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Mapping 45S Ribosomal DNA on Somatic Chromosomes of two *Disporum* Species (Liliaceae) in Japan

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Abstracts. Ribosomal DNA (rDNA) sites on somatic chromosomes of *Disporum smilacinum* and *D. sessile* were compared by the fluorescence *in situ* hybridization (FISH) method. The two species of *Disporum* commonly showed the chromosome number of 2n = 16 and the chromosome complements consisted of four **m** and twelve **sm** chromosomes. The present FISH respectively detected two terminal and two pericentric rDNA sites in *D. smilacinum*; and six terminal rDNA sites in *D. sessile*. The present study implicates that the rDNA sites situated at the pericentric region in *D. smilacinum* must be an useful marker for explaining karyotype evolutions and for examining chromosome genomes in a hybrid between the two *Disporum* species.

Introduction

Disporum is a genus in Liliaceae (Melchior 1964, Tamura 2003) or Colchicaceae (Kubitzki 1998, Angiosperm Phylogeny Group 2003). The genus consists of 22 species and is primarily distributed in the temperate in the eastern Asia (Kawano and Takatsu 2004), and four of the 22 species are recognized in Japan (Satake 1981). Out of the four species, *D. sessile* D. Don and *D. smilacinum* A. Gray are most widely distributed in Japan: *D. smilacinum* occurred from Hokkaido to Kyushu in Japan (Kawano and Takatsu 2004); and *D. sessile* occurred from Hokkaido to the Ryukyu Archipelago in Japan (Kawano and Hori 2004).

Previously some cytological studies have been performed in the two Japanese species, and confirmed that they commonly had the chromosome number of 2n = 16 with the basic chromosome number of x = 8 (Kayano 1960a, 1960b, Chao *et al.* 1963, Chuang and Hsu 1974, Noguchi and Kawano 1974, Utech and Kawano 1974, Tamura *et al.* 1992). Recently a molecular-cytological technique of the fluorescence *in situ* hybridization (FISH) has been applied to various wild plant taxa, and has been confirmed as one of powerful methods to analyze chromosomal genomes. In the present study, the FISH using ribosomal DNA (rDNA) probe is used to compare somatic chromosomes of the two *Disporum* species in Japan.

Materials and Methods

Plant materials

Taxonomic treatment was followed to Satake (1981) for the present cytological study. Plants of *D. smilacinum* (Fig. 1A), and *D. sessile* (Fig. 1B) were collected from Amakubo, Tsukuba, Ibaraki, Japan.

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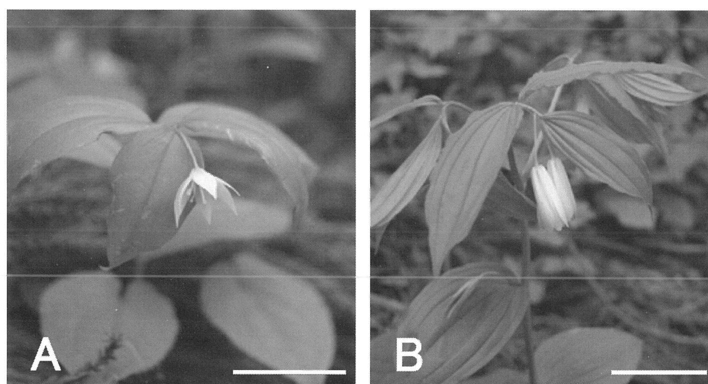

Fig. 1. Plants of two *Disporum* species. Bar indicate 5 cm. **A**. *D. smilacinum*. **B**. *D. sessile*. Voucher specimens are deposited in the herbarium of National Science Museum, Tokyo (TNS).

They were transplanted to the experimental greenhouse of the Tsukuba Botanical Garden, National Science Museum, Tokyo for the present cytological study. Voucher specimens of *D. smilacinum* (*GK 1725*) and *D. sessile* (*GK 8440*) were deposited in the herbarium of National Science Museum, Tokyo (TNS).

