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DEVELOPMENT OF MICROSATELLITE MARKERS FOR THE SEMI-NATURAL GRASSLAND HERB *VERONICASTRUM JAPONICUM* (PLANTAGINACEAE)¹

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- **Premise of the study:** *Veronicastrum japonicum* (Plantaginaceae) grows in grasslands on Honshu Island, Japan, and is threatened by habitat loss because of rapid land development over recent decades. For the genetic characterization of the remaining populations, microsatellite markers were developed.
- **Methods and Results:** Twelve polymorphic microsatellite loci were developed using next-generation sequencing. The number of alleles per locus ranged from two to 24 (mean 7.7), and the expected heterozygosity per locus ranged from 0.35 to 0.94 (mean 0.68).
- **Conclusions:** These markers can be used for genetic studies in conservation, such as the evaluation of genetic diversity and genetic structure.

Key words: conservation; grassland herb; next-generation sequencing; Plantaginaceae; *Veronicastrum japonicum*.

Veronicastrum japonicum (Nakai) T. Yamaz. (Plantaginaceae) is a perennial plant species growing in the grasslands of mountainous regions on Honshu Island, Japan (Ohwi, 1978). In recent decades, the number of *V. japonicum* populations has rapidly decreased, and this species is now classified as near threatened or higher in five of the 47 Prefectural Red Lists of Japan (Association of Wildlife Research and EnVision, 2007). The decline of *V. japonicum* populations can be attributed to shrinking grasslands resulting from land development or the abandonment of traditional management. Furthermore, serious grazing damage by deer, which has explosively increased in the past few decades, has threatened *V. japonicum* populations (Takatsuki, 2009; Nagaike et al., 2014). *Veronicastrum japonicum* is also the main larval food plant species of *Melitaea ambigua* (Butler) (Nymphalidae), a rare butterfly species classified as endangered in the Japanese Red List (Ministry of the Environment of Japan, 2012). Accordingly, knowledge of the genetic diversity and structure of *V. japonicum* populations is important for the conservation management of *V. japonicum* and *M. ambigua*. Thus, 12 nuclear microsatellite markers for *V. japonicum* were developed using next-generation sequencing.

METHODS AND RESULTS

A fresh leaf sample of *V. japonicum* was collected from an individual plant growing in Gunma Prefecture, Japan, and genomic DNA was extracted using

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TABLE 1. Characteristics of microsatellite loci for *Veronicastrum japonicum*. All values are based on 48 samples from two populations in Gunma and Nagano prefectures in Japan.^a

Locus	Primer sequences (5'–3')	Repeat motif	Fluorescent label ^b	Allele size range (bp)	GenBank accession no.
Veja004	F: AGGAGCCCAGTTTGCCTAC R: CAAATGCTAATTGATCTCGACC	(CT) ₉	FAM	211–221	LC030210
Veja005	F: CCCTCTTTCTGGAAGTTTGAGC R: ACGACTCCTCCTTGCTTAGG	(TGT) ₆	VIC	232–237	LC030211
Veja007	F: GGGACATTGGAATGGAGGC R: ATTGATGCTGAACCAGGGC	(TCT) ₆	FAM	166–176	LC030212
Veja009	F: CATTAAATACTCTCTCCGCAGG R: GCTCTTCCACAGGTTTCC	(CT) ₉	NED	233–253	LC030213
Veja011	F: TGATGCTTGCCATCGTTCC R: AACTCAGCTGTTCTCCCG	(GCT) ₉	VIC	200–221	LC030214
Veja012	F: CTTCCCTTACCAACCAAGGG R: AACAGGACTCGCTCGTCAG	(GT) ₉	NED	308–332	LC069371
Veja015	F: CTGTCCACCTTTAGATTCCC R: GTCCCTTACAAGCTTCCC	(TC) ₉	NED	187–193	LC030215
Veja018	F: CACCAATCATTTCCGCAAACC R: ACAACCGCCCTTTATGATCTAC	(GA) ₁₂	NED	251–321	LC030216
Veja021	F: TGGACACATCAACCAGATTTC R: TCCCTGGATTCAACGAGAACC	(TAT) ₇	NED	194–209	LC030217
Veja025	F: AGGCCAATCAAATAATTACGC R: ACGCTTGTAGAGAGGTTAGGC	(CA) ₉	FAM	173–192	LC030218
Veja029	F: GCGCACTGTTAAGGACAGG R: CGTGAACAACGGCATAACC	(TGT) ₆	VIC	178–196	LC030219
Veja032	F: CAACGCCAGCCATCCATAC R: TTGGAGGAGAATGACACCCA	(GT) ₁₀	VIC	238–265	LC030220

^aAnnealing temperature was 57°C for all loci.

