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Comparison of Ribosomal DNA Distribution Patterns on Somatic Chromosomes between *Aster miyagii* and *Erigeron thunbergii* (Asteraceae)

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Abstract Somatic chromosomes of *Aster miyagii* (=*Erigeron miyagii*) being endemic to the Ryukyus and *Erigeron thunbergii* were compared by the conventional aceto-orcein staining method and the fluorescent *in situ* hybridization using 45S ribosomal DNA (rDNA) probes. The two species commonly had a chromosome number of 2n=18, and a rDNA site in a pair of chromosomes in a complement. The Ryukyu plants exhibited the rDNA site at a secondary constriction between the short arm and satellite. On the other hand, the plant of *E. thunbergii* exhibited the rDNA site at a secondary constriction and satellite. The present cytological investigation concludes that the Ryukyu plants must be treated as *Aster miyagii* agreeing with Hatusima (1975), Walker (1975), Kitamura (1937), and Ito and Soejima (1995).

Key words: *Aster*, chromosomes, *Erigeron*, FISH, rDNA, Ryukyus.

Introduction

Aster miyagii Koidz. (=*Erigeron miyagii* (Koidz.) Honda) is perennial herb, and distributes in Amami, Tokuno-shima, Kakeroma-jima and Okinawa Islands as endemic to the central part of Ryukyu Archipelago (the Ryukyus) (Hatusima, 1975). In 1914, Koidzumi described *A. miyagii* for the Ryukyu plants, and his taxonomic concept has been supported by most taxonomists (e.g. Hatusima, 1975). However, it is not necessary that all taxonomists accept the taxonomic concept of Koidzumi (1914), namely Honda (1931) transferred *A. miyagii* to the genus *Erigeron*, and treated it as *E. miyagii* (see detail in Discussion).

Recently the florescent *in situ* hybridization (FISH) method, which is one of molecular-cytological techniques has been applied to taxonomic study in the genus *Aster*, and verified a useful method (e.g. Kokubugata *et al.*, 2003, 2008; Saito and Kokubugata, 2004; Matoba *et al.*, 2005, 2007). In the present study, we compare rDNA distribution patterns on somatic chromosomes of *A. miyagii* (=*E. miyagii*) and *E. thunbergii* using the FISH method using 45S rDNA probes, and reconsider taxonomic status of the Ryukyu plants.

Materials and Methods

Plant materials

Plants of *Aster miyagii* (=*Erigeron miyagii*) collected from seven localities in the central Ryukyus, and that of *Erigeron thunbergii* collected from Japan proper were investigated in the present study (Table 1). These plants were cultivated in the experimental greenhouse of Tsukuba Botanical Garden, and then were used as the present cytological materials. The voucher specimens were deposited in the herbarium of the

Table 1. Localities and vouchers of *Aster miyagii* (=*Erigeron miyagii*) and *E. thunbergii*

Species*	Locality	Voucher (TNS no.)**
A. miyagii	Japan, Ryukyus: Ichi, Amami-shi, Amami Is., Kagoshima.	*GK 5829* (9518622)
	Japan, Ryukyus: Toguchi, Tatsugo-cho, Amami Is., Kagoshima.	*GK 5836* (9518629)
	Japan, Ryukyus: Ankyaba, Setouchi-cho, Kakeroma-jima Is., Kagoshima.	*GK 5942* (9518819)
	Japan, Ryukyus: Inunojo-futa, Isen-cho, Tokuno-shima Is., Kagoshima.	*GK 5865* (9518718)
	Japan, Ryukyus: Kametoku, Tokunoshima-cho, Tokuno-shima Is., Kagoshima.	*GK 5886* (9518606)
	Japan, Ryukyus: Oku, Kunigami-son, Okinawa Is., Okinawa.	*GK 5340* (9518977)
	Japan, Ryukyus: Ada, Kunigami-son, Okinawa Is., Okinawa.	*GK 5345* (9518981)
E. thunbergii	Japan, Honshu: Mt. Kanfu-yama, Oga-shi, Akita.	*GK 9790* (9531823)

*Taxonomic treatment followed to Ito and Soejima (1995).
**Voucher specimens are deposited in TNS.

National Museum of Nature and Science (TNS; Table 1).

