Development of microsatellite markers and the genetic diversity of *Myocastor coypus* introduced to South Korea

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ABSTRACT. The nutria (*Myocastor coypus*) was introduced to South Korea in 1987 for breeding of individuals for fur and meat industry, and was accidentally released into the wild. Here, we report the development of microsatellites for the nutria collected from South Korea using Illumina MiSeq genome sequencing to identify the genetic variability and demographic history of these introduced populations. A total of 626,282 microsatellite sequences were identified, and nine polymorphic loci were characterized. We used four novel loci developed and three previously known loci to investigate the genetic diversity of twelve South Korean populations. A low level of diversity was found, and no signature of genetic structuring was revealed among populations, indicating that Korean nutria individuals originated from a single population or a highly inbred reared herd.

KEY WORDS: genetic structure, Illumina MiSeq, invasive species, microsatellites, *Myocastor coypus*

The nutria (*Myocastor coypus*) is a large herbivorous rodent species belonging to Echimyidae [11, 19]. Originally native to subtropical and temperate South America [2, 3], this species was introduced via Bulgaria to South Korea in 1987 primarily for breeding of individuals that could be used for fur and meat industry [3, 9]. This species was accidentally released into the wild, and has become a mammalian species that is frequently found in nature. The Ministry of Environment in South Korea designated this species as an ecological disturbance species in 2009, and with the local governments, started to control the population in nature [9]. This species is still considered as pests in many areas of South Korea, especially on the southern part, destroying aquatic vegetation, marshes and irrigation systems [9].

There are no basic ecological and historical records in South Korea on how many individuals are currently living in the wild and how many or diverse broodstocks have been introduced to South Korea. The absence of information on introduced wildlife also means that it is difficult to control the disease or the risks that this organism may cause. Insights can be obtained into contemporary demographic history by the practice of hypervariable and codominant neutral molecular loci such as microsatellites for the estimation of genetic diversity and structure among populations [1, 17]. Primers for microsatellites of this species are already known [2]. Given that introduced species are likely to be genetically very monomorphic [15], however, novel polymorphic loci are required to be characterized from these introduced individuals. Here we report the development of microsatellite markers for the nutria collected from South Korea using Illumina MiSeq genome sequencing technology. The obtained polymorphic loci were used to identify the genetic variability and demographic history of these introduced populations. Many individuals of this species are found mainly in the southeastern part of South Korea, and the sampling was accomplished throughout most of the known areas (Fig. 1).

For the development of microsatellite markers, total genomic DNA of 48 individuals collected from two localities (Goryeong and Haman) in South Korea (Table 1; Fig. 1) was extracted with a DNeasy Blood and Tissue Kit (Qiagen, Dusseldorf, Germany). A high-quality DNA sample was selected and sent to Macrogen Inc. (Seoul, South Korea), where library construction, quality check and sequencing were performed. One microgram was processed in a hydrodynamic shearing system (Covaris, Woburn, CA,
U.S.A.) to produce fragments of less than 800 bp. The fragments were blunt-ended and phosphorylated, and the appropriate library sizes (around 450 bp) were selected by purification beads. A paired-end library was constructed using the TruSeq DNA Sample Prep kit (Illumina, San Diego, CA, U.S.A.). The library constructed was electrophoresed on 1% agarose gel to extract fragments of approximately 500 bp, and the purity of eluted fragments was checked using a Quant-iT™ dsDNA High Sensitivity Assay Kit (Invitrogen, Carlsbad, CA, U.S.A.). The final library preparation was sequenced using the MiSeq sequencing (Illumina) platform to generate 300-bp paired-end reads (600-cycle MiSeq Reagent Kit v3). Raw image files obtained in the platform were processed to generate base calling through RTA (Real Time Analysis v1.18, Illumina), which was converted into FASTQ utilizing MSR (Miseq Reporter, Illumina).

A total of 31,805,658 high-quality reads, consisting of 9,545,204,206 bp (Q20=72.90%), were generated, and the reads with Phred quality scores of less than 20 and lengths of less than 20 bp were removed. De novo genome assembly was performed with the high quality filtered reads using the de Bruijn graph algorithm and the SOAPdenovo2 software package (v. 2.04) under the default settings [10]. Consequently, 626,282 microsatellite sequences were identified using the MicroSAtellite Identification Tool (MISA) [18]; the composition of di-, tri- and tetranucleotide repeats were 32.5% (203,662 loci), 40.2% (251,719 loci) and 24.3% (152,147 loci), respectively. All of the microsatellite information are stored in Yeungnam University database (http://suk.yu.ac.kr/english.html) and can be released freely to anyone who needs this. Of these, 169 loci were selected among 15–50 repeat types for design of primer pairs using Primer3 software [16]. Primer pairs were synthesized for those loci and were examined on whether successfully generating PCR products in eight randomly selected individuals. All PCR amplifications were performed in a 10 µl volume containing 1 µl DNA template, 1X Taq Buffer, 0.2 mM dNTPs, 2.5 µM of each primer and 2.5 units of Taq DNA

