

A chromosome study of two centromere differentiating *Drosera* species, *D. arcturi* and *D. regia*

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Abstract — Using sequential fluorescent staining method and fluorescence *in situ* hybridization (FISH) technique, karyomorphological and molecular cytogenetic investigations of two centromere differentiating *Drosera* species, *D. arcturi* and *D. regia* were carried out. *Drosera arcturi* had chromosome number of $2n=58$, while *D. regia* had chromosome number of $2n=34$. Many chromosome bands stained with CMA positive and DAPI positive (CMA+DAPI+) were the most common in both species. CMA positive and DAPI negative (CMA+DAPI-) sites were shown in two chromosomes of both species. Four sites stained with CMA+DAPI-appeared on both sides of the constrictions of two larger chromosomes in *D. arcturi*, while two CMA+DAPI-sites appeared at terminal positions of two chromosomes in *D. regia*. Two-color FISH of 5S and 45S rDNAs showed two regions with major 45S rDNA signals in the both species, and four sites with clear 5S rDNA signals in *D. arcturi*. *Drosera arcturi* did not show any primary constriction in all chromosomes, except for two larger chromosomes. In contrast, *D. regia* had localized-centromeric position or well-differentiated primary constrictions in most metaphase chromosomes.

Key words: 45S rDNA, 5S rDNA, diffuse centromere, *Drosera arcturi*, *Drosera regia*, FISH.

INTRODUCTION

The carnivorous plant family Droseraceae historically includes four genera, *Aldrovanda*, *Dionaea*, *Drosera* and *Drosophyllum* (DIELS 1906; CRONQUIST 1981), although some morphological studies (TAKAHASHI and SOHMA 1982; JUNIPER *et al.* 1989; CONRAN *et al.* 1997) and molecular phylogenetic analyses (ALBERT *et al.* 1992; WILLIAMS *et al.* 1994; RIVADAVIA *et al.* 2003) suggest that *Drosophyllum* does not belong to the Droseraceae. Only the genus *Drosera* comprises more than 100 species distributed mainly in the Southern Hemisphere, with some in the Northern Hemisphere (JUNIPER *et al.* 1989; LOWRIE 1998). The latest extensive phylogenetic analysis of *Dros-*

era makes a new insight that *D. arcturi* Hook. and *D. regia* Stephens cluster more basally than the other *Drosera* species in the molecular trees (RIVADAVIA *et al.* 2003). The monophyly of three monotypic genera and the genus *Drosera* is accepted according to the morphological and molecular data (WILLIAMS *et al.* 1994; FAY *et al.* 1997; MEIMBERG *et al.* 2000; RIVADAVIA *et al.* 2003). The basal relationships in *Drosera*, however, are still ambiguous.

Drosera arcturi is native to New Zealand and southeastern Australia, including Tasmania (ALLAN 1961; LOWRIE 1998). In contrast, *D. regia* occurs in a single mountain valley in South Africa (OBERMEYER 1970), and has traditionally been treated as a different group from the other *Drosera* (WILLIAMS *et al.* 1994).

In spite of limited karyomorphological information of *Drosera*, the previous studies clarified that conspicuous chromosome diversity of the genus was caused by both aneuploidization and polyploidization (e.g. SHEIKH and KONDO 1995;

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HOSHI and KONDO 1998a, 1998b). Especially, to justify the relationship between chromosome number and distribution pattern in *Drosera*, some chromosome investigations were performed in wide spread groups distributed in the Northern Hemisphere (SIMAMURA 1941; WOOD 1955; KONDO 1971; KONDO and SEGAWA 1988; HOSHI and KONDO 1998a) and endemic groups distributed in the Southern Hemisphere (KONDO 1976; KONDO *et al.* 1976; KONDO and OLIVIER 1979; KONDO and LAVARACK 1984; SHEIKH and KONDO 1995; HOSHI and KONDO 1998b). These investigations indicated that the species grown mainly in the Northern Hemisphere formed a polyploid series with the basic chromosome number of $x=10$, while the species grown in the Southern Hemisphere, particularly common in Australian species, formed aneuploid series with the basic chromosome numbers of $x=3, 4, 5, 6, 7, 8, 9, 10, 11$ and 13 (KONDO 1976; KONDO *et al.* 1976; KONDO and OLIVIER 1979; KONDO and LAVARACK 1984; SHEIKH and KONDO 1995; SHEIKH *et al.* 1995). Recent molecular phylogenetic study demonstrated that the Northern Hemisphere group and the Southern Hemisphere group were not closely related to each other (RIVADAVIA *et al.* 2003). Drawing of the molecular phylogenetic tree with basic chromosome number, thus, led us to propose that the chromosome diversity was established early in the evolution of the plant taxa in *Drosera*. Therefore, karyomorphological data of the two basal species, *D. arcturi* and *D. regia* are essential to clarify chromosome differentiation in *Drosera*. Until now, however, *D. arcturi* and *D. regia* do not have any karyomorphological information, except for a somatic chromosome count in each species (BEHRE 1929; KONDO and WHITEHEAD 1971).

