Development and Characterization of SSR Markers for Aster savatieri (Asteraceae)

Authors: Ishikawa, Naoko, Sakaguchi, Shota, and Ito, Motomi

Source: Applications in Plant Sciences, 4(6)

Published By: Botanical Society of America

URL: https://doi.org/10.3732/apps.1500143

Applications in Plant Sciences 2016 4(6): 1500143

PRIMER NOTE

DEVELOPMENT AND CHARACTERIZATION OF SSR MARKERS FOR *ASTER SAVATIERI* (ASTERACEAE)[1]

NAOKO ISHIKAWA[2,4], SHOTA SAKAGUCHI[3], AND MOTOMI ITO[2]

[2]Laboratory of Plant Evolution and Biodiversity, Department of General Systems Studies, Graduate School of Arts and Sciences, The University of Tokyo, Meguro, Tokyo 153-0041, Japan; and [3]Graduate School of Human and Environmental Studies, Kyoto University, Yoshida-nihonmatsu-cho, Sakyo-ku, Kyoto 606-8501, Japan

- *Premise of the study:* Simple sequence repeat (SSR) markers were developed for *Aster savatieri* (Asteraceae) and the serpentine variety *A. savatieri* var. *pygmaeus* to re-evaluate their taxonomic status.
- *Methods and Results:* Using RNA-Seq data, 22 expressed sequence tag (EST)–SSR markers were developed. Polymorphisms were assessed in *A. savatieri* and in *A. savatieri* var. *pygmaeus*. The average number of alleles ranged from four to 15, and expected heterozygosity ranged from 0.417 to 0.870. Transferability was examined in six representative species of Japanese *Aster* and in *Solidago virgaurea* subsp. *asiatica* var. *asiatica*, a member of the tribe Astereae (Asteraceae); most of the loci were transferable to these examined species.
- *Conclusions:* These markers will be useful for genetic studies of variation in *A. savatieri* and other *Aster* species that occur in Japan.

Key words: *Aster*; Asteraceae; EST-SSR; serpentine plant.

Aster savatieri Makino (Asteraceae) is a perennial herb endemic to Japan (Makino, 1898). It grows in the understory of forests on the islands of Honshu, Shikoku, and Kyushu and is distinguishable from other Japanese congeners by the lack of pappus in its achene and its spring flowering habit (flowering of other species occurs from summer to fall). *Aster savatieri* var. *pygmaeus* Makino was originally recognized as a dwarf form occurring on Mt. Asama, in Mie Prefecture, Honshu, Japan (Makino, 1913). However, the taxonomic treatment of this variety is controversial. Dwarf forms have been reported from other localities in southwestern Honshu and Shikoku, and these were sometimes considered as var. *pygmaeus* (Makino, 1918; Kitamura, 1936). In contrast, Iwatsuki et al. (1995) considered var. *pygmaeus* to be a dwarf form endemic to serpentine areas in Aichi Prefecture, Mie Prefecture (= Mt. Asama), and Shikoku. Ploidy levels may be considered in taxonomic studies because differences in ploidy can affect plant size (Kondorosi et al., 2000; Tsukaya, 2013). Although few studies have examined ploidy levels in *A. savatieri*, a nonserpentine population of var. *pygmaeus* has been reported to be diploid and polymorphisms have often been found in western Honshu populations of *A. savatieri* ($2n = 2x = 18$, $2n = 3x = 27$, $2n = 4x = 36$; Huziwara, 1954; N. Ishikawa, T. Fukuda, S. Sakaguchi, and M. Ito, unpublished data). Therefore, the taxonomic discrimination of *A. savatieri* var. *savatieri* from *A. savatieri* var. *pygmaeus* requires analyses of the genetic relationships among serpentine and nonserpentine populations, as well as among populations with different ploidy levels.

Although eight simple sequence repeat (SSR) markers have been reported for *A. amellus* L. (Mayor and Naciri, 2007), only two polymorphic markers have been successfully amplified by PCR in *A. savatieri* (Y. Morishita and M. Ito, unpublished data). Thus, additional markers are needed to investigate the population divergence in greater detail. We developed 22 polymorphic expressed sequence tag (EST)–SSR markers for *A. savatieri* and evaluated their polymorphisms in, and transferability to, multiple species of *Aster* L. and a related genus.

