Chromosomal Variation in Populations of *Chamaelirium hisauchianum* (Melanthiaceae) with Holocentric Chromosomes

Noriyuki Tanaka*

98–11 Otsuka, Hachioji, Tokyo 192–0352, Japan

Received November 15, 2019; accepted December 14, 2019

Summary Chromosomal traits and their variation were investigated for a total of 435 plants from 23 local populations of the three subspecies of *Chamaelirium hisauchianum*. 334 out of 340 plants of subsp. *kurohimense* had $2n=44=12L+32S$ (where L and S denote ‘long’ and ‘short’ respectively) with the base number 22 (x_1), and six others were all hyperploids with $2n=45$ ($2x_1+1$; five plants) or 46 ($2x_1+2$; one plant). In subsp. *hisauchianum*, 46 out of 48 plants had $2n=42=14L+28S$ with the base number 21 (x_2), and two others had $2n=42$ ($2x_2$) with an aberrant karyotype or $2n=43$ ($2x_2+1$). In subsp. *minoense*, 45 out of 47 plants, like subsp. *hisauchianum*, possessed $2n=42=14L+28S$ ($x_2=21$), and two others showed $2n=43$ ($2x_2+1$) or 45 ($2x_2+3$). All of the observed chromosomal variants exhibited structural heterozygosity and presumably arose by fission, fusion, and duplication probably derived from irregular chromosomal disjunction. The chromosomal variants found in all populations examined amounted to 2.3% on average. Pollen stainability with cotton blue in four chromosomal variants of subsp. *kurohimense* and *minoense* was lower than in chromosomally normal plants. Some topics relevant to the results were also discussed, comparing with the data previously reported for *C. japonicum* and *C. koidzumianum* that markedly differ in the mating system as well as some chromosomal traits.

Keywords Aneuploidy, *Chamaelirium hisauchianum*, Holocentric chromosome, Karyotype, Mating system, Pollen fertility.

Chamaelirium hisauchianum (Okuyama) N. Tanaka is one of the four species belonging to subsect. *Chionographis* in sect. *Chionographis* (Tanaka 2017). It is a perennial endemic to Japan, comprising three subspecies, *hisauchianum*, *minoense* and *kurohimense*. Subspecies *kurohimense* is distributed on the Japan Sea side of central and northern Honshu, while two other subspecies, *hisauchianum* and *minoense*, are confined respectively to small areas on the Pacific side of central Honshu. These subspecies are allopatric in distribution (Tanaka 1985, 2003, 2017). The populations of *C. hisauchianum* are reported as either hermaphroditic or gynodioecious, hence the individuals are hermaphroditic (including male or andromonoecious plants) or female (Tanaka 1985, 2003, 2016). The hermaphrodites are highly self-compatible (Tanaka 2003), whereas those of most populations of *C. japonicum* and all populations of *C. koidzumianum* of the same subsection investigated are highly self-incompatible (Tanaka 2003, 2020).

Chromosomal traits, such as chromosome number and karyotype, of the three subspecies of *C. hisauchianum* have been reported by Kurosawa and Hara (1960), Hara and Kurosawa (1962), Ajima (1976), and Tanaka and Tanaka (1977, 1979, 1980). According to these

reports, subsp. *kurohimense* has $2n=44$, while subsp. *hisauchianum* and *minoense* have $2n=42$, thus markedly differing from *C. japonicum* and *C. koidzumianum* that possess $2n=24$ (e.g. Hara and Kurosawa 1962, Tanaka and Tanaka 1977, 1979, Tanaka 2020). Except for subsp. *minoense*, all of the taxa so far investigated have been reported to have holocentric chromosomes.

Despite these investigations, we still have very limited knowledge of chromosomal variation at the population level of *C. hisauchianum*. In species having holocentric chromosomes, chromosomal variants originated by chromosomal fission are expected to occur, for fragmented chromosomes are likely to survive through mitoses and gametes with such fragments may participate in sexual reproduction. This deduction has recently been explored for *C. japonicum* and *C. koidzumianum* (Tanaka 2020), but not yet for *C. hisauchianum* that is contrastingly distinct from them in the mating system as well as some chromosomal traits such as karyotype and ploidy level. To fill this lack of knowledge, many individuals of *C. hisauchianum* were sampled from different local populations and their chromosomal traits and variation were investigated. In this paper, some topics relevant to the results are also discussed, comparing with the data previously reported for *C. japonicum* and *C. koidzumianum*.