PCR amplification and DNA labeling

Total DNA isolated from *D. smilacinum* (*GK 1725*) by the DNeasy Plant Mini Kit (QIAGEN Cat. No. 69104) was used as a template for polymerase chain reaction (PCR) as FISH probes. The PCR amplification of part of the 18S rDNA array being a part of 45S rDNA was performed using primer NS1 (5'-GTA GTC ATA TGC TTG TCT-3') and NS4 (5'-CTT CCG TCA ATT CCT TTA AG-3') designed by White *et al.* (1990). The PCR profile for 30 cycles of DNA amplification was 30 sec at 94°C, 30 sec at 56°C and 1 min at 72°C. The amplified DNA fragments were labeled with digoxigenin- (DIG) dUTP by the Nick Translation Kit following the manufacturer's protocol (Roche, Cat. No. 976776). The labeled probe was dissolved in 50% formamide and 10% dextran sulfate (w/v) in 2 × SSC, and adjusted to a final DNA-concentration of 5 μg/ml. This hybridization mixture was denatured at 75°C for 10 min before being immediately chilled in ice-cold water for 10 min.

FISH using rDNA probes

Root tips were harvested from the plants cultivated, and then pretreated in 2 mM 8-hydroxyquinoline at 20°C for 2h. Fixed root tips were digested in a mixture of 2% cellulase "Onozuka" RS (Yakult) and 1% pectolyase "Y-23" (Seisin) dissolved in distilled water (w/v ; pH 4.5) at 36°C for 20 min, and then washed in distilled water for 10 min. A single digested root tip was placed on a glass slide, the meristematic apex dissected and squashed in 45% acetic acid under a glass cover slip. The cover slip was removed from the slide by the dry-ice method. The air-dried root meristem preparation was treated with 0.1% RNase (w/v) in 2 × SSC at 37°C for 1h. After a wash in 2 × SSC at room temperature for 10 min, it was submerged in 4% paraformaldehyde (w/v) in phosphate-buffered saline (PBS) at room temperature for 5 min, and then rinsed in distilled water at room temperature for 10 min. The preparation was then dehydrated through an ethanol series (75%, 80% and 100% ethanol at room temperature for 3 min each), and dried at 36°C for 30 min. The preparation was covered with 10 μl of the hybridization mixture, and mounted with a silicone-

coated cover slip before being sealed with rubber solution. Then, the preparation was denatured at 80°C for 10 min and placed in a humid chamber at 37°C overnight for DNA hybridization to occur. Following the hybridization, the slide was rinsed in 4 × SSC at 40°C for 10 min twice. The hybridization signals on the chromosomes were detected with 20 μg/ml Anti-digoxigenin-rhodamine, Fab-fragments (Roche, Cat. No. 1207750) in 1% bovine serum albumin dissolved in 4 × SSC at 37°C for 1h. The slide was rinsed in 4 × SSC at room temperature for 10 min twice in a dark box, and received 100 μl of 4,6-diamidino-2-phenylindole (DAPI; Sigma Cat. No. D9542) for counter-staining at 4°C for 30 min before being mounted with a coverslip. The hybridization signals fluoresced red while the non-hybridized region fluoresced blue when visualized using a triple band pass excitation filter (Zeiss, filter set No. 25).

Description of chromosomes

Chromosomes at mitotic metaphase were classified by arm ratio (R = long arm length / short arm length ratio) following Levan *et al.* (1964). Median- (R = 1.0 to 1.7), submedian- (R = 1.8 to 3.0), subterminal- (R = 3.1 to 7.0) and terminal- (R = 7.1 to ∞) centromeric position were respectively designated and symbolized as "**m**", "**sm**", "**st**" and "**t**".

Results and Discussion

Cytological characters

Two species of *Disporum smilacinum* and *D. sessile* investigated commonly showed the chromosome number of 2n = 16 at mitotic metaphase (Fig. 2). The chromosome number of 2n = 16 in *D. smilacinum* was corresponded with the previous reports by Utech and Kawano (1974) and Tamura *et al.* (1992; Fig. 2A); and that of 2n = 16 in *D. sessile* was corresponded with the previous reports by Kayano (1960a, 1960b), Noguchi and Kawano (1974), and Tamura (1992; Fig. 2B). The two species had commonly the chromosome complements consisted of four **m** and twelve **sm** chromosomes (Fig. 3). However, there were positional differences in the ordinal number by their length in **m** and **sm** chromosomes between the two species: four **m** chromosomes were aligned at the 7th, 8th, 11th and 12th in *D. smilacinum* (Fig. 3A), while those were aligned at the 3rd, 4th, 11th and 12th in *D. sessile* (Fig. 3B). These karyotypes in the present