Table 1. The nine novel microsatellite loci developed in this study and the diversity estimates obtained from two *Myocastor coypus* populations, Goryeong and Haman (N=48)

| Name   | A   | A_R | H_O | H_E | F_IS | F_ST | R_ST | NCBI ACCN # |
|--------|-----|-----|-----|-----|------|------|------|-------------|
| Mck044 | 5   | 4.929 | 0.704 | 0.661 | −0.091 | 0.034 | −0.018 | MH346456    |
| Mck055 | 3   | 2.998 | 0.576 | 0.445 | −0.253 | 0.088 | 0.103 | MH346457    |
| Mck087 | 4   | 3.925 | 0.774 | 0.661 | −0.152 | 0.009 | −0.003 | MH346458    |
| Mck101 | 3   | 2.975 | 0.591 | 0.558 | −0.087 | −0.020 | −0.020 | MH346459    |
| Mck130 | 5   | 4.993 | 0.691 | 0.743 | 0.068  | 0.076 | 0.017  | MH346460    |
| Mck141 | 3   | 2.970 | 0.313 | 0.301 | −0.017 | 0.017 | −0.022 | MH346461    |
| Mck226 | 3   | 2.986 | 0.626 | 0.571 | −0.100 | 0.008 | 0.006  | MH346467    |
| Mck250 | 4   | 3.637 | 0.695 | 0.657 | −0.066 | 0.007 | −0.017 | MH346469    |
| Mck291 | 3   | 3.000 | 0.636 | 0.583 | −0.068 | 0.076 | 0.009  | MH346471    |

Data include total number of alleles (A), allelic richness (A_R), observed (H_O) and expected (H_E) heterozygosities, fixation indices (F_IS), and NCBI GenBank accession numbers (NCBI ACCN #).

Fig. 1. Sampling location of the 12 *Myocastor coypus* populations on the Korean Peninsula. See Table 3 for locality numbers.
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Table 2. The seven microsatellite loci developed in this study (Mck044, Mck087, Mck130 and Mck250) and previously reported (McoD212, McoD215 and McoD69) and the diversity estimates obtained from twelve Myocastor coypus populations (N=269)

| Name    | A  | A_R | H_O | H_E | F_IS | F_ST | R_ST |
|---------|----|-----|-----|-----|------|------|------|
| McoD212 | 3  | 2.066 | 0.528 | 0.456 | -0.171 | 0.053 | 0.057 |
| McoD215 | 4  | 3.941 | 0.233 | 0.662 | 0.592 | 0.111 | 0.251 |
| McoD69  | 3  | 2.782 | 0.549 | 0.536 | -0.018 | 0.049 | 0.048 |
| Mck044  | 5  | 3.977 | 0.647 | 0.655 | 0.004 | 0.043 | 0.056 |
| Mck087  | 6  | 4.292 | 0.721 | 0.668 | -0.061 | 0.078 | 0.062 |
| Mck130  | 6  | 5.544 | 0.817 | 0.763 | -0.095 | 0.060 | 0.045 |
| Mck250  | 5  | 3.606 | 0.626 | 0.629 | 0.001 | 0.037 | 0.024 |

Data include total number of alleles (A), allelic richness (A_R), observed (H_O) and expected (H_E) heterozygosities, fixation indices (F_IS), F_ST and R_ST.

Table 3. List of twelve Myocastor coypus populations on the Korean Peninsula and the diversity estimates obtained from the genetic structure analysis with seven microsatellites (see Table 2)

| Name    | Locality     | N  | A  | A_R | H_O | H_E | F_IS | F_ST | R_ST |
|---------|--------------|----|----|-----|-----|-----|------|------|------|
| Goryeong| 35°42’N 128°21’E | 32 | 3  | 3.545 | 0.692 | 0.656 | -0.055 | 0.040 | 0.031 |
| Gimhae  | 35°11’N 128°51’E | 23 | 3  | 3.460 | 0.596 | 0.631 | 0.066 | 0.052 | 0.039 |
| Daegu   | 35°51’N 128°44’E | 27 | 3  | 3.446 | 0.677 | 0.629 | -0.078 | 0.068 | 0.074 |
| Milyang | 35°23’N 128°50’E | 24 | 3  | 3.555 | 0.625 | 0.664 | 0.060 | 0.058 | 0.038 |
| Busan   | 35°06’N 128°54’E | 34 | 3  | 3.288 | 0.542 | 0.586 | 0.075 | 0.075 | 0.052 |
| Yangsan | 35°19’N 129°01’E | 22 | 3  | 3.495 | 0.552 | 0.583 | 0.054 | 0.080 | 0.064 |
| Uiryeong| 35°21’N 128°22’E | 10 | 3  | 3.526 | 0.543 | 0.637 | 0.155 | 0.083 | 0.042 |
| Jinju   | 35°12’N 128°09’E | 25 | 3  | 3.032 | 0.560 | 0.614 | 0.090 | 0.068 | 0.039 |
| Changnyeong | 35°30’N 128°28’E | 22 | 3  | 3.152 | 0.610 | 0.627 | 0.028 | 0.079 | 0.106 |
| Changwon| 35°18’N 128°40’E | 20 | 3  | 3.348 | 0.507 | 0.596 | 0.153 | 0.057 | 0.030 |
| Haman   | 35°18’N 128°23’E | 21 | 3  | 3.268 | 0.612 | 0.623 | 0.017 | 0.068 | 0.061 |
| Hapcheon| 35°30’N 128°10’E | 9  | 3  | 3.714 | 0.556 | 0.631 | 0.126 | 0.086 | 0.107 |

