



DNA Contents and Karyotypes of the Natural Hybrids in *Taraxacum* (Asteraceae) in Japan

Watanabe, Kuniaki
Shibaike, Hiroyuk
Suzuki, Takeshi
Ito, Motomi
Hoya, Akihiko

(Citation)

Acta Phytotaxonomica et Geobotanica, 72(2):135-144

(Issue Date)

2021-06-30

(Resource Type)

journal article

(Version)

Version of Record

(Rights)

© 2021 The Japanese Society for Plant Systematics

(URL)

<https://hdl.handle.net/20.500.14094/0100477450>



DNA Contents and Karyotypes of the Natural Hybrids in *Taraxacum* (Asteraceae) in Japan

KUNIAKI WATANABE^{1,*}, HIROYUKI SHIBAIKE², TAKESHI SUZUKI³, MOTOMI ITO⁴
AND AKIHIKO HOYA⁴

¹Department of Biology, Graduate School of Science, Kobe University, Kobe, Hyogo 657-8501, Japan, nabekuni@kobe-u.ac.jp (author for correspondence); ²Division of Biodiversity, Institute for Agro-environmental Sciences, NARO, Tsukuba, Ibaraki 305-8604, Japan; ³Institute of Natural and Environmental Sciences, University of Hyogo, Yayoigaoka 6, Sanda, Hyogo 669-1546 Japan; ⁴Department of General Systems Studies, Graduate School of Arts and Science, The University of Tokyo, Meguro-ku, Tokyo 153-8902, Japan

The DNA content and karyotype of the native Japanese *Taraxacum platycarpum* subsp. *hondoense* and members of the *T. officinale* complex are reported. Members of the *T. officinale* complex were easily distinguished from each other by their DNA content and karyotype. The Japanese diploid *Taraxacum* and the exotic triploid *T. officinale*, are distinct in chromosome number, chromosome size, and the number, size and morphology of satellite chromosomes. The karyotypes of the 3x and 4x hybrids are invariably contain one large Japanese *Taraxacum* chromosome set and two or three small *T. officinale* chromosome sets, suggesting that the native Japanese species, as the haploid ovule donor (with the Japanese chloroplast DNA haplotype), hybridized asymmetrically with the (reduced) 2x or (unreduced) 3x pollens of the introduced *T. officinale*.

Keywords: Asteraceae, DNA content, hybrid, karyotype, satellited chromosome, *Taraxacum officinale*, *Taraxacum platycarpum* subsp. *hondoense*

Taraxacum (Asteraceae) comprises 2,500 species grouped into 40 sections (Kirschner & Stepanek 1996, 1997, Kirschner *et al.* 2007, Richards, 1985). Although most of the sections harbor both sexual diploids and agamosperous polyploids, the latter are clearly predominant in many species and areas of the world. Japanese sexual diploids of *Taraxacum* sect. *Mongolica* are widespread in the rural areas, except in the northern Tohoku District and on Hokkaido (Morita 1976, 1995). The European or North American *T. officinale* F. H. Wigg. (sect. *Taraxacum*) [= the common *Taraxacum* based on the lectotype designated by Kirschner & Stepanek (2011), Kirschner *et al.* (2007), and Majesky *et al.* (2017)] was introduced into Japan more than 120 years ago (Makino 1904). Its numbers have increased drastically around urban areas since 1970 and it has come into close contact with native diploid species (Hotta 1977, Nehira *et al.* 1977a, b, 1979, Ogawa

& Mototani 1985, 2001). Based on an analysis of allozyme markers, Morita (1988) reported natural hybrids between Japanese species of *Taraxacum* and *T. officinale*. Watanabe *et al.* (1997a, b, c, d), and Hamaguchi *et al.* (2000), also using allozyme data, determined that plants previously identified as *T. officinale* were hybrids between *T. officinale* and native Japanese species. Shibaike *et al.* (2002) found differences in the length of the intergenic region between the *trnL* (UAA)3' exon and *trnF* (GAA) in the chloroplast DNA (cpDNA) of Japanese *Taraxacum* and *T. officinale*. Shibaike *et al.* (2002) also found that some strains in the *T. officinale* complex had the same chloroplast haplotype as in Japanese diploid species. They were regarded such plants to be natural hybrids between a Japanese species of *Taraxacum* (as the female parent) and *T. officinale*. Based on that report, Yamano *et al.* (2002, 2003), Shibaike *et al.* (2002), and Ide *et al.* (2005) hypothesized that

Taraxacum officinale was rather locally restricted in Japan, and not as widespread as previously estimated. Sato *et al.* (2004) reported tetraploids in addition to triploids in the *T. officinale* complex.

Nisioka (1956), Takemoto (1961), Yamaguchi (1986) and Sato *et al.* (2007b), after analyzing the karyotype of Japanese diploid species of *Taraxacum*, reported the karyotypes of Japanese lowland diploid species, except *T. maruyamanum* Kitam. (endemic to Okinoshima Isls., Shimane Pref., western Japan) to be similar and indistinguishable between species. Sorensen & Gudjonsson (1946), Takemoto (1961) and Sato *et al.* (2007a, c, 2008, 2014) reported the karyotypes of *T. officinale*. Previous cytologists, however, have never considered natural hybridization between native Japanese species of *Taraxacum* and the introduced *T. officinale*, and even have provided results contradictory to those based on molecular markers. Specifically, for example, Sato *et al.* (2007c) examined the karyotypes of the triploid hybrids identified by an allozyme marker and reported three chromosomes with satellite, instead of the expected four, two from a Japanese diploid species and two from *T. officinale*.

In this paper, we show that the DNA contents and the karyotypes of triploid and tetraploid hybrids between Japanese diploid species of *Taraxacum* and *T. officinale* are congruent with those to be expected from crosses between their putative parents, indicating that hybrids occur with certainty in Japan.

