東海丘陵要素トウカイコモウセンゴケとその両親種モウセンゴケとコモウセンゴケの系統学的位置づけ

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Yasunori Ichihashi[1, 2] and Motoyasu Minami[1] : **Phylogenetic positions of Tokai hilly land element, *Drosera tokaiensis* (Droseraceae) and its parental species, *D. rotundifolia* and *D. spatulata***

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The peculiar flora of the Tokai area were classified as 'Tokai hilly land element', which is defined as the local endemic, semi-endemic and relict taxa growing in small peat-less mires (swamp and marsh) in the Circum-Ise Bay area and surrounding areas (Ueda 1989). In the present study, we address *Drosera tokaiensis* (Komiya et C. Shibata) T. Nakamura et K. Ueda, which is a member of the Tokai hilly land element and amphidiploid species between *D. rotundifolia* L. and *D. spatulata* Labill. (Nakamura and Ueda 1991).

The genus *Drosera* (Droseraceae) consists of carnivorous plants, and includes approximately 150 species (Rivadavia et al. 2003). Although some species are distributed around the world, most are distributed throughout the Southern Hemisphere (Marchant and George 1982). The origin of *Drosera* is inferred to be Africa or Australia, and expansion of its distribution to the Northern Hemisphere occurred in different lineages (Rivadavia et al. 2003). In the 1950 s, *D. tokaiensis* was classified as the Kansai type of *D. spatulata* (Komiya 1978). However, it was eventually defined as an independent species and shown to be amphidiploid by cytological and morphological studies (Nakamura and Ueda 1991). It was then proved to be a hybrid species (2 n=60) between *D. rotundifolia* (2 n=20) and *D. spatulata* (2 n=40) (Hoshi et al. 1994). The parental species of *D. tokaiensis* are distributed around the world. *Drosera rotundifolia* is distributed widely in temperate and subarctic regions of the Northern Hemisphere (Crowder et al. 1990). In contrast, *D. spatulata* is distributed in Tasmania, Australia, New Zealand, Indonesia, Malaysia, the Philippines, Taiwan, southern China and Japan (van Steenis 1953 ; Marchant and George 1982 ; Chen et al. 1984). However, the distribution of these two species overlaps only in Japan. *Drosera rotundifolia* is distributed throughout Japan, except for the Ryukyu Islands (Komiya and Shibata 1978). *Drosera spatulata* is distributed within the Ryukyu Islands and the regions of the Pacific Ocean side (Komiya and Shibata 1978 ; Nakamura and Kadono 1993 ; Nakamura 2000 ; Mitsuta and Murata 2001 ; Seno 2002). In contrast, *D. tokaiensis* is distributed only within a specific area in Japan : the Circum-Ise Bay area, Kansai and Shikoku districts, and on the Japan Sea side of central Honshu (Komiya and Shibata 1978 ; Ueda 1989 ; Nakamura and Kadono 1993 ; Sugino 1994 ; Yoshida et al. 1996 ; Nakamura 2000 ; Mitsuta and Murata 2001).

The present distribution of *D. tokaiensis* is thought to have been established by geographic history rather than the present climate (Ueda 1994) ; that is, *D. tokaiensis* has expanded its distribution depending on the geographic history of the Tokai area, so that this species is now distributed in its local endemic area. Therefore, the phylogenetic relationship between *D. tokaiensis* and its parental species could provide a model case to understand the process of establishment of the flora in the Tokai area. Although the phylogenetic relationship is known among the main species of the Droseraceae (Albert et al. 1992 ; Rivadavia et al. 2003), the phylogenetic positions of *D. tokaiensis* and its parental species are unknown. Here, to reveal the phylogenetic positions of *D. tokaiensis* and its parental spe-

cies collected in Japan and Russia, molecular phylogenetic analysis of these *Drosera* species was conducted on chloroplast DNA.

Materials and methods

Plant materials

Four *Drosera* species were collected at a total of 23 localities in Japan and Russia (Table 1). The four Drosera species were : *D. tokaiensis* (10 loc.), *D. rotundifolia* (7 loc.), *D. spatulata* (5 loc.) and *D. anglica* Huds. (1 loc.). *Drosera anglica* is a putative amphidiploid species between *D. rotundifolia* and *D. linearis* Goldie (Wood 1955) and Rivadavia et al. (2003) suggested that *D. rotundifolia* is a maternal parent of *D. anglica* based on the cpDNA sequences. Therefore, we also included *D. anglica* to understand the relationship between *D. tokaiensis* and its parental species in detail. All plants used in this experiment were identified by morphology. In particular, three species (*D. tokaiensis*, *D. rotundifolia* and *D. spatulata*) were identified by the morphological traits of stipules, according to Nakamura and Ueda (1991). Leaves were sampled from plants and kept at −20℃ until direct sequencing. Plant specimens were deposited in the Graduate School of Bioscience and Biotechnology, Chubu University, Japan.