METHODS AND RESULTS

Total RNA was extracted from *A. savatieri* (Appendix 1; Aichi population) and *A. savatieri* var. *pygmaeus* (Appendix 1; Kochi population) using the Agilent Plant RNA Isolation Mini Kit (Agilent Technologies, Santa Clara, California, USA). Normalized cDNA libraries of shoots and roots of *A. savatieri* were constructed and sequenced using the HiSeq 2000 system (Illumina, San Diego, California, USA). De novo assembly of 37,253,459 cleaned 100-bp reads using Trinity (Grabherr et al., 2011) produced 162,360 contigs (N50: 1678 bp). A cDNA library of *A. savatieri* var. *pygmaeus* inflorescences was constructed and sequenced using the Ion Torrent Personal Genome Machine (Thermo Fisher Scientific, Waltham, Massachusetts, USA). De novo assembly of 8,280,151 cleaned reads (≥400 bp) with CLC Genomics Workbench version 7.5.1 software (CLC bio, Aarhus, Denmark) produced 81,275 contigs (word size 43, bubble size 40, N50: 502 bp).

Microsatellite regions (≥10 dinucleotide repeats, ≥7 trinucleotide repeats) were searched using MSATCOMMANDER (Faircloth, 2008). Primer pairs with an

[1]Manuscript received 21 December 2015; revision accepted 2 March 2016.

The authors thank Dr. Y. Watanabe (Chiba University, Japan), Dr. S. Nakagawa (Kyoto University, Japan), and T. Nishino (Osaka Prefecture University, Japan) for providing plant material. This research was partly supported by the Environment Research and Technology Development Fund (no. 4-1403) from the Ministry of the Environment, Grants-in-Aid for Scientific Research (no. 25291085) from the Japan Society for the Promotion of Science, and the National BioResource Project of the Ministry of Education, Culture, Sports, Science, and Technology.

[4]Author for correspondence: naishikawa@gmail.com

doi:10.3732/apps.1500143

TABLE 1. EST-SSR markers for *Aster savatieri* and *A. savatieri* var. *pygmaeus*.

