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MICROSATELLITE LETTERS

Development and characterization of 30 novel microsatellite markers for Grant's gazelle (*Nanger granti*)

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Abstract We isolated and characterized a set of 30 novel microsatellite loci for Grant's gazelle (*Nanger granti*). Loci were screened in 24 individuals from a population in Laikipia County, Kenya. The mean number of alleles per locus was 3.73 (range 1–10), and observed heterozygosity ranged from 0.00 to 0.870 (mean 0.404). The Grant's gazelle is currently listed as a species of least concern by the IUCN, but declining numbers across a large part of its range are a cause for concern. These new loci will facilitate basic behavioral, ecological, and population genetic studies of a species facing declining populations.

Keywords *Nanger granti* · Grant's gazelle · Illumina · Microsatellite · PCR primers

Grant's gazelles (*Nanger granti*) are distributed across East Africa from Ethiopia and South Sudan, across Kenya and into Uganda and central Tanzania. Although general features of Grant's gazelle ecology and social organization have been described, key characteristics of the species' biology remain unknown. In the face of downward trends in Grant's gazelle population numbers (IUCN 2008), insights drawn from microsatellite-based studies can contribute to a deeper understanding of gazelle behavior, ecology, and conservation.

We collected blood and ear punch samples from Grant's gazelles at the Mpala Research Centre, Laikipia, Kenya (0°17′N, 37°52′E) in 2009 and 2011. Blood samples were frozen at −20 °C and tissue samples were kept in 95 % EtOH until DNA extraction. DNA extractions were performed using DNeasy blood and tissue kits (Qiagen) according the manufacturer's instructions. Total DNA extracted from the tissue of a single individual was used for isolation and identification of microsatellite loci. An Illumina paired-end shotgun library was prepared by following the standard protocol of the Illumina Nextera DNA Sample Preparation kit and using a dual index identifier adaptor. This library was pooled with those from other species and Illumina sequencing was conducted on the HiSeq with 100 bp paired-end reads. Five million of the resulting reads were analyzed with the program *PAL_FINDER_v0.02.03* (Castoe et al. 2012) to extract those reads that contained di-, tri-, tetra-, penta-, and hexanucleotide microsatellites. Once positive reads were identified they were batched to a local installation of the program Primer3 (version 2.0.0) for primer design. We tested forty-eight primer pairs for amplification and polymorphism following the methods detailed in O'Bryhim et al. (2013), and assessed the variability of 30 loci in 24 individuals (12 from 2009 and 12 from 2011). Conditions and characteristics of the loci are provided in Table 1. Tests for deviations from Hardy–Weinberg equilibrium (HWE) and for linkage

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Table 1 Details for 30 microsatellite loci developed for *Nanger granti*

