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Rapid microsatellite development in *Gekko japonicus* using sequenced restriction-site associated DNA markers

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ABSTRACT. Twelve polymorphic microsatellite loci were isolated in the Japanese gecko, *Gekko japonicus*. We genotyped one population from Wenzhou, Zhejiang Province, China (N = 36). The mean number of observed alleles per locus was 7.3 (range 4 to 13). Observed and expected heterozygosity values ranged from 0.200 to 0.944 and from 0.395 to 0.797, respectively. One locus (GJ20) showed significant departure from Hardy-Weinberg equilibrium; no linkage disequilibrium was found between any two loci. These informative microsatellite markers will be useful for population genetic analyses of *G. japonicus* and other species in the genus *Gekko*.

Key words: Microsatellite; *Gekko japonicus*; Genetic diversity; Polymorphism

INTRODUCTION

The Japanese gecko, *Gekko japonicus*, is distributed throughout east China, South Korea, and Japan (Zhao and Adler, 1993). *G. japonicus*, in common with other reptile species, has temperature-dependent sex determination, that is, the sex of the embryo depends on the temperature of the environment in which it develops (Ding et al., 2012). In recent years, many studies on *G. japonicus* have focused on reproductive traits (e.g., Xu and Ji, 2001; Ikeuchi, 2004; Zhang et al., 2009; Zhu et al., 2009), thermoregulation, and locomotion (Hu and Du, 2007). Twenty-one microsatellite DNA primers were isolated from *G. swinhonis* and 10 of these successfully amplified targets in *G. japonicus* (Li and Zhou, 2007). However, the lack of sufficient polymorphic molecular markers has limited the analyses of molecular phylogeny, population structure, and genetic diversity in this species. Therefore, isolation and development of additional novel polymorphic microsatellite or other molecular markers are necessary to better investigate the genetics of *G. japonicus.* Due to their high abundance, high rate of polymorphism, and codominant inheritance, microsatellite markers are often used in population genetics and population history analyses. In this study, we describe the isolation and characterization of 12 new microsatellite loci in *G. japonicus* using restriction-site associated DNA sequencing technology. These new markers will be of value for advancing our molecular knowledge of the phylogenetics of *G. japonicus*.

MATERIAL AND METHODS

Genomic DNA was extracted from the muscle tissue of one *G. japonicus* individual using the DNeasy Tissue Kit (QIAGEN). We used the Hiseq 2000 platform to sequence the partial genome of *G. japonicus*; this sequencing was performed by the Novogene Bioinformatics Technology Co., Ltd (Beijing) following standard protocols. Simple sequence repeats (SSRs) were identified from the assembled sequences using the SSR Hunter software, Version 1.3 (Li and Wan, 2005) and primer pairs were designed for selected microsatellites using the Primer 5.0 software. Genomic DNAs from eight *G. japonicus* individuals were used to optimize conditions for PCR amplification and to screen for polymorphic SSRs. For each primer pair developed, the forward primer was labeled with FAM, HEX or TAMARA fluorescent dye.

In total, 36 individuals were genotyped using the optimized conditions. The total volume of each PCR mixture was 15 µL and contained 100 ng DNA template, 0.5 µM forward primer, 0.5 µM reverse primer, 0.5 mM of each dNTP, 0.1 U hot-start *Taq* DNA polymerase (QIAGEN), and 1.2 mM $MgCl_2$. Amplification was typically performed with an initial denaturation at 95°C for 15 min, followed by 34 cycles at 94°C for 30 s, T_a (the optimal annealing temperatures, see Table 1) for 30 s, 72°C for 30 s, and a further extension step at 72°C for 20 min. The PCR products were genotyped on an ABI 3730 sequencer (Applied Biosystems) and analyzed with the GeneMarker v2.2 software. Observed and expected heterozygosity values, and tests for deviations from Hardy-Weinberg equilibrium and linkage disequilibrium were performed using GENEPOP 4.0 (Rousset, 2008) and FSTAT 2.9.3.2 (Goudet, 1995), respectively.

