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TECHNICAL NOTE

Isolation and characterization of 25 polymorphic microsatellites of the large Japanese wood mouse (*Apodemus speciosus*)

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Abstract The large Japanese wood mouse (*Apodemus speciosus*) is common, but endemic to Japan, and its population structure was affected by habitat fragmentation because of urbanization. It suggested that the species might be one of the important models for the conservation of ecosystems and biodiversity affected by humans, including the effect of radioactive discharge caused by nuclear power plant accidents at Fukushima. We developed and characterized 25 novel polymorphic microsatellite markers from the next-generation sequencing data in an effort to provide an effective tool for genetic studies on this species. In 8 individuals from Aomori, Japan, the number of alleles and expected heterozygosities ranged from 5 to 13 and from 0.795 to 0.991, respectively, suggesting the availability of these markers for genetic studies in this species.

Keywords *Apodemus speciosus* · Wood mouse · Microsatellite · NGS

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The Japanese wood mouse (*Apodemus speciosus*) is common in forests throughout Japan. Although it was categorized as Least Concern in the version 3.1 of the Red List (IUCN 2012), habitat fragmentation by urbanization has affected the genetic population structure of this species (Hirota et al. 2004). This may make certain local populations vulnerable in the near future, and the conservation problems that may arise for this species are representative of those for all other small mammals. In addition, it is an important model species for evaluating the effects of humans on wildlife. The Fukushima Daiichi Nuclear Power Plant accident in 2011 possibly damaged ecosystems and biodiversity even in areas that were far from the power plant, and it is among the most disquieting problems in biological conservation in Japan. We started investigating the impact of the low level but continuous radiation discharge after the accident on the wildlife. The target species for the assessment includes *A. speciosus*. To evaluate the effect of irradiation on the rate of reproductive success, subsequent population stability, and unusual mutations over generations, a genetic survey should be conducted for which molecular genetic markers are indispensable.

However, reliable co-dominant genetic markers such as microsatellites have not been reported for this species. Thus, we needed to develop new microsatellite markers. A large data set of genome sequences provided by next-generation sequencing (NGS) was used to obtain a sufficient number of candidates for marker development.

Genomic DNA for NGS was extracted from frozen liver tissue of *A. speciosus* captured in Tateyama (N 36°35′N, 137°24′E), Toyama Prefecture, central Japan, in June 2012, following the standard phenol–chloroform protocol (Sambrook and Russell 2001). Approximately 200 μg of DNA were sent to Hokkaido System Science Co., Ltd. (Sapporo, Japan) and sequenced using the paired-ends sequencing

Table 1 Primer sequence, repeat motif from the original sequence, allele size, observed number of alleles, and expected and observed heterozygosities (H_E and H_O) for 25 polymorphic microsatellite loci in *Apodemus speciosus*