Materials and Methods

Living plants of the native Japanese species *Taraxacum platycarpum* subsp. *hondoense* and members of the *T. officinale* complex were collected in various localities in Japan, and cultivated at the University of Tokyo (Meguro-Ku, Tokyo Met.) and the Institute for Agro-environmental Sciences (Tsukuba City, Ibaraki Pref.). The floral morphology, the cpDNA marker, DNA content, chromosome number, and karyotype of seven accessions (Table 1), including two of *T. platycarpum* subsp. *hondoense*, one of *T. officinale*,

two of triploid putative hybrids, and two of putative tetraploid hybrids, were examined. The length of the cpDNA marker was examined following the method of Shibaike *et al.* (2002). For the measurements of DNA contents, approximately 5 mm² of leaf tissue from mature plants was cut with a new razor blade in a petri dish containing 400 µL chopping buffer. A piece of leaf of *Lotus japonicus* (Regel) K. Larsen was also chopped for inclusion as an internal standard. The suspension containing the nuclei was kept for 5 min at room temperature, then filtered using a 30 µm nylon mesh (Partec, Grlitz, Germany). The filtrate was incubated for 10 min at room temperature. The fluorescence of the nuclei stained by DAPI was measured using a Partec PAS flow cytometer. The 2C DNA content of each sample was calculated as the sample peak mean divided by the *L. japonicus* peak mean and multiplied by the amount of DNA in the *L. japonicus* internal standard (Ito *et al.* 2000). For karyotype analysis, excised root tips about 1 cm in length were pre-treated with ice water at 0 °C for 24 hr, fixed in a 3 : 1 ethanol-acetic acid solution at 5 °C for 1hr, and then stained in 2% aceto-orcein solution for three to seven days. In the photographs and idiograms, the chromosomes of Japanese native diploid species and *T. officinale* are denoted by alphabets 'J' and 'E', respectively.

Results

DNA content and karyotype of Japanese native diploid Taraxacum, T. platycarpum subsp. hondoense

Accessions (*Hoya* 723 & 763) of *T. platycarpum* subsp. *hondoense* are characterized by the erect, ovate outer involucre bracts, the brown achenes, the cpDNA haplotype with the length of 482 bp long and a DNA content of ($2C = 2x =$) 2.18–2.20 pg (Table 1). The total karyotype length ($2n = 2x = 16$) of the accession *Hoya* 723 was 50.6 µm long (Fig. 1A, Table 2). Chromosomes are arranged in order of size, from 1 to 8, in the diploid idiogram (Fig. 2A, 1J–8J). The idiogram is unimodal. The chromosomes gradually de-

TABLE 1. Collection localities and characteristics of *Taraxacum platycarpum* subsp. *hondoense*, *T. officinale* and the putative natural 3x and 4x hybrids.

Accession numbers and localities of the samples	Outer involucre bracts	The length of <i>trnL-F</i> region of cpDNA (bp)	2C DNA content (pg)	Chromosome number (2n)	Estimated genome constitution
<i>T. platycarpum</i> subsp. <i>hondoense</i> (Nakai ex. Koidz.) Morita (Japanese native dandelion)					
Hoya 723 (Hokuto City, Yamanashi Pref.)	erect	482	2.22	16	JJ
Hoya 765 (Hokuto City, Yamanashi Pref.)	erect	482	2.18	16	JJ
<i>T. officinale</i> F. H. Wigg.					
Hoya 1224 (Sapporo city, Hokkaido Pref.)	recurved completely, stiffly	405	1.94	24	EEE
Natural 3x hybrid (putative hybrid between Japanese native dandelion × <i>T. officinale</i>)					
Hoya 88 (Suginami-Ku, Tokyo Metrop.)	recurved incompletely, irregularly	482	2.53	24	J (EE)*
Hoya 395 (Ina, Kitaadachi Gun, Saitama Pref.)	recurved incompletely, irregularly	482	2.40	24	J (EE)*
Natural 4x hybrid (putative hybrid between Japanese native dandelion × <i>T. officinale</i>)					
Hoya 271 (Meguro-Ku, Tokyo Metrop.)	recurved incompletely, irregularly	482	2.96	32	JEEE
Hoya 2071 (Kawagoe City, Saitama Pref.)	recurved incompletely, irregularly	482	2.94	32	JEEE

J, Japanese dandelion genome. E, *T. officinale* genome

*, EE in parenthesis means that the genomic constitution of EE in 3x hybrids should be variable through the “reductive” meiosis.

crease in size. This order of the chromosomes in the idiogram is not consistent in all cells and accessions examined due to the small differences in length between the constituent chromosomes. The longest chromosome, Chromosome 1J, was determined to be a longer satellited chromosome than Chromosome 4J. The fourth chromosome, Chromosome 4J, also has a satellite.

DNA content and karyotype of *Taraxacum officinale*

The Hoya 1224 accession of *Taraxacum officinale* is characterized by the stiffly recurved outer involucre bracts, the yellowish brown achenes, the cpDNA haplotype 405 bp long and a DNA content of ($2C = 3x \Rightarrow$) 1.94 pg (Table 1). The total karyotype ($2n = 3x = 24$) of this accession was 54.2 μm long (Fig. 1B, Table 3). The chromosomes are arranged in order of size, from 1 to 8, in this triploid complement (Fig. 2B, 1E–8E). The idiogram consists of three sets of chromosomes. Their chromosome sets differ slightly from one another in size and arm ratio (= long arm length / short arm length) (Table 3). The idiogram is unimodal, and the chromosomes gradually decrease in size. The size differences between chromosomes in a complement are small. The order of

the chromosome was inconsistent in all the cells examined. The three longest chromosomes, Chromosomes 1E, have satellites within the long arm. The proximal segment of the long arm with a large satellite is shorter than the short arm.

