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Full Length Research Paper

Polymorphic microsatellite markers for genetic studies of African antelope species

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Many wild animal species lack informative genetic markers for analysing genetic variation and structure, which is essential for effective long term conservation and management. We present heterologous microsatellite markers in six Tanzanian antelope species including: grant's gazelle, hartebeest, eland, roan, impala and topi. Thirty eight primer pairs from cattle, sheep, goat and wildebeest were tested. Thirty three revealed polymorphisms in one or more of the six antelope species. Six were polymorphic across all tested species, providing evidence for high genetic variability across species. These sets of microsatellites are of particular usage in population genetic analyses of antelope species.

Key words: Antelopes, genetic study, microsatellites, Tanzania.

INTRODUCTION

In Tanzania, an increasing habitat loss and fragmentation is becoming a major conservation concern for many wildlife species (Bolger et al., 2008; Voeten et al., 2009). Studies have shown that wildlife is declining both inside and outside the National Parks (Caro et al., 1998; Caro and Scholte 2007; Stoner et al., 2007). The causes of these declines are principally anthropogenic, resulting from growth of human population, coupled with intensified agriculture and active elimination of wildlife on adjacent lands (Serneels and Lambin, 2001; Caro and Scholte, 2007; Bolger et al., 2008; Voeten et al., 2009). Consequently, many protected areas in Tanzania are rapidly becoming isolated, which may disrupt or prevent dispersal and gene flow (Kuehn et al., 2007; Heller et al., 2010). Small isolated populations are associated with increased genetic drift, inbreeding and loss of adaptive genetic variation (Soulé and Mills, 1992; Amos and Harwood, 1998; Keller and Waller, 2002), which calls for increased effort through genetic conservation programs.

Establishment of conservation programs requires an identification of any grouping of a species into distinct populations and an understanding of their genetic differences. However, the analysis of the genetic diversity and structure of natural populations requires availability of genetic maker systems with a relatively high degree of variability, to be able to resolve differences between individuals and populations (Røed and Midthjell, 1998). Microsatellites have been described as ideal makers for this purpose. These markers are highly polymorphic, codominantly inherited, abundant throughout the genome, and are thus widely used in population genetics and conservation studies (Tautz, 1989; Vial et al., 2003; DeYoung and Honeycutt, 2005). Although having species-specific primers for genetic studies may be the most accurate technique (Cosse et al., 2007), the characterization of species-specific primers is relatively laborious and expensive, involving cloning and sequencing (Ostrander et al., 1992; Galan et al., 2003). Microsatellite flanking sequences offer, however, conserved regions across closely related species and even across families, an advantage that has been utilized

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by several cross-species amplification studies (Røed, 1998; Lorenzini, 2005; Lorenzen et al., 2006). Antelope species are the most abundant among wild ungulate species in Tanzania and the objective of this study was to develop a panel of highly polymorphic microsatellites by the use of cross-species primers that can be used to efficiently study the genetic structure of the Tanzanian antelopes: Grant's gazelle (*Nanger granti)*, hartebeest (*Alcelaphus buselaphus)*, eland (*Taurotragus oryx)*, roan antelope (*Hippotragus equines)*, impala (*Aepyceros melampus)* and topi (*Damaliscus lunatus*).

MATERIALS AND METHODS

Study area and samples collection

Serengeti Ecosystem (SE) is a geographical region located in northern Tanzania between 34° 45' to 35° 50' E and 2° to 3° 20' S, often defined by high diversity of wildlife and seasonal ungulates migrations. The ecosystem mainly includes the Serengeti National Park (SNP), the Ngorongoro Conservation Area (NCA), the Maswa Game Reserves (MGR) and surrounding game controlled areas which act as important buffer zones between human and wildlife. Peripheral blood samples were obtained from 15 individuals of each of Grant's gazelle, hartebeest, eland, impala and topi from Serengeti National Park (SNP) after immobilization using etorphine hydrochloride in combination with xylazine hydrochloride. Immobilization was done by a qualified veterinarian during park routine procedures, taking into consideration the animals' welfare. Eight roan muscle samples were obtained from hunted animals in the MGR. Prior to laboratory procedures, blood samples were frozen in EDTA tubes, while muscle samples were stored in absolute ethanol at room temperature.

