Development of microsatellite markers for the Siberian weasel *Mustela sibirica*

Baojun Zhao^{1†}, Shuyu Xue^{1†}, Wanchao Zhu¹, TianShu Lv¹, Qinguo Wei¹,
Shuai Shang² and Honghai Zhang^{1*}

¹College of Life Science, Qufu Normal University, No. 57 Jingxuan West Road, Qufu 273165, China

²College of Marine Life Sciences, Ocean University of China, No. 238 Songling Road,
Qingdao 266100, China

(Received 8 April 2018, accepted 20 September 2018; J-STAGE Advance published date: 19 December 2018)

The Siberian weasel (*Mustela sibirica*) is widely distributed in mainland Asia, but its introduction into Japan and subsequent expansion have affected the Japanese weasel (*M. itatsi*). To provide a useful tool for population genetic studies and control of *M. sibirica*, we developed 10 polymorphic microsatellite markers. Among 40 individuals of *M. sibirica* collected in Hubei Province, China, the number of alleles per locus varied from 2 to 19, with the observed heterozygosity ranging from 0.050 to 1.000 and the expected heterozygosity ranging from 0.049 to 0.920. None of the loci deviated from Hardy–Weinberg equilibrium. These markers will be useful in further studies investigating the population structure and natural history of *M. sibirica*, and may thus provide new insights for the efficient management of this species.

Key words: genetic diversity, management, microsatellite, *Mustela sibirica*, population

INTRODUCTION

The Siberian weasel (*Mustela sibirica*) is distributed in many areas of Asia, including Pakistan, Nepal, Bhutan, Laos, Myanmar, Vietnam, Thailand, China, Mongolia, North Korea, South Korea, Russia and Japan (Suzuki et al., 2011, 2013; Shalabi et al., 2016). Like many other members of the genus *Mustela*, *M. sibirica* is an efficient and fierce predator. It plays an important role in controlling rodent and other small mammal populations in ecosystems (Zöller et al., 2008). In Japan, *M. sibirica* occurs naturally only on Tsushima Island, located between Kyushu and the Korean Peninsula (Shalabi et al., 2016). However, it was introduced to Honshu and Kyushu from the Korean Peninsula in the last century. It has adapted to Japanese environments, and is expanding its distribution in Kyushu, Shikoku and western Honshu (Kurose et al., 2000). The expansion of *M. sibirica* has affected the Japanese weasel (*M. itatsi*), a species endemic to Japan's main islands (Kyushu, Shikoku and Honshu) excluding Hokkaido (Ohdachi et al., 2009). Currently, these two species are sympatric in the western part of Japan, and *M. itatsi* is gradually disappearing from low-

lands because of the expansion of *M. sibirica* (Suzuki et al., 2011; Sasaki et al., 2014).

Invasions by nonindigenous species can have dramatic and devastating effects on ecosystems (Bryan et al., 2005). The application of genetic techniques to investigate biological invasions is potentially useful for the management of nonindigenous species (Colautti et al., 2005). Microsatellites, also known as simple sequence repeats (SSRs), are a kind of simple short tandem repeat sequence. They are dispersed in eukaryotic genomes and have proven to be an effective tool in molecular ecology and genetic diversity studies. Microsatellites can show the tempo and mode of population expansion of a species (Zhivotovsky et al., 2000). Microsatellite markers have been used to assess the genetic diversity and patterns of invasion of many introduced species, such as the sea lamprey *Petromyzon marinus* and the olive fruit fly *Bactrocera oleae* (Bryan et al., 2005; Zygouridis et al., 2009). In addition, microsatellites can also provide a description of the boundaries between populations and an accurate representation of their historical relationships, which will contribute to understanding the natural history of *M. sibirica*.

To provide a useful tool for population genetic studies and control of *M. sibirica*, we developed 10 polymorphic microsatellite markers.

Edited by Hideki Innan

* Corresponding author. E-mail: zhanghonghai67@126.com

† B. Z. and S. X. are joint first authors.

DOI: <http://doi.org/10.1266/ggs.18-00018>

MATERIALS AND METHODS

Sample collection and DNA extraction Tissue samples (skin or skeletal muscle) were obtained from 40 individuals of *M. sibirica*, which were collected from a wide range around Macheng of Hubei Province in February and March 2016. All the individuals were killed by poachers and were confiscated by the forest public security administration. We do not know the exact geographical source of each sample. All of them were donated to our laboratory and stored at -80°C . Genomic DNA of *M. sibirica* was extracted from tissue samples using a DNeasy Blood and Tissue Kit (QIAGEN), following the manufacturer's guidelines. All experimental procedures were approved by Qufu Normal University Institutional Animal Care and Use Committee (Permit Number: Qufu20170210001).

