Chromosome Studies of *Taraxacum denudatum* and *T. pectinatum* in Japan

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Summary We report on the occurrence of karyotypes of two species and one variety of dandelion, *Taraxacum denudatum* var. *denudatum*, var. *arakii*, and *T. pectinatum*, in western Japan. The two varieties of *T. denudatum* had two polyploid numbers of $2n=32$ (4x) and 40 (5x). In *T. denudatum* var. *denudatum*, the tetraploid plants had a karyotype form expressed as $2n=32=3M+25m+2m_{cs}+1sm+1sm_{cs}$, and the pentaploid plants comprised two karyotype forms of $2n=40=2M+32m+2m_{cs}+3sm+1sm_{cs}$ and $2n=40=5M+32m+3m_{cs}$. In *T. denudatum* var. *arakii*, the tetraploids had a karyotype form expressed as $2n=32=27m+5m_{cs}$ and the pentaploids had a karyotype form of $2n=40=3M+33m+3m_{cs}$. *Taraxacum pectinatum* was a tetraploid dandelion with a karyotype expressed as $2n=32=23m+5m_{cs}+2sm+2sm_{cs}$. The present study by their karyotype analyses suggests that *T. denudatum* var. *denudatum*, *T. denudatum* var. *arakii*, and *T. pectinatum* are all allopolyploid dandelions.

Key words Allopolyploid, Karyotype, Polyploidy, *Taraxacum denudatum* var. *denudatum*, *T. denudatum* var. *arakii*, *T. pectinatum*.

Taraxacum plants producing pale yellow flowers in Japan were first categorized into the following four taxa: *T. shinanense* H. Koidz. (Koidzumi 1933a), *T. denudatum* H. Koidz. (Koidzumi 1933a), *T. hideoi* Nakai ex H. Koidz. (Koidzumi 1933b), and *T. albidum* var. *igaense* H. Koidz. (Koidzumi 1936). After that, Kitamura (1957) treated *T. hideoi* as a synonym of *T. albidum* Dahlst., and *T. denudatum* as a synonym of *T. shinanense*. The classification proposed by Ohwi and Kitagawa (1992) was followed by that of Kitamura (1957). Morita (1995), however, divided the *Taraxacum* with pale yellow flowers occurring in Japan into *T. denudatum* and *T. hideoi*, and treated *T. shinanense* as a synonym of *T. venustum* H. Koidz., and *T. arakii* Kitam. as a synonym of *T. pectinatum* Kitam. Thereafter, Serizawa (2006) treated the *Taraxacum* plants with pale yellow flowers occurring in Japan as one species two varieties of *T. denudatum* var. *denudatum* and var. *arakii* (Kitam.) Seriz. Recently, Morita (2017) treated that *T. shinanense*, *T. denudatum*, *T. nambuense*, and *T. albidum* var. *igaense* are conspecific with *T. hideoi*. However, he did not describe the taxonomic statuses of *T. arakii* and *T. ceratolepis*.

Chromosome information provides a useful means to elucidate systematic relationships among polyploid plants, as well as intraspecific diversity. Thus, the present study examined the chromosome numbers and karyotypes of *Taraxacum* species with pale yellow flow-

ers and *T. pectinatum*.

Materials and methods

The taxonomic treatment of *Taraxacum* was followed Serizawa (2006), using the three taxa of *T. denudatum* H. Koidz. var. *denudatum*, *T. denudatum* var. *arakii* (Kitam.) Seriz., and *T. pectinatum* Kitam. The plants used in this study were collected from the natural habitats listed in Table 1 and, were grown and preserved in an experimental garden of the University of Toyama. Newly formed root tips were collected from each potted plant and pretreated in a 2.2 mM 8-hydroxyquinoline at 20–25°C for about 1 h and kept at 5°C for 15 h. Root tips were fixed in a mixture of glacial acetic acid and absolute ethanol (1:3) at room temperature for 1 h, macerated in 1 M HCl at 60°C for 10 min, washed in tap water, stained in a drop of 1.5% lacto-propionic orcein on a glass slide, and subjected to the ordinal squash technique. In this study, chromosome form was expressed on the basis of the nomenclature of Levan *et al.* (1964) and Tanaka (1977) with modification, that is, the chromosome with arm ratio 1.7 is categorized to the median centromere and expressed as “m”. Fully spread metaphase chromosomes used for the karyotype analyses came from some localities (Table 1).

Results and discussion

Taraxacum denudatum var. *denudatum*

Of the 154 plants examined (Table 1), 105 collected

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With Supplementary data of Tables (Tables S1–S6).

Table 1. Chromosome number, collection locality, and number of individuals examined of *Taraxacum*.