Laboratory procedures and data analysis

Genomic DNA was isolated using Qiagen®, DNeasy Blood and Tissue kit (Qiagen GmbH, Germany) following the manufacture's instruction. Samples were genotyped using 38 microsatellites primer pairs, 16 from bovine, six from ovine/caprine and 16 from blue wildebeest. The bovine/ovine/caprine primers were selected from previous successful cross-species amplification studies, while the wildebeest primers were novel. PCR assays were performed in a final volume of 10 µl at approximately 30 to 40 *ng* genomic DNA template, 2.3 µl of 10 x buffer, 1 µl dNTP, 2 pm of each primer, 50 mM KCl, 1.5 mM $MgCl_2$, 10 mM Tris-HCl and 0.5 U of *Taq* polymerase (Amplicon).

Amplification was done in Gene Amp® PCR 9700 system (Applied Biosystems), with annealing temperatures varying between 56 and 58°C to suit individual primers. Control samples (species specific for the primers used) were included in each PCR run. Forward primers were fluorescently labeled for electrophoresis on an ABI 3100 DNA (Applied Biosystems) sequencer. Commercially prepared size standard (ROX GENESCAN® 400HD) was run with every sample. Alleles were scored using GeneMapper v3.7 (ABI 3100, Applied Biosystems), and new PCR's were performed for samples where genotypes were unclear. Furthermore, 10% of all the samples were selected at random and PCR and electrophoresis on ABI 3100 processes was repeated to verify the consistence of genotype scoring. Genetic diversity was estimated from the mean number of alleles per locus, allelic richness, observed and expected heterozygosity (Nei, 1978) using FSTAT 2.9.3 (Goudet, 2001). Deviations from Hardy-Weinberg equilibrium (HWE) were assessed using GENEPOP v4.0 (Rousset, 2008). The demenorization number was 10,000 and the number of batches was 5,000. The significance level was sequentially Bonferroni adjusted for repeated tests (Rice, 1989). We used Micro-Checker 2.2.3 (Van Oosterhout et al., 2004) to test for null alleles, scoring errors and allele drop-outs. Only readable and polymorphic loci were considered during analyses.

RESULTS AND DISCUSSION

Of the 38 microsatellite primers tested, 33 were amplified alleles of the expected size range and were polymorphic on at least one of the six antelope species tested. Six microsatellites revealed polymorphism across all tested antelope species (Table 1). Four wildebeest primer pairs (CT08, CT14, CT18 and CT30) and one bovine primer pair (BM4208) were not polymorphic in any of the antelope species tested, though amplified products were present in the control samples which were DNA from wildebeest and cattle. The percentage of polymorphic loci were relatively high in topi and hartebeest (71.1%), medium in eland (52.6%), roan (52.6%) and impala (47.4%) and low in grant gazelle (26.3%). Mean number of alleles per locus, observed and expected heterozygosity are given in Table 1. After Bonferroni adjustment, none of the loci showed deviation from HWE in topi, roan and grant gazelle, however two loci in eland and impala, three in hartebeest retained significant values (Table 1), and were generally due to an excess of homozygotes.

The use of highly polymorphic microsatellites loci has greatly increased the potential for understanding population structure of species across the landscape. Despite the relatively low sample size, our results showed that the amount of genetic diversity was substantially high for all the six species analysed. Six loci (BM804, ETH10, BM1009, MCM38, BM2113 and TGLA122) were polymorphic across all tested species and five additional loci were polymorphic in five of the six species analyzed (BM1824, BMC3224, MAF209, MCM58 and SPS115). These sets of primers have proven to have sufficient levels of polymorphism and heterozygosity and they provide enough power to analyse population structure of antelope species in Tanzania.

