Comparative karyomorphological studies in four species and one variety of *Mosla* Buch.-Ham. ex Maxim. (Lamiaceae) in Japan

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ABSTRACT. Karyomorphology of four species and one variety of Japanese *Mosla* were observed. They had commonly chromosomal characters at resting nuclei of the complex chromocenter type, and the mitotic metaphase cells showed $2n=36$ for *M. japonica*, $2n=18$ for *M. chinensis*, *M. scabra*, *M. dianthera* and *M. dianthera* var. *nana*. The chromosome numbers of $2n=18$ and 36 were considered diploid and tetraploid, respectively if the basic number $x=9$ was accepted. The chromosome number of $2n=18$ for *M. dianthera* var. *nana* was reported here for the first time, and that of the other four species were verified with the previous reports. The karyotypes of five taxa had commonly similar karyomorphology to each other except for chromosome numbers; small sized chromosomes (2.2 to $1.1\ \mu\text{m}$ long), mono-modal (gradual) decrease in chromosome length from the longest to the shortest chromosomes, and consists of median- and submedian-centromeric chromosomes.

KEYWORDS: Japan, Karyomorphology, Lamiaceae, *Mosla*, Subtribe Perillinae

The genus *Mosla* Buch.-Ham. ex Maxim. is one of the three genera in the subtribe Perillinae Briq., the family Lamiaceae (Labiatae), consists of about 22 species (Wu and Li 1977b; Li and Hedge 1994), that are commonly annual herbs and aromatic plants, and grow in grassy slopes, forest margins, hills thickets, open hills and stream-sides in temperate regions of India, Southeast Asia and East Asia. Among the species of this genus, 12 species are found in China (Li and Hedge 1994) and four species and one variety grow in Japan (Murata and Yamazaki 1993).

Seven species (including a synonym) of *Mosla* have been cytologically studied previously and documented their chromosome numbers as $n=9$, $2n=18$ and $2n=36$ (e.g., Sugiura 1931; Suzuka 1953; Mehra and Gill 1968; Gill 1971, 1984; Probatova and Sokolovskaya 1986; Sokolovskaya *et al.* 1986; Zhang and Xu 1988; Hsieh and Huang 1999), and these studies are only chromosome numbers reports.

This paper reports detailed karyomorphologies (the resting nucleus and the mitotic metaphase chromosomes) in the Japanese species of *Mosla*.

MATERIALS AND METHODS

Sixty-nine plants of four species and one variety of *Mosla* were collected in 14 localities in Japan (Fig. 1; Table 1), and cultivated in unglazed plant-pots in shade place in the experimental garden of Showa Pharmaceutical University. Chromosome observations were made in meristematic cells of fresh root tips. They were cut off 5–10 mm long and pretreated in 2 mM 8-hydroxyquinoline at ca 20°C for 4 h and then fixed in 45% acetic acid at ca 2°C for 10 min. They were macerated in a mixture of 45% acetic acid and

1N hydrochloric acid (1:1) at ca 60°C for 20–23 sec and were then stained with 2% aceto-orcin at room temperature in moist chamber with 45% acetic acid for ca 30 min and were prepared by the conventional squash method. The slides were faintly heated under an alcohol flame for 1–2 sec before observation. Measurements of chromosome length were made in each average length in several good metaphase cells.

Morphological type of the resting nuclei was classified for Tanaka (1971, 1977), and classifications of mitotic metaphase chromosomes followed Levan *et al.* (1964). Taxonomical treatment followed Murata and Yamazaki (1993). The voucher specimens of the plants used in this study were deposited in Funamoto's Herbarium in Showa Pharmaceutical University.

RESULTS AND DISCUSSION

Five species of *Mosla* in Japan such as *M. japonica* (Benth. ex Oliv.) Maxim., *M. chinensis* Maxim., *M. scabra* (Thunb.) C. Y. Wu et H.W. Li, *M. dianthera* (Buch.-Ham. ex Roxb.) Maxim. and *M. dianthera* var. *nana* (Hara) Ohwi had common karyomorphological characters in the resting nucleus as the complex chromocenter type which had numerous chromomeric granules, fibrous threads and chromatin blocks and the small chromatin blocks were irregular in shape and varied in number in the whole region (Fig. 2A, D, F, H and J). This resting nucleus type is the same type as the other genera in Lamiaceae (eg; Funamoto 2007; Funamoto *et al.* 2008, 2009, 2011 and 2012). The mitotic metaphase chromosomes in these five taxa had commonly small sized chromosomes and mono-modal (gradual) degraded in the chromosome length from the longest to the shortest chromosomes, and thus, formed

Fig. 1. Map of collection sites of five taxa of *Mosla* in Japan.

