Development and characterization of microsatellite markers in the small Indian mongoose (Urva auropunctata)

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Abstract

Background The small Indian mongoose (Urva auropunctata) is one of the world’s worst invasive alien species and eradication programs are ongoing worldwide. The development of individual and sex identification markers will improve their management.

Methods and results We searched for novel mongoose microsatellite markers using genome-wide screening and identified 115,265 tetra-nucleotide repeat loci. Of 96 loci tested, 17 were genotyped in 28 mongooses from the Okinawa population. The genetic diversity analysis showed that the average expected and observed heterozygosity and number of alleles were 0.55, 0.56, and 2.94, respectively. Of 17 loci, one deviated from Hardy–Weinberg equilibrium and six loci pairs were likely linked to each other. However, we succeed in identifying all individuals using all of the microsatellite loci. The novel sex identification markers worked successfully in a test using sex known samples.

Conclusion Our novel microsatellite and sex identification markers should be useful in studies of individual identification and population genetics of the mongoose.

Keywords Genome wide screening · Introduced species · Okinawa · Population genetics · Microsatellite

Introduction

The small Indian mongoose Urva auropunctata (Hodgson, 1836) is a generalist predator that naturally occurs from the Arabian Peninsula to Southeast Asia [1]. It was introduced to 64 islands in the Pacific and Indian Oceans and Caribbean and Adriatic Seas and to mainland Europe, South America, Australia, and North America for rat or snake control, causing enormous damage to the native biodiversity [2]. Ecological niche modelling of the mongoose suggests that it will spread globally by 2050 [3].

Mongoose eradication programs are underway using trapping and poisoning. On Amami Oshima Island, Japan, the program is close to success [4], and the populations and distributions of the endemic Amami rabbit Pentalagus furnessi (Stone, 1900) and Amami spiny rat Tokudaia osimensis (Abe, 1933) have expanded [5, 6]. To continue these eradication programs, methods to estimate the mongoose population are needed.

The application of genetic analysis is essential for wildlife management. Microsatellite markers are often used to reveal the genetic diversity and origin of invasive species through population genetic analysis [e.g., 7, 8]. Single nucleotide polymorphisms (SNPs) are also powerful markers in population genetics; however, SNP analysis requires many markers to be analysed than microsatellite analysis [9], and it is unsuitable for analysing additional samples. Therefore, microsatellite markers were chosen in the present study.

Previously, eight mongoose microsatellite markers were developed [10]; however, there is a problem with their use. Three of these loci could not be scored reliably [11], implying that the loci included unstable repeat characters. This problem leads to mis-genotyping and reduces the resolution power of markers.

This study developed novel microsatellite markers for the small Indian mongoose using genome-wide screening in a mongoose from the Okinawa, Japan, population. We selected tetra-nucleotide repeats with short amplification lengths as markers. We also developed sex identification markers. The
novel markers were verified with 28 mongooses from the Okinawa population.

**Materials and methods**

**Sample collection**

We obtained tissue samples from 28 mongooses in the Okinawa population (Fig. 1; Table 1). All samples were taken from euthanized animals through the mongoose eradication program.

**Genome sequences and identification of microsatellite loci**

Genomic DNA was extracted from a small Indian mongoose tissue sample (Nago1; Table 1) and the genome was sequenced by Macrogen Japan (Kyoto, Japan). Whole-genome shotgun sequencing was performed on an Illumina NovaSeq 6000 sequencer (150 paired ends). The raw sequence data was deposited in the DNA Data Bank of Japan (DDBJ) Sequence Read Archive (DRA; https://www.ddbj.nig.ac.jp/dra/) with the accession number DRR294934. The sequence quality was checked by the FastQC program, and noise and adapters were trimmed with fastp [12] under the default configurations. We then mapped the sequences onto the genome of a banded mongoose *Mungos mungo* (acc. no. GCA_004023785) using BWA-mem2 [13] with the default parameters. Among the species with genome information, *M. mungo* is most closely related to the small Indian mongoose [14]. To produce the consensus sequence, the output BAM file was sorted using SAMtools [15] and the variants were called with bcftools. In total, 52,502 sequences were obtained, with a total valid sequence length of 1,435,707,730 bp.

The microsatellite loci were identified from the consensus sequences using Krait [16] with the following configuration: perfect microsatellite search mode; minimum repeat number of tetra-nucleotide repeats = 7; and flanking sequence length = 200. We selected tetra-nucleotide repeats because the repeats are easy to determine and the genotype is less prone to slippage [17]. To search for primers, Primer3 [18] implemented in Krait was used with following
configuration: primer product size range = 80–270; primer GC content = 40–60; and Primer Max Ns accepted as 0. The other parameters were set to the default values. In total, 115,265 tetra-nucleotide repeat loci were found and 10,224 primer pairs were exposed. Of these, 96 primer pairs were selected for multiplex PCR amplification based on their product sizes, which were around 80, 120, 160, or 200 bp. To avoid linkage disequilibrium, a marker was selected from each scaffold.