None of the wildebeest primers showed cross-species amplification success across all species despite the closer taxonomic relationship of the analyzed antelopes towards the wildebeest as compared with the bovine, ovine and caprine. This could be due to the fact that the wildebeest primers used in this study are novel (Røed et al., 2011) and have not so far been tested in any cross-species amplifications, while most bovine/ovine/caprine primers used here were not randomly chosen, but have proven to be useful also in other cross-species amplification studies (Vaiman et al., 1996; Lorenzen and Siegismund, 2004; Lorenzini, 2005; Lorenzen et al., 2006; Cosse et al., 2007). Since species specific microsatellites for many wild animals are lacking, and the cost of cloning and sequencing is relatively high,

Table 1. Polymorphisms of microsatellite loci in the six antelope species including marker name, number of alleles per locus (Na), observed (Ho) and expected (He) heterozygosity and their mean values for the polymorphic loci. The number of individuals is given in brackets.

Marker	Topi (15)			Eland (15)			Hartebeest (15)			Grant Gazelle (15)			Impala (15)			Roan (8)		
	Na	Ho	He	Na	Ho	He	Na	Ho	He	Na	Ho	He	Na	Ho	He	Na	Ho	He
BM804††	8	0.867	0.802	11	0.786	0.849	6	0.786	0.728	2	0.533	0.497	3	0.133	0.301	5	0.500	0.533
BM203††	10	0.933	0.880	11	0.667	0.922*	10	0.867	0.768	-	-	-	3	0.267	0.246	3	0.571	0.615
BM1824††	8	0.667	0.749	4	0.133	0.193	8	0.733	0.814*	-	-	-	3	0.133	0.297	3	0.125	0.242
BM757††	2	0.133	0.129	-	-	-	6	0.857	0.735	-	-	-	8	0.800	0.834	-	-	-
BM1009††	2	0.133	0.239	8	0.800	0.830	2	0.667	0.522	4	0.733	0.706	5	0.733	0.729	6	0.875	0.850
BM2113††	8	0.733	0.772	3	0.133	0.131	5	0.600	0.618	4	0.667	0.710	4	0.400	0.352	8	0.875	0.858
BMC3224§	8	0.867	0.832	11	0.933	0.890	11	0.933	0.906	5	0.533	0.637	4	0.667	0.616	-	-	-
BM1818††	-	-	-	10	0.750	0.909	4	0.900	0.600	-	-	-	-	-	-	-	-	-
BM4107††	-	-	-	9	0.667	0.830	-	-	-	-	-	-	4	0.933	0.756	6	0.875	0.833
ETH10‡‡	3	0.600	0.577	8	0.867	0.832	7	0.600	0.717	5	0.692	0.766	7	0.600	0.747	4	0.665	0.783
INRA23¥	8	1.000	0.837	9	0.600	0.779	6	0.786	0.836	3	0.133	0.131	-	-	-	-	-	-
TGLA53†	13	0.867	0.936	-	-	-	6	0.867	0.759	7	0.867	0.782	3	0.600	0.618	2	0.375	0.325
TGLA122†	9	0.867	0.890	13	1.000	0.940	2	0.200	0.186	5	0.857	0.791	6	0.667	0.692	7	0.750	0.892
CSSM003‡	8	1.000	0.858	-	-	-	8	0.800	0.768	-	-	-	-	-	-	5	0.714	0.791
CSSM066**	2	0.071	0.071	-	-	-	4	0.500	0.492	6	0.933	0.763	-	-	-	7	0.500	0.775
SPS115§§	7	0.600	0.818	6	0.929	0.852	8	0.867	0.860	-	-	-	3	0.643	0.685	3	0.500	0.508
MCM58¥¥	7	0.667	0.823	7	0.933	0.747	6	0.867	0.798	-	-	-	8	0.667	0.834	6	0.750	0.792
MCM38¥¥	10	0.867	0.917	9	1.000	0.902	8	0.933	0.825	4	0.733	0.692	5	0.571	0.696	5	0.625	0.792
MCM104#	-	-	-	-	-	-	5	0.750	0.761	-	-	-	-	-	-	-	-	-
MAF209Ŧ	4	0.867	0.674	5	0.600	0.766	7	0.800	0.830	-	-	-	3	0.357	0.370	3	0.375	0.425
OarFCB48Ŧ	7	0.692	0.855	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
CT02	8	0.467	0.660	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
CT03	-	-	-	10	0.667	0.903*	-	-	-	-	-	-	-	-	-	-	-	-
CT07	10	0.800	0.871	-	-	-	6	0.733	0.644	-	-	-	-	-	-	9	0.750	0.917
CT10	8	0.733	0.669	5	0.533	0.497	-	-	-	-	-	-	4	0.692	0.711	5	0.625	0.775
CT12	2	0.133	0.129	-	-	-	7	0.467	0.738*	-	-	-	-	-	-	7	0.750	0.867
CT13	8	0.867	0.864	-	-	-	5	0.467	0.713	-	-	-	-	-	-	-	-	-
CT17	7	0.615	0.849	9	0.733	0.846	7	0.692	0.818	-	-	-	-	-	-	3	0.375	0.625
CT19	-	-	-	-	-	-	3	0.143	0.624	-	-	-	-	-	-	-	-	-
CT21	-	-	-	9	0.643	0.847	7	0.429	0.751*	-	-	-	-	-	-	-	-	-
CT23	8	0.933	0.874	-	-	-	3	0.357	0.537	-	-	-	4	0.467	0.609	-	-	-
CT25	8	0.417	0.855*	-	-	-	8	0.385	0.474	-	-	-	5	0.643	0.701*	5	0.625	0.767
CT27	9	0.733	0.846	5	0.429	0.709	-	-	-	-	-	-	-	-	-	-	-	-
Mean	7.11	0.671	0.718	8.10	0.690	0.759	6.11	0.666	0.698	4.50	0.668	0.648	4.56	0.554	0.599	5.10	0.610	0.698

