Determination of Ploidy Level and Nuclear DNA Content in the Droseraceae by Flow Cytometry

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Summary   2C-values of nine species of the genus *Drosera*, and two monotypic genera *Aldrovanda* and *Dionaea* were estimated to provide an overview of the genome diversity and chromosome differentiation in the Droseraceae. The measured DNA contents of all species used in this study ranged over nine-fold from 2C=0.63 pg in *D. burmannii* to 5.67 pg in *D. anglica*. In the genus *Drosera*, even though the polyploid species were excluded, the difference of the 2C DNA contents among diploid species was still high, ranging 4.3-fold from 0.66 pg in *D. spatulata* to 2.85 pg in *D. intermedia*. In subgenus *Drosera*, especially the polyploidal group, two chromosome types were identified by means of their size; this therefore made it possible to discriminate two groups of the genomes: one group was of a smaller genome size (S genome group) consisting of a total of 10 small-sized chromosomes (x=10s), and the other group was of a larger genome size (M genome group) consisting of a total of 10 middle-sized chromosomes (x=10 m). The Cx-value of the S genome group was less than 0.4 pg (*ca.* 400 Mbp). On the other hand, the Cx-value of the M genome group showed a range of 1.3–1.5 pg (1270–1470 Mbp). Moreover, the 2C DNA content of the hexaploid species *D. tokaiensis* ($2n=6x=20$ m+40s, 2C=3.57 pg), which originated from naturally occurring interspecific hybridization event between *D. rotundifolia* ($2n=2x=20$ m, 2C=2.73 pg) and the tetraploid *D. spatulata* ($2n=4x=40$s, 2C=1.38 pg), was less (86.9%) than the sum of their putative parental species.

Key words   Nuclear DNA content, Genome size, 2C-Value, Droseraceae, *Aldrovanda*, *Dionaea*, *Drosera*.

Evolutionary changes of both genome and chromosome size with species differentiation, particularly in angiosperms, have been a topic of intense interest in plant cytogenetics (*e.g.* Bennett and Leitch 2005, Leitch *et al.* 2007, Leitch and Bennett 2007, Grover and Wendel 2010). Among eukaryotes, angiosperms show an unusually wide range of genome size with an almost 2000-fold variation (Leitch *et al.* 2009). The largest genomic DNA quantity measured is found in *Fritillaria* species (Liliaceae) having around 130 pg (130000 Mbp) (Leitch *et al.* 2009, Zonneveld 2010). These plants possess gigantic chromosomes of 10 to 20 μm (Kamari and Phitos 2006), while the smallest genome is found in two carnivorous plant species of the *Genlisea* (Lentibulariaceae) family, with only 0.06 pg (63 Mbp), possessing tiny chromosomes less than 1 μm (Greilhuber *et al.* 2006). Most angiosperms have small- and middle-sized genomes (*ca.* 1 and 3 pg, respectively) and chromosomes (*ca.* 1 and 3 μm, respectively). The plant groups with wide genome ranges belong to some large families such as Asteraceae, Brassicaceae, Fabaceae, Liliaceae, Orchidaceae and Poaceae. In contrast, the sundew family Droseraceae, which is a representative family of carnivorous plants, is a relatively compact group (about 250 species) showing a high variation in genome size with small to large chromosomes. Having such wide genome range within such a small family readily lends itself to research into mechanisms of genome and chromosome changes associated with plant speciation.