Locus	Primer sequences (5′–3′)[a]	Repeat motif	Allele size range (bp)	Fluorescent dye	BlastX top hit description	*E*-value	GenBank accession no.
Ast_comp41702_c0_seq1	F: TGTGGAATTGTGAGCGGTGGCCAACACAACGAAACG R: GTTTCTTCTGCTTCTTCATCACCACCC	$(AAC)_7$	329–335	D3	PREDICTED: probable WRKY transcription factor 14 [*Vitis vinifera*]	5E-86	FX983032
Ast_comp53978_c4_seq1	F: CACGACGTTGTAAAACGACCAAAGTGTTGGTTCCGAGACC R: GTTTCTTTCATGGATGTCGCTGAACAAC	$(AAG)_7$	197–203	D2	Polyphenol oxidase [*Taraxacum officinale*]	0.0	FX983033
Ast_comp54189_c0_seq15	F: TGTGGAATTGTGAGCGGATTCACAATGTCCAGCCAGC R: GTTTCTTATGTAGGTCGAAAGGGTGGC	$(ACC)_7$	182	D3	Hypothetical protein PHAVU_006G115800g [*Phaseolus vulgaris*]	4E-12	FX983034
Ast_comp22325_c0_seq1	F: TGTGGAATTGTGAGCGGTGTGAATCGGTTGCATAGCC R: GTTTCTTCACCAGTCCAACACAAAGCC	$(ACC)_7$	136–148	D3	PREDICTED: transcription factor HEC2-like [*Sesamum indicum*]	9E-75	FX983035
Ast_comp37017_c0_seq1	F: CACGACGTTGTAAAACGACTCAGATCCAACAGGCAAGTG R: GTTTCTTAAACCACCATGTCCCTGCC	$(ACC)_7$	166–181	D2	PREDICTED: zinc finger CCCH domain-containing protein 14-like [*Nelumbo nucifera*]	9E-103	FX983036
Ast_comp36481_c0_seq1	F: CTATAGGGCACGCGTGGTGAGGTTCTTGAAGACTGCTGC R: GTTTCTTGCCCTCCCACTTCTACCTTC	$(AGC)_8$	302–332	D4	*S*-adenosylmethionine synthase 2 [*Cucumis melo*]	0.0	FX983037
Ast_comp55030_c0_seq87	F: CACGACGTTGTAAAACGACTCACAAATCAAACCACCGGC R: GTTTCTTCCATGGAAGTATAGAGCGCG	$(CCG)_7$	267–279	D2	PREDICTED: N(6)-adenine-specific DNA methyltransferase 2 isoform X1 [*Nicotiana tomentosiformis*]	2.00E-103	FX983038
Ast_comp41314_c0_seq1	F: CTATAGGGCACGCGTGGTAGACCACCCAGATCTCTTTGTC R: GTTTCTTTCGCACGGTTAGATTCTCAC	$(AAC)_7$	159–210	D4	PREDICTED: uncharacterized protein LOC104099663 [*Nicotiana tomentosiformis*]	2E-37	FX983039
Ast_comp48897_c0_seq1	F: TGTGGAATTGTGAGCGGCACCAACATCATCCTCAGGG R: GTTTCTTAAATTGATGCCCACAACGCC	$(AGC)_7$	190–220	D3	Predicted protein [*Nematostella vectensis*]	0.15	FX983040
Ast_comp51216_c2_seq2	F: CACGACGTTGTAAAACGACCGATTTGGCTCACTGGAACG R: GTTTCTTTCCCACTCGAACCAGGTTTC	$(AAC)_7$	350–374	D2	No significant similarity found.		FX983041
Ast_comp50838_c2_seq2	F: CACGACGTTGTAAAACGACGTGCTGATCCGGTGTTCTTC R: GTTTCTTGCTTCAAAGGGTGGTTCAGG	$(ACC)_7$	204–210	D2	PREDICTED: uncharacterized protein LOC105170415 [*Sesamum indicum*]	0.00002	FX983042
Ast_comp55875_c0_seq1	F: TGTGGAATTGTGAGCGGGCCCGAGCCTTTAATCCAAC R: GTTTCTTTGTTCCACGCTCATCTCTCC	$(CCG)_7$	167–191	D3	PREDICTED: probable prefoldin subunit 5 [*Nicotiana tomentosiformis*]	3E-79	FX983043
Ast_comp53959_c2_seq2	F: CACGACGTTGTAAAACGACGAAGAAGAAGGTGGTGTGGC R: GTTTCTTAGGCGGGTTCTCATTCTCTAC	$(ATC)_7$	155–173	D2	Hypothetical protein PRUPE_ppa002546mg [*Prunus persica*]	2E-122	FX983044
Ast_comp46752_c1_seq1	F: CACGACGTTGTAAAACGACATACTCTCGGGTCTGCACAG R: GTTTCTTGGACTTTCCCTAGGCTTCCG	$(AGG)_7$	181–199	D2	PREDICTED: UPF0503 protein At3g09070, chloroplastic-like [*Solanum tuberosum*]	9E-69	FX983045
Ast_33509	F: CACGACGTTGTAAAACGACTTTCATCATGGGCCTGTCAC R: GTTTCTTTTTGCATCTTCTGGTGGCTC	$(AAG)_{10}$	201–225	D2	Unnamed protein product [*Vitis vinifera*]	9.00E-14	FX983024
Ast_19559	F: CACGACGTTGTAAAACGACACGACGATGAACATAGCAGC R: GTTTCTTTACCACGCTCAGCCAGTATC	$(ATC)_{12}$	220–235	D2	Hypothetical protein MIMGU_mgv1a003121mg [*Erythranthe guttata*]	1.00E-10	FX983025
Ast_44410	F: TGTGGAATTGTGAGCGGAGATCCAGAACCAACCACCG R: GTTTCTTACTTACGGTGTCAACAAACTTG	$(ATC)_{11}$	248–257	D3	No significant similarity found.		FX983026
Ast_65237	F: CTATAGGGCACGCGTGGTAGGGTCGATACTACTGTGGC R: GTTTCTTCATTCACCCAAAGCCCGTAC	$(AC)_{11}$	213–221	D4	No significant similarity found.		FX983027

TABLE 1. Continued.