Standard orcein staining squash method

Root tips of the two species plants were harvested, pretreated in 2 mM 8-hydroxyquinoline at 15°C for 3 h, and fixed in acetic ethanol (1 : 3) at 4°C for 2 h at least. The root tips were macerated in a mixture of 1N hydrochloric acid and acetic acid (2 : 1) at 60°C for 10 sec, put on glass slides, and then were stained in 2% aceto-orcein at room temperature for 4 h and squashed.

FISH method

FISH protocol in the present study was following Kokubugata *et al.* (2003). A 18S rDNA sequence, being a part of 45S rDNA, amplified from the total genomic DNA of *A. ageratoides* (*GK 2810*; Kokubugata *et al.*, 2003) by the polymerase chain reaction (PCR) using the primers of NS1 and NS4 designed by White *et al.* (1990). The PCR-amplified rDNA was labeled with digoxigenin- (DIG-) dUTP. The labeled probes hybridized on somatic chromosome were detected with Anti-digoxigenin-fluorescein, Fab-fragmentavidin with PI revealing non-hybridized regions with orange or dark red fluorescence.

Karyotype analyses

Chromosomes at mitotic metaphase were classified by arm ratio (R=long arm length/short arm length) following Levan *et al.* (1964). Median-centromeric ($1.0 \leqq R < 1.7$), submedian-centromeric ($1.8 \leqq R < 3.0$), subterminal-centromeric ($3.1 \leqq R < 7.0$), and terminal-centromeric chromosomes ($7.1 \leqq R$) are abbreviated as **m**, **sm**, **st** and **t** chromosomes in this paper, respectively.

Results and Discussion

Seven plants of *Aster miyagii* (=*Erigeron miyagii*) collected from the Ryukyus and one plant of *E. thunbergii* commonly showed a chromosome number of 2n=18 (Fig. 2). In the Ryukyu plants, the present counts were consistent with the previous reports of Matsuda (1970) for plants collected from Tokuno-shima Island; and those of Shimotomai and Huziwara (1942), Matsuda (1970), Miyagi (1973) and Kanemoto (2000) for plants collected from Okinawa Island. In *E. thunbergii*, the chromosome number of 2n=18 were consistent with most previous references, for example Ishikawa (1916), Matsuura and Suto (1935), and Huziwara (1954). In karyotype analysis, most chromosomes in a comple-

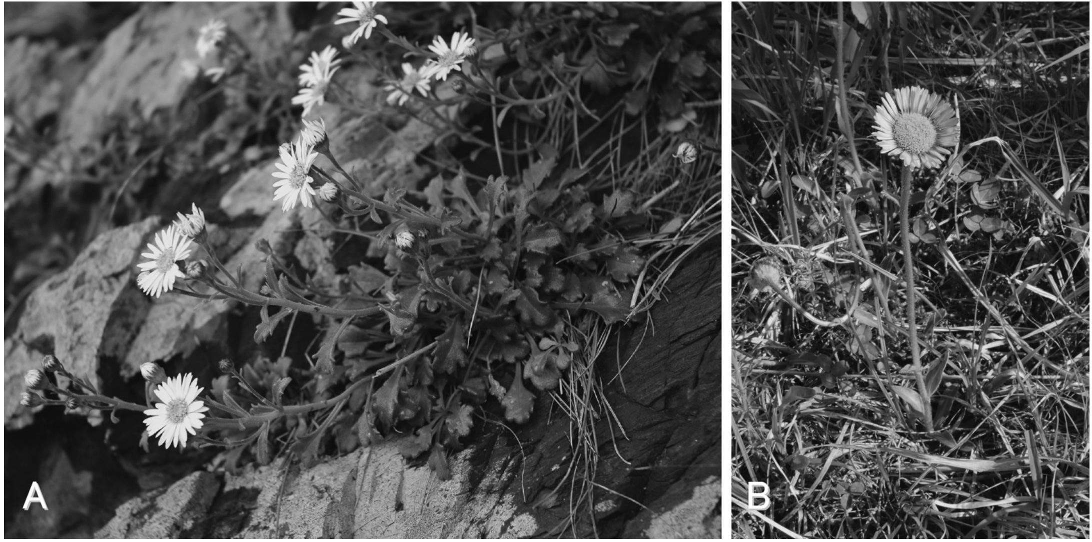

Fig. 1. Habit of *Aster miyagii* (=*Erigeron miyagii*) (A) and *E. thunbergii* (B).