* Corresponding author, e-mail: yukinofude@yahoo.co.jp
DOI: 10.1508/cytologia.85.115

Materials and methods

Plants of *C. hisauchianum* were collected in the 1980s from 23 localities (Table 1). These were individually potted and cultivated in the experimental nursery of Teikyo University at Hachioji, Tokyo. Herbarium specimens were prepared from many of them to serve as vouchers and for phenotypical studies and are currently preserved at the author's herbarium at Hachioji, Tokyo. Taxonomic identification followed Tanaka (2017).

Root tip meristematic cells were used to observe chromosome traits. The method for making chromosomal preparations is the same as stated in Tanaka (2020). For observation of meiosis, very young spikes were excised and fixed in a 2:1:1 mixture of ethanol, acetic acid, and chloroform for over 1 h. A few immature anthers fixed were placed onto a glass slide, squeezed with a dissecting needle tip, stained with 1 or 2% aceto-orcein, and carefully pressed after a coverslip was mounted on the samples. For the method for investigating pollen stain-

Table 1. Variation in chromosome number and karyotype in three subspecies of *C. hisauchianum* from Honshū, Japan.

Taxon/Locality code: Locality	Total no. of plants examined	Chromosome no. and karyotype (2n)	Ploidy ¹⁾
Subsp. <i>kurohimense</i>			
T: Tohoku District			
YK: Akita Pref., Yuri-honjyō-shi, Mt. Yakushi	18	44=12L+32S	2x ₁
TO: Akita Pref., Yuri-honjyō-shi, Mt. Tôkô	4	44=12L+32S	2x ₁
OS: Akita Pref., Yuri-honjyō-shi, Ôsawakyô	3	44=12L+32S	2x ₁
C: Chûbu District			
WK: Niigata Pref., Murakami-shi, Wakigawa	10	44=12L+32S	2x ₁
IM: Niigata Pref., Murakami-shi, Imagawa	22	44=12L+32S	2x ₁
KW: Niigata Pref., Murakami-shi, Kuwagawa	40	44=12L+32S	2x ₁
	1	45=12L+32S+1f	2x ₁ +1
MA: Niigata Pref., Murakami-shi, Nogata, Majima	8	44=12L+32S	2x ₁
TG: Niigata Pref., Shibata-shi, Tagai	18	44=12L+32S	2x ₁
NI: Niigata Pref., Shibata-shi, Mt. Ninôji	8	44=12L+32S	2x ₁
TK: Niigata Pref., Gosen-shi, Takôji	12	44=12L+32S	2x ₁
GM: Niigata Pref., Minami-kanbara-gun, Tagami-machi, Gomadôyama	31	44=12L+32S	2x ₁
OG: Niigata Pref., Santô-gun, Izumozaki-cho, Oginojyô	8	44=12L+32S	2x ₁
HK: Niigata Pref., Nagaoka-shi, Hakama	21	44=12L+32S	2x ₁
SU: Niigata Pref., Kashiwazaki-shi, Suganuma, Kanakura*	1	44=12L+32S	2x ₁
	1	45=12L+33S	2x ₁ +1
HA: Niigata Pref., Nagaoka-shi, Mt. Hachikoku	11	44=12L+32S	2x ₁
KO: Niigata Pref., Kashiwazaki-shi, Kôchi	14	44=12L+32S	2x ₁
	1	45=12L+31S+2f	2x ₁ +1
YO-1: Niigata Pref., Kashiwazaki-shi, Tan'ne, Mt. Yoneyama	35	44=12L+32S	2x ₁
	1	45=12L+32S+1f	2x ₁ +1
YO-2: Niigata Pref., Jyôetsu-shi, Kakizaki-cho, Mt. Yoneyama (Shimomaki-guchi)	11	44=12L+32S	2x ₁
	1	45=12L+33S	2x ₁ +1
KH: Niigata Pref., Itoigawa-shi, Mt. Kurohime	30	44=12L+32S	2x ₁
	1	46=11L+35S	2x ₁ +2
MJ: Niigata Pref., Itoigawa-shi, Mt. Myôjyô	21	44=12L+32	2x ₁
OA: Nagano Pref., Azumi-gun, Otari-mura, Kita-otari, Ôami	8	44=12L+32	2x ₁
Total 1	340		
Subsp. <i>hisauchianum</i>			
K: Kantô District			
KA : Saitama Pref., Han'nô-shi, Kawamata	46	42=14L+28S	2x ₂
	1	42=13L+1L'+27S+1f	2x ₂ -ab ²⁾
	1	43=14L+29S	2x ₂ +1
Total 2	48		
Subsp. <i>minoense</i>			
C: Chûbu District			
NE: Gifu Pref., Motosu-shi, Neo	45	42=14L+28S	2x ₂
	1	43=14L+29S	2x ₂ +1
	1	45=14L+31S	2x ₂ +2
Total 3	47		

*Material collected by Shun'itsu Iwano.