In the last two decades, base-specific fluorochromes such as chromomycin A₃ (CMA) and 4',6-diamidino-2-phenylindole (DAPI) have been often used to stable banding methods for chromosome identification in important crops and wild plants (e.g. FUKUI *et al.* 1994; PLÄDER *et al.* 1998; HAYASAKI *et al.* 2001; FUKUSHIMA *et al.* 2008). The fluorescent staining methods have been employed to obtain well-stained chromosomes with reproducibly characteristic bands for *Drosera* (SHEIKH and KONDO 1995; SHEIKH *et al.* 1995; HOSHI and KONDO 1998a, 1998b; HOSHI *et al.* 2008). Moreover, fluorescence *in situ* hybridization (FISH) using tandem DNA repeats such as the 5S and 45S ribosome DNAs (rDNAs) have become universally-applicable markers for chromosome and genome characterizations in many plants (FUKUSHIMA *et al.* 2011).

To offer further insight into chromosome differentiation in *Drosera*, karyomorphological and molecular cytogenetic investigations of *D. arcturi* and *D. regia* were carried out using sequential fluorescent staining method with CMA and DAPI, and cytomolecular technique with FISH.

MATERIALS AND METHODS

Plant materials - Plant accessions of *Drosera arcturi* Hook. from State of Tasmania, Australia, and *D. regia* Stephens from Wellington District, South Africa, used in this study are shown in Table 1. These plant materials were cultured on hormone-free 1/2 Murashige and Skoog basal medium (MURASHIGE and SKOOG 1962) supplemented with 0.35% gellan gum and 3% sucrose for *in vitro* culture, and maintained in the plant culture room of Department of Plant Science, School of Agriculture, Tokai University.

Slide preparation - After root tips were pretreated with 0.2 mM 8-hydroxyquinoline for 2 h at 18°C, they were fixed in 70% ethanol for 1 h on ice, washed with distilled water for 60 min, and then macerated in an enzymatic mixture containing 4% Cellulase Onozuka RS (Yakult Pharmaceutical Industry Co., Ltd., Tokyo, Japan) and 2% Pectolyase Y-23 (Seishin Pharmaceutical Co., Tokyo, Japan) for 1 h at 37°C. After washing with distilled water for 1 h, root tips were placed onto glass slide, and spread with ethanol-acetic acid (3:1). The preparations were air-dried for 24 h at room temperature.

Fluorescent staining with CMA and DAPI - Chromosome preparations were stained with 25 µg/ml chromomycin A₃ (CMA) (Sigma-Aldrich Inc., MO, USA) in McIlvaine's buffer (pH 7.0) containing 5 mM MgSO₄ and 50% glycerol. These chromosome preparations stained with CMA were observed with a BV filter. Then, the slides were used for sequential 4',6-diamidino-2-phenylindole (DAPI) (Nacalai Tesque, Inc., Kyoto, Japan) staining. The slides were destained in 45% acetic acid for 30 min, dehydrated in a series of ethanol, and air-dried for 30 min. They were stained with 1 µg/ml DAPI in McIlvaine's buffer containing 50% glycerol. The chromosomes stained with DAPI were observed with a U filter.

DNA extraction, PCR amplification and labeling - Total genomic DNAs were isolated from young leaves following modified cetyltrimethylammonium bromide (CTAB) method (DOYLE and DOYLE 1990). The samples were ground