Primer design Microsatellite loci were screened according to the method described by Refseth et al. (1997). Isolated DNA was digested by Sau3AI restriction enzyme (Thermo Fisher Scientific) and ligated to double-strand linkers (Sau3AI F: 5'-GGCCAGAGACCCCAAGCTTCG-3' and Sau3AI R: 5'-GATCCGAAGCTTGGGGTCTCTGGCC-3') for making the enriched library. 5'-Biotinylated (AC)₁₂ probes were used to hybridize with products containing repeat regions. Dynabeads M-280 Streptavidin (Invitrogen) was used to capture the target fragments. The enriched fragments were ligated into pMD18-T Simple Cloning Vector (Takara), which was then used to transform Trans1-T1 Phage Resistant Chemically Competent Cells (TransGen Biotech). Recombinants were screened by polymerase chain reaction (PCR) using three primers: vector primers (M13F and M13R) and (AC)₁₂ oligonucleotide. The products were examined by 1% agarose electrophoresis. We selected clones that showed two or more bands as positive clones. The positive clones were sequenced by Sangon Biotech (Shanghai). We sequenced 63 positive clones from 432 plasmids. In addition, we downloaded nucleotide sequences of *M. sibirica* from the GenBank database (Kuang et al., 2007), and we used SSRHunter 1.3 software to find regions containing SSRs, where the parameters were set for detection of di-, tri-, tetra-, penta- and hexanucleotide motifs with a minimum of six repeats (Li et al., 2012). We designed 54 primer pairs using Primer Premier 5.0 (Lalitha, 2000), 36 of which were designed according to positive clones.

PCR amplification and genotyping PCR was performed in a 25- μl reaction volume containing 7.5 μl 2 $\times$ Easy Taq PCR Supermix (TransGen Biotech), 100–200 ng genomic DNA and 2.0 pM of each primer pair (with the forward primer fluorescently labeled with FAM, HEX). Amplification was carried out in an Applied Biosystems (ABI) 9700 Thermal Cycler under the following conditions: 5 min at 95°C ; 35 cycles of denaturation (30 s

at 95°C), annealing (30 s at the annealing temperature (Table 1)) and extension (30 s at 72°C); and, finally, a 10-min extension at 72°C . PCR products were separated on an ABI 3500 Genetic Analyzer (Applied Biosystems) with a 10- μl reaction volume including GeneScan 600 liz size standard. GeneMapper (version 5.0, Applied Biosystems) was used to analyze allele sizes.

Data analysis We used GenAlEx 6.5 (Peakall and Smouse, 2012) to calculate numbers of alleles (N_A), observed heterozygosity (H_O) and expected heterozygosity (H_E). CERVUS 3.0 (Kalinowski et al., 2007) was applied to assess polymorphic information content (PIC) values. GENEPOP version 4.0 (Rousset et al., 2008) was used for testing linkage disequilibrium and deviation from Hardy–Weinberg equilibrium.

RESULTS AND DISCUSSION

In the present study, 63 positive clones from 432 plasmids were sequenced. Although we obtained many sequences with repeats, a proportion of them could not be used to design primers for several reasons, such as annealing temperatures that were too high or low (Zhi et al., 2014), or repeats that were too short. In addition, some sequences could not be amplified with the primers. Finally, 36 primer pairs were designed from the positive clones, and six primer pairs (H1, H16, Ms13, Ms18-1, Ms21, Ms45) successfully yielded clear bands. These six microsatellite loci were screened and all of them showed polymorphism. Six of the 18 primer pairs designed according to the sequences from GenBank database produced clear bands, four of which (Ms57, Ms59, Ms63, Ms65) showed polymorphism. The number of alleles of 10 polymorphic microsatellite markers ranged from two to 19, with the observed heterozygosity ranging from 0.050 to 1.000 and the expected heterozygosity ranging from 0.049 to 0.920. None of the loci deviated from Hardy–Weinberg equilibrium; the PIC values ranged from 0.048 to 0.915; two loci showed low polymorphism ($\text{PIC} < 0.25$), two showed moderate polymorphism ($0.25 < \text{PIC} < 0.5$), and six were highly polymorphic ($\text{PIC} > 0.5$) (Table 1). Therefore, most of the loci can be utilized to increase the effectiveness of genetic diversity analysis (Zhu et al., 2016).