DNA content and karyotypes of the putative natural 3x hybrids between Japanese native *Taraxacum* and *T. officinale*

Accessions (Hoya 88 & 395) of the putative natural 3x hybrids are characterized by the incompletely recurved and irregular outer involucre bracts, the yellowish brown achene, the cpDNA haplotype 482 bp long and a DNA content of ($2C = 3x \Rightarrow$) 2.40–2.53 pg (Table 1). The total karyotype length ($2n = 3x = 24$) of accession Hoya 88 was 80.7 μm long (Table 4). Chromosomes 1J–8J, presumed to be derived from the diploid Japanese *Taraxacum*, were longer than those from the triploid *T. officinale* (Fig. 1C). Thus, the chromosomes of the Japanese *Taraxacum* are arranged first, in order of size from 1J to 8J, then, according to the relative length and the arm ratio, two sets of chromosomes (1E–8E) of *T. officinale* are arranged in the triploid idiogram (Fig. 2C). They were identified as being 3x hybrids comprising one chromosome set (1J–8J)

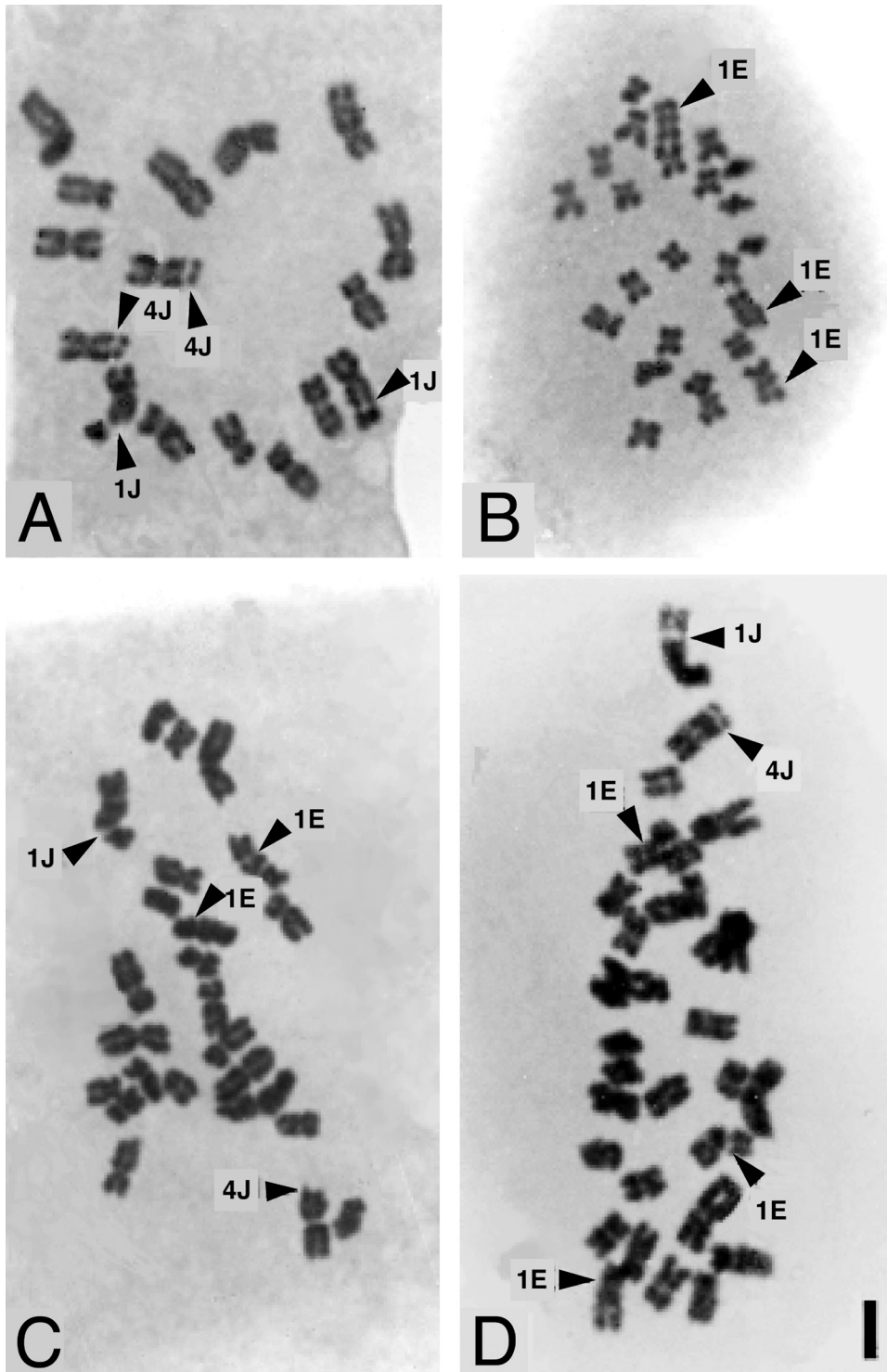


FIG. 1. Photomicrographs of somatic metaphase chromosomes of the *Taraxacum officinale* complex. Arrowed Chromosomes 1J, 4J and 1E are satellite chromosomes. Bar = 2.5 μ m. **A**, *Taraxacum platycarpum* subsp. *hondoense* (Hoya 723; $2n = 2x = 16$). **B**, *T. officinale* (Hoya 1224; $2n = 3x = 24$). **C**, putative natural 3x hybrid (Hoya 88; $2n = 3x = 24$). Chromosome 4J has an ill-defined secondary constriction in this plate (Fig. 1C). **D**, putative natural 4x hybrid (Hoya 271; $2n = 4x = 32$).