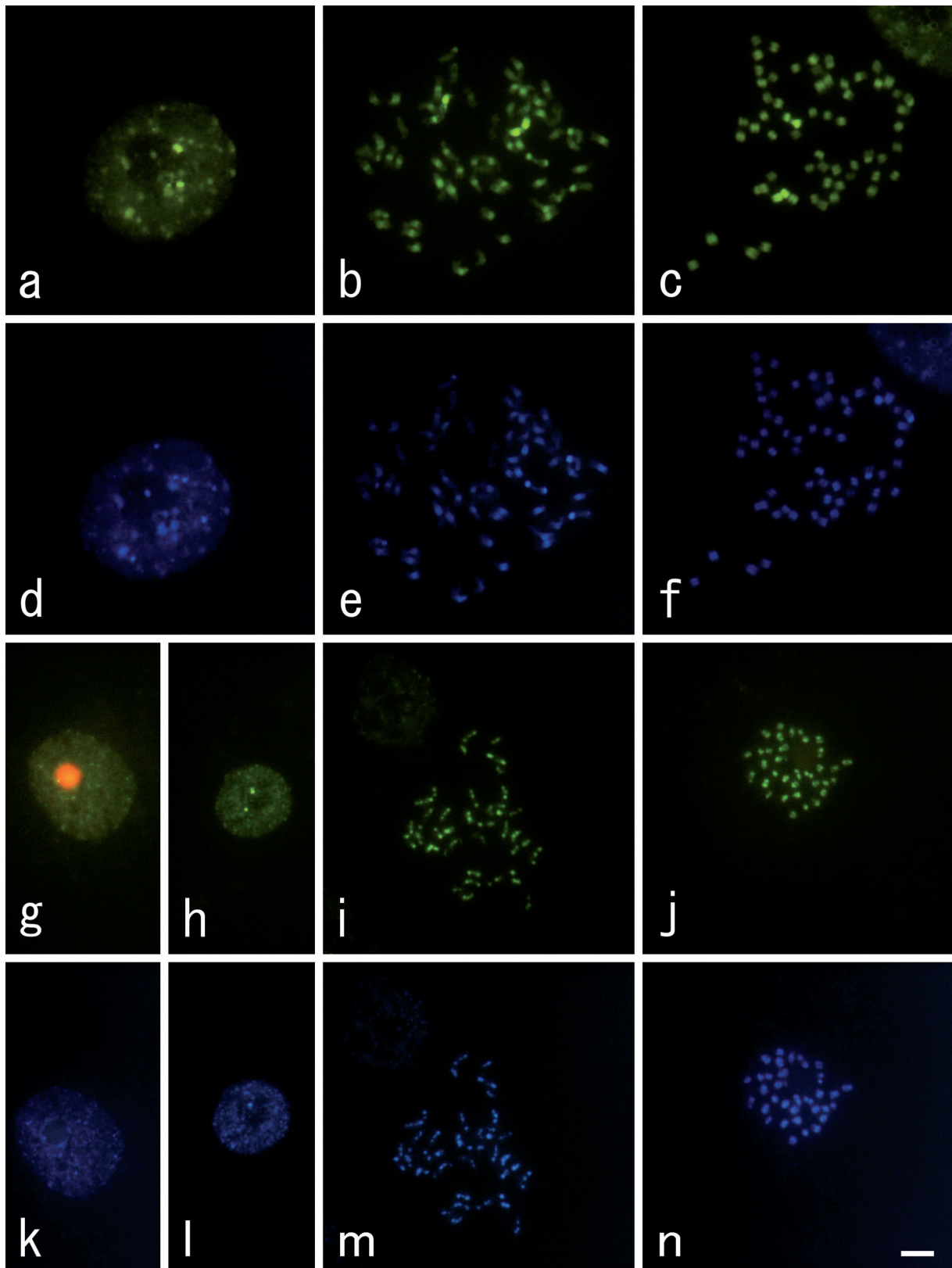


Fig. 1 — Interphase nuclei (a, d, g, h, k and l) and mitotic chromosomes at prophase (b, e, i and m) and metaphase (c, f, j and n) in *D. arcturi* (a-f) and *D. regia* (g-n) stained with CMA (a-c and g-j) and DAPI (d-f and k-n). Bar = 5 μm.

into powder with liquid nitrogen and homogenized in the buffer containing 0.1 M Tris-HCl (pH 8.0), 20 mM EDTA (pH 8.0), 1.4 M NaCl, 2% CTAB and 0.5% mercaptoethanol. The homogenates were extracted twice with an equal volume of chloroform-isoamyl alcohol (24:1) for 10 min each and the DNAs were precipitated with an equal volume of isopropyl alcohol at room temperature. The DNAs were treated with DNase-free RNase A (10 µg/ml) at 37°C for 1 h followed by extractions with chloroform. To track the chromosomal locations of the 5S ribosome DNA (rDNA) and 45S rDNA, the 5S rDNA unit and the 18S rDNA were used as fluorescence *in situ* hybridization (FISH) probes, respectively. With extracted DNA, the 5S rDNA and the 18S rDNA sequences were amplified by polymerase chain reaction (PCR) using the universal primer sets as follows: 5'-CGGTGCATTAATGCTGGTAT-3' and 5'-CCATCA-GAACTCCGCAGTTA-3' for the repeating units in 5S rRNA gene clusters, and 5'-AACCTGGTTGATCCTGCCAGT-3' and 5'-TGATCCTTCTGCAGGTTTCACCTAC-3' for the 18S rRNA coding regions. The cycle profile was an initial denaturation of 94°C (4 min), 35 cycles with 94°C (30 sec), 48°C (30 sec) and 72°C (60 sec), and a final extension step of 72°C for 5 min. The 5S and the 18S rDNA fragments were DIG-labeled or Biotin-labeled by random primed labeling technique (FEINBERG and VOGELSTEIN 1983) using DIG-High Prime (Roche Applied Science, IN, USA) or Biotin-High Prime (Roche Applied Science, IN, USA), respectively.