Population genetic studies have brought important insights into the understanding of natural history of species. The microsatellite markers that we developed can be used to examine patterns of genetic diversity among different geographic populations of *M. sibirica*. This will help to elucidate population dynamics processes such as population expansion and contraction, as well as immigration and emigration routes. The genetic data will show whether there are bottlenecks in the introduced populations and may provide useful information for the

Table 1. Characteristics of microsatellite loci for *Mustela sibirica*

Locus	Primer sequences (5'–3')	Repeat motif	T_a (°C)	Size range (bp)	N_A	H_O	H_E	PIC	HWE	Accession no.
H1	CGATGGCATGGTCAGCTC AGGTCTCCTCCCCGTCTC	(CA) ₁₁	58	85–103	6	0.825	0.588	0.543	1.000	KY678236
H16	TAGGTCTCCTCCCCGTCT CAGGTCTCTTTACACTCAG	(TG) ₁₁ GC(TG) ₅	56	82–106	7	1.000	0.638	0.575	1.000	KY678237
Ms13	ATCATCTCATCTGCTT CTAGGTCTCCTCCCCATC	(CA) ₁₃	54	126–130	2	1.000	0.500	0.375	1.000	KY678238
Ms18-1	TAGGTATCTCCTCCCATCT GGTCAGGTCCTCTTTACAC	(TG) ₁₄	54	85–113	5	1.000	0.547	0.446	1.000	KY678239
Ms21	TGGTCAGGTCCTCTTACA TTCCATCCAGGTCATTGC	(CA) ₁₁	54	114–118	5	0.775	0.678	0.619	0.448	KY678240
Ms45	TTTATCTTTGACTGGGTG AGAACTGAGCCTTGAAC	(AC) ₁₇	52	124–143	19	0.950	0.920	0.915	0.546	KY678241
Ms57	ACATATTAAGATTTTCGACCCAG GGCAGACACATCCAACAG	(GGT) ₈	56	156–166	4	0.075	0.049	0.097	0.076	KY678242
Ms59	AATACGGTGCCAAACATA ACCAGTGACCATCCCAGA	(CT) ₇	52	192–196	2	0.050	0.049	0.048	1.000	KY678243
Ms63	GAAAACTTTGAGAATAGCATAA ACAGTCCAGTGGGTAGCA	(TG) ₁₆	50	138–162	19	0.875	0.842	0.833	0.799	KY678244
Ms65	AGTGGCTCAGTTGGCTAA GATCAGGATGAAAAGGTCTAT	(CT) ₁₀	52	200–217	7	0.600	0.616	0.566	0.244	KY678245

T_a , annealing temperature; N_A , number of alleles; H_E , expected heterozygosity; H_O , observed heterozygosity; PIC, polymorphic information content; HWE, Hardy–Weinberg equilibrium.

government to make management decisions. In addition, *M. itatsi* and *M. sibirica* are most closely related among *Mustela* species, and their divergence is estimated to have occurred around 1.19 Mya (Shalabi et al., 2016). It remains to be determined by further experiments whether the primers developed for *M. sibirica* can also be applied to *M. itatsi*.

In conclusion, these 10 polymorphic microsatellite markers will be useful for population genetic studies and management of *M. sibirica*.

We thank everyone who contributed to this work. This project was supported by the Special Fund for Forest Scientific Research in the Public Welfare (201404420), the National Natural Science Fund of China (31672313, 31372220), and the Science and Technology Research Plan of Shandong Province (2013GSF11707).