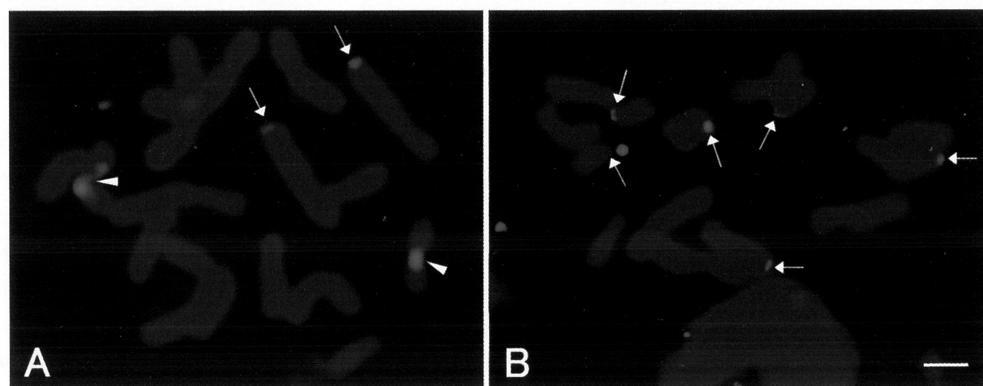

Fig. 2. FISH-detected chromosomes at mitotic metaphase in two *Disporum* species investigated. **A**. *D. smilacinum*. **B**. *D. sessile*. Arrows indicate rDNA sites, arrow heads indicate pericentric rDNA region. Bar indicates 10 μm.

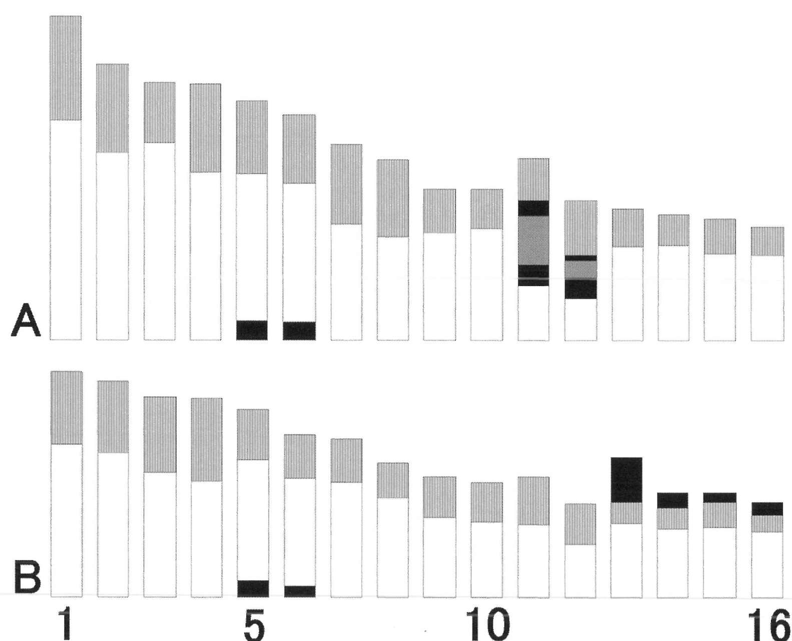

Fig. 3. Ideograms of two *Disporum* species investigated. **A**. *D. smilacinum*. **B**. *D. sessile*. Opened areas indicate long arms; solid areas indicate short arms; grayish areas indicate rDNA sites.

study were similar to those reported by Tamura *et al.* (1992) and Kogi and Suzuki (1988).