Locus	Primer sequences (5′–3′)[a]	Repeat motif	Allele size range (bp)	Fluorescent dye	BlastX top hit description	*E*-value	GenBank accession no.
Ast_47436	F: CACGACGTTGTAAAACGACGGTCTTTCTCCCTCCTTTGAAG R: GTTTCTTGGTATCTCCTGTTTCTGCGG	$(AAG)_{11}$	131–185	D2	PREDICTED: heat shock cognate 71 kDa protein-like [*Amphimedon queenslandica*]	3.00E-04	FX983028
Ast_34501	F: CACGACGTTGTAAAACGACGGGTGCATCAGAATCCGTAC R: GTTTCTTTGGCGGTAATCTAGGTGTCC	$(AAC)_{10}$	292–307	D2	PREDICTED: uncharacterized protein LOC104095266 [*Nicotiana tomentosiformis*]	0.23	FX983029
Ast_59032	F: CACGACGTTGTAAAACGACTTGTTAATGGCGGCATCTC R: GTTTCTTGCGACGACTGCAGAATTGG	$(AGC)_{11}$	247–253	D2	No significant similarity found.		FX983030
Ast_26109	F: CACGACGTTGTAAAACGACCGTGAGTCAAACCCGAGAAC R: GTTTCTTCGCCTTCAAATCCTCCAACTC	$(AC)_{11}$	462–498	D2	PREDICTED: interactor of constitutive active ROPs 2 [*Vitis vinifera*]	3.00E-146	FX983031

[a]Forward and reverse primer sequence (with tag sequence).

optimal annealing temperature of 60 ± 2°C, a GC content of 30–70%, and a product size range of 100–500 bp were generated by Primer3 (Rozen and Skaletsky, 1999). We obtained 118 and 284 primer sets for *A. savatieri* and *A. savatieri* var. *pygmaeus*, respectively. Each of the 48 primer sets was selected from the two taxa based on the repeat numbers. For all loci, the forward primer was synthesized with one of three different M13 sequences (5′-CACGACGTTGTAAAACGAC-3′, 5′-TGTGGAATTGTGAGCGG-3′, or 5′-CTATAGGGCACGCGTGGT-3′) and the reverse primer was tagged with a PIG-tail (5′-GTTTCTT-3′). A similarity search of each contig against the National Center for Biotechnology Information (NCBI) nr database was conducted using the BLASTX algorithm. PCR reactions were performed using a QIAGEN Multiplex PCR Kit (QIAGEN, Hilden, Germany) in a 10-μL volume containing 5–10 ng DNA, 5 μL 2× Multiplex PCR Master Mix, 0.01 μM forward primer, 0.2 μM reverse primer, and 0.1 μM fluorescently labeled M13 primer. The PCR protocol was as follows: 95°C for 3 min; followed by 35 cycles of 95°C for 30 s, 57°C for 3 min, 68°C for 1 min; and a 20-min extension at 68°C. The PCR product was loaded with DNA Size Standard 600 (Beckman Coulter, Brea, California, USA) onto a GenomeLab GeXP Genetic Analysis System (Beckman Coulter), and fragment size was determined with CEQ fragment analysis software (Beckman Coulter).

For PCR amplification trials, we used two individuals from each of the two *A. savatieri* populations (Appendix 1; Aichi and Nagano populations) and the two *A. savatieri* var. *pygmaeus* populations (Appendix 1; Mie and Kochi populations). For the 22 primer pairs that showed clear peaks (Table 1), 24 individuals from each population (Aichi, Kyoto, and Mie) were evaluated for polymorphisms. All of the 24 individuals were considered to be diploid because no more than two alleles were found in any loci. We also confirmed the diploid status of these samples by microscopic chromosome counting of one individual from the Mie population, which showed that it was diploid ($2n = 2x = 18$). Flow cytometer (BD Biosciences, Franklin Lakes, New Jersey, USA) analyses of 10 individuals from each population revealed that all were diploid. Summary statistics were generated using GenAlEx 6.5 software (Peakall and Smouse, 2012), i.e., number of alleles per locus (A), expected heterozygosity (H_e), and observed heterozygosity (H_o). The significance of Hardy–Weinberg equilibrium and genotypic equilibrium was tested by 1000 randomizations with adjustment of the resulting P values through the Bonferroni correction using FSTAT 2.9.3 software (Goudet, 1995).