¹⁾ For chromosome base number, see the text.

²⁾ 2x₂-ab: 2x₂ with aberrant karyotype.

Table 2. Occurrence of chromosome variants in populations of three E. Asian species of *Chamaelirium*.

Taxon	2n (rarely found)	Total no. of examined plants (populations)	Total no. of chromosomal variants (%)	Type of chromosomal variant (total no. of plants)
<i>C. hisauchianum</i>				
subsp. <i>kurohimense</i>	44 (45, 46)	340 (21)	6 (1.8)	Hyperploid (6)
subsp. <i>hisauchianum</i>	42 (42*, 43)	48 (1)	2 (4.2)	Hyperploid (1), structural heterozygote* (1)
subsp. <i>minoense</i>	42 (43, 45)	47 (1)	2 (4.3)	Hyperploid (2)
Total 1		435 (23)	10 (2.3)	
<i>C. japonicum</i> **	24 (25, 36)	334 (34)	3 (0.9)	Hyperploid (1), triploid (2)
<i>C. koidzumianum</i> **	24	20 (3)	0 (0)	—
Total 2		354 (37)	3 (0.8)	

*Structural heterozygote without change in chromosome number.

**Data from Tanaka (2020).

Fig. 1. Somatic chromosomes at metaphase of *C. hisauchianum* subsp. *kurohimense*. A. $2n=44=12L+32S$ from C-YO-1 (Yoneyama-saru 3, 1 Apr. 1987). B. $2n=45=12L+32S+1f$ from C-YO-1 (Yoneyama-ko A-3, 1 Apr. 1987). C. $2n=45=12L+33S$ from C-YO-2 (no. 400-6, 20 June 1980). D. $2n=45=12L+33S$ from C-SU (no. 2, June 1980). E. $2n=45=12L+31S+2f$ from C-KO (no. 4, June 1981). F. $2n=45=12L+32S+1f$ from C-KW (no. J200-13, 13 June 1980). G. $2n=46=11L+35S$ from C-KH (no. 1040-1, 11 July 1980). A, C–E: Fixed without pretreatment with 8-hydroxyquinoline. B, F, G: Treated with 8-hydroxyquinoline for 3 h prior to fixation. A–C, E, F: ♂. D: Sex unknown. G: ♀. Arrows indicate L. Longer arrows in B, E, F indicate fragmentary chromosomes. Scale bar=5 μ m.

ability with cotton blue and the information about the microscope and camera used, see Tanaka (2020).

Results

Chromosomal traits and variation in populations of C. hisauchianum

Subsp. *kurohimense*

A total of 340 plants of subsp. *kurohimense* from 21 localities were chromosomally examined (Tables 1, 2). Of them, 334 plants had 44 chromosomes, of which 12 were relatively long (L) and 32 were short (S), although the distinction between the shortest L and the longest S was slight. The somatic chromosome composition or karyotype can be formulated as $2n=44=12L+32S$ (Fig. 1A), coinciding with previous reports (Ajima 1976, Tanaka and Tanaka 1979). At least five plants from five localities (C-GM, -HA, -HK, -KW, -NI; Table 1) observed regularly formed 22 bivalents at meiotic metaphase I ($n=22$, Fig. 2A), and the subsequent meiotic process and microsporogenesis proceeded normally, forming tetrad cells with $n=22$ (Fig. 2B) and sound pollen grains (Fig. 2C, Table 3). As two other species of the same subsection, *C. japonicum* and *C. koidzumianum*, are diploid, having $2n=24$ with the base number (x) 12 (Tanaka and Tanaka 1979, 1980, Tanaka 2020), subsp. *kurohimense* with $2n=44$ is interpreted as a hypotetraploid ($4x-4$) if assumed to share the same base number with the two diploids. On the other hand, the chromosomes in meiosis of subsp. *kurohimense* behave regularly like those of diploids, in this respect, this subspecies is viewed as a secondarily balanced diploid with the base

Fig. 2. Meiosis and pollen grains stained with cotton blue of *C. hisauchianum* subsp. *kurohimense*. A. Pollen mother cell at metaphase I with $n=22$ (C-GM: no. 5, ♀, 2 Apr. 1987). B. Tetrad with $n=22$ (plant same as A). C. Pollen grains well stained (C-KH: no. 1040-5, ♀, 11 July 1980). D. Pollen grains well stained and unstained (C-KW: no. J200-13, ♀, 13 June 1980). The scale bar at B= $5\mu\text{m}$, applying also to A, at D= $60\mu\text{m}$ also to C.