Fluorescence *in situ* hybridization (FISH) - Chromosome preparations were treated with 250 µg/ml proteinase K (Nacalai Tesque, Inc., Kyoto, Japan) for 45 min at 37°C in a humid chamber. They were treated with 100 µg/ml RNase A (Nippon Gene Co., Ltd., Tokyo, Japan) for 60 min at 37°C in a humid chamber. After dehydration in a graded series of ethanol, a hybridization mixture containing 50% formamide, 10% dextran sulfate and DNA probes were dropped onto the slides. The preparations were sealed, denatured for 3 min at 78°C, and then incubated for 16 h at 37°C. Subsequently, the slides were rinsed in 2×SSC at 42°C for 10 min, 0.2×SSC at 42°C for 10 min, and 2×SSC/0.2% Tween20 at room temperature for 10 min twice. The slides were blocked with 5% bovine serum albumin in 2 × SSC/0.2% Tween20 for 60 min at 37°C. Biotin-labeled and DIG-labeled probes were detected with streptavidin-Alexa Fluor 488 (Invitrogen, CA, USA) and anti-dig-

oxigenin-rhodamine (Roche Applied Science, IN, USA) in 2×SSC, respectively, for 2 h at 37°C in a humid chamber. The slides were washed in 2×SSC/0.2% Tween20 for 10 min twice, and 2×SSC for 10 min twice at room temperature. The preparations were then mounted in Vectashield mounting medium containing 1.5 µg/ml DAPI (Vector Laboratories, Inc., CA, USA). Chromosome images were taken by a digital camera (CoolSNAP: Roper Scientific, Inc., Chiba, Japan) on a microscope (Olympus BX51; Olympus, Tokyo, Japan).

RESULTS

Interphase nuclei of CMA and DAPI staining of *D. arcturi* and *D. regia* are shown in Fig. 1. *Drosera arcturi* had many brightly-stained chromocenters with irregular distribution pattern in the nucleus, while *D. regia* had many small chromocenters with almost homogeneous chromatin-distribution in the nucleus. Especially, the chromocenter shapes of *D. arcturi* were amorphous, sometime took a fibrous structure of densely-packed chromatins (Fig. 1a and d). During the course of the investigation, more than 70% of nuclei in *D. regia* during observation had nucleoli with orange-colored autofluorescence property, when nuclei were irradiated with BV (blue violet) filter cassette (Fig. 1g). In both species, except for CMA positive and DAPI negative fluorescent intensities (CMA+DAPI-) sites, no major differentiation as number and distribution pattern of the chromocenters in nuclei was observed between CMA and DAPI staining (Fig. 1a, d, h and l).

At mitotic prophase and prometaphase, many chromosomes of the two *Drosera* species studied here formed at least one heterochromatin segments (Fig. 1b, e, i and m). In these stages, *D. arcturi* showed delays of chromatin condensations or thread-like structures at the both distal-ends of most chromosomes. In contrast, *D. regia* had distinct heterochromatin segments at the distal regions in most chromosomes. Moreover, *D. regia* possessed major heterochromatin blocks at the inner parts on 14 chromosomes.

Karyomorphological characters at mitotic metaphase of the two species are shown in Figs. 1 and 2 and Table 1. *Drosera arcturi* had chromosome numbers of $2n=58$, total chromosome length of 132 µm, and average chromosome lengths of 2.3 µm. *Drosera regia* had chromosome numbers of $2n=34$, total chromosome length of

60 μm , average chromosome lengths of 1.5 μm . Thus, these observed values indicated that both of the species were quite different from each other. *Drosera arcturi* had larger chromosome number and larger chromosome size than those of *D. regia*, suggesting more decondensed chro-

mosomes or higher DNA amount of *D. arcturi* genome. Additionally, *D. arcturi* was bimodal, while *D. regia* was more or less symmetric and monomodal. The result with different modality suggested that *D. arcturi* was more advantage karyomorphological character. As same as other