Direct sequencing

To select regions with many DNA sequence variations among *D. tokaiensis* and its parental species, screening was carried out for a total of 31 regions, including 25 chloroplast DNA (cpDNA), four mitochondrial DNA (mtDNA) and two nuclear DNA (nrDNA) regions shown in Table 2. As for the *rbc*L gene region, we designed three internal primers (1 F : 5'−CACGGTATCCAAGTTGAGAG−3', 2R : 5'−ACGCAGAGCTTTGAACCCAA−3' and 3F : 5'−CATCACCTTAGGCTTCGTTG−3') using GENETYX-MAC software, and determined the entire sequence of the region amplified by PCR. After the screening, we selected

Table 1. Sampling sites for the four *Drosera* species

Species	Herbarium specimen number	Locality	Longitude, latitude	Altitude (m)	Date of plants collection
D. tokaiensis	DT−1	Takenami-cho, Ena, Gifu, Japan	35°25' N, 137°21' E	300	Apr. 2005
	DT−2	Tokitsu-cho, Toki, Gifu, Japan	35°20' N, 137°11' E	190	May 2006
	DT−3	Matsumoto-cho, Kasugai, Aichi, Japan	35°16' N, 137°00' E	70	Jul. 2004
	DT−4	Hirako-cho, Owariasahi, Aichi, Japan	35°14' N, 137°01' E	70	Apr. 2006
	DT−5	Meito-ku, Nagoya, Aichi, Japan	35°09' N, 137°01' E	80	Apr. 2006
	DT−6	Agui-cho, Chita-Gun, Aichi, Japan	34°56' N, 136°56' E	50	Apr. 2006
	DT−7	Kosugaya, Tokoname, Aichi, Japan	34°49' N, 136°52' E	20	Jun. 2006
	DT−8	Iwakura-cho, Toyota, Aichi, Japan	35°02' N, 137°11' E	70	Jun. 2005
	DT−9	Shinjo, Kosai, Shizuoka, Japan	34°44' N, 137°29' E	40	Apr. 2006
	DT−10	Seta, Otsu, Shiga, Japan	34°57' N, 135°56' E	170	May 2006
D. rotundifolia	DR−1	Pokrovka-Sakhalinskaya, Sakhalin Oblast, Russia	47°19' N, 142°42' E	10	Aug. 2005
	DR−2	Nakatsugawa, Nakatsugawa, Gifu, Japan	35°26' N, 137°30' E	920	Apr. 2006
	DR−3	Takenami-cho, Ena, Gifu, Japan	35°25' N, 137°21' E	300	Apr. 2005
	DR−4	Tokitsu-cho, Toki, Gifu, Japan	35°20' N, 137°11' E	190	May 2006
	DR−5	Hirako-cho, Owariasahi, Aichi, Japan	35°14' N, 137°01' E	70	Apr. 2006
	DR−6	Kosugaya, Tokoname, Aichi, Japan	34°49' N, 136°52' E	20	Jun. 2006
	DR−7	Nagakusa, Aso, Kumamoto, Japan	32°52' N, 131°03' E	1,130	Apr. 2006
D. spatulata	DS−1	Toyosawa, Fukuroi, Shizuoka, Japan	34°43' N, 137°58' E	90	Apr. 2006
	DS−2	Ono, Hamamatsu, Shizuoka, Japan	34°50' N, 137°46' E	140	Apr. 2006
	DS−3	Kanza, Kosai, Shizuoka, Japan	34°44' N, 137°29' E	50	Apr. 2006
	DS−4	Kosugaya, Tokoname, Aichi, Japan	34°49' N, 136°52' E	20	Jun. 2006
	DS−5	Ano-cho, Tsu, Mie, Japan	34°45' N, 136°28' E	20	Jun. 2005
D. anglica	DA−1	Pokrovka-Sakhalinskaya, Sakhalin Oblast, Russia	47°19' N, 142°42' E	10	Aug. 2005

Table 2. Description of 31 amplified regions of chloroplastDNA, mitochondrialDNA and nuclearDNA

