Polymorphic Microsatellite Markers for the Harvest Mouse (*Micromys minutus)* in Taiwan

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ABSTRACT: Seven polymorphic microsatellite loci are described for the harvest mouse, *Micromys minutus* in Taiwan. For a panel of 38 individuals with unknown relationship, the numbers of alleles per locus ranged from 3 to 17. The observed and expected heterozygosities averaged 0.433 and 0.656, respectively. These seven markers should offer potentials to investigate the harvest mouse population genetic structure.

KEY WORDS: harvest mouse, *Micromys minutus*, microsatellite.

INTRODUCTION

The harvest mouse (*Micromys minutus*) is widely distributed across much of Eurasia. The species inhabits grassy vegetation, including grassland, reeds, cereal crops, roadside verges, and salt marshes, throughout its range (Churchfield et al., 1997). Populations of the harvest mouse could fluctuate dramatically, both within and between years (Trout, 1976). In Taiwan, the harvest mouse (Fig. 1) is often found in habitats at early succession stages, such as grassy fields developed after fire or cultivation disturbance. A harvest mouse population inhabits the salt marsh in the Guandu Nature Park at suburban Taipei, Taiwan. The salt marsh underwent rapid succession as indicated by aerial photos during 2000-2004. Particularly, suitable habitats (dense vegetation) for the harvest mouse seemed to have declined, and become fragmented (Fig. 2). Such changes would affect genetic structure of the local harvest mouse population (Layme et al., 2004; Kearney et al., 2007). We developed microsatellite markers with an aim to study the effects of succession on population genetic structure of harvest mice.

MATERIALS AND METHODS

The DNA was extracted according to the standard phenol-chlorophorm extraction procedures described in Sambrook et al. (1989). Genomic DNA was digested with Sau3AI and fractioned on a 1% agarose gel. DNA of size range 300-1200bp was eluted, purified with GFX^{TM} Band Purification Kit (Amersham) and ligated into plasmids PUC118/BamHI/BAP (TaKaRa) according to manufacturer's protocols. Ligated plasmids were transformed into the competent ECOS 101 cells (Yeastern Biotech). Recombinant clones containing inserts were transferred to Hybond-N$^+$ nylon membranes (Amersham), which were hybridized to a set of oligonucleotide probes, including $(AC)_{15}$, $(AT)_{15}$, $(AG)_{15}$, $(AAT)_{10}$, $(AAG)_{10}$, and $(GATA)_6$. Probes were labeled with Digoxigenin (DIG) Oligonucleotide 3'-End Labeling Kit (Roche). Hybridization was performed at 50-53℃ for 16 hours in a standard hybridization buffer, consisting of 5X SSC, 0.1% Sodium N-lauroylsarcosine, 0.02% SDS, and 1% Blocking Reagent (Roche). The membranes were washed twice, each for 5 min at 45℃ with a solution of 2X SSC, 0.1% SDS, and then twice, each for 15 min at 65℃ with a solution of 0.1X SSC, 0.1% SDS. Chemiluminescent detection was performed with DIG Luminescent Detection Kit (Roche). A total of 64 positive clones were sequenced using a MegaBACE 1000 automated sequencer. Twenty-two clones containing repeat motifs with more than 6 repeats and sufficient flanking region were selected to design primers. About 4% of screened clones yielded positives clones, which was higher than the average of 2-3% in many other taxa (Zane et al., 2002).

Primers were designed with the on-line program Primer 3.0 (Rozen and Skaletsky, 2000) and FastPCR 1.2 (Kalendar, 2007). Polymerase chain reaction (PCR) conditions were optimized for each primer pair. Each PCR reaction mixture (10 μL) contained 50-100 ng template DNA, 0.5 units of *Taq* DNA polymerase (Bioman, Taipei, Taiwan), 2.0 mM of Mg^{2+}, 0.2 mM dNTP, 10X buffer (20 mM of Tris-HCl (pH8.8), 10 mM KCl, 10 mM $(NH_4)_2SO_4$, and 0.1% Triton X-100, Bioman), and 0.25 μM primer, with the forward or reverse primer being end-labeled with

fluorescent dyes (FAM, HEX or TAMRA). Amplification was carried out by the thermal profile: 94℃ 5 min, followed by 40 cycles of 94℃ 30 s, optimal annealing temperature for 30 s, 72℃ for 30 s, and a final extension step at 72℃ for7 min. PCR products were electrophoresised on a MegaBACE 500 automated sequencer with ET-400 Size Standard (Amersham). Individual genotypes were determined and individuals with ambiguous genotypes or homozygote were amplified and scored at least twice to determine the allele sizes.

