aR^aSSJJ

$(24-30)_{II}+(18-6)_{I}$

M. aquatica ($2n=60$) $R^aR^aSS+J/2J/2$

$(6-12)_{II}+(30-18)_{I}$ $(14-17)_{II}+(26-20)_{I}$

M. rotundifolia ($2n=24$) RR *M. spicata* var. *longifolia* ($2n=48$) RRSS

Fig. 4 Diagrammatic illustration of meiotc chromosome pairing in F_1 hybrids, *M. aquatica* (2n=60) 2x, 4x and 6x species. 30+6 were counted in most cases in *M. aquatica* ($2n=60$) 6x.

Table 3 Classification of mint species (by Briquet 1897) and their genome constitutions

Accession no.	Species studied	$2n$	Genome constitution
	Subgenus *Pulegium*		
2	*M. requienii* BENTH.	18	—
1	*M. pulegium* L.	48	—
3	*M. gattefosei* MAIRE	48	—
	Subgenus *Menthustrum*		
	Section *Spicatae*[a)]		
4	*M. rotundifolia* (L.) HUDS.	24	RR
9	*M. spicata* L. var. *longifolia* L.	24	RR
11	〃	48	RRSS
8	*M. spicata* L.	48	RRS^cS^c
13	var. *crispata* (SCHRAD.) KOCH	48	RRSS
14	var *crispa* BENTH.	48	RRS^cS^c
15	〃	54	RRS^cS^c+6B
6	*M. tomentosa* D'URV.	48	RRSS
16	*M. japonica* MAKINO[d)]	48	R^aR^aJJ
	Section *Capitatae*[b)]		
23	*M. aquatica* L.	60	$R^aR^aSS+J/2J/2$
24	〃	96	$R^aR^aSSJJA^{aq}A^{aq}$
	Section *Verticillatae*[c)]		
	M. arvensis L.		
17	var. *agrestis* (SOLE) SM.	72	R^aR^aSSJJ
22	var. *piperascens* MAL.	96	$R^aR^aSSJJAA$
19	var. *canadensis* BRIQ.	96	$R^aR^aSSJJAA$

a) With terminal spike of inflorescence.
b) With globular head of inflorescence.
c) With axillary type of inflorescence.
d) Not classified by Briquet.

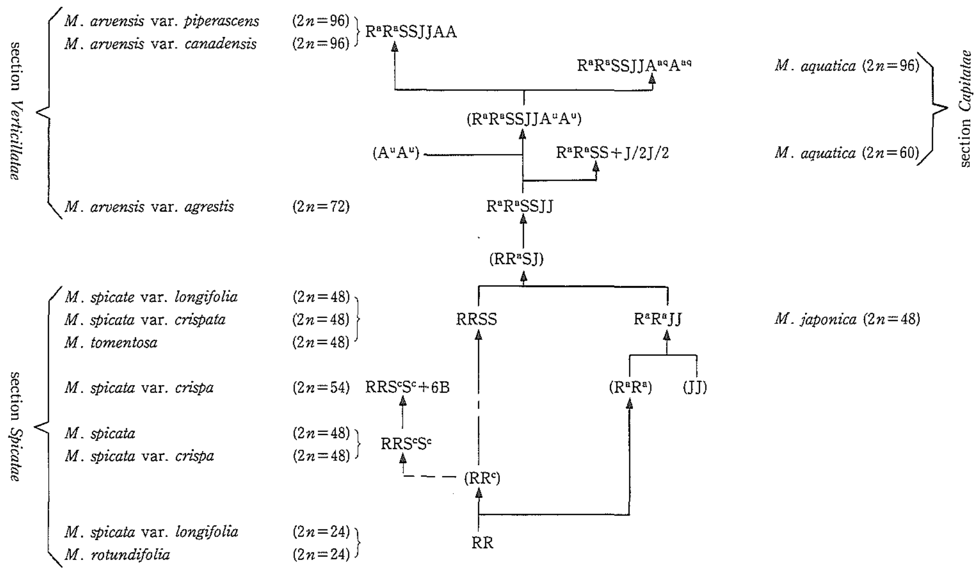

Fig. 5 Evolutionary tree for subgenus *Menthustrum* in the genus *Mentha*. Species with genomes in parentheses are not found at present J/2 of *M. aquatica* (2n=60) may consist of 6 from 12 chromosomes of the J genome.