Locus	Primer Sequence 5′– > 3′	Repeat motif	Size (bp)	N	*K*	H_o	H_e	PI	TD
Nagr2	F: *AGATTTCTGGAACTCTCTTGCC	TGC	240–264	24	3	0.125	0.119	0.78	TD 65
	R: AAATTGGCAACATCCACTGC								
Nagr5	F: *TTTAGCTTGAATTAAACCTGCTGC	AAAG	169–181	24	3	0.458	0.500	0.3	TD 65
	R: AATCCCATGAACGGAGATGC								
Nagr6	F: *GCAAATTCTTTACCGCTGGC	TGC	234–243	24	2	0.458	0.478	0.39	TD 65
	R: AATTTGACGGGCAAACAACC								
Nagr8	F: *TTTCTTTCAGTTCATTCATAGGGC	TGC	139–160	24	4	0.375	0.647	0.19	TD 65
	R: ACCTGGTGAGCTGCTATGGG								
Nagr12	F: *TCTTGTTTCTGTGCTTACATTGG	TGC	285–351	23	9	0.782	0.827	0.52	TD 65
	R: AGCTCTTCTGCTTTCCAGCC								
Nagr13	F: *AGTCGCATAGGGTCGGACG	TGC	248–266	24	3	0.292	0.254	0.58	TD 65
	R: ATTCTGGGTTCTGGAGACGC								
Nagr17	F: *CCACAGGACTATCCCATAAGGC	ATGG	208–212	23	2	0.217	0.364	0.47	TD 65
	R: CATTCACCTACTCAACCCACCC								
Nagr18†	F: *AAATTAGAATTTCTGTAGCTATGGCG	ATCT	245–265	24	5	0.375	0.753	0.10	TD 65
	R: CCCTCTATGATGAGGCACTCC								
Nagr20	F: *CTTCGAAAGAGTTCTCTCTGATGC	AATAG	200–210	24	3	0.375	0.362	0.46	TD 65
	R: CTGGAATTTCACACCCGTCC								
Nagr21	F: *TTGTTCTTCTATTTGGTATCCTATTGC	TTC	245–444	24	5	0.333	0.644	0.19	TD 65
	R: GAACAGAAACCTGTCCCTTGG								
Nagr22†	F: *CGACATCTTTGTTCTTCTGTAGTGG	TCTG	170–227	24	5	0.500	0.681	0.15	TD 65
	R: GAGACATGGCTTCTATCCCTGG								
Nagr23	F: *GGTTCAATGCCCTCCTCTGG	TTCC	259–283	24	4	0.458	0.609	0.22	TD 65
	R: GAGCCTGTCCCTACAGATCCC								
Nagr24	F: *CCCTCCTGAAATGTCCTCC	TCTG	237	24	1	0.000	0.000	0.10	TD 65
	R: GATATTTACTTTGGCACCCTTGG								
Nagr26	F: *CAACTTCCTATACAGATCCTCTGTTACC	ATGG	211–219	24	2	0.208	0.187	0.68	TD 65
	R: GCTCATTGTTTCTCCCATAAAGG								
Nagr27	F: *CACAGAAGACAGAGATGTTAAGTGC	TTC	207–261	23	10	0.870	0.846	0.40	TD 65
	R: GGAAACCCTTCTGCTTACTAGC								
Nagr28	F: *CCACCTGCCATGAAAGTTCC	ATGG	297	24	1	0.000	0.000	0.10	TD 65
	R: GGCTTTGGTAAGTATTGGGTGG								
Nagr29	F: *TCACCATGCTGCCTTAGACC	AAC	253-265	24	3	0.625	0.565	0.28	TD 65
	R: GGGAGCTTTGAAATCTAAAGAGG								
Nagr30	F: *AAGAAACAGGAGTTCAAATATGGG	AAGT	114–218	24	4	0.375	0.574	0.26	TD 65
	R: GGGTCCCAAAGAGTAGGGC								
Nagr31	F: *CTGCTGTTCCTGTGAGGGC	ATGG	206–214	24	2	0.375	0.353	0.48	TD 65
	R: GGTAGAGACATTTAGGGTTAGCTAGGG								
Nagr32	F: *TTAGACAGGCATCAATCTCTGC	ATC	193–203	24	4	0.333	0.640	0.19	TD 65
	R: GGTATAGAGCAAGCACTTAACTCG								
Nagr33	F: *CAGACTGAAGTCTTTCCCACCC	AAC	402–417	24	4	0.625	0.666	0.18	TD 65
	R: GGTCTCCCAGATAGCATCCC								
Nagr35	F: *GGTTTGCACAGAGTAGGACACG	TGC	233–263	23	4	0.565	0.575	0.24	TD 65
	R: GTGCTGTGATTTGTTGCTGC								
Nagr36	F: *TTCTTTCTTCCTATCTGCCTGC	ATCT	203–223	24	4	0.542	0.532	0.32	TD 65
	R: GTTTGGCCTCCAAATAGAGC								
Nagr38†	F: *CTTTATAGCAAGTCCACCGAAGG	TGC	271–305	24	4	0.125	0.666	0.18	TD 65
	R: TCCTAGGTTGCAGTCCCTGG								

Table 1 continued

Locus	Primer Sequence 5′– > 3′	Repeat motif	Size (bp)	N	*K*	H_o	H_e	PI	TD
Nagr40†	F: *AAGGGTATGTTGTGCCGC	TGC	255–276	23	4	0.261	0.598	0.23	TD 65
	R: TCTGCTGCTATTTGGATATTTAGAGG								
Nagr41	F: *CCACAAACAGTCAGGCACG	AAAG	220–244	24	4	0.833	0.727	0.12	TD 65
	R: TCTTAACTGTTACTGCCTTCATCTCC								
Nagr42	F: *TGGGAAGAGAGTGTGGATGC	ATGG	304–348	24	4	0.417	0.563	0.25	TD 65
	R: TCTTTGGTAGATGAAGAGATACATGG								
Nagr44	F: *TGCTATGTGTTTGACATTGTGC	ATGG	374–390	19	4	0.632	0.691	0.15	TD 65
	R: TGTTGAGAACTGGCTAATGACG								
Nagr45†	F: *TATGCATGCTCAAGGTTGCC	AAAC	309–313	24	2	0.000	0.330	0.5	TD 65
	R: TTCCAGGTCCTACCTCCTAAGC								
Nagr48	F: *GGATGTGACTGAAACCCTGG	ATGG	354–362	24	3	0.583	0.617	0.22	TD 65
	R: TTTCTCATGGATTGCCTCCC								

The size indicates the range of observed alleles in base pairs and includes the length of the CAG tag; number of individuals genotyped is *N*, *k* is number of alleles observed, H_o and H_e are observed and expected heterozygosity, respectively, *PI* is the probability of identity for each locus, and *TD* refers to the touchdown protocol used for PCR

* indicates CAG tag (5′-CAGTCGGGCGTCATCA-3′) label

† indicates significant deviations from Hardy–Weinberg expectations after Bonferroni correction

disequilibrium were conducted using GENEPOP v4.0 (Rousset 2008). After Bonferroni correction, five loci showed significant deviations from HWE. No linkage disequilibrium was detected. These new loci add to eight microsatellites described previously for *N. granti* (Huebinger et al. 2006).

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