Genes and intergenic regions of chloroplast DNA		
Amplified region	Nucleotide sequence of primer (Forward and Reverse)	References
*atp*F (intron)	5'–TTCATTTGGCTCTCACGCTC–3'	Nishizawa and Watano 2000
	5'–AATGCTGAATCGACGACCTA–3'	
*pet*B (intron)	5'–GTTCTAGTATGAATCTGAGG–3'	Nishizawa and Watano 2000
	5'–ACTTTCATCTCGTACAGCTC–3'	
*pet*B	5'–TGGGGAACTACTCCTTTGAT–3'	Tsumura et al. 1996
	5'–CCCGAAATACCTTGCTTACG–3'	
*pet*D-*rpo*A	5'–GGGCATTGGTGCAACATTAC–3'	Nishizawa and Watano 2000
	5'–CAGCCAAGAAGATCTTATGA–3'	
*psa*A-*trn*S	5'–ACTTCTGGTTCCGGCGAACGAA–3'	Demesure et al. 1995
	5'–AACCACTCGGCCATCTCTCCTA–3'	
*psb*C-*trn*S	5'–GGTCGTGACCAAGAAACCA–3'	Demesure et al. 1995
	5'–CGTTCGAATCCCTCTCTCTC–3'	
*rbc*L	5'–TGTCACCAAAAACAGAGACT–3'	Tsumura et al. 1995
	5'–TTCCATACTTCACAAGCAGC–3'	
*rpo*B	5'–CTAAGGGGTTGTTGTGTAAC–3'	Tsumura et al. 1996
	5'–AATATGCAACGTCAAGCAGT–3'	
rps 16 (intron)	5'–CCCCCTAGAAACGTATAGGA–3'	Nishizawa and Watano 2000
	5'–CGAAGTAATGTCTAAACCCA–3'	
rpl 16 (intron)	5'–GTTTCTTCTCATCCAGCTCC–3'	Nishizawa and Watano 2000
	5'–GAAAGAGTCAATATTCGCCC–3'	
rpl 16-*rpl* 14	5'–AAAGATCTAGATTTCGTAAACAACATAGAGGAAGAA–3'	Nakamura et al. 1997
	5'–ATCTGCAGCATTTAAAAGGGTCTGAGGTTGAATCAT–3'	
*trn*C-*trn*D	5'–CCAGTTCAAATCTGGGTGTC–3'	Demesure et al. 1995
	5'–GGGATTGTAGTTCAATTGGT–3'	
*trn*G (intron)	5'–ATATTGTTTTAGCTCGGTGG–3'	Nishizawa and Watano 2000
	5'–GTTTCATTCGGCTCCTTTAT–3'	
*trn*H-*trn*K (exon 1)	5'–ACGGGAATTGAACCCGCGCA–3'	Demesure et al. 1995
	5'–CCGACTAGTTCCGGGTTCGA–3'	
*trn*K	5'–TGGGTTGCTAACTCAATGG–3'	Yang et al. 2004
	5'–AACTAGTCGGATGGAGTAG–3'	
*trn*K (intron)	5'–GGGTTGCCCGGGACTCGAA–3'	Demesure et al. 1995
	5'–CAACGGTAGAGTACTCGGCTTTTA–3'	
*mat*K	5'–TTATACCCCCTTATCTTTCAGGAAT–3'	Yang et al. 2004
	5'–AACGTTCTAGCACAAGAAAGTCGA–3'	
*trn*M-*rbc*L	5'–TGCTTTCATACGGCGGGAGT–3'	Demesure et al. 1995
	5'–GCTTTAGTCTCTGTTTGTGG–3'	
*trn*S-*trn*fM	5'–GAGAGAGAGGGATTCGAACC–3'	Demesure et al. 1995
	5'–CATAACCTTGAGGTCACGGG–3'	
*trn*S-*trn*T	5'–CGAGGGTTCGAATCCCTCTC–3'	Demesure et al. 1995
	5'–AGAGCATCGCATTTGTAATG–3'	
*trn*T-*trn*L (5'exon)	5'–CATTACAAATGCGATGCTCT–3'	Taberlet et al. 1991
	5'–TCTACCATTTCGCCATATC–3'	
*trn*L (intron)	5'–CGAAATCGGTAGACGCTACG–3'	Taberlet et al. 1991
	5'–GGGGATAGAGGGACTTGAAC–3'	
*trn*L (3'exon) -*trn*F	5'–GGTTCAAGTCCCTCTATCCC–3'	Taberlet et al. 1991
	5'–ATTTGAACTGGTGACACGAG–3'	
*trn*V-*trn*M	5'–TGTAAACGAGTTGCTCTACC–3'	Nishizawa and Watano 2000
	5'–CTAACCACTGAGTTAAGTAG–3'	
*trn*W-*trn*P	5'–GATTTGAACCTACGACATCG–3'	Nishizawa and Watano 2000
	5'–GATGTGGCGCAGCTTGGTAG–3'	
Genes and intergenic regions of mitochondrial DNA		
Amplified region	Nucleotide sequence of primer (Forward and Reverse)	References
*nad*1 (exonB) -*nad*1 (exonC)	5'–GCATTACGATCTGCAGCTCA–3'	Demesure et al. 1995
	5'–GGAGCTCGATTAGTTTCTGC–3'	
*nad*4 (exon1) -*nad*4 (exon2)	5'–CAGTGGGTTGGTCTGGTATG–3'	Demesure et al. 1995
	5'–TCATATGGGCTACTGAGGAG–3'	
*nad*4 (exon2) -*nad*4 (exon 3)	5'–TGTTTCCCGAAGCGACACTT–3'	Demesure et al. 1995
	5'–GGAACACTTTGGGGTGAACA–3'	
rps 14-*cob*	5'–CACGGGTCGCCCTCGTTCCG–3'	Demesure et al. 1995
	5'–GTGTGGAGGATATAGGTTGT–3'	
Genes and intergenic regions of nuclear DNA		
Amplified region	Nucleotide sequence of primer (Forward and Reverse)	References
18 S	5'–CAACCTGGTTGATCCTGCCAGT–3'	Yang et al. 2004
	5'–CTGATCCTTCTGCAGGTTCACCTAC–3'	
18 S-26 S (ITS)	5'–CGTAACAAGGTTTCCGTAGGTGAAC–3'	Zhao et al. 2001
	5'–TTATTGATATGCTTAAACTCAGCGGG–3'	