The Droseraceae family contains the worldwide-distributed genus *Drosera* with flypaper-trap leaves, and two monotypic genera *Aldrovanda* and *Dionaea* with snap-trap leaves (Takahashi and Sohma 1982, Juniper *et al.* 1989, Conran *et al.* 1997, Williams *et al.* 1994, Rivadavia *et al.* 2003). *Drosera* is the largest carnivorous group, including aneuploidy, ployploidy and hybrid-derived species with different genome sizes (Hoshi *et al.* 2010). Although the proactive chromosome research of *Drosera* and two other genera in this family has been performed since the early 1900s (*e.g.* Rosenberg 1903, 1904, 1909), little work on nuclear DNA content measurements for genome size determination has focused specifically on this group. For example, only a few pa-

pers have dealt with C-value estimations of *Drosera* species, and with one exception (Rothfels and Heimburger 1968), these have all been published since 2000 (Greilhuber 2008, Shirakawa *et al.* 2011, Veselý *et al.* 2012, Záhumenická *et al.* 2013). It is indeed over 30 years since the first *Drosera* genome size was recorded. Thus, there is still a lack of genome-size (C-value) data and chromosome information in the Droseraceae, although nuclear DNA content is a specific karyological feature that is very useful for systematic purposes and evolutionary considerations (Bennett and Leitch 1995).

In this study, 2C-values of nine *Drosera* species and two monotypic genera *Aldrovanda* and *Dionaea* were estimated to provide an overview of the genome diversity and chromosome differentiation in the Droseraceae.

Materials and methods

Plant materials

Plant strains of species in the Droseraceae used are listed in Table 1.

Plant strains of *Aldrovanda vesiculosa* L. originating from two world populations were used. The temperate populations were from southwest Hungary (HUN strain: Lake Baláta-tó, Somody County, 46°19′N, 17°12′E), while the subtropical one was from southwest Australia (SWA strain: Coastal lake near Esperance, west Australia, 33°48′S, 121°49′E; see Elansary *et al.* 2010).

Taxonomic treatment followed basically Seine and Barthlott (1994). Two diploid strains of *Drosera spatulata* Labill. from New Zealand ('common' and 'SIWC,' see Table 1) were used. Although the flower morphologies of these strains clearly differ from typical Japanese tetraploid *D. spatulata* (Ueda *et al.* 2008), previous papers have customarily described these dip-

loid strains as a group in the *D. spatulata* complex (*e.g.*, Hoshi and Kondo 1998, Shirakawa *et al.* 2012), and hence we conventionally call these strains 'diploid *D. spatulata*.' For additional taxonomical information, the hexaploid species *Drosera tokaiensis* (Komiya & C. Shibata) T. Nakamura & Ueda was currently treated as a distinct species (Nakamura and Ueda 1991), as distinguished from *D. spatulata* or as the 'Kansai Type' of *D. spatulata* (Kondo 1971, Komiya 1978, Nakamura and Ueda 1991, Hoshi *et al.* 1994).

All strains used were cultured *in vitro* in the Laboratory of Plant Environment Science, Department of Plant Science, School of Agriculture, Tokai University.

In vitro *culture*

Aldrovanda strains were initially grown aseptically *in vitro* in a meristem culture of half-strength Gamborg's B5 (1/2 B5) liquid medium (Gamborg *et al.* 1968) (pH 5.5) with $500 \, mg \, mL^{-1}$ KNO_3, microelements, vitamins, and 2.5% sucrose, but without other organic substances (see Adamec and Pásek 2000, 2009), for growing the plants for one to two months. After the initial culture, these strains were then subcultured in 1/2 B5 liquid medium with 2.5% sucrose (pH 5.7 before autoclaving). In contrast, plant strains *in vitro* of *Dionaea muscipula* Ellis and all *Drosera* species were obtained from tissue culture seedlings germinated from aseptic-treated seeds sown on half-strength Murashige-Skoog (1/2 MS) medium (Murashige and Skoog 1962), supplemented with 3.0% sucrose and 0.2% gellan gum (pH 5.7 before autoclaving), and were subcultured in the same medium.

Flow cytometry and genome size estimation

To determine DNA contents, 10 fully developed leaves cultured *in vitro* were separated from the shoot

Table 1. Chromosome numbers and genome sizes of 11 species in the Droseraceae in this study.