Collection locality	No of plants with chromosome number (2n)		Total
	32	40	
<i>Taraxacum denudatum</i> var. <i>denudatum</i>			
Fukushima Pref.			
Ojima, Kawamata-machi, Date-gun		6 (2)*	6
Aichi Pref.			
Utsumi, Minamichita-cho, Chita-gun		8 (3)	8
Mie Pref.			
Katadatanakacho, Tsu city		5 (3)	5
Takajayakomoricho, Tsu city		14	14
Okayama Pref.			
Imazu, Tsugawamachi, Takahashi city	3		3
Kashinishi, Maniwa city	12		12
Katsuyama, Maniwa city	10		10
Minagi, Nagi-cho, Katsuta-gun	1	3	4
Miyagochi, Tettacho, Niimi city	7	1	8
Myobara, Tsuyama city	5		5
Nishi, Kibichuo-cho, Kaga-gun	8	6	14
Nogamicho, Ibara city	2	5 (1)	7
Oza, Kagamino-cho, Tomata-gun	1		1
Ushiroyama, Mimasaka city	7	1	8
Hiroshima Pref.			
Fukushiro, Tojocho, Shobara city	5		5
Kushiro, Tojocho, Shobara city	6		6
Matsusaki, Jogecho, Fuchu city	4		4
Momodani, Kamocho, Fukuyama city	10		10
Shimoryoke, Soryocho, Shobara city	12		12
Shinmen, Jinsekikogen-cho, Jinseki-gun	12 (2)		12
<i>Taraxacum denudatum</i> var. <i>arakii</i>			
Kyoto Pref.			
Oyogicho, Ayabe city	2 (1)		2
Uesugicho, Ayabe city	1		1
Okayama Pref.			
Hataki, Tesseicho, Niimi city	24		24
Shimane Pref.			
Fukuura, Mihonosekicho, Matsue city		6	6
Iruma, Kakeyacho, Unnan city		9	9
Kakeya, Kakeyacho, Unnan city		2	2
Kawauchi, Kawamoto-machi, Ohchi-gun		8	8
Nonami, Shimanecho, Matsue city		15	15
Okamotocho, Matsue city		21	21
Otsukamiya, Mitoyacho, Unnan city		8	8
Owashi, Shimanecho, Matsue city		9 (1)	9
Sen'yama, Asayamacho, Ohda city		2 (2)	2
Tamatsukuri, Tamayucho, Matsue city		5	5
Yawatabara, Sadacho, Izumo city		10	10
<i>Taraxacum pectinatum</i>			
Hyogo Pref.			
Okuyama, Izushicho, Toyooka city	8 (2)		8

*Numbers in parentheses are the individuals used in karyotype analysis.

from 16 localities both in Okayama Pref. and Hiroshima Pref., adjoining Prefectures of the Chugoku Region on Honshu (the main island), were all tetraploids with $2n=32$ chromosomes (Fig. 1A). The remaining 49 plants, collected in nine localities across the main island of Honshu, from Fukushima Pref. of the Tohoku Region, Aichi and Mie Prefs. of the Chubu Region, and Okayama Pref. of the Chugoku Region, were pentaploids with $2n=40$ chromosomes (Fig. 1B). The two chromosome counts of $2n=32$, 40 found in this taxon were consistent with previous reports ($2n=32$: Okabe 1951, Takemoto

1961, 1970, $2n=40$: Okabe 1951, Yamaguchi 1976). The chromosome count of $2n=38$ as reported by Miyaji (1932) was not observed in any of the plants from this study.

Tetraploid ($2n=32$): The two plants collected at Shinmen, Jinsekikogen-cho, Hiroshima Pref., showed very similar karyotypes. Their 32 chromosomes at metaphase ranged from 1.5 to 3.4 μm in length and 1.0 to 1.8 in arm ratio (Fig. 2A). The 32 chromosomes were divided into 30 metacentric chromosomes and two submetacentric chromosomes (Table S1). With regard to chromosome

Fig. 1. Photographs at somatic metaphase chromosomes of A) *Taraxacum denudatum* var. *denudatum* with $2n=32$. B) var. *denudatum* with $2n=40$. C) var. *arakii* with $2n=32$. D) var. *arakii* with $2n=40$. E) *T. pectinatum* with $2n=32$. Bars = $5\mu\text{m}$.

complement, each of the short arms of two metacentric chromosomes and the long arm of one submetacentric chromosome had a secondary constriction. A total length of the somatic complement was $78.7\mu\text{m}$. Chromosome complements were formulated as $2n=32=3M+25m+2m^{cs}+1sm+1sm_{cs}$.