Genotyping microsatellite loci

To verify the novel microsatellite markers, 28 tissue samples were used (Table 1). Total DNA was extracted with the DNeasy Blood & Tissue Kit (QIAGEN, Hilden, Germany) according to the manufacturer’s protocol. All PCRs were conducted with QIAGEN Multiplex PCR kits in a total volume of 10 µL, with 5 µL Multiplex PCR Master Mix, 1 µL primer mix (0.2 µM each of the forward and reverse primers), 3 µL dH2O, and 1 µL genomic DNA. The PCR amplification comprised 95 °C for 15 min; 35 cycles at 94 °C for 30 s, 60 °C for 90 s, and 72 °C for 60 s; then 60 °C for 30 min on a MiniAmp Plus thermal cycler (Applied Biosystems [ABI], Foster City CA, USA). The amplicons were checked by electrophoresis on 3% agarose gels. Based on Schuelke [19], we ordered universal primers with four different fluorescent dyes at the 5’ ends (5’-CACGACGTTGTA AACGAC-3’ for 6-FAM, 5’-GTGTGAAATTGAGCGG-3’ for VIC, 5’-CGGAGAGCGAGGTG-3’ for NED, or 5’-CTATAAGGCACGGTGTT-3’ for PET), and new forward primers tagged with one of the four different universal primers at the 5’ end for the successfully amplified loci. The primer mixes were prepared according to Culley et al. [20]. Fragment analysis was conducted by the FASMAC DNA sequencing service (FASMAC, http://fasmac.co.jp/) on an ABI 3730xl DNA Analyzer with GeneScan 600 LIZ Size Standard (ABI). For genotyping, Peak Scanner 1.0 (ABI) was used. We repeated the PCR and genotyping twice for heterozygotes and three times for homozygotes to confirm repeatability.

Development of sex identification markers

Based on the sequences of zinc finger protein genes on the X (ZFX: AB848712) and Y (ZFY: AB848711) chromosomes from Murata et al. [21], new mongoose sex identification markers were designed (Table 2). The PCR product length was 174 bp for ZFX and 221 bp for ZFY. We used the same PCR volumes as for microsatellites and the following PCR conditions: 95 °C for 15 min; 35 cycles at 94 °C for 30 s, 58 °C for 90 s, and 72 °C for 30 s; then 72 °C for 10 min. For the PCR, 0.2 µM each of primers was used. This sex identification process was confirmed by PCR amplification with sex known six samples and a negative control via electrophoresis on a 3% agarose gel.

Molecular data analyses

For all microsatellite loci, the observed (Ho) and expected (He) heterozygosity, deviation from Hardy–Weinberg equilibrium (HW), inbreeding coefficient (Fis), and linkage disequilibrium were analysed using GENEPOP [22]. The presence of null and dropout alleles was tested with Microchecker [23]. The probabilities of identity (PID and PIDsib) were calculated by GIMLET [24].

Results and discussion

Of 96 loci tested, 18 produced single bands of the target size. All loci except one successfully scored the genotypes by fragment analysis, and two to five alleles were found in the Okinawa mongoose population (Table 2). The 17
Table 2  Characteristics of novel microsatellite and sex identification markers of the small Indian mongoose