Table 1 Contd.

SD	2.8	0.28	0.25	2.7	0.25	0.23	2.2	0.23	0.15	1.4	0.23	0.20	1.7	0.22	0.19	1.9	0.19	0.19

*Deviation from HWE. ††, Bishop et al. (1994); §, Kappes et al. (1997); ‡‡, Solinas-Toldo et al. (1993); ¥, Vaiman et al. (1994); †, Georges and Massey (1992); ‡ Moore et al. (1994); **, Barendse et al. (1994); §§, Moore and Byrne (1993); ¥¥, Hulme et al. (1994); #, Smith et al. (1995); ₣, Buchanan et al. (1992); all CT primers: Røed et al. (2011).

the use of heterologous primer pairs may be a cost effective alternative in conservation management programs specifically in many developing regions.

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REFERENCES

Amos W, Harwood J (1998). Factors affecting levels of genetic diversity in natural populations. Philos. Trans. R. Soc. Lond. B Biol. Sci., 353: 177-186.

Bolger DT, Newmark WD, Morrison TA, Doak DF (2008). The need for integrative approaches to understand and conserve migratory ungulates. Ecol. Lett., 11: 63-77.

Caro T, Scholte P (2007). When protection falters. Afr. J. Ecol., 45: 233-235.

Caro TM, Pelkey N, Borner M, Campbell KLI, Woodworth BL, Farm BP, OleKuwai J, Huish SA, Severre ELM (1998). Consequences of different forms of conservation for large mammals in Tanzania: preliminary analyses. Afr. J. Ecol., 36: 303-320.

Cosse M, González S, Maldonado JE (2007). Cross-amplification tests of ungulate primers in the endangered Neotropical pampas deer (*Ozotoceros bezoarticus*). Genet. Mol. Res., 6: 1118-1122.

DeYoung RW, Honeycutt RL (2005). The molecular toolbox: genetic techniques in wildlife ecology and management. J. Wildl. Mgmt., 69: 1362–1384.

Galan M, Cosson JF, Aulagnier S, Maillard JC, Thévenon S, Hewison JM (2003). Cross-amplification tests of ungulate primers in roe deer (*Capreolus capreolus*) to develop a multiplex panel of 12 microsatellite loci. Mol. Ecol. Notes., 3: 142–146.