three variable regions of cpDNA : *rbc*L gene, *rpl* 16-*rpl* 14 spacer and *trn*W-*trn*P spacer. These sequences of the three regions were determined for all samples shown in Table 1. Additionally, *pet*B gene intron sequence was also determined for the sample of *D*. *anglica* (DA−1). All sequences were determined based on direct sequencing of PCR-amplified DNA fragments following the protocols described previously (Minami et al. 2009). Novel sequences have been deposited to DDBJ/EMBL/GenBank under accession numbers AB 298083 to AB 298100.

Phylogenetic analysis

Sequences for homologous regions of the *rbc*L gene of 56 Droseraceae taxa in DDBJ/EMBL/GenBank were obtained (Table 3). Molecular phylogenetic trees based on 1,227 bp of *rbc*L gene sequences of *D*. *tokaiensis* (accession number AB 298088), *D*. *rotundifolia* (AB 298089), *D*. *spatulata* (AB298090) and *D*. *anglica* (AB298091) obtained in this study and 56 Droseraceae taxa were constructed using four different methods : Bayesian, maximum likelihood (ML), maximum parsimony (MP) and neighbor-joining (NJ) methods. For Bayesian, ML and NJ analysis, we applied a GTR+I+G model selected by a hierarchical likelihood ratio test (hLRT) using MrModeltest 2.2 software (Nylander 2004) (Nylander, J. A. A. 2004. MrModeltest 2.1. Program distributed by the author. Evolutionary Biology Centre, Uppsala University, Uppsala.). Bayesian analysis was performed using MrBayes 3.1.2 software (Ronquist and Huelsenbeck 2003). Two runs with four chains of Markov Chain Monte Carlo (MCMC) interactions were performed for 10^6 generations, keeping one tree every 100 generations. The first 25% of the generations were discarded as burn-in, and the remaining trees were used to calculate a 50% majority-rule tree and to determine the posterior probabilities of the individual branches. The standard deviation for the two MCMC interaction runs was below 0.01, indicating convergence. Bootstrap probabilities were calculated using PAUP 4.0 b 10 software (Swofford 2002) (Swofford, D. L. 2002. PAUP* : Phylogenetic Analysis Using Parsimony (* and other methods). Version 4.0 b 10. Sinauer Associates, Sunderland.). Bootstrap probabilities for ML analysis were calculated based on 100 replications of heuristic searches with the subtree pruning regrafting branch-swapping algorithm (Swofford 2002). Bootstrap probabilities for MP analysis were calculated based on 10,000 replications of heuristic searches with the tree bisection

Table 3. Accession numbers of *rbc*L gene sequence of 56 Droseraceae taxa in the DNA Data Bank of Japan