Twenty-two primer pairs were polymorphic; A ranged from four to 15 alleles, while H_e and H_o ranged from 0.417 to 0.870 and 0.174 to 0.690, respectively (Table 2). No significant departures from Hardy–Weinberg equilibrium were detected for any of the populations or loci after correcting for multiple tests (nominal level of significance: 0.05). No significant genotypic equilibrium was detected for any pair of loci. We examined the transferability of these primers to six representative Japanese *Aster* species and *Solidago virgaurea* L. subsp. *asiatica* Kitam. ex H. Hara var. *asiatica* Nakai ex H. Hara, a member of the tribe Astereae (Asteraceae). The *Aster* species were selected to cover the main lineages of Japanese *Aster* (Table 3; Appendix 1; Ito et al., 1998). The *Solidago* L. species was included to assess the general applicability of the primers. The PCR protocol was as follows: 95°C for 3 min; 40 cycles of 95°C for 30 s, 57.5°C for 3 min (with reductions of 0.1°C per cycle), 68°C for 1 min; with a 20-min extension at 68°C. Of the 22 EST-SSR primer pairs tested, 14–20 and 16 loci were successfully amplified in the six *Aster* species and *S. virgaurea* subsp. *asiatica* var. *asiatica*, respectively (Table 3, Appendix 1). Thus, most of the loci were transferable to the examined species.

CONCLUSIONS

The 22 EST-SSR markers developed were substantially polymorphic within and between populations. Thus, these markers will be useful for investigations of intraspecific relationships among *A. savatieri* var. *savatieri* and *A. savatieri* var. *pygmaeus* populations occurring at serpentine and nonserpentine sites. Transferability analyses were conducted with six representative species of Japanese *Aster* and *S. virgaurea* subsp. *asiatica* var. *asiatica*, a member of the tribe Astereae (Asteraceae). Of the 32 Japanese *Aster* species, 20 are endemic to Japan and 11 are regarded as endangered (Iwatsuki et al., 1995; Ministry of the Environment, 2012). Thus, our markers should also prove useful in conservation-directed investigations of genetic variation in endangered *Aster* species that occur in Japan.

TABLE 2. Characteristics of the 22 polymorphic EST-SSR markers for *Aster savatieri* and *A. savatieri* var. *pygmaeus*.