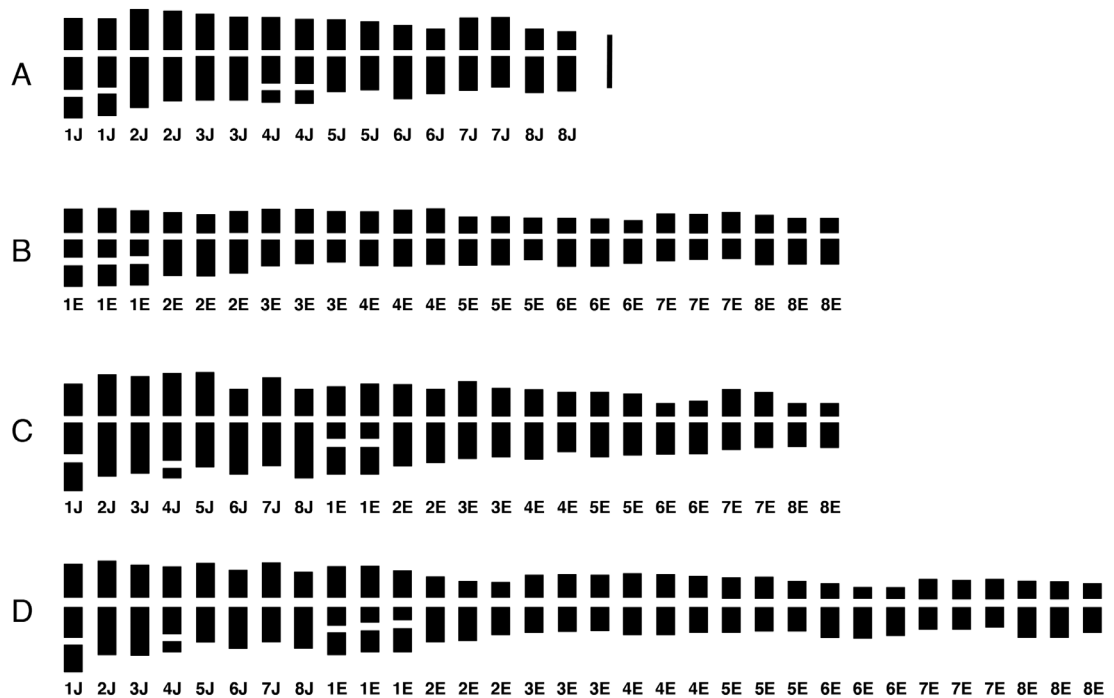


FIG. 2. Idiograms of somatic metaphase chromosomes in the *Taraxacum officinale* complex. Bar = 2.5 μ m. A, *Taraxacum platycarpum* subsp. *hondoense* (Hoya 723; $2n = 2x = 16$). B, *T. officinale* (Hoya 1224; $2n = 3x = 24$). C, putative natural $3x$ hybrid (Hoya 88; $2n = 3x = 24$). D, putative natural $4x$ hybrid (Hoya 271; $2n = 4x = 32$).

from the diploid Japanese *Taraxacum* and two chromosome sets (1E–8E) of $3x$ from *T. officinale*. Although two sets of Chromosomes 1E are in this idiogram, we were unable to determine if the two chromosome complements were derived from the triploid *T. officinale*, because the male gamete was derived from *T. officinale* through irregular meiosis. The data in Table 4 and the idiogram in Fig. 2C enable a preliminary interpretation of chromosome identity, phenetically, with respect to the chromosome length and the arm ratio. The longest chromosome is clearly Chromosome 1J with a large satellite. The shorter chromosome with a satellite, Chromosome 4J, shows an ill-defined secondary constriction in this plate (Fig. 1C). This satellite is the smallest among the four chromosomes with satellites. Two other chromosomes with satellites with short proximal segments of the long arm, are easily recognizable (Figs. 1C & 2C). Since the chromosomes with satellite, Chromosomes 1E, are the longest chromosomes in *T. officinale*, the remaining small

chromosomes are derived from *T. officinale*, 2E–8E.

DNA content and karyotypes of the putative natural 4x hybrids between Japanese native Taraxacum and T. officinale

Accessions (Hoya 271 & 2071) of the putative $4x$ hybrids are characterized by the incompletely and irregularly recurved outer involucre bracts, the yellowish brown achenes, cpDNA haplotype 482 bp long and DNA contents of ($2C = 4x =$) 2.94–2.96 pg (Table 1). The total karyotype length ($2n = 4x = 32$) of the accession Hoya 271 was 86.2 μ m (Table 5). In the plates of the karyotype (Fig. 1D) examined, most of the Chromosomes 1J–8J, presumed to be derived from diploid Japanese *Taraxacum* are longer than those derived from the triploid *T. officinale*. Thus, chromosomes derived from Japanese *Taraxacum* are arranged first, in order of size from 1J to 8J, then three sets of chromosomes (1E–8E) of *T. officinale* are arranged in a tetraploid complement

TABLE 2. Measurements of somatic chromosomes of *Taraxacum platycarpum* subsp. *hondoense* (Hoya 723).

Chromosome	Short arm + long arm (μm)	Total length (μm)	Arm ratio (L/S)
1J	1.5 + 2.3 (1.4 + 0.9)*	= 3.8	1.53
1J	1.4 + 2.2 (1.3 + 0.9)*	= 3.6	1.57
2J	2.0 + 2.2	= 4.2	1.10
2J	1.7 + 2.0	= 3.7	1.18
3J	1.6 + 2.0	= 3.6	1.25
3J	1.5 + 2.0	= 3.5	1.33
4J	1.5 + 1.7 (1.2 + 0.5)*	= 3.2	1.13
4J	1.4 + 1.7 (1.2 + 0.5)*	= 3.1	1.21
5J	1.4 + 1.6	= 3.0	1.14
5J	1.4 + 1.4	= 2.8	1.00
6J	1.0 + 1.9	= 2.9	1.90
6J	0.9 + 1.7	= 2.6	1.89
7J	1.3 + 1.6	= 2.9	1.23
7J	1.2 + 1.5	= 2.7	1.25
8J	0.9 + 1.7	= 2.6	1.89
8J	0.8 + 1.6	= 2.4	2.00
Total	50.6 (25.3 μm/1x)		

*, the lengths of proximal and distal parts of the long arm with a satellite are given in parenthesis, respectively.

(Fig. 2D). The longest chromosome was clearly Chromosome 1J with a large satellite. Chromosome 4J with the smallest satellite on the long arm is easily recognized. Three satellite chromosomes with the shorter proximal segments of the long arm, Chromosomes 1E, derived from *Taraxacum officinale*, are easily recognized (Figs. 1D & 2D).