Species	Accession number for nucleotide data	Species	Accession number for nucleotide data	Species	Accession number for nucleotide data
Dionaea muscipula	L 01904	*D. ericksoniae*	AB 072507	*D. pauciflora*	AB 072552
Aldrovanda vesiculosa	AB 072550	*D. esmeraldae*	AB 072526	*D. peltata*	L 01912
Drosera alba	AB 072515	*D. felix*	AB 072527	*D. petiolaris*	L 01913
D. aliciae	AB 072516	*D. gigantea*	L 19528	*D. platypoda*	AB 072547
D. anglica	AB 072517	*D. glanduligera*	AB 072511	*D. prostratoscaposa*	AB 072554
D. arcturi	AB 072512	*D. graminifolia*	AB 072528	*D. pygmaea*	AB 072505
D. biflora	AB 072518	*D. graomogolensis*	AB 072529	*D. regia*	L 01914
D. binata	AB 072922	*D. hamiltonii*	AB 072921	*D. rosulata*	AB 072555
D. brevifolia	AB 072519	*D. hilaris*	AB 072530	*D. rotundifolia*	AB 072538
D. burkeana	AB 07250	*D. hirtella*	AB 072531	*D. scorpioides*	AB 072509
D. burmannii	L 01908	*D. indica*	L 19529	*D. sessilifolia*	AB 072551
D. caduca	AB 072510	*D. kaieteurensis*	AB 072532	*D. spatulata*	L 19530
D. capensis	L 01909	*D. macrantha*	AB 072549	*D. stenopetala*	AB 072539
D. capillaris	AB 072521	*D. madagascariensis*	AB 072533	*D. stolonifera*	L 19531
D. chrysolepis	AB 072522	*D. montana* var. *montana*	AB 072534	*D. trinervia*	AB 072548
D. cistiflora	AB 072523	*D. montana* var. *schwackei*	AB 072535	*D. uniflora*	AB 072540
D. collinsiae	AB 072524	*D. montana* var. *tomentosa*	AB 072536	*D. villosa* var. *ascendens*	AB 072542
D. cuneifolia	AB 072525	*D. natalensis*	AB 072537	*D. villosa* var. *villosa*	AB 072541
D. dichrosepala	L 01910	*D. occidentalis*	AB 072506		

reconstruction branch-swapping algorithm (Swofford 2002). Bootstrap probabilities for NJ analysis were calculated based on 10,000 replications. To compare the inferred phylogenetic relationship with the present species distribution, information on the known distribution of the Droseraceae was obtained from the Carnivorous Plants Database (http://www.omnisterra.com/bot/cp_home.cgi).

Results and discussion

Interspecific variation between *D. tokaiensis* and its parental species, *D. rotundifolia* and *D. spatulata*

We examined a total of 31 regions on cpDNA, mtDNA and nrDNA (Table 2). The DNA regions variable among *D. tokaiensis*, *D. rotundifolia* and *D. spatulata* were screened. As a result, four regions on cpDNA (*pet*B gene intron, *rbc*L gene, *rpl*16-*rpl*14 spacer and *trn*W-*trn*P spacer) showed high DNA sequence variation among *D. tokaiensis*, *D. rotundifolia* and *D. spatulata*. The DNA sequence variations detected in these four regions are shown in Table 4. The portion of the *pet*B gene selected for this study was a partial intron sequence. The sequence of the *pet*B gene intron was found to be from 314 to 319 bp in length in *D. tokaiensis*, *D. rotundifolia* and *D. spatulata*. In this region, eight sites of base substitution and one site of deletion/insertion (5 bp) were detected among the three *Drosera* species. The portion of the *rbc*L gene selected for this study was a partial exon sequence. The sequence of the *rbc*L gene was found to be 1,348 bp in length in *D. tokaiensis*, *D. rotundifolia* and *D. spatulata*. In this region, 17 sites of base substitution were detected among the three *Drosera* species. The *rpl*16-*rpl*14 spacer selected for this study included a partial 3' exon of the *rpl*16 gene and the complete *rpl*16-*rpl*14 spacer region. The sequence of the *rpl*16-*rpl*14 spacer of *D. tokaiensis* and *D. spatulata* included 356 bp of the 3' exon of the *rpl*16 gene between positions 1 and 356, and a 230 bp spacer region between positions 357 and 586. In contrast, for *D. rotundifolia*, the sequence included 359 bp of the 3' exon of the *rpl*16 gene between positions 1 and 359 and 228 bp of the spacer region between positions 360 and 587. In this region, 14 sites of base substitution and one site of deletion/insertion (1 bp) were detected among the three *Drosera* species. The *trn*W-*trn*P spacer selected for this study included partial sequence of *trn*W and *trn*P and the complete spacer region (complementary sequences). The sequence of the *trn*W-*trn*P spacer of *D. tokaiensis* and *D. spatulata* included 42 bp of the 5' region of the *trn*W gene between positions 1 and 42 (from the 3' end region), 179 bp of the spacer region between positions 43 and 221 and 51 bp of the 3' region of the *trn*P gene between positions 222 and 272. The sequence of *D. rotundifolia* included 42 bp of the 5' region of the *trn*W gene between positiosn 1 and 42, 174 bp of the spacer region between positions 43 and 216 and 51 bp of the 3' region of the *trn*P gene between positions 217 and 267. In this region, eight sites of base substitution and one site of deletion/insertion (5 bp) were detected among the three *Drosera* species. To summarize, *D. tokaiensis* had sequence perfectly identical to those of *D. spatulata* in all four regions examined. These results suggested that *D. spatulata* was the maternal parent of *D. tokaiensis* in all samples examined, because chloroplasts are maternally inherited in almost all angiosperms (Sears 1980). Nakano et al. (2004) suggested that the maternal parent of *D. tokaiensis* was *D. spatulata* based on flowering phenology. Our molecular data confirmed this suggestion.