M. japonica (■); *M. chinensis* (*); *M. scabra* (●); *M. dianthera* (☆); *M. dianthera* var. *nana* (★).

Table 1. Collection sites, sample numbers and chromosome numbers in four species of *Mosla* in Japan

Species	Collection site	Sample number	Chromosome number (2n)
<i>M. japonica</i>	Okayama Prefecture, Kurashiki City, Mizue, Mt. Hachiman, alt. 100 m	4	36
	Tokushima Pref., Miyoshi City, Ikeda-cho, Mt. Wakasamine, alt. 770 m	2	36
<i>M. chinensis</i>	*Okayama Pref., Kurashiki City, Ikusaka, 34° 37' 91" N, 133° 46' 84" E, alt. 50 m	6	18
	Yamaguchi Pref., Yamaguchi City, Aio-futajima, Senbo-gawa Kasen Park, 34° 02' 16" N, 131° 26' 97" E, alt. 30 m	12	18
<i>M. scabra</i>	Yamagata Pref. Sakata City, Soukouji-Sawa, 38° 51' 09" N, 139° 58' 44" E, alt. 260 m	6	18
	Yamagata Pref., Higashine City, Izumigo, 38° 27' 33" N, 140° 29' 75" E, alt. 470 m	2	18
	Tokyo Metro., Machida City, Higashi-Tamagawagakuen, 35° 33' 12" N, 139° 28' 38" E, alt. 460 m	2	18
	Fukui Pref., Ono City, Shimo-Hanbara	4	18
	Fukui Pref., Obama City, Oya, 35° 35' 65" N, 136° 07' 57" E, alt. 160 m	4	18
	Tokushima Pref., Miyoshi City, Ikeda-cho, Mt. Wakasamine, alt. 770 m	3	18
<i>M. dianthera</i>	Kyoto Pref., Kameoka City, Motoume-machi, Higashikasya, 35° 00' 08" N, 135° 29' 12" E, alt. 220 m	3	18
	Tokushima Pref., Miyoshi City, Ikeda-cho, Mt. Wakasamine, alt. 770 m	8	18
<i>M. dianthera</i> var. <i>nana</i>	Okayama Pref., katsuta-Gun, Shohoku-cho, Oiwa, 35° 08' 65" N, 134° 07' 47" E, alt. 510 m	4	18
	Okayama Pref., Tomata-Gun, Kagamino-cho, Yamashiro, 35° 06' 34" N, 133° 53' 46" E, alt. 180 m	3	18
	Okayama Pref., Maniwa City, Yonoshita, Akameiwa-rindo, 35° 06' 04" N, 133° 48' 85" E, alt. 230 m	5	18
	Okayama Pref., Kume-Gun, Misaki-cho, Utanokita, 34° 59' 25" N, 133° 55' 23" E, alt. 290 m	1	18

*Collection site by Mr. Hiroshi Koyano (Head Curator; now Emeritus Curator) in Sept. 1985, and cultivated in Shigei Medicinal Garden, Kurashiki, Okayama Pref.

similar karyotypes to each other except for chromosome numbers and chromosome complement with respect to centromeric positions.

***Mosla japonica* (Benth ex Oliv.) Maxim.** is distributed in Japan and South Korea. This species is found in Hokkaido, Honshu, Shikoku and Kyushu in Japan, and grows in sunny meadows on hills or mountains; lowlands to 1,000 m (Murata and Yamazaki 1993). Six plants of this species were collected in two localities in sunny place on the rock and a forestry road-sides in Japan (Fig. 1; Table 1). They had common chromosome numbers of $2n=36$ in all plants studied (Fig. 2B and C). This chromosome numbers verified with the previous report (Suzuka 1953). This

species could be tetraploid if the basic chromosome number $x=9$ was accepted. The chromosome sizes ranged from 2.1 to 1.1 μm long, total chromosome length of 55.2 μm and average chromosome length of 1.54 μm . The chromosome complement in centromeric positions consisted median- and submedian-centromeric chromosomes. The first to fourth chromosomes were median-centromeric chromosomes (Table 2; Fig. 3A). Small dot like satellites were rarely observed on short arms in medium sized chromosomes.