Name of the marker	Primer sequence	Repeat motif	Allele size	Number of alleles	H_E	H_O
AMS03	F TGTGGGATAGAGAACCATCCA	GT	269–295	9	0.911	1.000
	R GCAGGTCACCAACTCCACAA					
AMS07	F CAATGGCTTTGGGTAGAGGA	CA	332–354	8	0.911	0.750
	R AGCAAAGGAATTTGGAAAGAA					
AMS08	F CATTTGCACACATTTCAGTCA	CA	334–354	9	0.920	0.875
	R ATCATCCATTGGCAAGCTCT					
AMS12	F GACAACAGCTTGGAGGGAGT	GT	296–331	13	0.973	1.000
	R ACCCAGCACCTCATACATGG					
AMS14	F AAACAGCACCTGGAGCCTTA	GT	265–287	9	0.911	1.000
	R AGCCCGAACCTTTAAGCAGT					
AMS15	F CCTGCTTCTGGCTGTTCTCT	GT	274–300	11	0.911	0.875
	R TGTTTGTCAGGGGTTTCTGC					
AMS20	F ACGTGAACAAGGCATGTGAA	GA	238–266	10	0.911	1.000
	R ACAACGGATGCCTCAACTCT					
AMS25	F CAGTGTTCAGAAGGCTGCAA	CA	282–298	9	0.893	0.875
	R GCCAGCAGAGGGAACCTTAT					
AMS31	F CCCTAAGTGAACCAATTTCATCTC	GT	214–236	9	0.929	0.750
	R CGTTCCTAGCTCAGCTCTCA					
AMS34	F TAGGCCTGAGATGGATCCTG	GT	184–194	5	0.795	0.875
	R TGGCTTCCATATTCACATGC					
AMS35	F CCCCATCTCCTTAGATTACAATG	CA	218–268	9	0.857	1.000
	R CATCAACCATATTGTCTTCCTTC					
AMS37	F GATCCACTTTACAATTGCTCTGA	GT	242–260	10	0.893	1.000
	R AGGTGCTGGGACCTAAACTCT					
AMS38	F TTCTGGGCAGAAACAAGTGTA	CA	213–239	11	0.955	1.000
	R TCTCCTTGGCTTAGTGTTTTCC					
AMS41	F AAATGCTGCTTTTATGCTGATTA	CA	254–272	6	0.830	1.000
	R TCCTTTTGCAGCAAGACAAT					
AMS44	F CCTCCACATCTGCTCACTCA	CA	193–209	6	0.804	1.000
	R TCACTGGAAGGCACAGAACA					
AMS46	F CAAATTGAATATAAGTCTGAGTGTGTT	GT	251–275	9	0.929	1.000
	R CTGAAGATGACCTTTGGCTTC					
AMS49	F GCAGGTTAAGTTACTCCCTTGG	GT	250–290	13	0.964	1.000
	R TTGGACTCAAGCTATACCACACA					
AMS50	F CGATGTGTTCTCTAGTCTCAAAAGA	CA	308–348	9	0.893	1.000
	R CTTGGTGCTTCAGAGTTTTGG					
AMS51	F TCAAGTTCTAACCGTCTATCCTGA	CT	216–254	11	0.946	1.000
	R TCACAGCAATCAGAGCGAGT					
AMS52	F ATTCCCAGCATCAACCTCTG	CA	213–257	13	0.991	0.750
	R CTACCCTGGGTTGCCAAAGT					
AMS58	F TGACATGGTGACAAACTGCTT	CA	272–290	9	0.920	0.875
	R TCTCCCATTCTTACTTCATAGCA					
AMS59	F CGAGAGACTAAAGTAATGCCAACA	CA	324–360	10	0.902	0.750
	R CTTTACAATGAACTGAATCTGAGAAC					
AMS60	F GCAATGTCGTATGAGACAGTGAAT	GT	292–318	10	0.911	1.000
	R GACCTTTGGCAGCTATGTGG					
AMS66	F GGAGAAACTAAGACATTCCATGA	CA	230–258	7	0.821	0.875
	R GAGCAGTTTTTGTTGATTCTTGTT					
AMS69	F ACCTAGGGCTATATAATGAAATCAAA	GT	224–254	10	0.946	1.000
	R GGAAGATGCAGACAGGATCG					

protocol on Illumina Hiseq 2000 (Illumina). We received 1,500,000 selected sequences that were candidates for designing microsatellite markers containing more than 9 repeats of CA, GA, or GAA (or their complements, GT, CT, or CTT). We checked approximately 1,000 sequences, selected 60 of them, and designed primer sets for these sequences by using Primer 3 (Rozen and Skaletsky 2000).

To optimize the utility of the new markers, we chose samples from a locality other than Toyama for PCR tests. Genomic DNA in 8 *A. speciosus* collected from Towada (40°35′N, 140°57′E), Aomori Prefecture, northern Japan, in September 2012, was extracted with DNeasy Blood & Tissue Kit (Qiagen) from ethanol-fixed liver samples. To check the availability of 60 candidate primer pairs, PCR was carried out using TaKaRa ExTaq Hot start version (TaKaRa) in a total volume of 15 μL following the manufacturer's instructions. The thermal profile included pre-cycling denaturation at 95 °C for 5 min, followed by 35 cycles of denaturation at 95 °C for 30 s, annealing at 58 °C for 90 s and extension at 72 °C for 30 s, and post-cycling extension at 72 °C for 10 min. PCR products were roughly sized on agarose electrophoresis. Thirty-four primer sets that were amplified in all 8 samples and that showed length polymorphism were selected, and the forward primer of each set was fluorescently labeled using 6-FAM, VIC, NED, or PET. Fluorescent PCR was carried out in the same manner as specified previously in the text, except that the final extension step was performed for 30 min so that adenine is added at the end of all the products. The PCR products were electrophoresed using size marker 500 LIZ (Applied Biosystems) on ABI 3130xl (Applied Biosystems), and the product sizes were estimated using Gene Mapper software (Applied Biosystems). The number of alleles and observed and expected heterozygosities were calculated using Genepop on the Web (Raymond and Rouset 1995; Rouset 2008), and deviations from Hardy–Weinberg equilibrium (HWE) and linkage disequilibrium were tested using the same website.

After the primer pairs showing multiloci or difficult genotyping with stutter peaks were eliminated, 25 pairs were found to be well scored (Table 1). The fragment lengths, allele numbers, and expected and observed heterozygosities showed ranges of 184–360, 5–13, and 0.795–0.991 and 0.750–1.000, respectively. No significant linkage disequilibrium or deviation from HWE was detected, suggesting that all the markers are available for genetic analyses in *A. speciosus.* We plan to investigate the population genetics of this species to evaluate the effects of humans on wild mammals.

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