	A. savatieri						*A. savatieri* var. *pygmaeus*					
	Aichi population ($N = 24$)			Kyoto population ($N = 24$)			(Mie population) ($N = 24$)			All ($N = 72$)		
Locus	A	H_e	H_o	A	H_e	H_o	A	H_e	H_o	A	H_e	H_o
Ast_comp41702_c0_seq1	2	0.080	0.083	6	0.800	0.750	3	0.119	0.125	6	0.707	0.319
Ast_comp53978_c4_seq1	3	0.385	0.417	4	0.490	0.524	2	0.478	0.542	4	0.633	0.493
Ast_comp54189_c0_seq15	1	0.000	0.000	4	0.580	0.542	2	0.080	0.000	4	0.417	0.181
Ast_comp22325_c0_seq1	2	0.469	0.500	5	0.468	0.458	2	0.153	0.167	6	0.607	0.375
Ast_comp37017_c0_seq1	5	0.659	0.708	5	0.493	0.375	3	0.559	0.458	7	0.758	0.514
Ast_comp36481_c0_seq1	3	0.612	0.500	6	0.700	0.583	3	0.405	0.458	7	0.741	0.514
Ast_comp55030_c0_seq87	2	0.041	0.042	7	0.740	0.714	2	0.041	0.042	8	0.679	0.246
Ast_comp41314_c0_seq1	5	0.654	0.458	10	0.857	0.625	6	0.655	0.667	13	0.858	0.583
Ast_comp48897_c0_seq1	5	0.722	0.333	11	0.828	0.500	4	0.650	0.167	13	0.870	0.333
Ast_comp51216_c2_seq2	2	0.478	0.542	10	0.774	0.429	3	0.569	0.583	10	0.658	0.522
Ast_comp50838_c2_seq2	2	0.444	0.333	8	0.741	0.783	2	0.117	0.125	11	0.666	0.408
Ast_comp55875_c0_seq1	2	0.353	0.375	6	0.715	0.792	3	0.559	0.417	11	0.848	0.528
Ast_comp53959_c2_seq2	3	0.226	0.167	5	0.642	0.304	3	0.471	0.083	8	0.789	0.183
Ast_comp46752_c1_seq1	4	0.609	0.333	4	0.560	0.522	4	0.617	0.458	9	0.837	0.437
Ast_33509	7	0.798	0.833	10	0.811	0.750	4	0.556	0.458	13	0.851	0.681
Ast_19559	4	0.606	0.542	4	0.430	0.417	3	0.288	0.333	6	0.696	0.431
Ast_44410	2	0.478	0.708	3	0.553	0.609	3	0.226	0.250	5	0.479	0.521
Ast_65237	4	0.630	0.667	9	0.820	0.667	3	0.525	0.542	15	0.868	0.625
Ast_47436	3	0.478	0.042	7	0.654	0.048	7	0.647	0.417	14	0.861	0.174
Ast_34501	5	0.749	0.875	7	0.787	0.739	6	0.423	0.292	10	0.793	0.634
Ast_59032	2	0.080	0.083	7	0.794	0.750	3	0.392	0.417	7	0.579	0.417
Ast_26109	6	0.718	0.625	6	0.721	0.609	9	0.813	0.833	14	0.859	0.690
Average	3.4	0.467	0.417	6.5	0.680	0.568	3.6	0.425	0.356	9.1	0.730	0.446

Note: A = number of alleles per locus; H_e = expected heterozygosity; H_o = observed heterozygosity; N = number of individuals genotyped.

LITERATURE CITED

FAIRCLOTH, B. C. 2008. MSATCOMMANDER: Detection of microsatellite repeat arrays and automated, locus-specific primer design. *Molecular Ecology Resources* 8: 92–94.

GOUDET, J. 1995. FSTAT: A computer program to calculate *F* statistics, version 1.2. *Journal of Heredity* 86: 485–486.

GRABHERR, M. G., B. J. HAAS, M. YASSOUR, J. Z. LEVIN, D. A. THOMPSON, I. AMIT, X. ADICONIS, ET AL. 2011. Full-length transcriptome assembly from RNA-Seq data without a reference genome. *Nature Biotechnology* 29: 644–652.

HUZIWARA, Y. 1954. Karyotype analysis in Gymnaster. *Japanese Journal of Genetics* 29: 76–82.

ITO, M., A. SOEJIMA, AND K. WATANABE. 1998. Phylogenetic relationships of Japanese *Aster* (Asteraceae, Astereae) sensu lato based on chloroplast-DNA restriction site mutations. *Journal of Plant Research* 111: 217–223.

IWATSUKI, K., T. YAMAZAKI, D. E. BOUFFORD, AND H. OHBA. 1995. Flora of Japan, Vol. IIIb: Angiospermae, Dicotyledoneae, Sympetalae (b). Kodansha, Tokyo, Japan.

KITAMURA, S. 1936. Expositiones plantarum novarum Orientali-Asiaticum 1. *Acta Phytotaxonomica et Geobotanica* 5: 243–244.

KONDOROSI, E., F. ROUDIER, AND E. GENDREAU. 2000. Plant cell-size control: Growing by ploidy? *Current Opinion in Plant Biology* 3: 488–492.

MAKINO, T. 1898. Plantae Japonenses novae vel minus cognitae. *Botanical Magazine (Tokyo)* 12: 45–52.

MAKINO, T. 1913. *Aster savatieri. In* C. Iinuma [ed.], Zotei Somoku Zusetsu 4, p. 1101. Seibido, Tokyo, Japan.

MAKINO, T. 1918. A contribution to the knowledge of the flora of Japan. *Journal of Japanese Botany* 2: 11–14.