Table 2. Arm ratios of eighteen chromosomes in a complement of *Aster miyagii* (=*Erigeron miyagii*) and *E. thunbergii*

Species	*A. miyagii* (=*E. miyagii*)							*E. thunbergii*
Island	Amami		Kakeroma-jima	Tokuno-shima		Okinawa		
Individual*	*5829*	*5836*	*5886*	*5952*	*5865*	*5340*	*5345*	*9790*
Chromosomes alighted by length 1	1.0	1.0	1.0	1.0	1.0	1.2	1.1	1.5
2	1.1	1.2	1.0	1.2	1.0	1.2	1.1	1.5
3	1.0	1.3	1.4	1.1	1.0	1.0	1.1	2.8
4	1.0	1.1	1.2	1.1	1.3	1.3	1.3	2.2
5	1.3	1.1	1.3	1.3	1.4	1.0	1.3	2.9
6	1.4	1.1	1.2	1.1	2.1	1.3	1.3	2.5
7	1.2	1.4	1.5	1.3	1.1	1.0	1.2	2.0
8	1.3	1.1	1.7	2.1	1.2	1.4	1.4	1.9
9	1.2	1.5	1.1	1.2	1.3	1.5	1.7	2.1
10	1.4	1.6	1.1	1.6	1.4	1.6	1.5	2.1
11	1.5	1.5	1.7	1.8	2.2	1.5	1.3	1.2
12	1.7	1.2	1.6	1.3	1.5	1.8	1.5	1.0
13	1.3	2.0	1.8	1.1	1.6	1.5	1.5	2.8
14	1.6	1.8	1.7	1.2	1.5	1.4	1.5	2.2
15	1.4	1.2	1.3	1.1	1.3	1.1	1.1	1.2
16	1.0	1.1	1.1	1.3	1.0	1.3	1.0	2.0
17	1.1	1.6	1.2	1.5	1.2	1.0	1.1	1.2
18	1.1	1.2	1.1	1.0	1.6	1.0	1.2	1.8

*Voucher number of Goro Kokubugata (TNS) indicated in Table 1.

ment of the seven Ryukyu plants were identified as **m**-chromosomes (Table 2). Although some of them were identified as **sm**-chromosomes, their arm ratio values of 1.8 to 2.2 were close to 1.7 being the highest extreme of the arm ratio range of the **m** chromosomes according to Levan *et al*. (1964; Table 2). On the other hand, a chromosome complement of *E. thunbergii* was consisted