Table 3. Pollen stainability with cotton blue in 20 individuals of three subspecies of *C. hisauchianum* from seven local populations.

Taxon/Locality code*	$2n$ /karyotype (ploidy)	Total no. of plants examined	Total no. of pollen grains examined/plant: range (mean)	Pollen grains well stained/plant: range (mean) (%)	Plants examined: Collection number, date
<i>Subsp. kurohimense</i>					
C-KW	$44=12L+32S (2x_1)$	1	823	98.1	No. 4, 20 May 1980
	$45=12L+32S+1f (2x_1+1)$	1	1103	57.8	No. J200-13, 13 June 1980
C-HK	$44=12L+32S (2x_1)$	1	825	99.4	No. 7, June 1981
C-KO	$44=12L+32S (2x_1)$	4	564–1236 (795.8)	98.9–99.3 (99.1)	No. J100-2, -4, -9, -10; 20 June 1980
	$45=12L+31S+2f (2x_1+1)$	1	919	44.4	No. 4, 19 June 1981
C-YO-1	$44=12L+32S (2x_1)$	1	726	98.5	Yoneyama-saru 3, 1 Apr. 1987
	$45=12L+32S+1f (2x_1+1)$	1	858	86.4	Yoneyama-ko A-3, June 1981
C-KH	$44=12L+32S (2x)$	1	783	98.9	No.1040-5, 11 July 1980
<i>Subsp. hisauchianum</i>					
C-KA	$42=14L+28S (2x_2)$	3	505–781 (666.3)	96.2–99.8 (98.4)	Nos. 235-1, -6, 260-11, 22 June 1980
<i>Subsp. minoense</i>					
C-NE	$42=14L+28S (2x_2)$	5	565–661 (608.4)	99.5–100.0 (99.8)	Nos. 210-4, -6, 220-2, -3, -4, 4 June 1980
	$43=14L+29S (2x_2+1)$	1	1828	93.9	No. 350-6, 4 June 1980

Fig. 3. Somatic chromosomes at metaphase of *C. hisauchianum*, subsp. *hisauchianum* from C-KA. A. $2n=42=14L+28S$ (no. 2, ♀, col. 1989). B. $2n=42=13L+1L'+27S+1f$ (no. 25, ♀, 22 May 1986). C. $2n=43=14L+29S$ (no. 260-7, ♀, 22 June 1980). All fixed without pretreatment with 8-hydroxyquinoline. Arrows indicate L. Two longer arrows in B indicate f and L', respectively. Scale bar = $5\mu\text{m}$.

number $x=22$ (x_1).

Six others deviated in chromosome number and karyotype from the plants with $2n=44=12L+32S$. All of the six variants were hyperploids, of which five were $2n=45$ ($2x_1+1$; Fig. 1B–F), and one was $2n=46$ ($2x_1+2$; Fig. 1G). Except for one plant whose sex is unknown, four plants were hermaphroditic (♀) and one was female (♀) (Fig. 1). These plants had the following karyotypes: — Two plants from the localities C-YO-1 (Yoneyama-ko A-3, ♀, 1 Apr 1987; Fig. 1B) and C-KW (no. J200-13, ♀, 13 June 1981; Fig. 1F) had $2n=45=12L+32S+1f$ (f: fragmented chromosome). Two plants from C-YO-2 (no. 400-6, ♀, 20 June 1980; Fig. 1C) and C-SU (no. 2, sex unknown, June 1980; Fig. 1D) possessed $2n=45=12L+33S$. As having one excessive S chromosome that probably originated by duplication resulted from irregular chromosomal disjunction, they may be trisomic as to one of S chromosomes. One plant from C-KO (no. 4, ♀, June 1981; Fig. 1E) appears to have $2n=45=12L+31S+2f$, the two fragments of which might have arisen by fission in one original S. One plant from C-KH (no. 1040-1, ♀, 11 June 1980; Fig. 1G) appears to have $2n=46=11L+35S$, of which the three excessive S chromosomes might have originated by fission in one original L chromosome and addition in S chromosomes.