MAYOR, R., AND Y. NACIRI. 2007. Identification and characterization of eight microsatellite loci in *Aster amellus* L. (Asteraceae). *Molecular Ecology Notes* 7: 233–235.

MINISTRY OF THE ENVIRONMENT, GOVERNMENT OF JAPAN. 2012. Red list of vascular plants in Japan. Website https://www.env.go.jp/press/15619.html [accessed 18 May 2016] (in Japanese).

PEAKALL, R., AND P. E. SMOUSE. 2012. GenAlEx 6.5: Genetic analysis in Excel. Population genetic software for teaching and research—An update. *Bioinformatics* 28: 2537–2539.

ROZEN, S., AND H. SKALETSKY. 1999. Primer3 on the WWW for general users and for biologist programmers. *In* S. Misener and S. A. Krawetz [eds.], Methods in molecular biology, vol. 132: Bioinformatics methods and protocols, 365–386. Humana Press, Totowa, New Jersey, USA.

TSUKAYA, H. 2013. Does ploidy level directly control cell size? Counterevidence from *Arabidopsis* genetics. *PLoS ONE* 8: e83729.

TABLE 3. Transferability of the 22 EST-SSR markers for Japanese *Aster* and *Solidago* species.

Locus	*A. ageratoides* var. *ageratoides* ($N = 3$)[a]	*A. glehnii* var. *hondoensis* ($N = 2$)[b]	*A. hispidus* var. *tubulosus* ($N = 2$)[b,c]	*A. rugulosus* ($N = 6$)[a]	*A. scaber* ($N = 3$)[b]	*A. sohayakiensis* ($N = 2$)[b]	*S. virgaurea* subsp. *asiatica* var. *asiatica* ($N = 4$)[a]	*A. savatieri* (Nagano population) ($N = 2$)[b,d]	*A. savatieri* var. *pygmaeus* (Kochi population) ($N = 2$)[b,d]
Ast_comp41702_c0_seq1	—	+	+	—	+	—	+	+	—
Ast_comp53978_c4_seq1	—	+	—	+	+	—	+	+	+
Ast_comp54189_c0_seq15	+	NG	NG	+	—	+	NG	+	—
Ast_comp22325_c0_seq1	NG	—	+	—	—	—	—	+	—
Ast_comp37017_c0_seq1	+	+	+	+	+	—	+	—	+
Ast_comp36481_c0_seq1	+	—	+	—	+	—	+	+	+
Ast_comp55030_c0_seq87	+	+	+	+	+	—	NG	—	+
Ast_comp41314_c0_seq1	NG	—	NG	NG	NG	NG	+	—	+
Ast_comp48897_c0_seq1	+	NG	+	NG	+	+	NG	+	+
Ast_comp51216_c2_seq2	+	—	+	+	+	+	NG	+	+
Ast_comp50838_c2_seq2	NG	—	—	+	—	+	+	NG	—
Ast_comp55875_c0_seq1	—	+	NG	—	—	NG	+	+	+
Ast_comp53959_c2_seq2	—	NG	+	+	—	NG	+	—	+
Ast_comp46752_c1_seq1	—	+	—	+	+	+	+	—	+
Ast_33509	+	+	+	—	+	—	—	+	+
Ast_19559	—	NG	NG	+	+	NG	+	+	+
Ast_44410	—	NG	NG	+	+	+	NG	+	+
Ast_65237	+	+	+	+	+	+	+	+	+
Ast_47436	NG	NG	NG	NG	NG	NG	NG	—	—
Ast_34501	+	NG	+	+	—	+	+	—	—
Ast_59032	+	NG	+	+	—	—	+	—	+
Ast_26109	+	—	NG	—	+	—	+	+	+
No. of successfully amplified loci	18	14	15	19	20	17	16	21	22

Note: — = monomorphic (only one allele was detected); + = polymorphic (more than one allele was detected); NG = no signal or nonspecific amplification was detected in PCR amplification.
[a] Individuals originated from more than one population.
[b] Individuals originated from a single population.
[c] Putative tetraploid.
[d] Samples used for initial PCR amplification trials.

Appendix 1. Voucher information for *Aster* and *Solidago* species used in this study.