Pentaploid ($2n=40$) karyotype 1: Karyotype examination of the nine pentaploid plants revealed the same karyotype from across the total of eight plants collected from three localities; Ojima, Kawamata-machi, Fukushima Pref.; Utsumi, Minamichita-cho, Aichi Pref.; and Katadatanakacho, Tsu city, Mie Pref. As shown in Fig. 2B, the 40 chromosomes at somatic metaphase ranged from 1.5 to $3.2\mu\text{m}$ in length and 1.0 to 1.8 in arm ratio and were divided into 36 metacentric chromosomes and four submetacentric chromosomes (Table S2). In the somatic metaphase chromosome complement, each of the long arms of two metacentric chromosomes and the long arm of one submetacentric chromosome had a secondary constriction. The total length of the somatic complement was $89.2\mu\text{m}$. This chromosome complement was formu-

lated as $2n=40=2M+32m+2m_{cs}+3sm+1sm_{cs}$.

Pentaploid ($2n=40$) karyotype 2: Somatic chromosomes at metaphase collected at Nogamicho, Ibara city, Okayama Pref., ranged from 1.4 to $3.7\mu\text{m}$ in length and 1.0 to 1.7 in arm ratio (Fig. 2C, Table S3). The 40 chromosomes were all metacentric chromosomes. With regard to the somatic metaphase chromosome complement, three chromosomes had a secondary constriction in their long arms. The total length of the somatic complement was $89.4\mu\text{m}$. The chromosome complement was formulated as $2n=40=5M+32m+3m_{cs}$.

Taraxacum denudatum var. *arakii*

The somatic chromosome counts of 122 plants were examined (Table 1); 27 plants collected from three localities in Kyoto Pref. and Okayama Pref. were tetraploids with $2n=32$ chromosomes (Fig. 1C), and the remaining 95 plants collected from 11 localities in Shimane Pref. were pentaploids with $2n=40$ chromosomes (Fig. 1D). Of the two counts, $2n=32$ was consistent with Takemoto (1970, as *T. ceratolepis*) and Yamaguchi (1976, as *T. cer-*

Fig. 2. Karyotypes of somatic metaphase chromosomes of *Taraxacum denudatum* var. *denudatum*. A) $2n=32$. B) $2n=40$ (Type 1). C) $2n=40$ (Type 2). Arrows indicate chromosome arms with secondary constrictions. Bar= $5\mu\text{m}$.

Fig. 3. Karyotypes of somatic metaphase chromosomes of *Taraxacum denudatum* var. *arakii*. A) $2n=32$. B) $2n=40$. Arrows indicate chromosome arms with secondary constrictions. Bar= $5\mu\text{m}$.

Fig. 4. Karyotype of somatic metaphase chromosomes of *Taraxacum pectinatum*. Arrows indicate chromosome arms with secondary constrictions. Bar=5 μ m.

Fig. 5. Geographic distribution of the two different polyploids of A) *Taraxacum denudatum* var. *denudatum* with $2n=32$. B) var. *denudatum* with $2n=40$. C) var. *arakii* with $2n=32$. D) var. *arakii* with $2n=40$. Detailed localities are given in Table 1. Bars=100 km.

atolepis), while $2n=40$ was a new count for this taxon.

Tetraploid karyotype: A total of three plants: one plant collected from Oyogicho, Ayabe city, Kyoto Pref.; and two plants collected at Hataki, Tesseicho, Niimi city, Okayama Pref., had the same karyotype form. The chromosomes at metaphase ranged from 1.6 to 3.6 μ m in length and 1.0 to 1.7 in arm ratio (Fig. 3A). Each of these five metacentric chromosomes had a secondary constriction in their long arms. A total length of the somatic complement was 79.5 μ m. The chromosome complement was expressed as a karyotype of $2n=32=27m+5m_{cs}$ (Table S4).

Pentaploid karyotype: Three plants (two collected at Sen'yama, Asayamacho, Ohda city, Shimane Pref., and one collected at Owashi, Shimanecho, Matsue city, Shimane Pref.) had the same karyotype. As shown in Fig. 3B, the 40 chromosomes ranged from 1.5 to 2.9 μ m in length and 1.0 to 1.7 in arm ratio. Each of the four chromosomes had a secondary constriction in their long arms. A total length of the somatic complement was 84.0 μ m. This chromosome complement was formulated

as $2n=40=3M+33m+4m_{cs}$ (Table S5).

Taraxacum pectinatum

All eight plants collected at Okuyama, Izushicho, Toyooka city, Hyogo Pref., had $2n=32$ chromosomes (tetraploid), consistent with the counts previously reported by Okabe (1951), Takemoto (1961), and Yamaguchi (1976) (Fig. 1E, Table 1). The two plants that were examined showed the same karyotype form. Metaphase chromosomes ranged in length from 1.8 to 3.4 μ m in length and 1.1 to 2.4 in arm ratio (Fig. 4). The 32 chromosomes were divided into 28 metacentric chromosomes and four submetacentric chromosomes. A total length of the somatic complement was 76.2 μ m. Of the 32 chromosomes, each of the long arms of five metacentric chromosomes and the long arms of two submetacentric chromosomes had a secondary constriction. The chromosome complement was formulated as $2n=32=23m+5m_{cs}+2sm+2sm_{cs}$ (Table S6).