Subsp. *hisauchianum*

A total of 48 plants of subsp. *hisauchianum* from one locality (K-KA) were chromosomally studied (Tables 1, 2). Of them, 46 plants had $2n=42=14L+28S$ (Fig. 3A), according with previous reports (Kurosawa and Hara 1960, Hara and Kurosawa 1962, Tanaka and Tanaka 1979). The chromosomes regularly form 21 bivalents at meiotic metaphase I, and the subsequent meiotic process and microsporogenesis proceed normally (Table 3), as reported by Tanaka and Tanaka (1980). Subsp. *hisauchianum* is, accordingly, regarded as a diploid with the base number of $x=21$ (x_2). On the other hand, it is also interpreted as a hypotetraploid ($2n=4x-6$) if assumed to share the same base number ($x=12$) with the two diploids, *C. japonicum* and *C. koidzumianum*.

Two others deviated from the plants with $2n=42=14L+28S$: — One (no. 25, ♀, 22 May 1986; Fig. 3B) was a diploid ($2x_2$) with $2n=42=13L+1L'+27S+1f$, including one particularly long L (designated as L') and one f. There is the possibility that one original S fragmented into two, of which one became f, and the other fused with one original L, forming one L'. The other (no. 260-7, ♀, 22 June 1980; Fig. 3C) was a hyperploid ($2x_2+1$) with $2n=43=14L+29S$. Possessing one excessive S, it may be trisomic as to one of S chromosomes.

Subsp. *minoense*

A total of 47 plants from one locality C-NE of ssp. *minoense* were chromosomally investigated (Tables 1, 2). Forty-five of them consistently had $2n=42=14L+28S$ (Fig. 4C, D). Chromosomes measured for one metaphase plate in Fig. 4D showed that they range in length from 3.0 to $1.3\mu\text{m}$ (L: 3.0 – $2.1\mu\text{m}$, S: 1.9 – $1.3\mu\text{m}$) with the mean $1.9\mu\text{m}$, and their total length was $80.0\mu\text{m}$. These measurements are well comparable to those for subsp. *hisauchianum* having the same chromosome number and a similar karyotype (Tanaka and Tanaka 1979). The 42 chromosomes regularly form 21 bivalents at metaphase I in meiosis, and the microsporogenesis proceeds normally (Table 3), as reported by Hara and Kurosawa (1962). Accordingly, subsp. *minoense* is regarded as a diploid with the base number $x_2=21$. It is also viewed as a hypotetraploid ($2n=4x-6$) if assumed to share $x=12$ with diploid species, *C. japonicum* and *C. koidzumianum*.

Two others deviated from the plants with $2n=42=14L+28S$: One (no. 350-6, ♀, 4 June 1980; Fig. 4F) was a hyperploid ($2x_2+1$) with $2n=43=14L+29S$. Having one excessive S, this plant may be trisomic regarding one of S chromosomes. The other (no. 350-7, sex unknown, 4 June 1980; Fig. 4G) was also a hyperploid ($2x_2+3$) with $2n=45=14L+31S$, including three excessive S. In the habitat these two plants occurred nearby (the distance between them was approximately less than 0.5m). It is hence probable that the plant no. 350-7 arose as a consequence of fertilization involving gametes with excessive S chromosomes originally derived from the

Fig. 4. Chromosomes in mitotic cell cycle of *C. hisauchianum* subsp. *minoense* from C-NE. A. Interphase nucleus (no. 7, ♀, 26 March 1987). B. Prophase (no. 1, male-sterile, 26 March 1987). C. Metaphase, $2n=42=14L+28S$ (no. 5, ♀, 26 March 1987). D. Metaphase, $2n=42=14L+28S$ (plant same as A). E. Transitional phase from metaphase to anaphase, $2n=42=14L+28S$ (plant same as B). F. Metaphase, $2n=43=14L+29S$ (no. 350-6, ♀, 4 June 1980). G. Metaphase, $2n=45=14L+31S$ (no. 350-7, sex unknown, 4 June 1980). A, C: Treated with 8-hydroxyquinoline for 3 h before fixation. B, D–G: Fixed without pretreatment. Arrows indicate L. Scale bar = $5\mu\text{m}$.

plant no. 350-6. Plant no. 350-7 might therefore be pentasomic as to one of S chromosomes.