Moreover, we also examined these four regions of *D. anglica*, which has been considered to be of hybrid origin between *D. rotundifolia* and *D. linearis* (Wood 1955). Rivadavia et al. (2003) suggested that *D. rotundifolia* is the maternal parent of *D. anglica* based on the similarity of the *rbc*L nucleotide sequences of *D. rotundifolia* and *D. anglica*. However, alignment between *D. rotundifolia* and *D. anglica* in these four regions showed 36 sites of substitution and one site of deletion/insertion (1 bp) (Table 4). This apparently contradicts the hypothesis that *D. rotundifolia* is the maternal parent of *D. anglica*, proposed by Rivadavia et al. (2003). Intraspecific variation of *D. rotundifolia* may not be the cause of the contradiction, because the *D. rotundifolia* cpDNA sequence was uniform in all samples from one Russian and six Japanese locali-

Table 4. Comparison of DNA sequence variation in four regions of cpDNA among *Drosera tokaiensis*, *D. rotundifolia* and *D. spatulata*, and between *D. rotundifolia* and *D. anglica*

*pet*B [a]	53	56	76	108	196	197	198	199	200	213	219	259	303					Total length (bp)
D. tokaiensis	T	G	C	A	A	A	A	A	A	G	T	T	A					319
D. rotundifolia	C	C	A	C	-	-	-	-	-	T	C	G	G					314
D. spatulata	T	G	C	A	A	A	A	A	A	G	T	T	A					319
*rbc*L [b]	8	36	47	48	250	429	669	714	825	831	880	937	1,055	1,278	1,324	1,325	1,345	Total length (bp)
D. tokaiensis	T	G	A	C	G	A	G	C	T	C	A	A	G	C	A	G	G	1,348
D. rotundifolia	G	T	C	T	T	C	A	G	G	T	C	C	C	G	G	C	T	1,348
D. spatulata	T	G	A	C	G	A	G	C	T	C	A	A	G	C	A	G	G	1,348
rpl 16-*rpl* 14 [b]	38	50	178	186	248	354	411	421	426	436	499	516	518	532	541			Total length (bp)
D. tokaiensis	C	T	T	A	G	T	-	G	A	C	T	T	G	T	C			586
D. rotundifolia	T	C	C	G	C	A	T	A	T	A	A	G	A	C	T			587
D. spatulata	C	T	T	A	G	T	-	G	A	C	T	T	G	T	C			586
*trn*W-*trn*P [b]	55	106	108	110	111	112	113	114	143	173	210	238	248					Total length (bp)
D. tokaiensis	A	G	A	T	G	C	A	T	A	G	A	T	G					272
D. rotundifolia	C	A	C	-	-	-	-	-	G	C	C	C	A					267
D. spatulata	A	G	A	T	G	C	A	T	A	G	A	T	G					272
*pet*B	31	93	98	108	169	207	208	254	290									Total length (bp)
D. rotundifolia	G	T	C	C	G	A	T	G	G									314
D. anglica	T	G	A	A	T	C	G	T	C									314
*rbc*L	403	413	825	1,055	1,278	1,344												Total length (bp)
D. rotundifolia	C	T	G	C	G	A												1,348
D. anglica	A	C	T	G	T	T												1,348
rpl 16-*rpl* 14	50	98	178	186	209	224	281	388	404	518	541							Total length (bp)
D. rotundifolia	C	A	C	G	G	A	G	G	G	A	T							587
D. anglica	T	C	A	A	C	G	A	T	A	G	C							587
*trn*W-*trn*P	106	108	143	146	162	168	179	206	234	244								Total length (bp)
D. rotundifolia	A	C	G	C	A	C	-	C	C	A								267
D. anglica	G	A	A	T	C	T	C	A	T	G								268

Numbers above DNA sequence variations are position from the 5' end of the forward primer. -: deletion. [a]habitats of samples examined were as follows: DT-1 (*D. tokaiensis*), DR-3 (*D. rotundifolia*) and DS-5 (*D. spatulata*). [b]habitats of samples examined were as follows: DT-1 to DT-10 (*D. tokaiensis*), DR-1 to DR-7 (*D. rotundifolia*) and DS-1 to DS-5 (*D. spatulata*).

ties (Table 4). Therefore, these DNA sequence variations presumably accumulated after the origin of *D. anglica* from *D. rotundifolia*, suggesting that *D. tokaiensis* originated from *D. spatulata* much more recently, at least before the divergence of *D. anglica* from *D. rotundifolia*. However, it is also considered that *D. anglica* could not have *D. rotundifolia*, but have *D. linearis* as its maternal species.