^bSequence of the fluorescent labels: FAM = 5'-CACGACGTTGTAACGAC-3', NED = 5'-CTATAGGGCAGCGTGGT-3', VIC = 5'-TGTG-GAATTGTGAGCGG-3'.

(mean 0.66) and from 0.35 to 0.94 (mean 0.68), respectively (Table 2). Only one locus (Veja009) in the Gunma population showed significant deviation from HWE ($P < 0.05$). Significant LD was found in the Nagano population for only one pair of loci (Veja004 and Veja021; $P < 0.05$).

CONCLUSIONS

The microsatellite markers described in this study will be useful for conservation genetic studies of *V. japonicum*, such as those evaluating genetic diversity and structure within and between populations. Assessment of their genetic information will also contribute to elucidating how *V. japonicum* genetic diversity and structure has been affected by declining populations

and habitat management practices. Understanding the impacts of habitat management will help the conservation of both *V. japonicum* and the endangered butterfly *M. ambigua*, because maintenance of *V. japonicum* population size is crucial for survival of this butterfly species.

LITERATURE CITED

- ASSOCIATION OF WILDLIFE RESEARCH AND ENVISION. 2007. Search system of Japanese Red Data. Website <http://www.jpnrdb.com/index.html> [accessed 1 February 2015] [in Japanese].
BOUTIN-GANACHE, I., M. RAPOSO, M. RAYMOND, AND C. F. DESCHÉPPER. 2001. M13-tailed primers improve the readability and usability of

TABLE 2. Results for primer screening of all samples for 12 microsatellite loci in two populations of *Veronicastrum japonicum*.^{a,b}

Locus	Gunma ($N = 21$)				Nagano ($N = 27$)			
	A	H_o	H_e	F_{IS}	A	H_o	H_e	F_{IS}
Veja004	7	0.81	0.75	-0.09	5	0.48	0.59	0.19
Veja005	2	0.43	0.39	-0.11	3	0.37	0.60	0.39
Veja007	6	0.62	0.71	0.13	6	0.70	0.65	-0.08
Veja009	8	0.48	0.70	0.32	8	0.70	0.80	0.12
Veja011	6	0.62	0.53	-0.17	5	0.59	0.64	0.08
Veja012	13	0.86	0.82	-0.05	12	0.78	0.77	-0.01
Veja015	4	0.52	0.67	0.22	4	0.30	0.35	0.15
Veja018	18	0.95	0.91	-0.04	24	1.00	0.94	-0.06
Veja021	7	0.62	0.73	0.15	6	0.67	0.71	0.06
Veja025	9	0.57	0.67	0.14	10	0.93	0.85	-0.09
Veja029	5	0.52	0.52	-0.01	3	0.70	0.60	-0.17
Veja032	5	0.86	0.73	-0.17	9	0.63	0.67	0.06

Note: A = number of alleles; F_{IS} = fixation index; H_e = expected heterozygosity; H_o = observed heterozygosity; N = sample size.

^aPopulations: Gunma Prefecture: 36°26'12"N, 138°25'27"E; Nagano Prefecture: 35°57'25"N, 137°37'49"E. Vouchers deposited in the Kyoto University Museum herbarium (accession numbers: KYO_00019997 and KYO_00019998, respectively).

^bNumbers in boldface show deviations from Hardy–Weinberg equilibrium ($P < 0.05$).

- microsatellite analyses performed with two different allele-sizing methods. *BioTechniques* 31: 24–26.
- 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.
- MINISTRY OF THE ENVIRONMENT OF JAPAN. 2012. The red list of insects of Japan. Web site http://www.biodic.go.jp/rdb/rdb_f.html [accessed 1 February 2015] [in Japanese].
- NAGAIKE, T., E. OHKUBO, AND K. HIROSE. 2014. Vegetation recovery in response to the exclusion of grazing by sika deer (*Cervus nippon*) in seminatural grassland on Mt. Kushigata, Japan. *ISRN Biodiversity* 2014: 493495.
- OHWI, J. 1978. Flora of Japan. Shibundo, Tokyo, Japan [in Japanese].
- PEAKALL, R., AND P. E. SMOUSE. 2006. GenAlEx 6: Genetic analysis in Excel. Population genetic software for teaching and research. *Molecular Ecology Notes* 6: 288–295.
- 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.
- TAKATSUKI, S. 2009. Effects of sika deer on vegetation in Japan: A review. *Biological Conservation* 142: 1922–1929.