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MICROSATELLITE LETTERS

Development of 12 new microsatellite markers for the naked mole-rat, *Heterocephalus glaber*

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Abstract Twelve novel microsatellite markers were developed for the naked mole-rat, *Heterocephalus glaber*, a species characterized by a social structure analogous to that seen in eusocial insects. All but one locus (Hglab01) were found to be polymorphic across the 33 samples screened, with the number of alleles ranging from one to seven (average 3.58). Average observed and expected estimates of heterozygosity were 0.328 and 0.533, respectively. These are the first microsatellites developed for *H. glaber* and provide a useful tool for further population and mating system studies.

Keywords *Heterocephalus glaber* · Naked mole-rat · Eusociality · Microsatellites · Inbreeding

The naked mole-rat, *Heterocephalus glaber*, is a species of fossorial rodent that occurs throughout semi-arid desert shrub regions of Ethiopia, Somalia, and Kenya. The discovery of its eusocial-like population structure, characterized by cooperative brood care and single female breeding in colonies, has stimulated considerable research related to how this complex system evolved and the genetic consequences of such a social system (Reeve et al. 1990; Honeycutt et al. 1991; Sherman et al. 1991). Recently, naked mole-rats are being promoted as model organisms for studies of aging and cancer (e.g., Kim et al. 2011). Previous molecular studies, based on mitochondrial DNA (Honeycutt et al. 1991) and minisatellite loci (Reeve et al. 1990) revealed significantly low levels of genetic diversity, possibly as a result of inbreeding and patterns of colony formation. The availability of markers, such as microsatellite loci with higher mutation rates, offer the opportunity for detailed studies of how the mole-rat's breeding system and dispersal capabilities have influenced the partitioning of genetic variation among colonies and regions. Such information is useful to conservation efforts focused on small, isolated populations of threatened and endangered species with limited dispersal capabilities.

A size-selected (400–1,500 bp) genomic library was constructed from a single individual (H034) using a *Pst*I-pBluescript plasmid, and transformed into DH10β electrocompetent *E. coli* (Stratagene). Inserts were confirmed using standard blue/white screening. Colonies with inserts were probed with the following repeat motifs: $(GT)_{18}$, $(CT)_{18}$, $(GTCT)_8$, $(CA)_{18}$, $(GA)_{18}$, and $(GTAA)_8$. For recombinants containing repeat motifs, insert DNA was sequenced using the pUC primers (pUC-F, pUC-R). Oligonucleotide primers (both forward and reverse) flanking each locus were designed using the program PRIMER v0.5 (Whitehead Institute, MIT).

Genotypes were determined for 33 individuals obtained from the following four localities in Kenya: Isiola District (23 individuals from 6 colonies), Mbuvu (4 individuals from 2 colonies), Nguni (4 individuals from 2 colonies), and Mtito Andei (6 individuals from 4 colonies). All genotyping was performed on an ABI 3130 automated

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Table 1 Characterization of twelve microsatellite loci isolated from *Heterocephalus glaber* −33 individuals