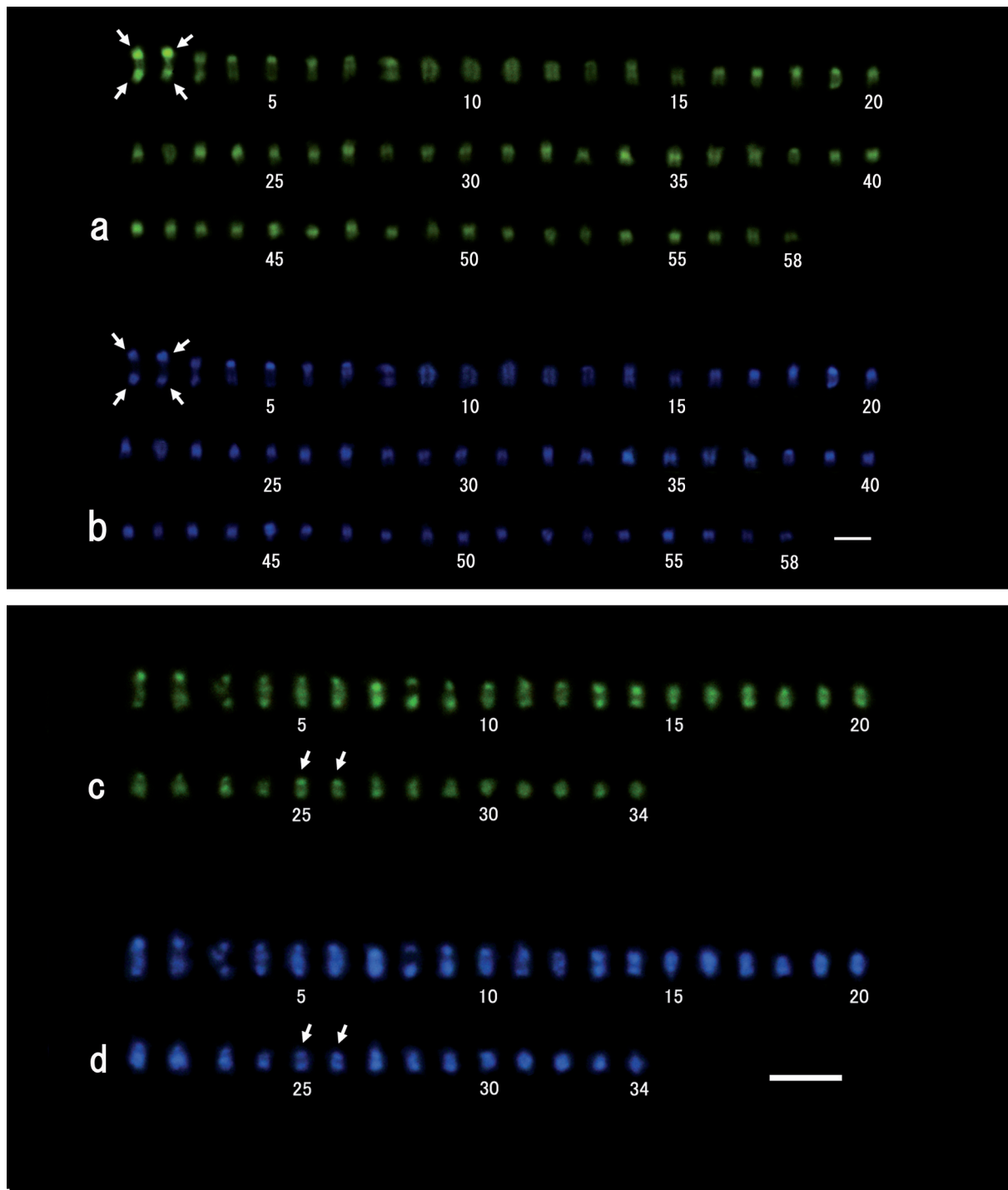


Fig. 2 — Chromosome alignments *D. arcturi* (a and b) and *D. regia* (c and d) stained with CMA (a and c) and DAPI (b and d). Arrows indicate CMA⁺ DAPI⁻ sites. Bar = 5 μm .

Drosera species, *D. arcturi* did not show any primary constriction in all chromosomes, except for two larger chromosomes (Fig. 2a and b). In contrast, *D. regia* had localized-centromeric position or well-differentiated primary constrictions in most metaphase chromosomes (Fig. 2c and d).

The results of fluorescence bands are shown in Figs. 1 and 2, and Table 1. Many chromosome bands stained with CMA positive and DAPI positive (CMA+DAPI+) were the most common at prometaphase and metaphase stages in both species. In *D. arcturi*, the CMA+DAPI+bands were observed in the inner parts of chromosomes. The CMA+ DAPI+bands in *D. regia*, on the other hand, were mainly on the distal regions, and some bands were located on the inner parts of certain chromosomes. In contrast to CMA+DAPI+, just a few of CMA positive and DAPI faint or DAPI negative (CMA+DAPI-) sites were shown in two chromosomes of both species. Four sites stained with CMA+DAPI- appeared on both sides of the constrictions of the two larger chromosomes in *D. arcturi* (Fig. 2a and b, arrows). Whereas, in *D. regia*, two CMA+DAPI-sites appeared at terminal positions of two chromosomes (Fig. 2c and d, arrows).