In FISH using rDNA probes, the two species of *Disporum* commonly exhibited an rDNA sites on the terminal region at the long arm of the two **sm** chromosomes (Fig. 2, arrows). Moreover, the rDNA sites were detected on stretched-pericentric region of another two **sm** chromosomes in a complement of *D. smilacinum* (totally four sites; Fig. 2A, arrowheads); and those exhibited were detected on the terminal region at the short arm of the four **sm** chromosomes in a complement of *D. sessile* (totally six sites; Fig. 2B, arrows). Kogi and Suzuki (1988) reported that the shortest pair of **m** chromosomes possessed stretched centromere in *D. smilacinum*. The pericentric rDNA sites of *D. smilacinum* must be consistent with the stretched centromere reported by Kogi and Suzuki (1988).

Interspecific variation between D. smilacinum *and* D. sessile

Previously rDNA sites at the pericentric region have been also reported in the other plant taxa, for instance *Microcycas* A. DC. (Zamiaceae; Kokubugata and Kondo 1998), *Oryza* L. (Gramineae; Caperta *et al.* 2002) and *Crepidiastrum*, × *Crepidiastrixeris* and *Paraixeris* (Compositae; Kokubugata and Matsumoto 1999, Saito *et al.* 2003). Bennett (1995) hypothesized that the rDNA position at pericentric region might be related with their origin of centromeric fusion in certain plant taxa. In the present study, there are not enough data to explain karyotype evolution from *D. sessile* to *D. smilacinum*, but it is possible to hypothesize that rDNA position at pericentric region may be an apomorphic character, and *D. smilacinum* may be speciated from a common-ancestral taxon to *D. sessile* having no pericentric rDNA sites with the centromeric fusion. Even if the **m** chromosomes with the pericentric rDNA of *D. similacinum* was created by the centromeric fusion, the same chromosome number of 2n = 16 of two

Disporum species implicates that not merely a simple centromeric fusion but also the other chromosome change, e.g. aneuploidization increasing chromosome number, could be related to speciation between the two species. Further studies including molecular phylogeny analysis and comparing with other *Disporum* species must clarify cytotaxonomic relationships between the two species.

Disporum smilacinum and *D. sessile* are found in similar habitats being shaded and slight moist, e.g., under forest beside of stream or pond. Previously a hybrid (*D.* × *hishiyamanum* Kogi & Suzuki) between *D. smilacinum* and *D. sessile* was found by Kogi and Suzuki (1988). They compared karyotype of a hybrid with those of the two parent species, and not only morphologically but also cytotaxonomically confirmed that *D.* × *hishiyamanum* could be a hybrid, because one of the shortest pair of **sm** chromosome had stretched centromere as heteromorphy (Kogi and Suzuki 1988). The present FISH using rDNA probes must be more useful marker to confirm the hybrid origin of *D.* × *hishiyamanum*.

In Taiwan, four *Disporum* species which is thought to be close to *D. sessile* occur, namely *D. kawakamii* Hayata, *D. nantouense* S.S. Ying, *D. shimadai* Hayata and *D. taiwanense* S.S. Ying. Previously chromosome numbers and karyotypes of *D. kawakamii* and *D. shimadai* were reported by Tamura *et al.* (1992). They mentioned that chromosome number of *D. shimadai* with 2n = 14 was different from that of *D. sessile*; and a pair of chromosomes of *D. kawakamii* was karyomorphologically different from that of *D. sessile* (Tamura *et al.* 1992). The FISH analysis might contribute to analyze the cytotaxonomic relationships between *D. sessile* and *Disporum* members in Taiwan.

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摘　要

チゴユリ属 *Disporum*（ユリ科）の2種、チゴユリ（*D. smilacinum*）、ホウチャクソウ（*D. sessile*）の体細胞分裂中期染色体を 18S ribosomal DNA (rDNA)をプローブとした蛍光 *in situ* hybridization 法で検出して比較した。2種の染色体数は 2n = 16 で、4個の中部動原体型染色体、12個の次中部動原体型染色体から構成されていた。チゴユリでは4個のシグナル部位のうち、2個は挟動原体部位に検出された。一方、ホウチャクソウでは6個のシグナル部位が検出された。本研究で観察を行ったチゴユリとホウチャクソウは、45S rDNA 部位の分布パターンにより明瞭に区別され、45S rDNA プローブを用いた FISH 法がチゴユリ属の染色体進化及びチゴユリとホウチャクソウを両親種とする雑種ホウチャクチゴユリの染色体観察に有効であることが示唆された。

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