Genus	Species[a]	Strain(s)	Chromosome number (2*n*)	Basic chromosome number (*x*)	Ploidy level (*x*)	Chromosome size type[b]	2C (pg)[c]	Amount of 2C (Mbp)	1C (pg)	1C*x* (pg)
Aldrovanda	*A. vesiculosa*	HUN	48	24	2	s	1.24±0.07	1213	0.62	0.62
		SWA	48	24	2	s	1.23±0.12	1203	0.62	0.62
Dionaea	*D. muscipula*	Shira M06-2	32	16	2	m	5.52±0.05	5399	2.76	2.76
Drosera Subgen. *Thelocalyx*										
	D. burmannii	KF-01	20	10	2	s	0.63±0.02	616	0.32	0.32
Subgen. *Drosera*										
	D. anglica	KF-01	40	10	4	m	5.67±0.19	5545	2.84	1.42
	D. capensis	wood×clbe-3	40	10	4	s	0.87±0.03	851	0.44	0.22
	D. communis	KF-03	60	10	6	s+m	3.05±0.07	2983	1.53	0.51
	D. intermedia	Florida	20	10	2	m	2.85±0.02	2787	1.43	1.43
	D. rotundifolia	010816-sera-1	20	10	2	m	2.73±0.15	2670	1.37	1.37
	D. spatulata	P94 common 22′	20	10	2	s	0.66±0.09	645	0.33	0.33
		P67 SIWC 14′	20	10	2	s	0.68±0.08	665	0.34	0.34
		Jpn Ha 4*x*-6	40	10	4	s	1.38±0.18	1350	0.69	0.35
	D. tokaiensis	Jpn Ha 6x-9	60	10	6	s+m	3.57±0.13	3491	1.79	0.60
Subgen. *Ergaleium*										
	D. peltata	Kasai 15-a	32	16	2	s	0.97±0.01	949	0.49	0.49

[a] Taxonomic treatment followed Seine and Barthlott (1994). [b] s: small-sized chromosome (*ca.* 1 μm). [c] 1 pg = 978 Mbp (Doležel *et al.* 2003)

and were chopped in 1.0 mL of nuclei extraction buffer, containing 50 mM Tris–HCl (pH 7.5), 50 mM Na_2SO_3, 140 mM 2-mercaptoethanol, 2 mM $MgCl_2$, 2% (w/v) PVP K-30 and 1% (v/v) Triton X-100. The samples were then filtered through a 48-μm nylon mesh and centrifuged at 12000 rpm for 2 min at room temperature (RT). After centrifuging, the supernatants including isolated nuclei were stained with a 0.2 mL propidium iodide solution (25 μg mL^{-1}) and dissolved again in the same buffer of nuclei extraction. After incubating the supernatant samples for 5 min at RT, the DNA content was measured using a flow cytometer (Guava EasyCyte 12HT microcapillary flow cytometer, Millipore). Five thousand cells acquired at a flow rate of 0.12 μL s^{-1} were used for each flow cytometry measurement and at least three replicates were measured for each strain. *Oryza sativa* L. 'Nipponbare' (2C genome content of 0.91 pg, Uozu *et al.* 1997) as a reference standard was used to estimate genome size in absolute units. *Oryza sativa* 'Hinohikari' was also used for the absolute size estimation. After confirmation of the 2C-value of *Drosera rotundifolia* strain 010816-sera-1 (2C=2.73) with *O. sativa* standards, the genome contents of some species were estimated using this strain. Conversion into base-pair numbers was also calculated using the value of 978 Mbp=1 pg (Doležel *et al.* 2003).