Figure 3 shows chromosomal positions of 5S and 45S rDNAs by two-color FISH. In mitotic chromosome complements, two regions with major 45S rDNA signals were shown in the both species (Fig. 3a and c), while four sites with clear 5S rDNA signals were only shown in *D. arcturi* (Fig. 3b). Any obvious 5S rDNA signal was not detected in *D. regia* (Fig. 3d).

DISCUSSION

Despite long history of cytological work of the genus *Drosera*, chromosome information of the two phylogenetically-basal species dealt with in this paper are quite limited. Each of the species has one previous report with cytogenetic investigation to figure out somatic chromosome number and metaphase chromosome size (BE-

HRE 1929; KONDO and WHITEHEAD 1971). One of the earliest reports in last century documented the chromosome number of $2n=34$ in *D. regia* (BEHRE 1929), suggesting putative basic chromosome number of $x=17$. The chromosome number of *D. regia* reported here corresponded to the previous one, whereas our chromosome count of *D. arcturi*, by contrast, was different from the previously determined number reported by KONDO and WHITEHEAD (1971). The past record of somatic chromosome number of *D. arcturi* was $2n=20$ (KONDO and WHITEHEAD 1971), while that of our record was $2n=58$. The plant materials of *D. arcturi* in the previous paper and our study were from the Australian state of Queensland and Tasmania, respectively. Although chromosome number information in our result, together with previous work, could not lead to determine a basic chromosome number of *D. arcturi*, we suggested that more than one cytotypes with different chromosome number might exist in this species. An intraspecific differentiation with chromosome number variation may occur independently in each population of *D. arcturi*.

In differential staining methods of CMA and DAPI, the most common fluorescent band type in both species was CMA+DAPI+. The CMA+DAPI+bands were reproducibly observed on metaphase chromosomes, and seemed to be heterochromatic. Chromomycin A₃ binds specifically to guanine regions in helical DNA (WARD *et al.* 1965) or heterochromatin (DEUMLING 1981; DEUMLING and GREILHUBER 1982), revealing the Guanine-Cytosine (GC) rich regions of the genome. In contrast to CMA, DAPI binds specifically to Adenine-Thymine (AT) base pairs in the minor groove of DNA (PORTUGAL and WARING 1988), revealing the AT rich regions of the genome. Our result of the differential fluorescent staining suggests that AT and GC base pairs are equally dispersed in heterochromatin of all chromosomes in these species.

Four sharp signals of 5S rDNA FISH were detected in *D. arcturi*, whereas any obvious 5S rDNA signal was not detected in *D. regia* (Fig. 3),

TABLE 1 — Comparison of karyotypes in *D. arcturi* and *D. regia*.

Species	Accession number	Chromosome number	Total chromosome length (μm) (mean ± SD)	Average chromosome length (μm)	The largest to the smallest chromosome (μm)
<i>D. arcturi</i>	YS-02	$2n = 58$	132.27 ± 32.31	2.28 ± 0.56	4.45-1.12
<i>D. regia</i>	YS-01	$2n = 34$	60.0 ± 10.79	1.49 ± 0.33	2.24-0.92

although 45S rDNA signals were shown in both species. As same as in 45S rDNA, 5S rDNA is an essential for life activity because this gene codes for rRNAs. They are component of ribosome to produces proteins. In higher eukaryotes, the both rDNAs are present in a high copy number and are clustered as tandem repeats at one or more chromosomal sites (ROGERS and BENDICH 1987; ÁLVAREZ and WENDEL 2003). Furthermore, the copy number and chromosomal location of rDNA are rapidly changed in plant genomes (SCHUBERT and WOBUS 1985; RAINA and MUKAI 1999). Therefore, dual rDNA FISH result in present study indicates that the copy number of 5S rDNA unit in *D. regia* may be too low to detect on chromosomes at the cytogenetic level, even though there is the loci on the genome in this species or the plant strains used here. Several evidences of the intraspecific variation sup-

ported that unequal crossing over was the main mechanism involved in size changes of rDNA sites (BUTLER and METZENBERG 1989; KOMMA and ATWOOD 1994). Determination of 5S rDNA locus in *D. regia*, thus, will be possible by using other individuals or strains corrected from different population.