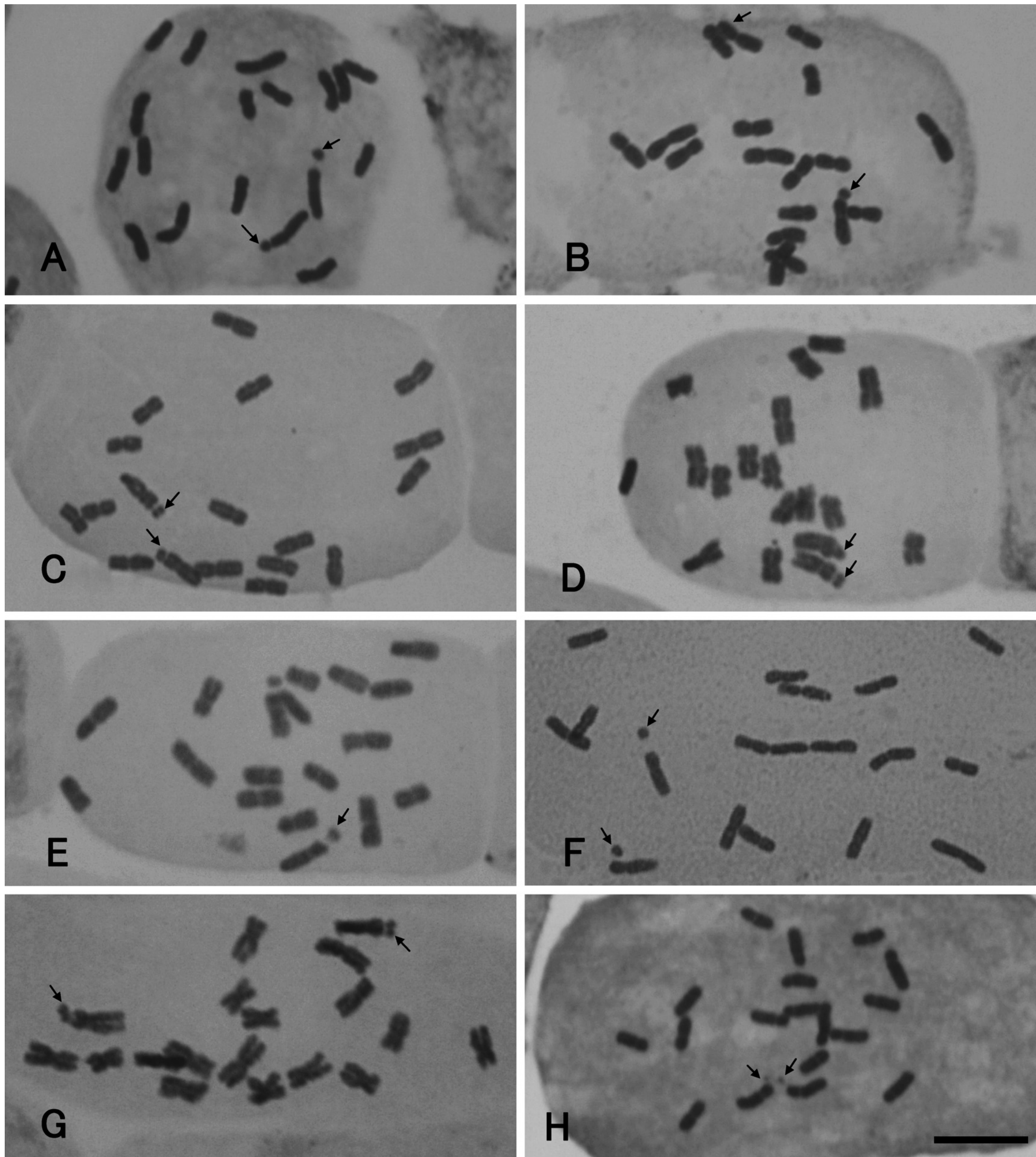

Fig. 2. Orcein-stained chromosomes of *Aster miyagii* (=*Erigeron miyagii*) (A–G) and *E. thunbergii* (H). A. Ichi (Amami Is.). B. Honohoshi (Amami Is.). C. Ankyaba (Kakeroma-jima Is.). D. Inunojo-futa (Tokuno-shima Is.). E. Kametoku (Tokuno-shima Is.). F. Oku (Okinawa Is.). G. Ada (Okinawa Is.). H. Mt. Kanfu-yama (Akita). Arrows show satellite. Bar indicates 10 μm.

of thirteen **m**-chromosomes and five **sm**-chromosomes (Table 2).

The seven Ryukyu plants and one plant of *E. thunbergii* commonly had a pair of **m** chromosomes with a satellite (sat-chromosome; arrows in Fig. 1). The two sat-chromosomes of the seven Ryukyu plants were aligned from the first to the fourth by chromosome lengths; while those of *E.*