***Mosla chinensis* Maxim.** is distributed in Japan, South Korea, Taiwan and Central to South China. This species is found in West Honshu (Okayama, Hiroshima (?)) and

Fig. 2. Karyomorphological comparisons of resting nuclei and mitotic metaphase chromosomes in five taxa of *Mosla*. A, D, F, H and J: The resting nucleus; B, C, E, G, I and K: The mitotic metaphase chromosomes. A-C: *M. japonica* ($2n=36$); D and E: *M. chinensis* ($2n=18$); F and G: *M. scabra* ($2n=18$); H and I: *M. dianthera* ($2n=18$); J and K: *M. dianthera* var. *nana* ($2n=18$). C is drawing of photograph for B. Arrows indicate satellites. Scale bar=5 μ m.

Yamaguchi Pref.) and Kyushu (Nagasaki Pref.) in Japan, and rise in sunny, more or less dry places on hills in Japan (Murata and Yamazaki 1993). Eighteen samples of this species were collected in two localities in sunny, more or less dry places in Japan (Fig. 1; Table 1). They had the common chromosome numbers of $2n=18$ (Fig. 2E). This chromosome numbers verified with the previous report (Zhang and Xu 1988). This species could be diploid if the basic chromosome number $x=9$ was accepted. The chromosome sizes ranged from 2.1 to 1.3 μ m long, total chromosome length of 29.2 μ m and average chromosome length of 1.62 μ m. The chromosome complement in centromeric positions was consisted of median- and submedian-centromeric chromosomes. The first and second chromosomes were submedian-centromeric (Table 2; Fig. 3B). Any satellited chromosome has never been observed.

Mosla scabra (Thunb.) C. Y. Wu et H. W. Li [= *M. punctulata* (J. F. Gmel.) Nakai] is distributed in Japan, South Korea, Taiwan, Central and South China and Vietnam. This species is found in Hokkaido, Honshu, Shikoku, Kyushu

and Ryukyu in Japan and rise in sunny meadows; lowlands to 1,000 m (Murata and Yamazaki 1993). Twenty-one samples of this species were collected in six localities in sunny meadows in Japan (Fig. 1; Table 1). They had common chromosome numbers of $2n=18$ in the individual plants studied (Fig. 2G). This chromosome number verified with the previous reports (Sugiura 1931, 1936; Zhang and Xu 1988; Hsieh and Huang 1999). This species could be diploid if the basic chromosome number $x=9$ was accepted. The chromosome sizes ranged from 2.0 to 1.1 μ m long, total chromosome length of 27.5 μ m and average chromosome length of 1.53 μ m. The chromosome complement consisted of median- and submedian-centromeric chromosomes. The first to fourth chromosomes were submedian-centromeric chromosomes (Table 2; Fig. 3C). A little large dot like satellites were rarely observed on short arms in medium sized chromosomes.

Mosla dianthera (Buch.-Ham. ex Roxb.) Maxim. (= *M. ocimoides* Bunch. -Ham. ex Benth.) is distributed in Japan, Korea, China, Far East in Russia, Southeast Asia and North India. This species is found from Hokkaido to