| Locus | Forward Primer sequence (5′-3′) | Reverse Primer sequence (5′-3′) | Label | Motif | Size range (bp) | k | He  | Ho  | HWE   | Fis   |
|-------|--------------------------------|--------------------------------|-------|-------|-----------------|---|-----|-----|-------|-------|
| Uau1  | TGGGAGCTATCAGAGTCCGG           | GCCACATTATCAACCCACTGC          | 6-FAM | GAAA  | 93–117          | 4 | 0.61| 0.68| 0.525 | −0.103|
| Uau2  | GCTTCCTATTCACGAGGCC            | GTAGGAGCACTCATGATGACG          | VIC   | CAAA  | 97–105          | 2 | 0.49| 0.43| 0.696 | 0.143 |
| Uau3  | ACAGGATCTCTCTGTGGGCC           | TGTCCTCTCTCCTCTCTTCC          | PET   | TGGT  | 95–99           | 2 | 0.50| 0.50| 1.000 | 0.018 |
| Uau4  | ACAGGATGATGATGATGATGATGCC      | CTGTCATGTGTCCACTCCGG          | 6-FAM | ATGA  | 114–122         | 3 | 0.51| 0.43| 0.243 | 0.179 |
| Uau5  | TCCCTGGTCCTACCTGTGGACC         | TGGACTCCTCCCTTCCATTCA         | NED   | TGAA  | 117–137         | 3 | 0.56| 0.61| 0.149 | −0.065|
| Uau6  | GCAATAAGAAGACTCAACTCAACAGC     | GCCATACGATGTCCTCTTGG          | VIC   | ATAC  | 138–150         | 3 | 0.60| 0.54| 0.155 | 0.122 |
| Uau7  | AAGGGAGAAGAGTGTGGGCG           | TGAGAGAAGAGTGGCTAAATCTGG      | NED   | GATG  | 193–205         | 2 | 0.50| 0.50| 1.000 | 0.013 |
| Uau8  | TCTTAAAGTATTGAGGCAGGG          | TGTATCGCAATTTACCTTCC          | PET   | TAA   | 176–184         | 3 | 0.63| 0.54| 0.427 | 0.168 |
| Uau9  | TCTATCATATCATCTCTTCCAGC        | AAGGTGCCAGAGGAGGAGG           | 6-FAM | CTA   | 175–179         | 2 | 0.48| 0.46| 1.000 | 0.059 |
| Uau10 | CCATGCTACACCTACCTCCAGC         | TCCTACATAGAAGAGAGACTGAGC      | VIC   | TCA   | 181–185         | 2 | 0.50| 0.64| 0.251 | −0.276|
| Uau11 | TGAGAGAAAGAAAGGCATGGG          | GTCAATACTCCCTTCCACTG          | NED   | CATT  | 180–192         | 4 | 0.65| 0.64| 0.733 | 0.036 |
| Uau12 | TGAACATCCCTCTGCCGAGCC          | ACTGATTTAAAGCTCTTGGAC         | PET   | TATC  | 171–187         | 3 | 0.57| 0.61| 0.217 | −0.055|
| Uau13 | TTGTCTTACCCTGTGTCCGC          | TCAGCTTTAATAGGGAATGCTGG       | 6-FAM | TCA   | 217–233         | 4 | 0.33| 0.39| 1.000 | −0.165|
| Uau14 | CTGTGCACTTATCCTCAGTGCC         | CTGACTGAGAGGAGGAGGG           | VIC   | ATCT  | 220–228         | 3 | 0.63| 0.61| 0.422 | 0.052 |
| Uau15 | GTGATGCTATGAGAGGGGG           | GGCAACACCAAGTAGGAAGG          | NED   | ATGA  | 203–219         | 3 | 0.57| 0.57| 0.533 | 0.010 |
| Uau16 | TGAGATCAGCGCCCTGCATGG          | CCCAGGAGGAAACAGCATGG          | 6-FAM | ATAG  | 211–251         | 5 | 0.67| 0.82| <0.003| −0.205|
| Uau17 | TGCCCTCATATCTCTTGGGCC          | AGGAAAAACAGGGCACTATGCG        | VIC   | AAGA  | 215–227         | 2 | 0.49| 0.61| 0.439 | −0.211|
| ZFX   | GAACCTGATGTAACTGAAAGGA         | ACTGATTTAATAGGGAAGG          | 174   |      |                 |   |      |      |       |       |
| ZFY   | GAACCTAGATGTAACTGAAAGGA        | CTGACTGAGAGGAGGAGGG           | 221   |      |                 |   |      |      |       |       |

_k_ number of alleles, _He_ expected heterozygosity, _Ho_ observed heterozygosity, _HWE_ Hardy Weinberg equilibrium _P_ value, _Fis_ inbreeding coefficient

A bold means statistically significant (_P_ < 0.05)
microsatellite sequences were reported in Supplementary file 1. *He*, *Ho*, and *Fis* were 0.33–0.67, 0.39–0.82, and −0.2756 to 0.1787, respectively (Table 2). Microchecker suggested that no loci showed evidence of null alleles, while one locus (Uau16) deviated from HWE expectations (*P* < 0.003). Based on linkage disequilibrium tests, six loci pairs were likely linked (*P* < 0.05): Uau1 and 17, Uau1 and 7, Uau1 and 8, Uau3 and 7, Uau6 and 13, and Uau7 and 10. Generally, the genetic diversity of invasive species is lower than in the native population owing to the small number of founders or bottlenecks. There were at most 17 founders of the Okinawa mongoose population [25]. This history suggests that the Okinawa population might not follow HWE expectations. Using all 17 loci, 28 individuals were identified genetically; the *PID* was 1.92 × 10^{-10} and the *PIDsib* 3.68 × 10^{-5}. At minimum, four loci were required to reach *PID* < 0.001 and seven loci were required to reach *PIDsib* < 0.01. This suggests that our novel microsatellite markers are useful for individual identification.

We also confirmed that the novel sex identification markers worked successfully in a test using known sex samples (Figure S1).

As a result, we successfully developed 17 microsatellite markers and sex identification markers. These markers should be powerful tools for managing mongooses in combination with DNA collected from non-invasive samples, though it needs to verify and optimize the protocols.

**Supplementary Information** The online version contains supplementary material available at https://doi.org/10.1007/s11033-021-06655-9.

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**Author contributions** TS and TJ contributed to the research idea and manuscript writing. TS contributed to experimental design and data analysis.

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**Declarations**

**Conflict of interest** All authors declare no conflicts of interest.

**Consent to participate** All the authors listed have approved the manuscript that is enclosed.

**Consent for publication** The manuscript is approved by all authors for publication.

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