Counting of somatic chromosome number

A conventional method was developed with the protocol of Hoshi and Kondo (1998). Fresh and healthy root tips *in vitro* were collected and pretreated with 0.05% M colchicine at 18°C for 2 h before fixation in 45% acetic acid (2 : 1) at 60°C for 7 s. Root meristems were cut and squashed in 45% acetic acid. After removing the coverslips, the preparations were air-dried at RT. They were then stained with 1 μg mL^{-1} DAPI (4′,6-diamidino-2-phenylindole) in McIlvaine's buffer containing 50% glycerol. The chromosomes stained with DAPI were observed with a U filter for chromosome counting. Chromosome sizes were defined as: small (s) *ca.* 1 μm, and middle (m) *ca.* 2 μm. Additionally, in polyploidal series with *x*=10 of subgenus *Drosera* (classified within series Eurossolis Diels in section Rossolis Planchon in subgenus Rorella DC., according to the taxonomic system of Diels 1906), genome types were defined as: S genome (consisted of 10 s-chromosomes) and M genome (consisted of 10 m-chromosomes).

Results

Our experimental data of nuclear DNA contents and mitotic chromosome numbers of the 14 strains of 11 species in the Droseraceae are presented in Table 1. The measured DNA contents across all species studied were within a nine-fold range, from 2C=0.63 pg in *Drosera burmannii* to 5.67 pg in *Drosera anglica* (Table 1,
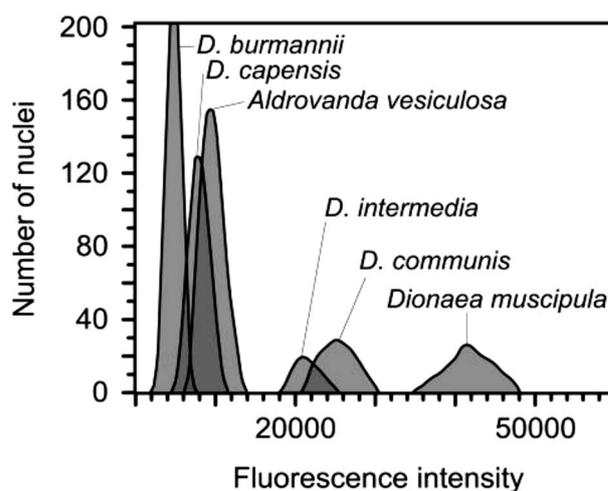

Fig. 1. The relative fluorescence intensity of 2C nuclei isolated from four *Drosera* species, and two monotypic genera *Aldrovanda* and *Dionaea*.
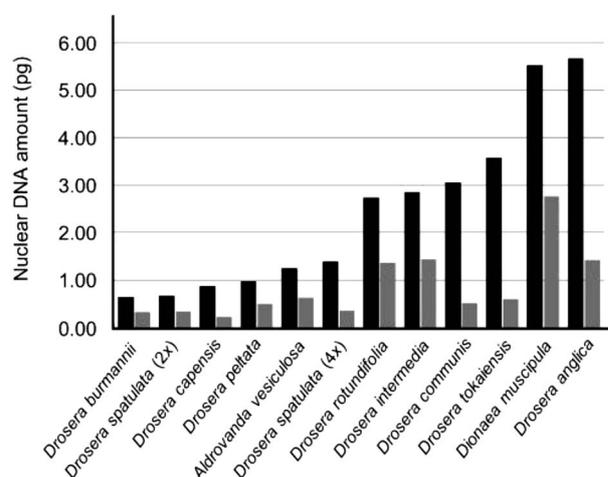

Fig. 2. DNA 2C- (black bars) and 1C*x*- (gray bars) values of 11 species (including two cytotypes of *D. spatulata*) in the Droseraceae, ordered according to size.