RESULTS

Using the Hiseq 2000 platform, a total of 21,144 contigs and 4,611,206 bp were obtained from the cluster tags with an average read length of 218 bp. A total of 16 microsatellite

primer pairs were designed and tested. Four pairs of primers showed monomorphic or poor amplification in *G. japonicus.* The remaining 12 pairs of primers were polymorphic in the 36 individuals (Table 1). The observed numbers of alleles ranged from 4 to 13 (mean 7.3) per locus (Table 1). Observed heterozygosity ranged from 0.200 to 0.944 and expected heterozygosity ranged from 0.395 to 0.797. One locus (GJ20) deviated significantly from Hardy-Weinberg equilibrium after Bonferroni's correction. No significant linkage disequilibrium was observed in pairwise loci.

Table 1. Characterization of the 12 microsatellite loci in *Gekko japonicus*.

Locus	GenBank accession No.	Repeat motif	Primer sequences (5′-3′)	Ta (°C)	N_A	Size range (bp)	H_O/H_E	P
GJ04	KR184141	$(CT)_8$	F: CCTTCCATCTCACCAAATCACT R: TAAGACTCTGCTGCTTGCTCCC	55	7	117-175	0.667/0.676	0.604
GJ05	KR184142	$(TG)_8$	F: TTCAAGCATTAGAGCATCC R: TGTTTACTGCTCACTACGC	55	7	119-145	0.412/0.688	1.000
GJ06	KR184143	$(TG)_7$	F: GTCTGGCATCCTTTTATTC R: CACCATCACCGTTACATTC	55	4	171-177	0.361/0.584	0.996
GJ09	KR184144	$(AG)_9$	F: AAACGCTCCAGCCATTCTT R: CCTCCTCTTTCCCTTCCTC	55	6	144-190	0.667/0.585	0.171
GJ12	KR184145	$(GT)_9$	F: TTTCCTTCCTGTATTCATAG R: TTTTCCTCCATCCTAGTGTT	50	4	236-242	0.303/0.692	1.000
GJ13	KR184146	$(GT)_8$	F: CAATGGAAAATAAAGGGAC R: TTCTTGCTATGCAACAATC	50	10	143-191	0.306/0.797	1.000
GJ16	KR184147	$(AC)_8$	F: AATGTAGAGGGAAATGGGT R: TGGCAGATGCTTTTGGAGT	55	5	212-224	0.639/0.577	0.250
GJ18	KR184148	$(GT)_7$	F: TCACTTGTTGTTTGGAGAA R: AAAAGCAGAGGATGGATTA	50	8	147-213	0.444/0.659	1.000
GJ20	KR184149	$(CA)_{10}$	F: TACTCTAGGCGAGACCAC R: ATTCAGGAGCATCCACCA	52	10	117-211	0.944/0.772	0.004*
GJ21	KR184150	$(TAG)_7$	F: TGTTATGGTGTCCTCGGTC R: TGGCTCGTAGTCGTCAGTG	52	8	111-183	0.528/0.712	0.996
GJ22	KR184151	$(CT)_{10}$	F: TTTCTATGCCACCTCTTCA R: TTCCTCATATTCCCAGTCA	55	13	173-231	0.583/0.719	0.992
GJ23	KR184152	$(TGA)_9$	F: TCAGTCATTTTGGTTTGTCTTG R: TTAATATCAGCCACAGCCCTTT	55	6	212-274	0.200/0.395	1.000

*Indicates significant deviation from Hardy-Weinberg equilibrium after Bonferroni's correction for multiple comparisons ($P < 0.005$). Ta = annealing temperature of primer pairs; N_A = number of alleles; H_O = observed heterozygosity; H_E = expected heterozygosity.

DISCUSSION

This study successfully isolated and developed 12 novel and highly polymorphic microsatellite markers in *G. japonicus.* Compared to a traditional library-based approach such as magnetic beads enrichment (Li and Zhou, 2007; Hua et al., 2014), the polymorphic microsatellite loci were isolated more quickly, more effectively, and at lower cost. This result is in agreement with recent studies in *Saiga tatarica* (Nowak et al., 2014) and *Microhyla ornata* (Wei et al., 2015). Therefore, these microsatellite loci will be helpful in studies of genetic diversity and population structure of *G. japonicus* as well as other species in the genus *Gecko*. We also believe that next-generation sequencing technology will help us develop powerful molecular tools for population genetic studies in the gecko.

Conflicts of interest

The authors declare no conflict of interest.

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