Fig. 1. The harvest mouse (*Micromys minutus*) is the smallest rodent in Taiwan, as well as many parts of the world. (Photo taken by Mr. Shao-Min Yang)

RESULTS AND DISCUSSION

Seven microsatellite loci were polymorphic among thirty-eight *M. minutus* individuals (Table 1). The number of alleles averaged 5.75 (3-10). The observed and expected heterozygosity averaged 0.6693 and 0.6936, respectively (Table 1). Hardy-Weinberg expectation and linkage disequilibrium for each locus were tested with the program GENEPOP 4.0 (Rousset, 2008) and FSTAT 2.9.3 (Goudet, 2001), respectively. Large allele drop out and error due to stutter were tested with MICROCHECKER (Van Oosterhout et al., 2004). There was no evidence of deviation from Hardy-Weinberg equilibrium or linkage ($P > 0.001786$, after Bonferroni correction). No evidence of large allele drop and error due to stutter. The levels of polymorphism uncovered at these loci suggested that they should be useful to study population structure of harvest mice (*Micromys minutus*).

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Fig. 2. An aerial photo showing habitat mosaics of the study site, a salt marsh in the Guandu Nature Park at suburban Taipei, Taiwan. (Curtsey of the Spacial Ecology Laboratory of the National Taiwan University)

Table 1. Characteristics of 7 polymorphic microsatellite loci developed for the harvest mouse, *Micromys minutus*.

Locus (accession no.)	Repeat motif	Primer sequences (5'-3')*	N	T_a (℃)	Allele size range(bp)	No. of alleles	H_O (H_E)	HW (P value)
MM-B03 (EU882038)	i(TG)$_5$GTA(TG)$_{16}$	TCCCTTCTGCTTTCACATCA CCACAGAGTGTCTCTCTATTGCAG-HEX	38	63	159-187	10	0.842 (0.870)	0.77
MM-C02 (EU882039)	i(TG)$_{17}$GTA(TG)$_5$	GCCTCCCATTTTTCACAGTC-TAMRA AGGCTTCCTCGTTCAAGACA	38	63	214-246	5	0.737 (0.760)	0.94
MM-E04 (EU882041)	(GT)$_7$	CGGGGATCTTCTCTTCTCTTTACG-HEX AGTTCCACATGCTTCAGCTACG	37	61	308-338	3	0.324 (0.341)	0.59
MM-D03 (EU882040)	(TG)$_{17}$	CACACGGGCCTTTGTTCTACCTGC TCAGACTAACTCTCTGGGTCACTGC-FAM	38	60	322-344	7	0.868 (0.780)	0.06
MM-E05 (EU882042)	(GT)$_{21}$	CACTGTTAAGTTCATCTCTGTGGTTG TCTTTGCTGAGGAATGAGACTGGTCTGTGG-TAMRA	38	57.7	228-240	5	0.658 (0.675)	0.87
MM-F03 (EU882043)	i(TG)$_3$C(TG)$_{16}$	GCCAGTCCTGAGACCCTTTG-FAM TCTTTGCCATCAATGTAGAGCTTGCAGG	38	56.5	128-146	6	0.632 (0.759)	0.20
MM-H04 (EU882044)	(TG)$_{23}$	AGTCTTCATAATTCAACCTCATGGT-HEX AATCCTCAGTTATTAGTGCATGTGC	38	60	106-120	4	0.737 (0.689)	0.17

* Primer labeled with fluorescent dye: FAM, HEX or TAMRA; 5' ends of MM-E05 and MM-F03 primers were modified by addition of the sequence TCTTTG to provide conditions for essentially full terminal nucleotide addition (see Brownstein et al. 1996).
i, interrupted repeat motif; H_O, observed heterozygosity; H_E, expected heterozygosity; HW, Hardy-Weinberg equilibrium; T_a, annealing temperature; and N, sample size.

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臺灣地區巢鼠的微隨體基因座

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摘要：本研究開發了 7 組臺灣地區巢鼠 (*Micromys minutus*) 具多型性的微隨體基因座。我們分析了同一族群中 38 隻巢鼠個體，結果顯示，該族群中各基因座具 3 至 17 個對偶子，而其異型合子的平均觀察值與預測值分別為 0.433 和 0.656，並無無效對偶子存在。未來將可據此 7 組微隨體基因座研究巢鼠的族群遺傳結構。

關鍵詞：巢鼠、微隨體、*Micromys minutus*。