Norihito Miura\(^1\) and Yoshikane Iwatsubo\(^2\): Polyploidy of *Glechoma hederacea* subsp. *grandis* (Labiatae) in Miyagi Prefecture, northeastern Japan

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*Glechoma* L., a small genus of Labiatae, comprises approximately 10 species (Mabberley 1997) and *G. hederacea* L. subsp. *grandis* (A. Gray) H. Harase is the only species within this genus native to Japan. Plants of this species have been collected in Kyushu, Honshu and Hokkaido (Kyushu: Omura City in Nagasaki Prefecture (Hara et al. 1954). Honshu: Kyoto City in Kyoto Prefecture (Tanaka 1953; Hara et al. 1954), Karuizawa-machi in Nagano Prefecture (Tanaka 1953; Hara et al. 1954), Mt. Takao in Tokyo Metropolitan Area (Tanaka 1953; Hara et al. 1954) and Shiki City in Saitama Prefecture (Hara et al. 1954). Hokkaido: Asahikawa City (Nishikawa 1985).), and the chromosome number of all of these is reportedly 2n=36. As such, *G. hederacea* subsp. *grandis* in Japan has been considered to be a 2n=36 plant (Hara et al. 1954). Recently, however, Iwatsubo et al. (2004) discovered that *G. hederacea* subsp. *grandis* distributed in Tohoku Prefecture, Chubu District of Honshu, central Japan, had the following three chromosome forms: 2n=36, 45 and 54. Furthermore, they clarified that its basic chromosome number was x=9, even though two basic numbers of x=6 (Hara et al. 1954) and x=9 (Sugiura 1940; Darlington and Wylie 1955; Skalinska. 1959; Morton 1973) had been proposed for *G. hederacea*.

Among plants distributed in Tohoku Prefecture, Japanese *G. hederacea* subsp. *grandis* has been extensively studied, but little is known regarding the distribution of the three chromosome forms 2n=36, 45 and 54 in other regions of Japan. In this study, we examined chromosomes of *G. hederacea* subsp. *grandis* collected from Miyagi Prefecture, in the Tohoku (northeastern) region of Honshu, in order to characterize the distributions of the three chromosome forms 2n=36, 45 and 54.

**Materials and methods**

This study was based on 86 individual plants of *G. hederacea* subsp. *grandis* collected from wild populations in Miyagi Prefecture, in the Tohoku (northeastern) region of Honshu, Japan. Only one individual from each locality was used, so as to avoid re-sampling of the same clone. All sampling localities of plants and their chromosome counts are listed in Appendix. Root tip cells were used to determine chromosome numbers. Newly-formed adventitious roots from the runners of these plants were gathered and pretreated in a 2 mM 8-hydroxyquinoline aqueous solution for 1 hr at 25°C and subsequently kept for 15 hr at 6°C. They were fixed in a mixture of glacial acetic acid and absolute ethyl alcohol (1:3) for 1 hr, soaked in 1 N HCl for a few hours, macerated in 1 N HCl at 60°C for approximately 10 min, and then immersed in tap water for a few minutes to several hours. They were stained and squashed in 1.5% lacto-propionic orcein. Fully-spread metaphase chromosomes were observed under microscope. Voucher specimens were deposited in the Toyama Science Museum (TOYA).

**Results and discussion**

As shown in Fig. 1, we found two chromosome forms: namely, tetraploid (2n=36 chromosomes) and hexaploid (2n=54 chromosomes). Appendix summarizes the chromosome numbers determined in the present study of *G. hederacea* subsp. *grandis* collected from Miyagi Prefecture, in the Tohoku (northeastern) region of Honshu,
Japan. Of the 86 individuals examined, 80 (93.0%) were tetraploids and 6 (7.0%) were hexaploids. We therefore demonstrate that tetraploid *G. hederacea* subsp. *grandis* is very common, whereas very few hexaploid plants are distributed in Miyagi Prefecture. The two chromosome forms do not show any differential geographical distribution (Fig. 2).