Figs. 1, 2). The DNA contents of the monotypic genera *Aldrovanda* and *Dionaea*, which share the snap-trap prey-catching mechanism of snap-traps, fell between the lowest and highest values of *Drosera*, which possess flypaper-traps (Fig. 2). A large difference in genome size was observed between *A. vesiculosa* and *Dionaea muscipula*. *Aldrovanda vesiculosa* had an unexpectedly small 2C value of 1.2 pg, while *Dionaea muscipula* had a 2C value of 5.5 pg (Table 1, Fig. 1), 4.5-times higher. For both of these snap-trap species, which were cytologically considered to be diploid species and showed symmetrical (monomodal) karyotypes with uniformly sized chromosomes, their chromosome features were quite different from each other. *Aldrovanda vesiculosa* had 2*n*=48 with small-sized chromosomes (s-chromosomes: *ca.* 1 μm) without any clear centromere position, while *Dionaea muscipula* had 2*n*=32 with middle-sized metacentric-chromosomes (m-chromosomes: *ca.* 2 μm) with localized centromeres (figure not shown).

The subgenus *Drosera*, especially the worldwide distributed members, was characterized by a high frequency of appearance of polyploidal series of x=10 and exhibited an 8.6-fold range of 2C DNA content (Table 1, Fig. 2). This range was quite high, almost the same as that of the lowest and highest values of all species in the Droseraceae. Even though the polyploid species was excluded, the difference of the 2C DNA contents among diploid species was still high (4.3-fold), and ranged from 0.66 pg in *D. spatulata* to 2.85 pg in *D. intermedia*. The difference could be due to chromosome size, because the size of difference was species-specific, and it was easy to distinguish the two types of chromosomes: s- (*ca.* 1 μm) and m- (*ca.* 2 μm) chromosomes. The two chromosome types were identified by means of their size, which therefore made it possible to discriminate two groups of genomes: one group was of a smaller genome size (S genome group) consisting of a total of 10 s-chromosomes (x=10s), and the other group was of a larger genome size (M genome group) consisting of a total of 10 m-chromosomes (x=10m). The Cx-value of the S genome group was 0.2–0.3 pg (*ca.* 200–300 Mbp/genome, 20–30 pg/chromosome). On the other hand, the Cx-value of the M genome group showed a range of 1.3–1.5 pg (*ca.* 1300–1400 Mbp/genome, 130–140 pg/chromosome). The species possessing S genomes were *D. capensis* ($2n$=$4x$=40s) and the diploid and tetraploid *D. spatulata* ($2n$=$2x$=20s and $2n$=$4x$=40s, respectively), while the species possessing M genomes were *D. anglica* ($2n$=$4x$=40m), *D. intermedia* ($2n$=$2x$=20m), and *D. rotundifolia* ($2n$=$2x$=20m). *D. spatulata* from New Zealand was the only diploid with S genomes, while *D. anglica* was the only tetraploid species with M genomes. Additionally, *D. tokaiensis* and *D. communis* shared both S-geomes and M-genomes (both species are $2n$=$6x$=20s + 40m).

The *D. spatulata* cytotypes with S genomes showed that the 2C DNA contents of the diploid ($2n$=$2x$=20s) and the tetraploid ($2n$=$4x$=40s) were 0.7 pg and 1.4 pg, respectively (Fig. 2). Consequently, the 1C DNA content value of 0.7 pg in the tetraploid cytotype was same as that of the 2C content of the diploid. In contrast, *D. anglica* possessed M genomes ($2n$=$4x$=40m, a tetraploid with genomes contributed from *D. rotundifolia*) showed the 2C-value of 5.67 pg, then the diploid (1C) and the haploid (1Cx) DNA contents were 2.84 pg and 1.42 pg, respectively (Fig. 2). The 1Cx DNA content of *D. anglica*, therefore, was very similar to those of the 1C-values of diploid species with $2n$=$2x$=20m such as *D. intermedia* and *D. rotundifolia* (Fig. 2). Moreover, the 2C DNA content of the hexaploid species *D. tokaiensis* ($2n$=$6x$=20m+40s, 2C=3.57 pg), which originated from a naturally-occurring interspecific hybridization event between *D. rotundifolia* ($2n$=$2x$=20m, 2C=2.73 pg) and the tetraploid *D. spatulata* ($2n$=$4x$=40s, 2C=1.38 pg), was less (86.9%) than the sum of their putative parental
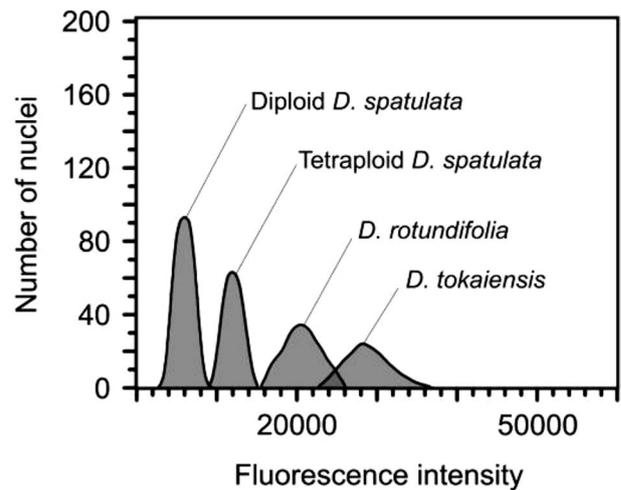