Discussion

We here report the cytological characteristics of *Taraxacum platycarpum* subsp. *hondoense*, *T. officinale* and putative 3x and 4x hybrids between them. The chromosome arrangement in their idiograms in previous reports on diploid species of *Taraxacum* (Nisioka 1956, Takemoto 1961, Yamaguchi 1974, 1986, Sato *et al.* 2007b) is inconsistent, due to the small differences of the length between the constituent chromosomes. Takemoto (1961) placed the chromosome with the smallest satellite, Chromosome 4J, second, which is fourth

TABLE 3. Measurements of somatic chromosomes of *Taraxacum officinale* (Hoya 1224).

Chromosome	Short arm + long arm (μm)	Total length (μm)	Arm ratio (L/S)
1E	1.1 + 1.7 (0.7 + 1.0)*	= 2.8	1.55
1E	1.1 + 1.7 (0.7 + 1.0)*	= 2.8	1.55
1E	1.1 + 1.6 (0.6 + 1.0)*	= 2.7	1.45
2E	0.9 + 1.7	= 2.6	1.89
2E	0.8 + 1.7	= 2.5	2.13
2E	0.9 + 1.6	= 2.5	1.78
3E	1.0 + 1.2	= 2.2	1.20
3E	1.0 + 1.2	= 2.2	1.20
3E	1.0 + 1.2	= 2.2	1.20
4E	1.1 + 1.2	= 2.3	1.09
4E	1.1 + 1.1	= 2.2	1.00
4E	1.0 + 1.1	= 2.1	1.10
5E	0.8 + 1.2	= 2.0	1.50
5E	0.8 + 1.2	= 2.0	1.50
5E	0.7 + 1.0	= 1.7	1.43
6E	0.7 + 1.4	= 2.1	2.00
6E	0.7 + 1.3	= 2.0	1.86
6E	0.6 + 1.2	= 1.8	2.00
7E	0.9 + 1.1	= 2.0	1.22
7E	0.9 + 1.0	= 1.9	1.11
7E	0.9 + 1.0	= 1.9	1.11
8E	0.8 + 1.3	= 2.1	1.63
8E	0.7 + 1.2	= 1.9	1.71
8E	0.7 + 1.2	= 1.9	1.71
Total	52.4 (17.5 μm/1x)		

*, the lengths of proximal and distal parts of the long arm with a satellite are given in parenthesis, respectively.

in our arrangement (Fig. 2A, Table2), even though it was fourth in his measurements and sixth according to Nisioka (1956) in diploid Japanese *Taraxacum*. Our chromosome arrangements are consistent with their order in the idiograms by Yamaguchi (1986) and Sato *et al.* (2007b). Except for *T. maruyamanum*, the karyotypes of the diploid species of Japanese lowland *Taraxacum* are similar and indistinguishable from one another (Sato *et al.* 2007b).

Sorensen & Gudjonsson (1946), Takemoto (1961), and Sato *et al.* (2007a, c, 2008, 2014) reported on the karyotypes of *T. officinale*. Although Sato *et al.* (2007a, c, 2008, 2014) studied the cytologically of exotic *Taraxacum* throughout Japan, they treated all of them as *T. officinale* without the discriminating *T. officinale* from the hybrids. The mitotic chromosomes they examined by their pretreatment methods were two-thirds of the lengths of chromosomes in our study and were too short and contracted to allow for analysis of the karyotype. The results from their preparations appear to have resulted in karyo-

TABLE 4. Measurements of somatic chromosomes of putative 3x hybrid (*Hoya 88*) between Japanese native dandelion and *Taraxacum officinale*.

Chromosome	Short arm + long arm (μm)	Total length (μm)	Arm ratio (L/S)
1J	1.5 + 2.9 (1.5 + 1.4)*	= 4.4	1.93
2J	2.1 + 2.6	= 4.7	1.24
3J	1.9 + 2.5	= 4.4	1.32
4J	1.9 + 2.3 (1.9 + 0.4)*	= 4.2	1.21
5J	1.9 + 2.2	= 4.1	1.16
6J	1.5 + 2.6	= 4.1	2.73
7J	1.8 + 2.2	= 4.0	1.22
8J	1.2 + 2.8	= 4.0	2.33
1E	1.6 + 2.2 (0.9 + 1.3)*	= 3.8	1.38
1E	1.6 + 2.2 (0.9 + 1.3)*	= 3.8	1.38
2E	1.4 + 2.3	= 3.7	1.64
2E	1.2 + 2.1	= 3.3	1.72
3E	1.3 + 1.9	= 3.2	1.46
3E	1.3 + 1.7	= 3.0	1.31
4E	1.7 + 1.8	= 3.5	1.06
4E	1.3 + 1.4	= 2.7	1.08
5E	1.1 + 1.8	= 2.9	1.64
5E	1.0 + 1.7	= 2.7	1.70
6E	0.6 + 1.7	= 2.3	2.83
6E	0.6 + 1.7	= 2.3	2.83
7E	1.1 + 1.5	= 2.6	1.36
7E	1.2 + 1.4	= 2.6	1.17
8E	0.8 + 1.4	= 2.2	1.75
8E	0.8 + 1.4	= 2.2	1.75
J chromosomes		33.9	
E chromosomes		46.8 (23.4 μm/1x)	
Total		80.7	

*, the lengths of proximal and distal parts of the long arm with a satellite are given in parenthesis, respectively.

types that prevented discrimination between *Taraxacum officinale* and its hybrids. Among eight plants identified as *T. officinale* in Sato *et al.* (2014), Karyotype 1 (Fig. 3-A in Sato *et al.* 2014) is similar to *T. officinale* in our Fig. 2B. The remaining seven karyotypes (Fig. 3-B–H in Sato *et al.* 2014) appear to be similar to those of the 3x hybrid between the Japanese *Taraxacum* and *T. officinale*, in our Fig. 2C. These karyotypes include one set of the satellite chromosomes of the Japanese diploid species, although the order of the chromosomes in their karyotypes is not consistent with that of our Fig. 2C. Sato *et al.* (2007c) reported the karyotypes of five plants (Plants1–5) morphologically identified as *T. officinale* by them. Nevertheless, four of them (Plants 2–5) had the specific genotypes at GOT locus of which were regarded to be hybrids between Japanese *Taraxacum* and *T. officinale*. The karyotype of

TABLE 5. Measurements of somatic chromosomes of putative 4x hybrid (*Hoya 271*) between Japanese native dandelion and *Taraxacum officinale*.