Chromatin in the interphase nucleus (Fig. 4A) formed numerous small darkly stained heteropycnotic bodies as well as highly dispersed lightly stained domains. The heteropycnotic bodies looked somewhat more distinct and often slightly larger than those found in *C. japonicum* and *C. koidzumianum* (Tanaka and Tanaka 1979, Tanaka 2020), and the number per nucleus varied among nuclei but consistently far exceeded the somatic chromosome number. Chromosomes at prophase (Fig. 4B) were unevenly contracted along the length, forming a beaded configuration. Chromosomes at metaphase (Fig. 4C, D, F, G) and anaphase (Fig. 4E) had no portion corresponding to a localized centromere in cells treated with 8-hydroxyquinoline (Fig. 4C) before fixation or fixed

directly without pretreatment (Fig. 4D–G). At anaphase, all pairs of sister chromatids separated and migrated parallel toward opposite spindle poles, suggesting the presence of diffuse centromeres along their length (Fig. 4E). There was no chromosome bearing a secondary constriction or satellite (Figs. 4C–G). These chromosomal traits in the mitotic cell cycle, including the holocentric nature of chromosomes, are reported here for the first time for this subspecies, and were quite similar to those of subsp. *hisauchianum* and *kurohimense* (Tanaka and Tanaka 1979).

C. hisauchianum as a species including three subspecies

Plants of *C. hisauchianum* are known to be sexually differentiated into two types, hermaphrodites and females, as stated earlier. However, no chromosomal dif-

ference was found between the two types in any of the three subspecies (Fig. 4C, D).

All of the ten chromosomal variants of the three subspecies showed no remarkable phenotypical difference from chromosomally normal plants of the respective subspecies.

Pollen stainability

Pollen stainability with cotton blue was examined for 11 plants of *C. hisauchianum* subsp. *kurohimense*, three plants of subsp. *hisauchianum*, and six plants of subsp. *minoense* (Table 3). In general, pollen grains stained well in blue were globose in shape and looked normal (fertile) (Fig. 2C, D), whereas those unstained or faintly stained were more or less distorted and apparently unviable (Fig. 2D). In eight plants of subsp. *kurohimense* with the normal karyotype $2n=44=12L+32S$ from C-KW, -HK, -KO, -YO-1, -KH (Fig. 2C), pollen stainability was high, ranging from 98.1 to 99.4% (98.9% on average). In contrast, in three chromosomal variants from C-KW (Fig. 2D), -KO, -YO-1, it was lower, ranging from 44.4 to 86.4% (62.9% on average). The percentage of well-stained pollen grains in chromosomally normal plants ($2n=42=14L+28S$) of subsp. *hisauchianum* from C-KA (three plants) and subsp. *minoense* from C-NE (five plants) were respectively high, ranging from 96.2 to 99.8% (the mean 98.4%) in the former, and from 99.5 to 100.0% (the mean 99.8%) in the latter. In one hyperploid with $2n=43=14L+29S$ of subsp. *minoense*, the percentage was 93.9 and only slightly lower than normal plants. The percentage of well-stained pollen grains in four chromosomal variants of both subsp. *kurohimense* and *minoense* varied widely from 44.4 to 93.9% (the mean 70.6%), probably reflecting varying degrees of chromosomal aberration.

In subsp. *kurohimense*, one hyperploid with $2n=45$ from C-KW (no. J200-1, 13 June 1980) had one large (dyad-sized), well-stained pollen grain (0.1%). One chromosomally normal plant with $2n=44$ from C-HK (no. 7, June 1981) also had one dyad-sized, well-stained pollen grain (0.1%). Another chromosomally normal plant with $2n=44$ from C-KH (no. 1040-5, 11 July 1980) had two dyad-sized, well-stained pollen grains (0.3%) and one monad-sized, faintly stained pollen grains (0.1%).

Discussion

Occurrence of chromosomal variants in populations and factors affecting it

The number of chromosomal variants found in the three subspecies of *C. hisauchianum* is summarized in Table 2. The percentage of the variants in populations varied from 1.8 to 4.3% (2.3% on average) between the three subspecies, and was slightly higher than that (0.8% on average) in two diploid species with $2n=24$, *C. koidzumianum* (no variants) and *C. japonicum* (0.9%)

(Table 2; Tanaka 2020). The hermaphrodites and andromonoecious plants of *C. hisauchianum* are highly self-compatible and hence deemed as autogamous, in contrast, those of *C. japonicum* and *C. koidzumianum* are highly self-incompatible and viewed as allogamous (Tanaka 2003). Accordingly, the rate of chromosomally anomalous seeds set by chromosomal variants of *C. hisauchianum* is expected to be higher than in the two self-incompatible plants. The higher occurrence of chromosomal variants in *C. hisauchianum* may partly be ascribed to the higher self-compatibility of the same species.