Fig. 3. The relative fluorescence intensity of 2C nuclei isolated from *D. tokaiensis* and its parental species of *D. rotundifolia* and tetraploid *D. spatulata*. Diploid *D. spatulata* showed a half intensity of tetraploid *D. spatulata*.

species (Fig. 3).

The species *D. peltata*, belonging to Subgenus *Ergaleium*, showed relatively small value of 2C nuclear DNA content (0.97 pg) (Table 1, Fig. 2).

Discussion

C-values of 11 species in the Droseraceae have previously been recorded (Rothfels and Heimburger 1968, Greilhuber 2008, Shirakawa *et al.* 2011, Veselý *et al.* 2012, Jensen *et al.* 2015, Tungkajiwangkoon *et al.* 2016). In this study, C-value data of *D. anglica*, *D. burmannii* and *D. communis* were measured for the first time. Except for new C-value records in this study, our result for the remaining species (Table 2) can be compared with the literature data, which correspond to different samples from those analyzed here.

With the genome size determination of *D. rotundifolia*, different C-values have been provided by researchers. Rothfels and Heimburger (1968) employed an original calculated value by using Feulgen cytophotometry, and gave *D. rotundifolia* the first C-value, which was later calibrated in absolute units (2C=1.75 pg) by Bennett and Smith (1976).

A similar value of 1.76 pg was obtained from flow cytometer measuring data by Shirakawa *et al.* (2011). On the other hand, Greilhuber (2008) and Tungkajiwangkoon *et al.* (2016) determined a 2C-value of 2.73 pg for this species, which is the same as our present data. Greilhuber (2008) mentioned that C-values in the Droseraceae published by Rothfels and Heimburger (1968) using an improved Feulgen method are likely underestimated due to tannin error, since secondary metabolites made flow cytometric determinations as well as Feulgen measurements problematic for genome size estimation. Up to the present, previous reports have indicated that

Table 2. Genome sizes of all the species investigated in this study and previously reported in the Droseraceae.