Chromosome	Short arm + long arm (μm)	Total length (μm)	Arm ratio (L/S)
1J	1.6 + 2.7 (1.4 + 1.3)*	= 4.3	1.69
2J	1.8 + 2.3	= 4.1	1.28
3J	1.6 + 2.3	= 3.9	1.44
4J	1.5 + 1.8 (1.3 + 0.5)*	= 3.3	1.20
5J	1.7 + 1.7	= 3.4	1.00
6J	1.2 + 2.1	= 3.3	1.75
7J	1.6 + 1.7	= 3.3	1.06
8J	1.1 + 2.1	= 3.2	1.91
1E	1.4 + 2.0 (0.9 + 1.1)*	= 3.4	1.43
1E	1.4 + 1.9 (0.9 + 1.0)*	= 3.3	1.36
1E	1.2 + 1.8 (0.8 + 1.0)*	= 3.0	1.50
2E	1.0 + 1.8	= 2.8	1.80
2E	0.9 + 1.8	= 2.7	2.00
2E	0.7 + 1.6	= 2.3	2.29
3E	1.2 + 1.5	= 2.7	1.25
3E	1.2 + 1.5	= 2.7	1.25
3E	1.0 + 1.3	= 2.3	1.30
4E	1.1 + 1.3	= 2.4	1.18
4E	1.2 + 1.2	= 2.4	1.00
4E	1.1 + 1.2	= 2.3	1.09
5E	0.9 + 1.3	= 2.2	1.44
5E	0.9 + 1.3	= 2.2	1.44
5E	0.7 + 1.2	= 1.9	1.71
6E	0.6 + 1.6	= 2.2	2.67
6E	0.6 + 1.6	= 2.2	2.67
6E	0.5 + 1.4	= 1.9	2.80
7E	1.0 + 1.1	= 2.1	1.10
7E	0.9 + 1.1	= 2.0	1.22
7E	1.0 + 1.0	= 2.0	1.00
8E	0.8 + 1.5	= 2.3	1.88
8E	0.8 + 1.5	= 2.3	1.88
8E	0.6 + 1.2	= 1.8	2.00
J chromosomes		28.8	
E chromosomes		57.4 (19.1 μm/1x)	
Total		86.2	

*, the lengths of proximal and distal parts of the long arm with a satellite are given in parenthesis, respectively.

their Plant 1 is certainly similar to our Fig. 2B of *T. officinale*, which include the three longest chromosomes with a large satellite. The remaining four triploids (Plants 2–5) had three satellite chromosomes, instead of the expected four (in hybrids): two from the Japanese diploid *Taraxacum* and two from *T. officinale*. Those chromosomes with satellite were arranged 3rd to 7th (Fig. 2-B–E. and Tables 3–6 in Sato *et al.* 2007c). Those four plants have karyotypes similar to our 3x hybrid with four chromosomes with satellites between Japanese *Taraxacum* and *T. officinale* (Fig. 1C, 2C & Table 2 in this paper). These karyotypes include one set of satellite chromosomes of the Japanese diploid, although the order of the chromosome in their karyotype is not inconsistent with our Fig. 2C. Sato *et al.* (2004,

2007a, 2008, 2014) repeatedly reported the 4x *Taraxacum* to be *T. officinale*. However, those 4x plants appear to be all the 4x hybrids between the Japanese *Taraxacum* and *T. officinale*, because of the karyotype includes one large chromosome set from Japanese native *Taraxacum*. The occurrence of tetraploid *T. officinale* in Japan was not reported previously (Watanabe, K. *et al.* unpublished).

All 3x hybrids have one large Japanese *Taraxacum* chromosome set and two small *T. officinale* chromosome sets and all 4x hybrids have one large Japanese *Taraxacum* chromosome set and three small *Taraxacum officinale* chromosome sets. Both types of hybrids examined had the Japanese haplotype of the chloroplast DNA, suggesting one directional crossing via reduced (2x) or unreduced (3x) pollens from *T. officinale* (Table 1) (Morita *et al.* 1990).

The DNA content of Japanese *T. platycarpum* subsp. *hondoense* (sect. *Mongolica* (1.10 pg/1x Table 1 in accessions Hoya 723 & 765) is 1.69 times that of *T. officinale* (sect. *Taraxacum*) (0.65 pg/1x Table 1 in accession Hoya 1224) in our study. The DNA contents of the 3x and 4x hybrids is 2.40–2.53 pg, and 2.94–2.96pg, respectively. These values are nearly equal to those calculated on the basis of their respective genomic constitutions suspected by the karyotype analysis: 2.40 pg [= 1.10 (J) + 0.65 × 2 (EE)] for 3x hybrid and 3.05 pg [= 1.10 (J) + 0.65 × 3 (EEE)] for 4x hybrid. The difference in DNA contents, generally, relates to the differences of total karyotype lengths between taxa (Rothfels *et al.* 1966, Watanabe 2020). The total karyotype length of the Japanese diploid *T. platycarpum* subsp. *hondoense*, 25.3 µm/1x, is 1.45 times that of *T. officinale* (17.5 µm/1x) (Tables 2, 3, Figs. 1A, 1B, 2A, 2B). The total haploid chromosome length of Japanese diploid *Taraxacum*, 33.9 µm/1x, is 1.45 times that of the presumed *T. officinale* (23.4µm /1x) in the 3x hybrid cell (accession Hoya 88, Table 4, Fig. 2C). A similar ratio of 1.51 times (28.8 µm/19.1µm/1x) is also estimated for 4x hybrid cells (accession Hoya 271, Table 5, Fig. 2D).