This study showed that *T. denudatum* var. *denu-*
datum comprises tetraploid ($2n=32=3M+25m+2m^{cs}+$

1sm+1sm_{cs}) and pentaploid plants (Fig. 5A, B), and that the pentaploid plants have two karyotype strains ($2n=40=2M+32m+2m_{cs}+3sm+1sm_{cs}$ and $2n=40=5M+32m+3m_{cs}$). *T. denudatum* var. *arakii* also comprises tetraploid ($2n=32=27m+5m_{cs}$) and pentaploid plants ($2n=40=3M+33m+4m_{cs}$) (Fig. 5C, D). *T. pectinatum* is a tetraploid plant ($2n=32=23m+5m_{cs}+2sm+2sm_{cs}$). These tetraploid and pentaploid karyotypes found in *T. pectinatum*, *T. denudatum* var. *denudatum*, and var. *arakii* suggest that they might be all allopolyploids with different karyotype forms.

By the results of this study, we found that the two varieties of *T. denudatum* cytologically cannot be distinguished. Plants described as *T. denudatum* H. Koidz. and recognized as *T. albidum* var. *igaense* are pentaploid. Plants recognized as *T. hideoi* have two different polyploids.

References

- Kitamura, S. 1957. Compositae Japonicae Pars sexta. Mem. Coll. Sci. Univ. Kyoto B **24**: 1–79 with pls. I–VII.
- Koidzumi, H. 1933a. *Taraxacum* novum orientali-Asiaticum I. Bot. Mag. Tokyo **47**: 89–124.
- Koidzumi, H. 1933b. *Taraxacum* novum Japonicum (1). Shokubutsu Kenkyu Zasshi **9**: 349–364.
- Koidzumi, H. 1936. *Taraxacum* novum Japonicum (4). Shokubutsu Kenkyu Zasshi **11**: 618–634.
- Levan, A., Fredga, K. and Sandberg, A. A. 1964. Nomenclature for centromeric position on chromosomes. Hereditas **52**: 201–220.
- Miyaji, Y. 1932. On the chromosome numbers in the genus *Taraxacum*. Bot. Mag. Tokyo **46**: 406–408. (*in Japanese*)
- Morita, T. 1995. *Taraxacum*. In: Iwatsuki, K., Yamazaki, T., Boufford, D. E. and Ohba, H. (eds.). Flora of Japan IIb. Angiospermae, Dicotyledoneae, Sympetaleae (b). Kôdansha, Tokyo. pp. 7–13.
- Morita, T. 2017. *Taraxacum*. In: Ohashi, H., Kadota, Y., Murata, J., Yonekura, K. and Kihara, H. (eds.). Wild Flowers of Japan 5. Convolvulaceae–Caprifoliaceae. Heibonsha, Tokyo. pp. 285–289 with pls. 173–177. (*in Japanese*)
- Ohwi, J. and Kitagawa, M. 1992. New Flora of Japan. Shibundô, Tokyo. pp. 1373–1376. (*in Japanese*)
- Okabe, S. 1951. On the Chromosome Numbers in Asiatic Species of *Taraxacum*. In: Okada, K. (ed.). Papers from the Committee for Research in Genetics 2. Coordinating Committee for Research in Genetics, Tokyo. pp. 3–6. (*in Japanese*)
- Serizawa, S. 2006. Classification and nomenclature of the pale-yellow-flowered dandelions in Japan. J. Phytogeogr. Taxon. **54**: 21–26. (*in Japanese*)
- Takemoto, T. 1961. Cytological studies on *Taraxacum* and *Ixeris* I. Some Japanese species of *Taraxacum*. Bull. Sch. Educ. Okayama Univ. **11**: 77–94.
- Takemoto, T. 1970. Chromosome studies on three tetraploid races of *Taraxacum*. Kromosomo **79–80**: 2539–2547. (*in Japanese*)
- Tanaka, R. 1977. Shin-kakugataron. In: Ogawa, K., Kurosumi, K., Koike, M. and Sato, S. (eds.). Plant Cytology. Asakura Publishing, Tokyo. pp. 293–326. (*in Japanese*)
- Yamaguchi, S. 1976. Chromosome numbers of Japanese *Taraxacum* species. Shokubutsu Kenkyu Zasshi **51**: 52–58.