The three regions on cpDNA (the *rbc*L gene, *rpl* 16-*rpl* 14 spacer and *trn*W-*trn*P spacer) were sequenced and aligned for all samples of *D. tokaiensis* (10 loc.), *D. rotundifolia* (7 loc.) and *D. spatulata* (5 loc.). All *D. tokaiensis* individuals used in this study were sampled in the Circum-Ise Bay area (Table 1), where this species is mainly distributed. Although the *D. spatulata* obtained in this study was limited to the Circum-Ise Bay area, the *D. rotundifolia* samples obtained in this study covered Japan from north to south (Table 1). However, intraspecific variation of *D. tokaiensis*, *D. rotundifolia* and *D. spatulata* used in this study was not detected in these informative regions on cpDNA (Table 4).

Phylogenetic relationship of *D. tokaiensis* and its parental species

The 1,227 bp of the *rbc*L gene sequence examined in 57 Droseraceae taxa including *D. tokaiensis*, *D. rotundifolia*, *D. spatulata* and *D. anglica* was used for construction of a phylogenetic tree. The Bayesian, ML, MP and NJ tree construction methods generated mostly congruent topologies for *rbc*L dataset. Thus, a Bayesian tree with the support of Bayesian posterior probabilities, ML bootstrap, MP bootstrap, and NJ bootstrap probabilities are shown in Fig. 1. *Drosera rotundifolia* and *D. anglica* from this study were included in the same clades as the *D. rotundifolia* (AB 072538) and *D. anglica* (AB 072517) that were already registered in sequence databases, respectively. While, the *D. spatulata* from this study was not included in the same clade as *D. spatulata* (L 19530) in these databases. It is suggested that *D. spatulata* might include cryptic species, so that further phylogenetic analyses and mating tests are necessary. Because accession L 19530 does not include clear information about the sampling site, however, we cannot do the detailed comparison of these two *D. spatulata* in this study. Our results showed that *D. tokaiensis* is in the same lineage as *D. spatulata*. *Drosera tokaiensis*, *D. rotundifolia*, *D. spatulata* and *D. anglica* were included in a robust clade (1.00 posterior probability, with 76, 84 and 89% bootstrap probability for ML, MP and NJ, respectively). In this clade, *D. spatulata* and *D. tokaiensis* were sister to the group from *D. kaieteurensis* Brumm.-Ding to *D. brevifolia* Pursh, which is moderately supported. Geographical distribution of each species is shown on the right of the *rbc*L tree (Fig. 1). It is apparent that some clades are characterized well by geographical distribution. Therefore, the parental species of *D. tokaiensis* were suggested to be relatively close to each other. Previous study indicated that expansion of distribution to the Northern Hemisphere from the Southern Hemispere occurred in a few different lineages in the genus *Drosera* (Rivadavia et al. 2003). One example was the lineage of *D. rotundifolia*, *D. anglica* and *D. brevifolia*, which are distributed in the Northern Hemisphere at the present time. This lineage inferred to have common ancestor originated at south America (Rivadavia et al. 2003) (Fig. 1), so that *D. rotundifolia* may have expanded to the Northern Hemisphere through the north American continent. On the other hand, our phylogenetic analysis showed that *D. spatulata* was the most basal within the clade from *D. kaieteurensis* to *D. tokaiensis*. Given the present distribution of *D. spatulata*, this result suggested that *D. spatulata* may have expanded to the Northern Hemisphere through southeast Asian islands independently of *D. rotundifolia*. Therefore, *D. rotundifolia* and *D. spatulata* could have reached Japan through independent migration routes from the Southern Hemisphere.