arvensis (R^aR^aSS+JJ) are similar in genome constitution but morphologically they are so different that they are placed in different sections (Table 3). On the other hand, octoploid and hexaploid *M. arvensis* are morphologically similar and so also are the octoploid and aneuploid *M. aquatica* ($2n=60$), in spite of a slight difference in their genomic constitutions. These findinigs are difficult to explain with our present knowledge.

Table 3 summerizes the available information on genome constitutions of different species and varieties of the genous *Mentha* .

4. Role of Polyploidy in Evolution

Base mainly on the genome constitution, an evolutionary tree for subgenus *Menthustrum* in the genus *Mentha* was tentatively established (Fig. 5). The genome R or its modified genome R^a is found in every species, because R is probably a primary genome. Therefore, *M. rotundifolia* and *M. spicata* with RR would be the most primitive diploid species, or close to the prototype. They would seen to have produced some tetraploid species (RRSS or RRS^cS^c) by introducing a new genome S or S^c in section *Spicatae*. In three triploid hybrids having genome formula RRS^c (*M. spicata* var. *crispa* (14, $2n=48$)× *M. rotundifolia* (4, $2n=24$)), *M. rotundifolia* (4, $2n=24$)× *M. spicata* (8, $2n=48$) RRS (*M. spicata* var. *crispata* (13, $2n=48$)× *M. rotundifolia* (4, $2n=24$)), some trivalents were occasionally formed (Table 2, Fig. 1). This fact indicates that S^c and S are not entirely independent of R. So we tentatively assume that the original R differentiated to R^c, and R^c differented to S^c or to S, in the course of the genomic evolution. Only an exceptional species, *M. japonica* has the different genome constitution of R^aR^aJJ, being indigenous to a limited district in Japan.

Hexaploid *M. arvensis* (R^aR^aSSJJ) in section *Verticillatae* would be produced by doubling chromosomes of *M. japonica* (R^aR^aJJ)× *M. spicata* (RRSS), though there is no support from geographical distribution for such a hypothesis.

Two octoloids *M. arvensis* ($R^aR^aSSJJAA$) and *M. aquatica* ($R^aR^aSSJJA^{aq}A^{aq}$) were probably obtained by further introduction of the A or A^{aq} genome into the hexaploid *M. arvensis*. It is possible that hexaploid *M. arvensis* naturally crossed with an unknown diploid species with A^u genome and produced octoploid species with $R^aR^aSSJJA^uA^u$ by chromosome doubling, and in the latter species, A^u evolved to A, forming octoploid *M. arvensis* and to A^{aq}, forming octoploid *M. aquatica*.

5. Concluding Remarks

Even though the research work reviewed in this chapter showed a considerable in the study of cytogenotics in relation with genome anlysis, phylogenetic investigation and exploration of basic chromosome number, some problems have not been completely solved yet. No cytogenetic relationship between the two subgenera of the genus *Mentha* has been studied, because they were cross- incompatible, though a few hybrids have been obtained through the embryo culture method.

Most species are included into the ploidy with a basic number $x=12$, excepting some species with $2n=18$, 54, 60. The origin or the genomic constitution of the latter species should be investigated in relation to evolution. In addition investigation of putative diploids species with J and A^u genomes is needed.

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ハッカ属植物の細胞遺伝

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世界各地から収集した２つの亜属（*Pulegium* と *Mentastrum*）にわたる多数のハッカ属植物ならびに，育成した多数の同質倍数体，種間雑種および複２倍体植物について細胞遺伝学的研究を行った．その結果，各種の染色体数を決定し，また，主要な種のゲノム分析による種間の近縁関係を明らかにした．両亜属の種の分化過程を解明し，ハッカ属植物の系統分類を明らかにした．