REFERENCES

- Bryan, M. B., Zalinski, D., Filcek, K. B., Libants, S., Li, W., and Scribner, K. T. (2005) Patterns of invasion and colonization of the sea lamprey (*Petromyzon marinus*) in North America as revealed by microsatellite genotypes. *Mol. Ecol.* **14**, 3757–3773.
- Colautti, R. I., Manca, M., Viljanen, M., Ketelaars, H. A. M., Bürgi, H., MacIsaac, H. J., and Heath, D. D. (2005) Invasion genetics of the Eurasian spiny waterflea: evidence for bottlenecks and gene flow using microsatellites. *Mol. Ecol.* **14**, 1869–1879.
- Kalinowski, S. T., Taper, M. L., and Marshall, T. C. (2007) Revisiting how the computer program CERVUS accommodates genotyping error increases success in paternity assignment. *Mol. Ecol.* **16**, 1099–1106.
- Kuang, G., Liu, Z., Lu, S., Liu, H., Zhang, J., and Xiao, T. (2007) Isolation and characterization of microsatellite loci of *Siniperca chuatsi* from GenBank database. *Acta Zool. Sin.* **53**, 184–189 (in Chinese with English summary).
- Kurose, N., Masuda, R., Aoi, T., and Watanabe, S. (2000) Karyological differentiation between two closely related mustelids, the Japanese weasel *Mustela itatsi* and the Siberian weasel *M. sibirica*. *Caryologia* **53**, 269–275.
- Lalitha, S. (2000) Primer Premier 5. Biotech. Softw. Internet Rep. **1**, 270–272.
- Li, M., Zhu, L., Zhou, C. Y., Lin, L., Fan, Y. J., and Zhuang, Z. M. (2012) Development and characterization of EST-SSR markers from *Scapharca broughtonii* and their transferability in *Scapharca subcrenata* and *Tegillarca granosa*. *Molecules* **17**, 10716–10723.
- Ohdachi, S. D., Ishibashi, Y., Iwasa, M. A., and Saitoh, T. (2009) The Wild Mammals of Japan. Shoukadoh Book Sellers, Kyoto, Japan.
- Peakall, R., and Smouse, P. E. (2012) GenAlEx 6.5: genetic analysis in Excel. Population genetic software for teaching and research—an update. *Bioinformatics* **28**, 2537–2539.

- Refseth, U. H., Fangan, B. M., and Jakobsen, K. S. (1997) Hybridization capture of microsatellites directly from genomic DNA. *Electrophoresis* **18**, 1519–1523.
- Rousset, F. (2008) GENEPOP'007: a complete re-implementation of the GENEPOP software for Windows and Linux. *Mol. Ecol. Resour.* **8**, 103–106.
- Sasaki, H., Ohta, K., Aoi, T., Watanabe, S., Hosoda, T., Suzuki, H., Abe, M., Koyasu, K., Kobayashi, S., and Oda, S. (2014) Factors affecting the distribution of the Japanese weasel *Mustela itatsi* and the Siberian weasel *M. sibirica* in Japan. *Mammal Study* **39**, 133–139.
- Shalabi, M. A., Abramov, A. V., Kosintsev, P. A., Lin, L. K., Han, S. H., Watanabe, S., Yamazaki, K., Kaneko, Y., and Masuda, R. (2016) Comparative phylogeography of the endemic Japanese weasel (*Mustela itatsi*) and the continental Siberian weasel (*Mustela sibirica*) revealed by complete mitochondrial genome sequences. *Biol. J. Linn. Soc. Lond.* **120**, 333–348.
- Suzuki, S., Abe, M., and Motokawa, M. (2011) Allometric comparison of skulls from two closely related weasels, *Mustela itatsi* and *M. sibirica*. *Zoolog. Sci.* **28**, 676–688.
- Suzuki, S., Peng, J. J., Chang, S. W., Chen, Y. J., Wu, Y., Lin, L. K., and Kimura, J. (2013) Insular variation of the craniodental morphology in the Siberian weasel *Mustela sibirica*. *J. Vet. Med. Sci.* **75**, 575–581.
- Zhi, Y. B., Ding, L., Wang, Y., Hu, L., Hua, Y., Zhao, K., He, J., Yang, C., and Zhang, B. (2014) Isolation and characterization of thirteen novel dinucleotide microsatellite loci from *Tetraena mongolica* Maxim. *Conserv. Genet. Resour.* **6**, 297–299.
- Zhivotovsky, L. A., Bennett, L., Bowcock, A. M., and Feldman, M. W. (2000) Human population expansion and microsatellite variation. *Mol. Biol. Evol.* **17**, 757–767.
- Zhu, W., Li, H., Zhang, H., Zhang, H., Lv, T., Xue, S., and Chen, J. (2016) Isolation and characterization of twenty-one novel microsatellite loci for the black cicada, *Cryptotympana atrata* (Hemiptera: Cicadidae). *Appl. Entomol. Zool.* **51**, 511–514.
- Zöller, M., Aeffner, F., Müller, J., Mätz-Rensing, K., and Kaup, F. J. (2008) Neoplasia in the Siberian weasel (*Mustela sibirica*): two case reports of fibrosarcoma and interstitial cell tumour. *Eur. J. Wildl. Res.* **54**, 15–20.
- Zygouridis, N. E., Augustinos, A. A., Zalom, F. G., and Mathiopoulos, K. D. (2009) Analysis of olive fly invasion in California based on microsatellite markers. *Heredity* **102**, 402–412.