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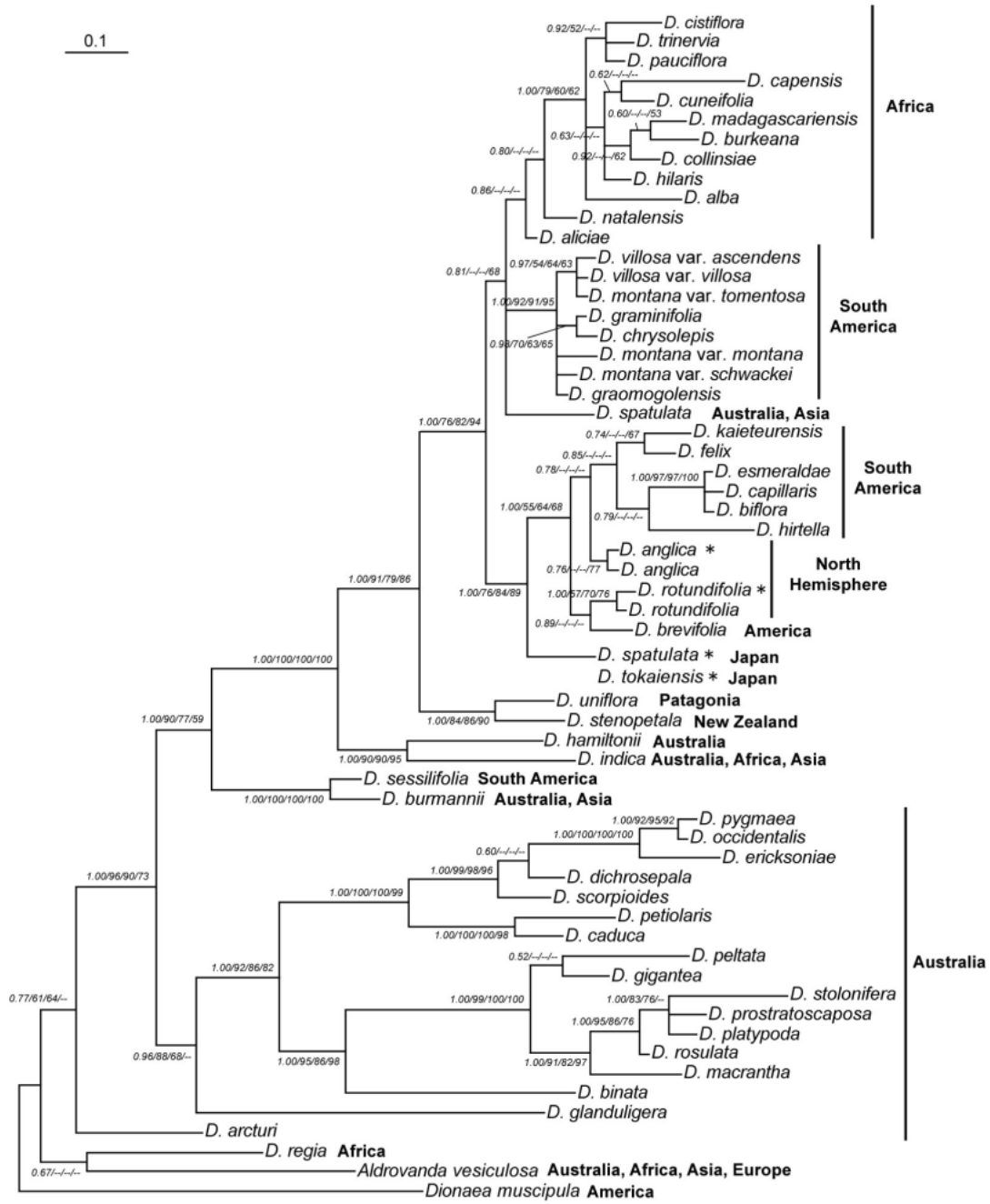

Fig. 1. Bayesian tree based on *rbc*L gene sequences of 57 Droseraceae taxa using information of DDBJ/EMBL/GenBank. Numbers beside branches indicate posterior probabilities from Bayesian analysis (left), bootstrap values from ML analysis (second left), bootstrap values from MP analysis (second right) and bootstrap values from NJ analysis (right). Only Bayesian posterior probabilities greater than 0.5 and bootstrap values greater than 50% are shown. Branch lengths represent nucleotide substitutions per site. Distribution is shown for several clades. Samples obtained in this study are shown by an asterisk. Known distribution of the Droseraceae was obtained from the Carnivorous Plants Database (http : //www.omnisterra.com/bot/cp_home.cgi).

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市橋泰範[1,2]・南　基泰[1]：東海丘陵要素トウカイコモウセンゴケとその両親種モウセンゴケとコモウセンゴケの系統学的位置づけ

東海地方には東海丘陵要素として知られる特徴的な植生がある。この東海丘陵要素のひとつである交雑起源種トウカイコモウセンゴケ（*Drosera tokaiensis*）とその両親種であるモウセンゴケ（*D. rotundifolia*）及びコモウセンゴケ（*D. spatulata*）のモウセンゴケ属内での系統学的位置づけを明らかにするため，葉緑体 DNA を用いた分子系統解析を行った。その結果，4 つの領域（*pet*B 遺伝子イントロン，*rbc*L 遺伝子，*rpl* 16-*rpl* 14 遺伝子間領域及び *trn*W-*trn*P 遺伝子間領域）において，トウカイコモウセンゴケとコモウセンゴケの DNA 配列が一致した。このことは，コモウセンゴケがトウカイコモウセンゴケの唯一の母親種であることを示唆する。また，*rbc*L 遺伝子配列を用いたベイズ法による系統解析を行い，現在のモウセンゴケ属の分布域を反映した系統樹を構築することができた。この系統樹から，トウカイコモウセンゴケの両親種であるモウセンゴケとコモウセンゴケは同じ単一系統群に属し，モウセンゴケ属内で近縁な関係であることが明らかになった。以上から，トウカイコモウセンゴケは遺伝的に近縁な両親種から起源したと考えられる。

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