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

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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-
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'-GATCGAAGCTTTGGGTCTCTGCCC-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×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 (PIC < 0.25), two showed moderate polymorphism (0.25 < PIC < 0.5), and six were highly polymorphic (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
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*.

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