Data include average number of alleles (A), allelic richness (A_R), observed (H_O) and expected (H_E) heterozygosities, fixation indices (F_IS), F_ST and R_ST.

polymerase (Genetbio, Daejeon, South Korea) under the following thermal cycling conditions (GenePro, Bioer, PR China): an initial denaturation at 94°C for 5 min followed by 30 cycles each of denaturation at 94°C for 30 sec, annealing at 58°C for 30 sec, and an extension at 72°C for 45 sec, with a final extension at 72°C for 10 min.

Only nine primer pairs showed polymorphism in the eight individuals, which is understandable considering that this species is a recently introduced species and its genetic variation would be extremely low. The information of the remaining 160 loci is stored in a recently introduced species and its genetic variation would be extremely low. The information of the remaining 160 loci is stored on the Korean Peninsula and the diversity estimates obtained from the genetic structure analysis with seven microsatellites (see Table 2).

The overall allelic diversity of the novel 9 loci was as low as 3 to 5 (Table 1; N=48). H_O ranged from 0.313 (Mck141) to 0.774 (Mck087) and H_E ranged from 0.301 (Mck141) to 0.743 (Mck130; Table 1). No locus deviated significantly from the expectation of Hardy-Weinberg equilibrium (HWE) were examined with the Fisher exact test following Markov chain parameters, with 1,000 batches and 10,000 iterations per batch [8] for each locus, implemented in GENEPOP 4.2 [13]. The linkage disequilibrium between each pair of loci was detected by Fisher exact tests under the Markov chain algorithm in GENEPOP. The level of statistical significance for simultaneous and multiple tests was corrected using sequential Bonferroni adjustments [14].

The seven microsatellite loci developed in this study (Mck044, Mck087, Mck130 and Mck250) and previously reported (McoD212, McoD215 and McoD69) and the diversity estimates obtained from twelve Myocastor coypus populations (N=269). The deviations from genotype frequencies expected under Hardy-Weinberg equilibrium (HWE) were examined with the Fisher exact test following Markov chain parameters, with 1,000 batches and 10,000 iterations per batch [8] for each locus, implemented in GENEPOP 4.2 [13]. The linkage disequilibrium between each pair of loci was detected by Fisher exact tests under the Markov chain algorithm in GENEPOP. The level of statistical significance for simultaneous and multiple tests was corrected using sequential Bonferroni adjustments [14].

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The five loci, except for Mck044, Mck087, Mck130 and Mck250, were very monomorphic in the other populations except Goryeong and Haman. For the investigation of genetic structure, the 15 alleles of these 5 loci were not used and replaced by 3 loci (McoD212, McoD215 and McoD69; Table 2) previously reported [2]. All 12 populations (N=269) had very similar levels and very low genetic variation (Table 3). No signature of genetic structure was found among populations, considering the low F_ST and R_ST values (mostly less than 0.1) in all loci examined (Table 2), probably indicating that Korean nutria individuals originated from a single population or a highly inbred reared herd, though it is premature to conclude only with our results yet. To identify the fine-
Bayesian clustering analysis was performed using Structure v2.3.4 [12]. The putative numbers of genetic clusters (K) were inferred by the delta K method [5] implemented in Structure Harvester v0.6.94 [4]. Although three was estimated to be the most predictable number of genetic clusters in delta K method (Fig. 2A), the analysis was performed for all K values from 2 to 6. Ten independent MCMC runs were performed for each number of genetic clusters with 4 × 10^5 iterations after a burn-in of 10^5 iterations. Evident signature of genetic structure was not found in Structure analysis, though there was a slight bias in the frequency of genetic characteristics among populations (Fig. 2B), which can be predicted from genetic drift or sampling error in populations with extremely low genetic variability. Our genetic data can be used to monitor the demographic change of Korean populations or for the population study in other areas.

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