As stated in a previous paper (Tanaka 2020), plants of *Chamaelirium* propagate themselves mostly by sexual means, and it takes a comparatively long period (at least three or four years) for their seedlings to reach sexual maturity, and hence it is highly probable that the offspring produced by sexual reproduction are subject to natural selection and many of them eventually die. That is, the survival rate of the seeds or seedlings is presumably very low. This low survival rate is supposedly one of the main causes of the comparatively low occurrence of chromosomal variants in populations not only of *C. japonicum* and *C. koidzumianum* but also of *C. hisauchianum* (Table 2).

A total of ten chromosomal variants were found in populations of the three subspecies (Tables 1, 2). Of them, eight had odd-numbered, heterozygous karyotypes (Figs. 1B–F, 3C, 4F, G), while two others had even-numbered, heterozygous karyotypes; $2n=46=11L+35S$ in subsp. *kurohimense* (Fig. 1G), and $2n=42=13L+1L'+27S+1f$ in subsp. *hisauchianum* (Fig. 3B). As evident from these data, all of the variants observed were chromosomally unstable, structural heterozygotes. Seldom propagating themselves by asexual means, such heterozygotes, like other normal individuals, is to be lost in due course from populations. Judging from the total absence of chromosomally balanced stable variants in populations examined, there may be only a very small chance that new structural homozygotes are generated by heterozygotes and established in populations (Tanaka 2020). Since the occurrence of chromosomal variants was a little higher in self-compatible *C. hisauchianum* (Table 2), the possibility of their generating new homozygotes is expected to be also stronger than in self-incompatible *C. japonicum* and *C. koidzumianum*. Heterozygotes found were quite a few even in *C. hisauchianum* (Table 2), yet their presence is still significant in that they are potential generators of new chromosomally balanced variants.

The low pollen viability in chromosomal variants (Table 3) must also be a key factor for reducing the chance of chromosomal variants being newly produced. In three chromosomally normal plants of subsp. *kurohimense* ($2n=44$) from C-KW, -HK, and C-KH, large, well stained, seemingly viable pollen grains were very rarely

observed (0.1, 0.1, 0.3%, respectively). The production of unreduced gametes in the form of dyads or monads may potentially lead to the formation of polyploids and serve as a factor for enhancing chromosome variation, but such large pollen grains appear to be too few to participate in sexual reproduction.

Types of chromosomal aberration observed

Of a total of ten chromosomal variants found in *C. hisauchianum* including three subspecies, nine were hyperploids (Figs. 1B–G, 3C, 4F, G) and one was a structural heterozygote without a change in chromosome number (Fig. 3B, Tables 1, 2). These variants appear to have arisen by chromosomal mutations such as fission, fusion including translocation, and duplication probably resulted from an irregular chromosomal disjunction in meiosis. When compared with the chromosome number and size in species with $2n=24$ (e.g. *C. japonicum*), there is no doubt that a single polyploidization event also occurred in the origination of *C. hisauchianum*. In plants with holocentric chromosomes, hyperploids originated by chromosome fragmentation are expected to occur, for fragmented chromosomes are likely to be retained through mitoses owing to their diffuse centromeres. Actually, in populations of *C. hisauchianum* several hyperploids with fragmented chromosomes were observed: —three plants of subsp. *kurohimense* with $2n=45$ from C-YO-1 (Fig. 1B), C-KO (Fig. 1E) and C-KW (Fig. 1F), and one plant of subsp. *hisauchianum* with $2n=42$ (including one fragment) from C-KA (Fig. 3B). Various types of chromosomal mutation observed in *C. hisauchianum* have also been reported to occur in species of Cyperaceae or Juncaceae known to have holocentric chromosomes: —e.g. polyploidization in *Carex siderosticta* (Tanaka 1939, Hoshino and Tanaka 1977) and *Luzula spicata* (Juncaceae) (Nordenskiöld 1951), chromosomal fission in *C. oxyandra* (Hoshino 1992), fusion in *C. conica* (Hoshino and Waterway 1994), duplication in *Eleocharis kamtschatica* (Yano and Hoshino 2006), and duplication, fission, and fusion in *C. blepharicarpa* (Hoshino and Okamura 1994).