Genus	Species	Ploidy level (x)	2C (pg)	DNA Amount of 2C (Mbp)	1C (pg)	1Cx (pg)	Reference[a]
Aldrovanda	*A. vesiculosa*	2	1.04	1017	0.52	0.52	Shirakawa *et al.* 2011
		2	1.24	1213	0.62	0.62	Present data
		2	1.23	1203	0.62	0.62	Present data
Dionaea	*D. muscipula*	2	1.44	1408	0.72	0.72	Shirakawa *et al.* 2011
		2	6.04	5912	3.02	3.02	Jensen *et al.* 2015[a]
		2	5.52	5399	2.76	2.76	Present data
Drosera Subgen. *Thelocalyx*							
	D. burmannii	2	0.63	616	0.32	0.32	Present data
Subgen. *Arcturi*							
	D. arcturi	2	1.10	1076	0.55	0.55	Shirakawa *et al.* 2011
Subgen. *Rediae*							
	D. regia	2	1.12	1095	0.56	0.56	Shirakawa *et al.* 2011
Subgen. *Drosera*							
	D. anglica	4	5.67	5545	2.84	1.42	Present data
	D. capensis	4	0.60	587	0.30	0.15	Rothfels and Heimburger 1968
		4	0.87	851	0.44	0.22	Present data
	D. communis	6	3.05	2983	1.53	0.51	Present data
	D. intermedia	2	1.90	1858	0.95	0.95	Rothfels and Heimburger 1968
		2	2.85	2787	1.43	1.43	Present data
	D. linearis	2	1.85	1809	0.93	0.93	Rothfels and Heimburger 1968
	D. rotundifolia	2	1.75	1712	0.88	0.88	Rothfels and Heimburger 1968
		2	2.73	2670	1.37	1.37	Greilhuber, 2008
		2	1.76	1721	0.88	0.88	Shirakawa *et al.* 2011
		2	2.73	2670	1.37	1.37	Tungkajiwangkoon *et al.* 2016
		2	2.73	2670	1.37	1.37	Present data
	D. spatulata	4	1.10	1076	0.55	0.28	Shirakawa *et al.* 2011
		4	1.41	1379	0.71	0.35	Tungkajiwangkoon *et al.* 2016
		2	0.66	645	0.33	0.33	Present data
		2	0.68	665	0.34	0.34	Present data
		4	1.38	1350	0.69	0.35	Present data
	D. tokaiensis	6	3.74	3658	1.87	0.62	Tungkajiwangkoon *et al.* 2016
		6	3.57	3491	1.79	0.60	Present data
Subgen. *Phycopsis*							
	D. binata	2	1.25	1223	0.63	0.63	Rothfels and Heimburger 1968
Subgen. *Ergaleium*							
	D. peltata	2	0.80	782	0.40	0.40	Veselý *et al.* 2012
		2	0.97	949	0.49	0.49	Present data
	D. menziesii	—	0.96	939	0.48	—	Veselý *et al.* 2012

[a] Genome size was estimated by DNA sequences

the most reliable data of 2C-value in *D. rotundifolia* seemed to be the 2.73 pg provided by Greilhuber (2008). Because our estimated 2C data of *D. rotundifolia* with the rice reference standard was the same as that of Greilhuber (2008), we believe that present C-values of all plant strains in the Droseraceae are also reliable.

The first C-value records of two monotypic genera, *Aldrovanda* and *Dionaea* were given by Shirakawa *et al.* (2011). Our 2C-value of *A. vesiculosa* (2C=1.2 pg) is similar to that of the previous report (2C=1.0 pg). On the other hand, the value based on current flow cytometric measurement of 2C=5.5 pg in *Dionaea muscipula* is about four times greater than the previously reported value (2C=1.4 pg). The transcriptome and genome size analyses of *Dionaea muscipula* were currently performed (Jensen *et al.* 2015). The authors estimated 2956 Mpb in the haploidal genome of *Dionaea muscipula*. Thus, the 2C-value of *Dionaea muscipula* converted

from the genomic DNA sequences could be estimated to be 6.0 pg. Our 2C-value of *Dionaea musipula* is roughly similar (about 8% lower) to the estimation of Jensen *et al.* (2015) than that of Shirakawa *et al.* (2011).