In Toyama Prefecture, Iwatsubo et al. (2004) reported that 95 (60.5%) of the 157 individuals examined were tetraploids (2n=36 chromosomes), 20 (12.7%) were pentaploids (2n=45 chromosomes) and 42 (26.8%) were hexaploids (2n=54 chromosomes).

In *G. hederacea* subsp. *grandis*, a pentaploid individual is considered to be a hybrid between the tetraploid and hexaploid individuals (Iwatsubo et al. 2004). Pentaploid individuals were not observed in this study, perhaps because of the low rate of crossing between tetraploid and hexaploid individuals, as very few hexaploid parent plants were found in Miyagi Prefecture.

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References
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(Appendix)

Chromosome numbers and collection localities of *Glechoma hederacea* subsp. *grandis* in Miyagi Prefecture.

2n=36

**Igusu** : Kamidakihigashi, Marumori-machi (alt. 50 m) ; Kaniyanagizawa, Marumori-machi (alt. 180 m) ; Minamida, Marumori-machi (alt. 70 m) ; Tanahita, Marumori-machi (alt. 30 m) ; Tanabata, Marumori-machi (alt. 30 m). **Ishinomaki City** : Higashifukuda (alt. 5 m) ; Kitakamicho-ona-gawa (alt. 90 m) ; Minowada (alt. 20 m) ; Yokokawa (alt. 5 m). **Kakuda City** : Domoki (alt. 30 m) ; Handa (alt. 30 m) ; Hatobara (alt. 5 m). **Kami-gun** : Hara, Kami-machi (alt. 200 m) ; Tsukizaki, Kami-machi (alt. 60 m) ; Shikama, Shikama-cho (alt. 30 m). **Kata-gun** : Hagizaki, Shichikashuku-machi (alt. 310 m) ; Seki, Shichikashuku-machi (alt. 340 m) ; Togeta, Shichikashuku-machi (alt. 460 m) ; Enda, Zao-machi (alt. 290 m) ; Togatta, Zao-machi (alt. 360 m). **Kesen-numa City** : Kamihigashigawane (alt. 70 m) ; Tanaka (alt. 70 m). **Kurihara City** : Betto (alt. 120 m) ; Hanaremori (alt. 180 m) ; Hanayama (alt. 320 m) ; Kannarihira (alt. 20 m) ; Nogawa (alt. 10 m) ; Shimizuzakazaki (alt. 30 m) ; Wakayanagi (alt. 10 m). **Kurokawa-gun** : Maruyama, Osato-cho (alt. 10 m) ; Yokosawa, Osato-cho (alt. 20 m) ; Mine, Taiwa-cho (alt. 40 m) ; Sawatari, Taiwa-cho (alt. 130 m) ; Shingyojimonzen, Taiwa-cho (alt. 30 m) ; Tanezawa, Taiwa-cho (alt. 330 m) ; Tannohara, Taiwa-cho (alt. 150 m). **Miyagi-gun** : Nemawari, Matsushima-machi (alt. 30 m) ; Akanuma, Rifu-cho (alt. 20 m). **Motoyoshi-gun** : Shidugawanakasemachi, Minamisanriku-cho (alt. 20 m) ; Hatanosawa, September 2008  J. Phytogeogr. Taxon.  Vol. 56, No. 1

Appendix

Chromosome numbers and collection localities of *Glechoma hederacea* subsp. *grandis* in Miyagi Prefecture.