In the both species, the number of fluorescent CMA+DAPI-segments, considered the chromosomal arias with GC-rich DNA sequences, was same to the signal number of 45S rDNA FISH. Since the chromosomes possessed CMA+DAPI-segments and 45S rDNA FISH signals were distinctive markers in especially *D. arcturi*, the locations of CMA+DAPI-sites seemed to correspond to those of 45S rDNA loci in the species, although simultaneous detections of fluorescent staining and FISH did not demonstrate on same chromosomes in our work. Generally, an angiosperm

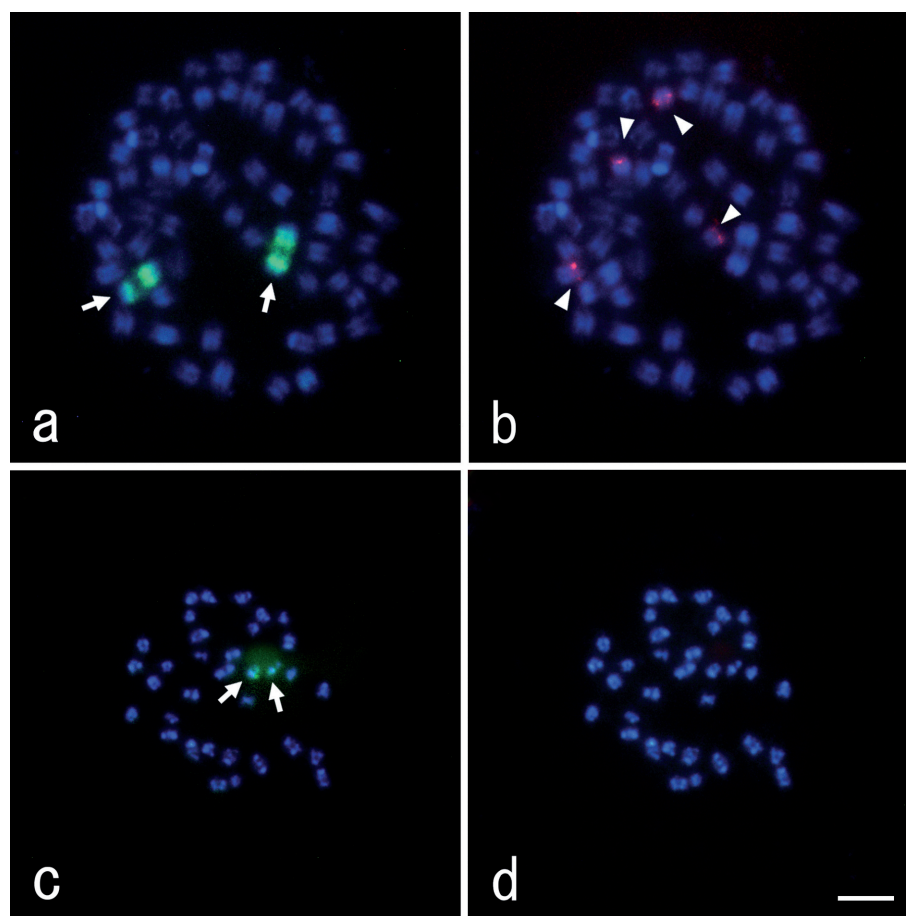


Fig. 3 — Fluorescence *in situ* hybridization of mitotic metaphase of *D. arcturi* (a and b) and *D. regia* (c and d). Green-fluorescent 45S rDNA (a and c) and red-fluorescent 5S rDNA (b and d) signals were detected on DAPI-counterstained chromosomes (blue fluorescence). Arrows and arrowheads indicate 45S and 5S rDNA signals, respectively. Bar = 5 μ m.

chromosome complement in somatic cell has at least two GC-rich segments of 45S rDNA loci or nucleolar organizer regions (NORs) to be detectable as CMA+ DAPI-sites (GALASSO *et al.* 1995). THOMAS *et al.* (2001) clarified the existence of extensive chromosome rearrangements based on the variation in the number and positions of rDNA sites in *Lolium rigidum*. SIROKY *et al.* (2003) reported that 45S rDNA was also involved in the chromosome breakage-fusion-bridge cycle and rearrangements in late generation telomerase-deficient *Arabidopsis*. HUANG *et al.* (2009) made cucumber genome draft by next generation sequencing, and demonstrated that five of the cucumber's seven chromosomes arose from fusions of ten ancestral chromosomes after divergence from melon. Especially, centromeric fusion hypothesis involving 45S rDNA repeats or NOR sites is strongly supported in two larger cucumber chromosomes, according to FISH studies (HUANG *et al.* 2009; HAN *et al.* 2011; HOSHI *et al.* 2011). Somatic chromosomes of melon, by contrast, are about half the size of those of cucumber, and the 45S rDNA repeats of melon are located at the ends of two sat-chromosomes (Chen *et al.* 1999). In our investigation, *D. arcturi* showed major 45S rDNA signals at both sides of the proximal regions of chromosomal gap or constriction parts of a pair of characteristically large chromosomes, which made karyotype bimodal (Fig. 3a). The large chromosomes were double size compared to all other chromosomes of *D. arcturi*. Therefore, we suggest that the large chromosomes are result of the rearrangement with chromosome fusion involving 45S rDNA. In the Droseraceae, section *Drosera* (classified by SEINE and BARTHOLOTT 1994) has been intensively used for cytogenetic research. All of the *Drosera* species of this section possess a basic chromosome number of $x=10$, and 45S rDNAs or NOR regions at the ends of two or four sat-chromosomes. Moreover, including the information of other section in the genus *Drosera*, there has been no previous report that *Drosera* chromosome has NOR site and secondary constriction at middle part of the chromosome. Except for the two larger chromosomes with major 45S rDNA repeats at the center parts, all chromosomes of *D. arcturi* were similar size of those of section *Drosera*. Taking into account previous chromosome number report of $2n=20$ for *D. arcturi* (KONDO and WHITEHEAD 1971), one possibility is that large chromosomes are formed by chromosome jointing at 45S rDNA regions on satellites of two sat-chromosomes of hexaploid ancestral species or cytotype with $2n=60$, pos-