The ratio of the longest chromosome to the shortest chromosome per one plate is 1.58

(3.8/2.4) in *T. platycarpum* subsp. *hondoense* (accession Hoya 723, Table 2, Fig. 2A), 1.47 (2.8/1.9) in *T. officinale* (accession Hoya 1224, Table 3, Fig. 2B), 2.14 (4.7/2.2) in the 3x hybrids between Japanese native *Taraxacum* and *T. officinale* (accession Hoya 88, Table 4, Fig. 2C), and 2.39 (4.3/1.8) in the 4x hybrids between Japanese native *Taraxacum* and *T. officinale* (accession Hoya 271, Table 5, Fig. 2D). The variance in chromosome size is more clearly revealed, due to mixing of both parental chromosomes, in the 3x and 4x hybrids (Figs. 1C & 1D).

The putative hybrids between Japanese diploid *Taraxacum* and *T. officinale* are characterized by the incompletely and irregularly recurvated outer involucre bracts. Those characteristics can be used to discriminate the hybrids from *T. officinale* in the field. DNA contents and the karyotype analyses are simple and useful for confirming hybrids between Japanese diploid *Taraxacum* and *T. officinale*, and the genomic constitution of the hybrids.

For further studies of habitat preference and geographical distribution, these four taxa should be treated separately because they differ from each other in the mode of microspore meiosis, seed reproduction and flowering phenology (unpublished).

We thank Prof. Emer. Morita, T. (Niigata Univ.) and Prof. Watano, Y. (Chiba Univ.) for providing references.

References

- Hamaguchi, T., M. Watanabe, N. Yamaguchi, & S. Serizawa. 2000. Distribution of hybridized alien dandelions in Hiratsuka City, Kanagawa Prefecture. Nat. Hist. Rep. Kanagawa 21: 7–21 (in Japanese).
- Hotta, M. 1977. On the distribution of dandelions (*Taraxacum*) in Kinki district. Studies on Natural History (Shizennshi-Kenkyu) 1: 117–134 (in Japanese with English summary).
- Ide, M., T. Uetake, H. Shibaike, Y. Kusumoto, S. Hiradate, H. Yano, A. Hoya, Y. Yoshimura & N. Shimizu. 2005. A study on the environmental characteristics of habitat and the genetic structure of *Taraxacum* species (dandelions) in the campus of National Institute