Primer name	Sequence 5′–3′	Repeats in cloned allele	Size range (bp)*	N_a*	H_0*	H_E*	HWE
Hglab01	F: TCAGAGTGCTACCCAGGATC	$(GTT)_6$	231	1	0.000	0.000	NA
	R: TACCAAAACTTGCAAAATTTCA						
Hglab03	F: GTCAGGTTGGCAGATTTTGA	$(GT)_{19}$ $(GA)_{16}$	295–323	7	0.424	0.736	0.001
	R: TGTGTGAGGGGGAGACAG						
Hglab07	F: AACTGAAGTTCACTGTGCTGG	$(GT)_{19}$ AT $(GT)_4$	169–179	2	0.091	0.088	NS
	R: TGAGGACACATTTCTTCTTGG						
Hglab08	F: CTGTGTTAAAAATGTGGCCC	$(GT)_{12}$	295–309	4	0.364	0.656	0.001
	R: ATCCCTGAAACACCCAGG						
Hglab09	F: AGATTTGTTCACCTCAATCC	$(GT)_{13}$	167–173	4	0.000	0.585	0.001
	R: GTTTTGGTAAAGGCTTCTTGG						
Hglab10	F: ACCAAGGGAAATAAACCTGC	$(GT)_{21}$	295–303	4	0.758	0.643	NS
	R: TTCTTCTTGTTCCTTGTGGC						
Hglab13	F: TCAGTTGGCTAGAGTGGGAG	$(GT)_{21}$	375–385	5	0.303	0.755	0.001
	R: CCAGGTTTCTGAGCGACTAA						
Hglab14	F: GACTAGAAGTCCTCCCCAGC	$(GT)_{13}$	221–227	4	0.606	0.598	NS
	R: CTTTCGGGACTTTAATTCAGG						
Hglab17	F: AGGAAGGTGCTAGAGAATTGG	$(GT)_{19}$	285–295	3	0.125	0.538	0.001
	R: GTTTCCCAGTCACATTTTGC						
Hglab18	F: GACAGTTCTGGGCTGAACC	$(GT)_{16}$	202–212	4	0.364	0.638	0.001
	R: AAGGACAGTGGAGAGTGTTAGC						
Hglab19	F: TACAGTGCTGCATACCATCG	$(GT)_{24}$	289–295	3	0.875	0.659	0.014
	R: AAGAGTCATTGAGCTGGGG						
Hglab22	F: ACCAATTCCACCATAAGAAGC	$(GT)_{14}$ G $(GA)_5$	233–237	2	0.031	0.495	0.001
	R: TGCAGTTGATGAAACTATCACC						

N_a number of alleles, *H_0** observed heterozygosity, *H_E* expected heterozygosity, *HWE* Hardy–Weinberg equilibrium

sequencer. Approximately 20–100 ng of template DNA was amplified in 25 μL reactions containing 250 μm dNTPs (TaKaRa), 2.5 pmol of fluorescent-labeled (forward) and unlabeled (reverse) primers, 0.88 U *Ex Taq* (TaKaRa) in 1× *Ex Taq* Buffer (w/2 mM $MgCl_2$)(TaKaRa). Reaction conditions included an initial 2 min denaturation at 95 °C, followed by 35 cycles of 95 °C for 30 s, 58 °C annealing temperature for 30 s, and 72 °C extension for 30 s, with a final extension of 7 min at 72 °C. The only exception to these conditions was Hglab18, with an annealing temperature of 57.4 °C. Fragment analysis was performed on an ABI 3130 Genetic Analyzer (Applied Biosystems, Carlsbad, CA), and allele sizes were determined with GeneMapper, version 3.7.

The primer sequence, number of alleles, allele size range, both observed (H_O) and expected (H_E) heterozygosities, and *p* value for Hardy–Weinberg equilibrium (HWE) test for each locus (Table 1) were calculated using GENEPOP v1.2 (Raymond and Rousset 1995). Initial analyses treated the 33 individuals as a single population. Although this pooling of localities probably compromised the test for Hardy–Weinberg equilibrium (HWE), it does allow for a better perspective of variation across the range of the species in Kenya.

The average number of alleles per locus was 3.4 and observed heterozygosity ranged from 0 % (Hglab01) to 28 % (Hglab19). Six loci (Hglab 03, 08, 09, 13, 17, 18, 22) were not in HWE, likely as a result of pooling all localities as a single population.

Overall, most loci had low numbers of alleles and low levels of heterozygosity. Most allelic differences were observed between colonies and regions, and within-colony variation was low. Although not highly variable, these microsatellite markers are sufficiently variable for detailed studies of the genetic structure of naked mole-rats on both local and broader geographic scales. They should also prove useful for future molecular ecological studies in this species.

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