Table 2. Karyotype comparisons in five taxa of *Mosla* observed here

Species	Chromosome number (2n)	Chromosome length (μm)				Form
		Longest ± SD (Range)	Shortest ± SD (Range)	Total ± SD (Range)	Average ± SD (Range)	
<i>M. japonica</i>	36	2.1 ± 0.23 (2.0-2.4)	1.1 ± -.06 (1.1-1.2)	55.2 ± 2.65 (52.5-57.8)	1.54 ± 0.08 (1.46-1.61)	m+sm
<i>M. chinensis</i>	18	2.1 ± 0.35 (1.7-2.6)	1.3 ± 0.23 (1.0-1.7)	29.2 ± 4.35 (23.9-36.8)	1.62 ± 0.23 (1.33-2.0)	m+sm
<i>M. scabra</i>	18	2.0 ± 0.29 (1.6-2.4)	1.1 ± 0.15 (1.0-1.4)	27.5 ± 3.38 (24.3-33.2)	1.53 ± 0.19 (1.35-1.84)	m+sm
<i>M. dianthera</i>	18	2.2 ± 0.18 (1.9-2.4)	1.2 ± 0.11 (1.0-1.3)	27.8 ± 2.24 (24.8-30.4)	1.55 ± 0.12 (1.38-1.69)	m+sm
<i>M. dianthera</i> var. <i>nana</i>	18	2.0 ± 0.25 (1.7-2.3)	1.1 ± 0.14 (0.9-1.2)	26.8 ± 2.75 (23.6-30.3)	1.49 ± 0.15 (1.31-1.68)	m+sm

SD: standard deviation; m: median-centromeric chromosome; sm: submedian-centromeric chromosome

Fig. 3. Karyotype comparisons in five taxa of *Mosla*. A: *M. japonica*; B: *M. chinensis*; C: *M. scabra*; D: *M. dianthera*; E: *M. dianthera* var. *nana*. Arrows indicate satellites. Scale bar=5 μm.

Ryukyu in Japan and rise in sunny meadows; lowlands to about 700 m (Murata and Yamazaki 1993). Eleven samples of the species were collected in two localities in sunny meadows in Japan (Fig. 1; Table 1). They had common chromosome numbers of 2n=18 in all plants (Fig. 2I). This chromosome numbers verified with the previous reports (e.g. Provatova and Sokolovskaya 1986; Sokolovskaya *et al.* 1986; Zhang and Xu 1988; Hsieh and Huang 1999). This species could be diploid if the basic chromosome number of x=9 was accepted. The chromosome sizes ranged from 2.2 to 1.2 μm long, total chromosome length of 27.8 μm and average chromosome length of 1.55 μm. The chromosome complement in centromeric positions consisted median- and submedian-centromeric chromosomes. The first to sixth chromosomes were submedian-centromeric (Table 2; Fig. 3D). A little large dot like satellites were rarely observed on the short

arms of the medium-sized chromosomes.

***Mosla dianthera* (Buch.-Ham. ex Roxb.) Maxim. var. *nana* (Hara) Ohwi (= *M. hirta* Hara)** is distributed in Japan, South Korea and Taiwan. This species is found in Honshu, Shikoku and Kyushu in Japan and rise in edge of forests in mountains (Murata and Yamazaki 1993). Thirteen samples of this species were collected in four localities in edge of forests in mountains in Japan (Fig. 1; Table 1). They had common chromosome numbers of 2n=18 in all plants (Fig. 2K). The chromosome numbers of this species was reported here for the first time. This species could be diploid if the basic chromosome number x=9 was accepted. The chromosome sizes ranged from 2.0 to 1.1 μm long, total chromosome length of 26.8 μm and average chromosome length of 1.49 μm. The chromosome complement in centromeric positions consisted of either

median- and submedian-centromeric chromosomes. The first to sixth chromosomes were submedian-centromeric chromosomes (Table 2; Fig. 3E). A little large dot like satellites were rarely observed on the short arms in the medium sized chromosomes.

Mosla is placed in subtribe Perillineae, tribe Saturejeae, subfam. Lamioideae taxonomically (Wu and Li 1977a) and divided in sect. *Orthodon* and sect. *Mosla* (Wu *et al.* 1965; Wu and Li 1977b). Japanese *Mosla*, section *Orthodon* included *M. japonica* and *M. chinensis*, while sect. *Mosla* included *M. scabra*, *M. dianthera* and *M. dianthera* var. *nana* (Murata and Yamazaki 1993). Thus, five taxa for Japanese *Mosla* had the same complex chromocenter type of the resting nuclei, and had the stable basic number of $x=9$ except for chromosome numbers, detailed forms for centromeric positions of large sized chromosomes and sizes of satellites between inter- or intra-sections as described above. Chromosome studies of many more species are necessary to clarify and justify cytotaxonomical relationships for *Mosla*.

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