sessing basic chromosome number of $x=10$.

We note that this paper is the first description of centromere-like primary constriction on somatic chromosomes in the genus *Drosera*. The chromosomes of most eukaryotes have 'localized centromere' which presents as a primary constriction. However, 'non-localized centromere' or 'diffuse centromere', which does not show any constriction or localized centromere position on chromosome, are known in some insects such as Heteroptera (HUGHES-SCHRADER and SCHRADER 1961), Homoptera (HUGHES-SCHRADER 1948) and in some plants such as *Luzula* (CASTRO *et al.* 1949), Cyperaceae (HÅKANSSON 1958). In *Drosera*, distinct primary constrictions, localized centromere or clear chromosomal gap between sister chromatid has not been observed in the past, supporting the diffuse centromere hypothesis (KONDO *et al.* 1976; KONDO and SEGAWA 1988). Since the diffused type possesses centromere function dispersed along the whole chromosome length, in theory all fragments of this type of chromosomes are stably transmitted after cell division. The experimental demonstration of the diffuse centromere has done well to test the ability of regular cell division and accurate segregation of each chromosome fragment, which induced by gamma- or x-ray irradiations in *Bombyx* (MURAKAMI and IMAI 1974) and *Luzula* (CASTRO *et al.* 1949). In *Drosera*, gamma-radiated plants propagated by *in vitro* culture of Australian aneuploid species, *D. dichrosepala* and *D. falconeri*, have also shown evidence that typical segregations of fragments and minute chromosomes at mitotic anaphase stages were observed in mitotic cell division (SHEIKH *et al.* 1995, FURUTA and KONDO 1999), confirming the validity of the hypothesis proposed by earlier workers (KONDO *et al.* 1976; KONDO and LAVARACK 1984). According to previous cytogenetic studies, localized centromere and diffused centromere have been seen in the Droseraceae, although centromeric type of chromosome is generally conservative in the plant genus level. In the Doroseraceae, *Dionaea muscipula* and *Drosophyllum lusitanicum* have the localized-centromeric chromosome, while *Aldrovanda vesiculosa* and all *Drosera* species studied previously show the chromosomes with no primary constriction. Here we present one *Drosera* species possessing somatic chromosomes with primary constrictions. Thus, finding of primary constriction in the genus *Drosera* suggests that centromere differentiation can be independently occur not only in the Droseraceae, but also in the genus *Drosera*.

The basal relationships of *Drosera* are still not clear, even with the recent molecular tree made by cluster analysis (RIVADAVIA *et al.* 2003). Dealing with the two phylogenetically basal species of *Drosera*, our chromosome study supports that *D. regia* has more ancestral character than that of *D. arcturi*, because majority of eukaryotes have localized-centromeric chromosomes, and thus non-localized centromeric type is a karyomorphologically derivative. As morphological status for traditionally taxonomic treatment, *D. regia* has some plesiomorphic characters similar to *Dionaea*, such as operculate pollen (TAKAHASHI and SOHMA 1982) and a lack of stipules (WILLIAMS *et al.* 1994). The basal clustering of *D. arcturi*, however, does not take on the same characters of *D. regia*. *Drosera arcturi* shares some morphological character with *D. stenopetala* and *D. uniflora*, such as solitary white flowers on relatively short scapes and reduced or absent stipules (DIELS 1906, 1936; SCHLAUER 1996; LOWRIE 1998), and then these species are thought to be closely related each other, whereas the chloroplast phylogenetic tree shows *D. arcturi* is distantly related to *D. stenopetala* and *D. uniflora*, (RIVADAVIA *et al.* 2003).

Further analyses of morphological and karyomorphological characters with molecular approach are necessary to clarify basal relationship of *Drosera*.

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