Among angiosperms in general, DNA content variation greater than two- to threefold is common among congeneric species (Johnston *et al.* 2005). In contrast, the genus *Drosera*, especially the subgenus *Drosera*, shows genomes ranging about tenfold. Although the quite large genome size gap among the three genera in the Droseraceae does not permit any clear insight into the evolutional trend of intergeneric genome size change, the discontinuous size variations detected in the subgenus *Drosera* point to a change of direction of genome and chromosome sizes, including polyploidization events, in a phylogenetic context. The latest molecular phylogenic tree (Rivadavia *et al.* 2003), together with our results, presents a 1Cx genome size of about 0.4 pg

(*ca.* 400 Mbp) at a root position of the subgenus *Drosera*, serving as a starting point to discuss genome and chromosome size evolutions of the polyploidal group in the genus *Drosera*. In the subgenus *Drosera*, 70% of the species are polyploids and many of them are tetraploids with small-sized chromosomes. The diploid *D. sptatulata* is thus an exceptional cytotype, which has the smallest genome size and possesses primitive cytogenetic property with s-chromosomes. Moreover, it is only found in New Zealand or some regions in south Australia, where it seems to be the origin of the Droseraceae. In contrast, the northern hemisphere species including *D. rotundifolia*, which have larger genome sizes and possess middle-sized chromosomes, possess cytogenetically derived characters. Hence, chromosome size and distribution patterns of these species show a strong support for the evidence of an increasing trend of the genome size change in the subgenus *Drosera*.

Two different types of events should be associated with genome-size dynamic changes: a polyploidization caused a double genome size change, while chromosome-size increase (from s- to m-chromosome) caused also the size increase without any doubling of genome set. In fact, m-chromosomes are observed in the four northern hemisphere species: *D. anglica*, *D. intermedia*, *D. rotundifolia* and *D. tokaiensis*. As with the case of the polyploidal group of the subgenus *Drosera*, the genus *Drosera* might hypothetically possess a small genome as a rooted species on the tree; therefore we would also expect that the general evolutionary trend should be towards larger genomes in the genus *Drosera*.

On the other hand, unexpected data of genome size are obtained in hybrid origin species sharing both s- and m-chromosomes. The DNA content of *D. tokaiensis* is proportionally less (86.9%) than the sum of the DNA contents of *D. rotundifolia* and *D. spatulata*, which are cytologically, genetically and morphologically demonstrated as the parental species (Nakamura and Ueda 1991, Hoshi *et al.* 1994, Hoshi *et al.* 2008). It could be estimated that the genome DNA loss of *D. tokaiensis* reached more than 0.5 pg (*ca.* 500 Mbp), equivalent to 1.5 times the total DNA amount of haploid genome of *D. spatulata*. Otherwise, the parental species might have such a huge interspecific variation. Up to the present, however, we have not detected such a wide range of 2C-value within both the parent species. Thus, we suggest that the decreases in DNA content have apparently occurred in amphidiploid hybrid species *D. tokaiensis* which originated from the natural crossing between *D. rotundifolia* and *D. spatulata*. Bennetzen and Kellogg (1997) proposed that plants had a 'one-way ticket to genomic obesity' as a consequence of retroelement accumulation and polyploidy. However, similar reductions are seen in the Brassicaceae. Johnston *et al.* (2005) investigated genome sizes and chromosome number of the 32 species of the Brassicaceae and found that the

genome size of the tetraploid *Arabidopsis suecica* was 4% less than the sum of its diploid ancestors. Narayan (1998) also reported that the genomes of these present-day allopolyploid *Brassica* species average more than 6% less than the expected value. Genome size reduction could occur after creating new species by an allopolyploidization, which is recognized as an important mechanism in plant speciation and genome evolution (Soltis *et al.* 2014). Thus, the non-additive change with DNA loss might be a universal event in allopolyploid species in plants, and seems to be due to a coexistence stress of heterogeneous genomes in a nucleus after allopolyploidal hybridization, which is discussed in *Triticum*, *Gossypium* and *Brassica* (Ozkan *et al.* 2003).

Further genome estimations with individual analyses within parental species are necessary to clarify a degree of intraspecific genome-size variation, and to figure out how non-additive genomic changes occur in hybrid origin species in the Droseraceae.

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