On the other hand, the phenomenon known as agmatoploidy (Malheiros-Gardé and Gardé 1950) or endonuclear polyploidy (Nordenskiöld 1951) was suggested to occur in *Luzula* as a special type of polyploidy or chromosome rearrangement that involves the fragmentation of an entire chromosome complement, generating a diploid with double its original chromosome number (Guerra 2016). However, this type of polyploidy in the strict sense (i.e., complete agmatoploidy excluding partial agmatoploidy, or endonuclear polyploidy excluding a half completed endonuclear polyploidy) has never been observed in *Chamaelirium*.

Acknowledgements

I cordially thank the late Mr. Shun'itsu Iwano, a botanist in Niigata Prefecture, Japan, who kindly provided me with living materials from a locality in the same prefecture. I also sincerely thank reviewers for valuable comments and corrections.

References

- Ajima, T. 1976. A new variety of *Chionographis japonica* Maxim. J. Geobot. **24**: 45–48.
- Guerra, M. 2016. Agmatoploidy and symploidy: A critical review. Genet. Mol. Biol. **39**: 492–496.
- Hara, H. and Kurosawa, K. 1962. Morphological and chromosomal variations in *Chionographis japonica* Maxim. Acta Phytotax. Geobot. **20**: 34–38. (in Japanese)
- Hoshino, T. 1992. Cytogeographical study of four aneuploids of *Carex oxyandra* Kudo in Japan. Bot. Mag. Tokyo **105**: 639–648.
- Hoshino, T. and Okamura, K. 1994. Cytological studies on meiotic configurations of intraspecific aneuploids of *Carex blepharicarpa* (Cyperaceae) in Japan. J. Plant Res. **107**: 1–8.
- Hoshino, T. and Tanaka, R. 1977. Karyomorphological studies of *Carex siderosticta* and its two allied species. Kromosomo **11**: 7–8: 191–194.
- Hoshino, T. and Waterway, M. J. 1994. Cytogeography and meiotic chromosome configurations of six intraspecific aneuploids of *Carex conica* Boott (Cyperaceae) in Japan. J. Plant Res. **107**: 131–138.
- Kurosawa, S. and Hara, H. 1960. Cytotaxonomical notes on some Japanese plants (I). Shokubutsu Kenkyu Zasshi **35**: 11–14.
- Malheiros-Gardé, N. and Gardé, A. 1950. Chromosome number in *Luzula multiflora* Lej. Genet. Iber. **4**: 91–94.
- Nordenskiöld, H. 1951. Cyto-taxonomical studies in the genus *Luzula* I. Somatic chromosomes and chromosome numbers. Hereditas **37**: 325–355.
- Tanaka, N. 1939. Chromosome studies in Cyperaceae, IV. Chromosome number of *Carex* species. Cytologia **10**: 51–58.
- Tanaka, N. 1985. Sexual variation in *Chionographis*. Biological research on species. Shuseibutsugaku-kenkyu **9**: 11–19. (in Japanese)
- Tanaka, N. 2003. New status and combinations for Japanese taxa of *Chionographis* (Melanthiaceae). Novon **13**: 212–215.
- Tanaka, N. 2016. The occurrence of gynodioecy in a Chinese species of *Chionographis* (Melanthiaceae). J. Jpn. Bot. **91**: 122–128.
- Tanaka, N. 2017. A synopsis of the genus *Chamaelirium* (Melanthiaceae) with a new infrageneric classification including *Chionographis*. Taiwania **62**: 157–167.
- Tanaka, N. 2020. High stability in chromosomal traits of *Chamaelirium japonicum* and *C. koidzumianum* (Melanthiaceae) with holocentric chromosomes. Cytologia **85**: 33–40.
- Tanaka, N. and Tanaka, N. 1977. Chromosome studies in *Chionographis* (Liliaceae) I. On the holokinetic nature of chromosomes in *Chionographis japonica* Maxim. Cytologia **42**: 753–763.
- Tanaka, N. and Tanaka, N. 1979. Chromosome studies in *Chionographis* (Liliaceae) II. Morphological characteristics of the somatic chromosomes of four Japanese members. Cytologia **44**: 935–949.
- Tanaka, N. and Tanaka, N. 1980. Chromosome studies in *Chionographis* (Liliaceae) III. The mode of meiosis. Cytologia **45**: 809–817.
- Yano, O. and Hoshino, T. 2006. Cytological studies of aneuploidy in *Eleocharis kamtschatica* (Cyperaceae). Cytologia **71**: 141–147.