Species	Population	Collection locality	Geographic coordinates (Altitude)	*N*	Voucher specimen accession no.[a]
Samples used for cDNA library construction					
Aster savatieri	Aichi	Hasso, Inuyama, Aichi Prefec., Japan	35°21′38″N, 137°01′34″E	2	TI00010644
Aster savatieri var. *pygmaeus*	Kochi	Hidaka, Takaoka, Kochi Prefec., Japan	33°32′48″N, 133°20′54″E	6	TI00010646
Samples used for initial PCR amplification trials					
Aster savatieri	Nagano	Togakushi, Nagano, Nagano Prefec., Japan	36°45′40″N, 138°04′09″E	2	TI00010645
Aster savatieri var. *pygmaeus*	Kochi	Hidaka, Takaoka, Kochi Prefec., Japan	33°32′48″N, 133°20′54″E	2	TI00010646
Samples used for initial PCR amplification trials and detailed evaluation for polymorphisms					
Aster savatieri	Aichi	Hasso, Inuyama, Aichi Prefec., Japan	35°21′38″N, 137°01′34″E	24	TI00010644
Aster savatieri var. *pygmaeus*	Mie	Asama, Ise, Mie Prefec., Japan	34°27′34″N, 136°47′05″E	24	TI00010647
Samples used for detailed evaluation for polymorphisms					
Aster savatieri	Kyoto	Ashiu, Miyama, Nantan, Kyoto Prefec., Japan	35°19′42″N, 135°43′42″E (528 m)	24	TI00010656
Samples used for transferability test					
Aster ageratoides Turcz. var. *ageratoides*	Ashio	Ashio, Nikko, Tochigi Prefec., Japan	36°43′00″N, 139°29′07″E	2	TI00010648
Aster ageratoides var. *ageratoides*	Chugushi	Chugushi, Nikko, Tochigi Prefec., Japan	36°43′28″N, 139°29′09″E	1	TI00010649
Aster glehnii F. Schmidt var. *hondoensis* Kitam.		Chugushi, Nikko, Tochigi Prefec., Japan	36°46′13″N, 139°27′17″E	2	TI00010654
Aster hispidus Thunb. var. *tubulosus* K. Asano		Shimoina, Nagano Prefec., Japan	—[b]	2	TI00010650
Aster rugulosus Maxim.	Tsugeno	Tsugeno, Shinshiro, Aichi Prefec., Japan	34°51′37″N, 137°34′45″E	2	TI00010652
Aster rugulosus	NAGN-a83	Naganoyama, Shinshiro, Aichi Prefec., Japan	35°00′02″N, 137°27′18″E	1	NA
Aster rugulosus	Bibai	Nishibibai, Bibai, Hokkaido, Japan	43°19′30″N, 141°48′39″E	1	NA
Aster rugulosus	KAWM-GH2	Kawaminami, Koyu, Miyazaki Prefec., Japan	32°12′15″N, 131°31′40″E	1	NA
Aster rugulosus	KIBG-A5	Shigaraki, Koga, Shiga Prefec., Japan	34°56′35″N, 135°57′29″E	1	NA
Aster scaber Thunb.		Onan, Ochi, Shimane Prefec., Japan	34°55′30″N, 132°28′31″E	3	TI00010651
Aster sohayakiensis Koidz.		Wadagawa, Shingu, Wakayama Prefec., Japan	33°45′56″N, 135°50′14″E	2	TI00010653
Solidago virgaurea L. subsp. *asiatica* Kitam. ex H. Hara var. *asiatica* Nakai ex H. Hara	Serpentine soil	Mukawa, Yufutsu, Hokkaido, Japan	42°51′18″N, 142°15′22″E (168 m)	2	TI00010655
Solidago virgaurea subsp. *asiatica* var. *asiatica*	Forest	Mukawa, Yufutsu, Hokkaido, Japan	42°51′26.9″N, 142°15′33.6″E (183 m)	2	NA

Note: *N* = number of individuals; NA = voucher unavailable.
[a]Vouchers deposited at the University of Tokyo (TI), Tokyo, Japan.
[b]GPS data are not shown because this variety is critically endangered, but are available from the authors upon request.