2n=36

**Igusu** : Kamidakihigashi, Marumori-machi (alt. 50 m) ; Kaniyanagizawa, Marumori-machi (alt. 180 m) ; Minamida, Marumori-machi (alt. 70 m) ; Tanahita, Marumori-machi (alt. 30 m) ; Tanabata, Marumori-machi (alt. 30 m). **Ishinomaki City** : Higashifukuda (alt. 5 m) ; Kitakamicho-ona-gawa (alt. 90 m) ; Minowada (alt. 20 m) ; Yokokawa (alt. 5 m). **Kakuda City** : Domoki (alt. 30 m) ; Handa (alt. 30 m) ; Hatobara (alt. 5 m). **Kami-gun** : Hara, Kami-machi (alt. 200 m) ; Tsukizaki, Kami-machi (alt. 60 m) ; Shikama, Shikama-cho (alt. 30 m). **Kata-gun** : Hagizaki, Shichikashuku-machi (alt. 310 m) ; Seki, Shichikashuku-machi (alt. 340 m) ; Togeta, Shichikashuku-machi (alt. 460 m) ; Enda, Zao-machi (alt. 290 m) ; Togatta, Zao-machi (alt. 360 m). **Kesen-numa City** : Kamihigashigawane (alt. 70 m) ; Tanaka (alt. 70 m). **Kurihara City** : Betto (alt. 120 m) ; Hanaremori (alt. 180 m) ; Hanayama (alt. 320 m) ; Kannarihira (alt. 20 m) ; Nogawa (alt. 10 m) ; Shimizuzakazaki (alt. 30 m) ; Wakayanagi (alt. 10 m). **Kurokawa-gun** : Maruyama, Osato-cho (alt. 10 m) ; Yokosawa, Osato-cho (alt. 20 m) ; Mine, Taiwa-cho (alt. 40 m) ; Sawatari, Taiwa-cho (alt. 130 m) ; Shingyojimonzen, Taiwa-cho (alt. 30 m) ; Tanezawa, Taiwa-cho (alt. 330 m) ; Tannohara, Taiwa-cho (alt. 150 m). **Miyagi-gun** : Nemawari, Matsushima-machi (alt. 30 m) ; Akanuma, Rifu-cho (alt. 20 m). **Motoyoshi-gun** : Shidugawanakasemachi, Minamisanriku-cho (alt. 20 m) ; Hatanosawa,
Motoyoshi-cho (alt. 20m); Magomemachikashira, Motoyoshi-cho (alt. 90m). **Osaki City**: Iwadeyamaiketsuki (alt. 110 m); Iwadeyamakaminome (alt. 60 m); Naruko-onseniwabuchi (alt. 30 m); Shimokanisawa (alt. 280 m). **Sendai City**: Imozawa, Aoba-ku (alt. 200 m); Kumagane, Aoba-ku (alt. 190 m); Kuryu, Aoba-ku (alt. 270 m); Okura, Aoba-ku (alt. 330 m); Sakunami, Aoba-ku (alt. 290 m); Shira-tori, Miyagino-ku (alt. 5 m); Futakuchionsen, Taihaku-ku (alt. 440 m); Nakayachi, Taihaku-ku (alt. 90 m); Otaki, Taihaku-ku (alt. 280 m); Takenouchi, Taihaku-ku (alt. 140 m); Tsubonuma, Taihaku-ku (alt. 110 m); Tanatsugi, Wakabayashi-ku (alt. 5 m). **Shibata-gun**: Maekawa, Kawasaki-machi (alt. 320 m); Okubo, Kawasaki-machi (alt. 180 m); Sasaya, Kawasaki-machi (alt. 480 m); Kitafunaoka, Shibata-machi (alt. 5 m). **Shiroishi City**: Arayashiki (alt. 120 m); Fukuokafukaya (alt. 110 m); Fukuokakuramototai (alt. 80 m); Fukuokanagafukuro (alt. 60 m); Fukuokayatsumiya (alt. 640 m); Kosugotaira (alt. 230 m). **Tome City**: Kawatsura (alt. 10 m); Koganenomiya (alt. 80 m); Matsugozawa (alt. 40 m); Nishikamisawa (alt. 30 m); Oinogake (alt. 10 m); Sanboe (alt. 10 m); Terakura (alt. 80 m); Yokomori (alt. 20 m); Yoneyamachonakatuyama (alt. 10 m). **Watari-gun**: Okumatazawa, Watari-cho (alt. 10 m); Kuboma, Yamamoto-cho (alt. 20 m); Washiashi, Yamamoto-cho (alt. 20 m).

2n=54

**Higashimatsushima City**: Kawakudari (alt. 20 m). **Kurihara City**: Kogawara (alt. 230 m); Miyaguchi (alt. 80 m); Osanagi (alt. 40 m). **Sendai City**: Akasaka, Aoba-ku (alt. 210 m). **Tome City**: Kajinuma (alt. 10 m).