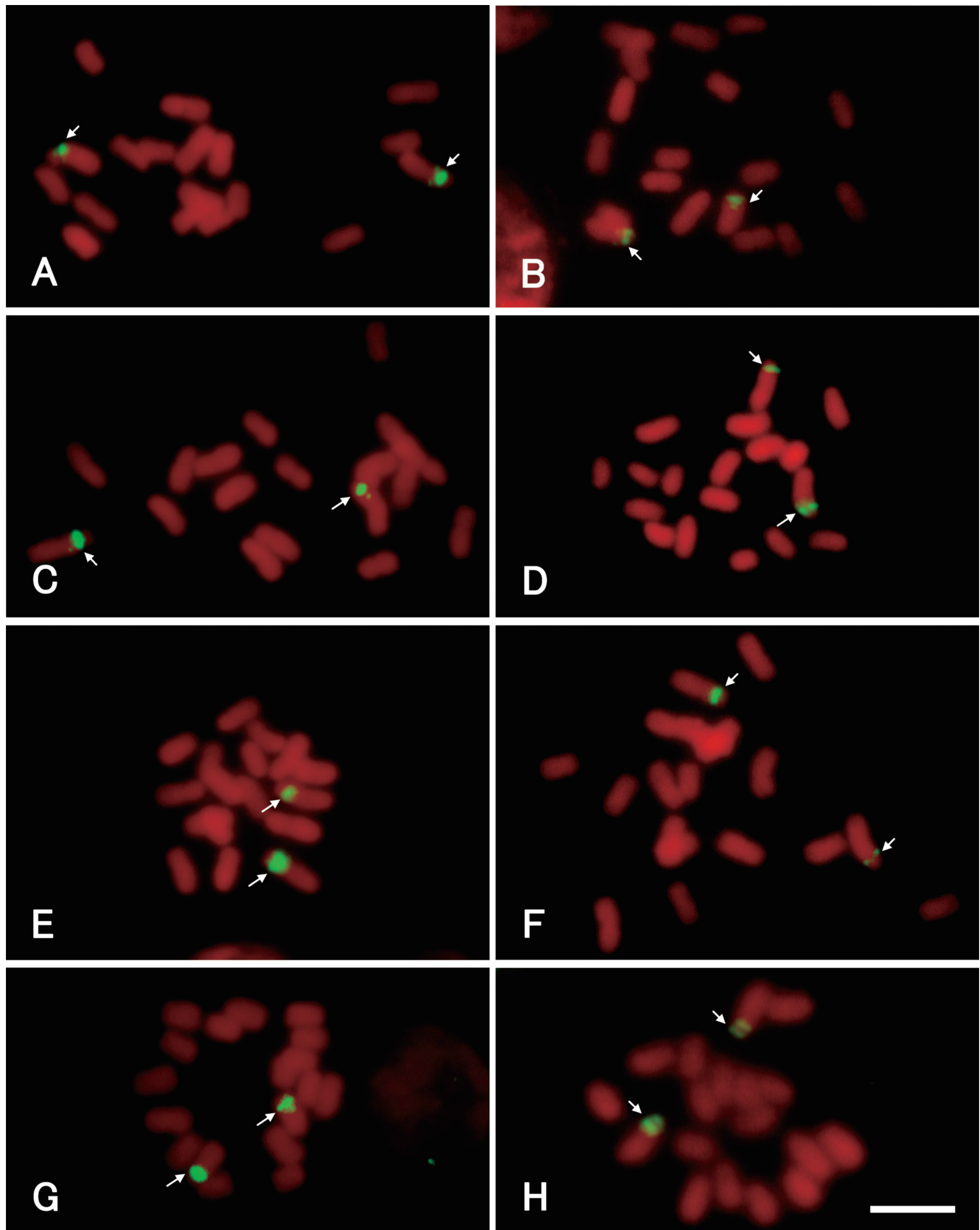

Fig. 3. FISH-detected chromosomes of *Aster miyagii* (= *Erigeron miyagii*) (A–G) and *E. thunbergii* (H). A. Ichi (Amami Is.). B. Honohoshi (Amami Is.). C. Ankyaba (Kakeroma-jima Is.). D. Inunojo-futa (Tokuno-shima Is.). E. Kametoku (Tokuno-shima Is.). F. Oku (Okinawa Is.). G. Ada (Okinawa Is.). H. Mt. Kanfu-yama (Akita). Arrows show satellite. Bar indicates 10 μm.