- of Livestock and Grassland Science. Landscape Res. J. 69: 545–548 (in Japanese with English abstract).
- Ito, M., J. Miyamoto, Y. Mori, S. Fujimoto, T. Uchiumi, M. Abe, A. Suzuki, S. Tabata & K. Fukui. 2000. Genome and chromosome dimensions of *Lotus japonicus*. J. Pl. Res. 113: 435–442.
- Kirschner, J. & J. Stepanek. 1996. Modes of speciation and evolution of the sections in *Taraxacum*. Folia Geobot. Phytotax. 31: 415–426.
- Kirschner, J. & J. Stepanek. 1997. A nomenclatural checklist of supraspecific names in *Taraxacum*. Taxon 46: 87–98.
- Kirschner, J. & J. Stepanek. 2011. Typification of *Leontodon Taraxacum* L. ($\equiv$ *Taraxacum officinale* F. H. Wigg.) and the generic name *Taraxacum*: A review and a new typification proposal. Taxon 60: 216–220.
- Kirschner, J., J. Stepanek & W. Greuter. 2007 onwards. *Taraxacum*. - In: Greuter, W. & E. von Raab-Straube (eds.), Compositae. Euro+MedPlantbase—The information resource for Euro-Mediterranean plant diversity. <<http://ww2.bgbm.org/EuroPlusMed/PTaxonDetail.asp?NameCache=Taraxacum>> [accessed Jan. 1, 2020].
- Majesky, L., F. Krahulec & R. J. Vasut. 2017. How apomictic taxa are treated in current taxonomy: A review. Taxon 66: 1017–1040.
- Makino, T. 1904. Dandelions in Japan (Nihon no tanpopo). Bot. Mag. Tokyo 18: 92–93 (in Japanese).
- Morita, T. 1976. Geographical distribution of diploid and polyploid *Taraxacum* in Japan. Bull. Natl. Sci. Mus. Ser. B. (Bot.) 2: 23–38 (in Japanese with English summary).
- Morita, T. 1988. Recent topics about *Taraxacum* agamospermy. Collecting & Breeding (Saishyu to Shiiku) 50: 128–132 (in Japanese).
- Morita, T. 1995. *Taraxacum*. In: Iwatsuki, K., T. Yamazaki, D. E. Boufford & H. Ohba (eds.), Flora of Japan IIIb, Angiospermae, Dicotyledoneae (b), pp. 7–13. Kodansha, Tokyo.
- Morita, T., A. A. Sterk & J. C. M. den Nijs. 1990. The significance of agamosperous triploid pollen donors in the sexual relationships between diploids and triploids in *Taraxacum* (Compositae). Pl. Spec. Biol. 5: 167–176.
- Nehira, K., M. Segawa, Y. Kobayashi & N. Kaneda. 1977a. On the distribution of dandelions (*Taraxacum*) in Hiroshima City. Plant and Nature (Shokubutsu to Shizen) 11: 18–20 (in Japanese).
- Nehira, K., M. Segawa, Y. Kobayashi & N. Kaneda. 1977b. On the distribution of dandelions (*Taraxacum*) around the Hiroshima Castle. Plant and Nature (Shokubutsu to Shizen) 11: 16–18 (in Japanese).
- Nehira, K., M. Nagahiro & K. Kondo. 1979. On the distribution of dandelions in Seto-Naikai Area. Stud. Env. Sci. Mem Integr. Arts Sci Hiroshima Univ. 55–64 (in Japanese with English summary).
- Nisioka, T. 1956. Karyotype analysis in Japanese Cichorieae. Bot. Mag. Tokyo 69: 586–591.
- Ogawa, K. & I. Mototani. 1985. Invasion of the introduced dandelions and survival of the native ones in the Tokyo Metropolitan Area of Japan. Jap. J. Ecol. 35: 443–452.
- Ogawa, K. & I. Mototani. 2001. Changes in the occurrence of native diploid and introduced triploid *Taraxacum* species in the southern Kanto District during the past decade. Wildlife Conservation Japan 6: 1–14 (in Japanese with English summary).
- Richards, A. J. 1985. Sectional nomenclature in *Taraxacum* (Asteraceae). Taxon 34: 63–3644.
- Rothfels, K., E. Sexsmith, M. Heimbürger & M. O. Krause. 1966. Chromosome size and DNA content of species of *Anemone* L. and related genera (Ranunculaceae). Chromosoma (Berlin) 20: 54–74.
- Sato, K., Y. Iwatsubo, M. Watanabe & M. Ohta. 2004. *Taraxacum officinale* complex in Mt Tateyama. Bull. Toyama Sci. Mus. 27: 53–60 (in Japanese with English summary).
- Sato, K., Y. Iwatsubo, M. Watanabe, S. Serizawa & N. Naruhashi. 2007a. Chromosome numbers of *Taraxacum officinale* (Asteraceae) in Tateyama Prefecture, central Japan. J. Phytogeogr. Taxon 55: 1–7.
- Sato, K., Y. Iwatsubo & N. Naruhashi. 2007b. Chromosome studies of native lowland diploid species of *Taraxacum* (Asteraceae) in Japan. Cytologia 72: 309–317.
- Sato, K., Y. Iwatsubo, M. Watanabe, S. Serizawa & N. Naruhashi. 2007c. Cytogenetic study of Japanese triploid *Taraxacum officinale* (common dandelion; Asteraceae). Cytologia 72: 475–482.
- Sato, K., Y. Iwatsubo, M. Ohta, T. Matsuhisa & N. Naruhashi. 2008. Chromosome numbers of *Taraxacum officinale* (Asteraceae) distributed in some high mountains in Central Honshu, Japan. J. Jap. Bot. 83: 115–120.
- Sato, K., T. Yamazaki & Y. Iwatsubo. 2014. Chromosome diversity of *Taraxacum officinale* Weber ex F. W. Wigg s.l. (common dandelion; Asteraceae). Cytologia 79: 39–5408.
- Shibaike, H., H. Akiyama, S. Uchiyama, K. Kasai & T. Morita. 2002. Hybridization between European and Asian dandelions (*Taraxacum* section *Ruderalia* and section *Mongolica*) 2. Natural hybrids in Japan detected by chloroplast DNA marker. J. Plant Res. 11: 321–328.
- Sorensen, T. H. & G. Gudjonsson. 1946. Spontaneous chromosome-abberrants in apomictic *Taraxaca*. Kongel. Danske Vidensk. Selesk. Biol. Skr. 4: 1–48.
- Takemoto, T. 1961. Cytological studies on *Taraxacum* and *Ixeris*. I. Some Japanese species of *Taraxacum*. Bull. School Educ. Okayama Univ. 11: 77–94.
- Watanabe, K. 2020. Index to Chromosome Numbers in Asteraceae. <http://www.lib.kobe-u.ac.jp/infolib/meta_pub/G0000003asteraceae> [accessed Jan. 1, 2020]

- Watanabe, M., Y. Maruyama & S. Serizawa. 1997a. Hybridization between native and alien dandelions in the Western Tokai district. (1) Frequency and morphological characters of the hybrid between *Taraxacum platycarpum* and *T. officinale*. J. Jap. Bot. 72: 51–57.
- Watanabe, M., Y. Maruyama & S. Serizawa. 1997b. Hybridization between native and alien dandelions in the Western Tokai district. (2) Frequency and morphological characters of the hybrid between *Taraxacum platycarpum* and *T. laevigatum*. J. Jap. Bot. 72: 352–356.
- Watanabe, M., M. Ogawa, S. Serizawa, M. Kanzaki & T. Mamakura. 1997c. Intrusion of hybridized alien dandelions into the native dandelion's habitats. Acta Phytotax. Geobot. 48: 73–78 (in Japanese with English summary).
- Watanabe, M., M. Ogawa, M. Naitou, M. Kanzaki, H. Shimomura & S. Serizawa. 1997d. Frequency and distribution of hybridized alien dandelion in Osaka Prefecture. Bull. Kansai Organ. Nature Conserv. (Kansai-shizenhogokikou-Kaisha) 19: 69–77 (in Japanese).
- Yamaguchi, S. 1974. Karyotype analysis in Japanese *Taraxacum*. I. *T. maruyamanum*, *T. japonicum* and *T. platycarpum*. Proc. Japan Soc. Plant Taxonomist 3: 15–16 (in Japanese with English summary).
- Yamaguchi, S. 1986. Karyotype analysis of the Japanese diploid species of *Taraxacum*, sec. *Mongolica* and sect. *Ceratophora*. Kromosomo II 43–44: 1386–1397.
- Yamano, M., H. Shibaike, T. Hamaguchi & M. Ide. 2002. Analysis on the distribution patterns of hybrid dandelions (*Taraxacum*) in Japan corroborated with the “Environmental indicator species survey” of common wildlife. Pap. Environ. Inform Sci. 16: 357–362 (in Japanese with English summary).
- Yamano, M., M. Ide & H. Shibaike. 2003. Analysis on relationships between landscape structures and distribution patterns for native and hybrid dandelions (*Taraxacum*) in Tsukuba-city, Ibaraki Pref. Landscape Research J. 67: 587–590 (in Japanese with English summary).

Received January 6, 2020; accepted September 3, 2020