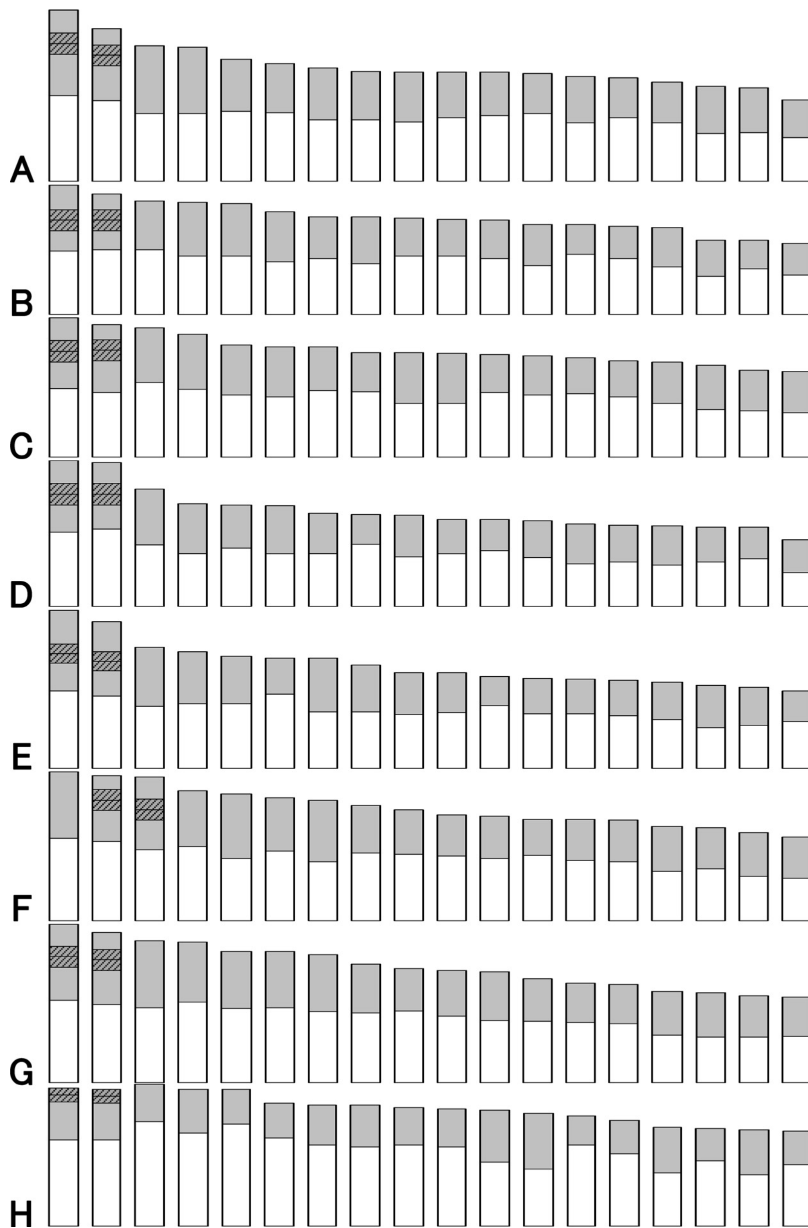

Fig. 4. Ideograms of *Aster miyagii* (=*Erigeron miyagii*) (A–G) and *E. thunbergii* (H). A. Ichi (Amami Is.). B. Honohoshi (Amami Is.). C. Ankyaba (Kakeroma-jima Is.). D. Inunojo-futa (Tokuno-shima Is.). E. Kametoku (Tokuno-shima Is.). F. Oku (Okinawa Is.). G. Ada (Okinawa Is.). H. Mt. Kanfu-yama (Akita). Arrows show satellite. Bar indicates 10 μm. Opened areas show long arms; grayish areas show short arms; hatched areas show rDNA sites.

thunbergii were alighted at the fifth and the sixth (Table 2). The satellites of the Ryukyu plants were two or three times longer than that of *E. thunbergii* (Figs. 2 and 4), and were clearly visible in the standard orcein staining method (Fig. 2).

In FISH observation, both of the Ryukyu plants and *E. thunbergii* commonly had a rDNA

site on a pair of **m**-chromosomes (arrows in Fig 3). However, there was a cytological difference between the Ryukyu plants and *E. thunbergii* in distribution pattern of rDNA site on chromosomes: in the Ryukyu plants, the rDNA site was situated at a secondary constriction between the short arm and the satellite (arrows in Figs. 3A–G and 4A–G); while it was situated at a secondary constriction and a satellite in *E. thunbergii* (arrows in Figs. 3H and 4H).

Taxonomic reconsideration

Aster miyagii was described by Koidzumi (1914) based on a type specimen collected from Okinawa Island. This taxonomic treatment has been supported by Hatusima (1975), Walker (1975), Kitamura (1937), and Ito and Soejima (1995). However, Honda (1931) transferred *A. miyagii* to the genus *Erigeron* and treated the Ryukyu plants as *E. miyagii* without detail explanation disagreeing with Koidzumi (1914) and most taxonomists mentioned above.

Kokubugata *et al*. (2008) previously reported rDNA distribution patterns on somatic chromosomes in nine *Aster* species in Japan, and resulted that the nine commonly had rDNA site situated at a secondary constriction between the short arm and satellite, and they commonly had clearly visible satellites in the standard orcein staining method. The cytological comparisons showed that the distribution pattern of rDNA site and visibility of satellite on somatic chromosomes in the seven Ryukyu plants were similar to those in the nine *Aster* species reported by Kokubugata *et al*. (2008), but were different from those in *E. thunbergii* investigated.

In conclusion, the present comparison based on the distribution pattern of rDNA site and visibility of satellite on somatic chromosome levels that *A. miyagii* must be treated the Ryukyu plants as a species of the genus *Aster* agreeing with Koidzumi (1914), Hatusima (1975), Walker (1975), Kitamura (1937), and Ito and Soejima (1995).

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