Goudet J (2001). FSTAT, a program to estimate and test gene diversities and fixation indices. Release 2.9.3.2. Available from http://www.unil.ch/izea/softwares/fstat.html

Heller R, Okello JBA, Siegismund H (2010). Can small wildlife conservancies maintain genetically stable populations of large mammals? Evidence for increased genetic drift in geographically restricted populations of Cape buffalo in East Africa. Mol. Ecol., 19: 1324-1334.

Keller LF, Waller DM (2002). Inbreeding effects in wild populations. Trends Ecol. Evol., 17: 230-241.

Kuehn R, Hindenlang KE, Holzgang O, Senn J, Stoeckle B, Sperisen C (2007). Genetic Effect of Transportation Infrastructure on Roe Deer Populations (*Capreolus capreolus*). J. Hered., 98: 13–22.

Lorenzen ED, Arctander P, Siegismund HR (2006). Regional Genetic Structuring and Evolutionary History of the Impala *Aepyceros melampus.* J. Hered., 97: 119–132.

Lorenzen ED, Siegismund HR (2004). No suggestion of hybridization between the vulnerable black-faced impala (*Aepyceros melampus petersi*) and the common impala (*A. m. melampus*) in Etosha National Park, Namibia. Mol. Ecol., 13: 3007–3019.

Lorenzini R (2005). A panel of polymorphic microsatellites in the threatened Apennine chamois (*Rupicapra pyrenaica ornata*). Mol. Ecol. Notes, 5: 372–374.

Marshall TC, Slate J, Kruuk LEB, Pemberton JM (1998). Statistical confidence for likelihood-based paternity inference in natural populations. Mol. Ecol., 7: 639-655.

Nei M (1978). Estimation of average heterozygosity and genetic distance from a small number of individuals. Genetics 155: 583–590.

Ostrander EA, Jong PM, Rine J, Duyk G (1992). Construction of small-insert genomic DNA libraries highly enriched for microsatellite repeat sequences. Proc. Nat. Acad. Sci. USA., 89: 3419-3423.

Rice WR (1989). Analyzing tables of statistical tests. Evolution. 43: 223-225.

Røed KH (1998). Microsatellite variation in Scandinavian Cervidae using primers derived from Bovidae. Hereditas., 129: 19-25.

Røed KH, Ernest EM, Midthjell L, Msoffe PLM (2011). Identification and characterization of 17 microsatellite loci in the blue wildebeest, *Connochaetes taurinus*. Conserv. Genet. Resour., 3: 181-183.

Røed KH, Midthjell L (1998). Microsatellites in reindeer, *Rangifer tarandus*, and their use in other cervids. Mol. Ecol. 7: 1771-1788.

Rousset F (2008). GenePop'007: a complete re-implementation of the GenePop software for Windows and Linux. Mol. Ecol. Resour., 8: 103–106.

Serneels S, Lambin E (2001). Impact of land-use changes on the wildebeest migration in the northern part of the Serengeti-Mara ecosystem. J. Biogeogr., 28: 391–407.

Soulé ME, Mills S (1992). Conservation genetics and conservation biology: a troubled marriage. In: *Conservation of biodiversity for sustainable development*. (Eds. OT Sandlund, K Hindarand and AHD Brown). Scandinavian University Press, Oslo, pp. 55-69.

Stoner CJ, Caro T, Mduma S, Mlingwa C, Sabuni G, Borner M (2007). Assessment of effectiveness of protection strategies in Tanzania based on a decade of survey data for large herbivores. Conserv. Biol., 21: 635–646.

Tautz D (1989). Hypervariability of simple sequences as a general source for polymorphic DNA markers. Nucleic Acids Res., 17: 6463-6471.

Vaiman D, Schibler L, Bourgeois F, Oustry A, Amigues Y, Cribiu EP (1996). A genetic linkage map of the male goat genome. Genetics., 144: 279-305.

Van Oosterhout C, Hutchinson WF, Wills DP, Shipley P (2004). MICRO-CHECKER: software for identifying and correcting genotyping errors in microsatellite data. Mol. Ecol. Notes, 4: 535–538.

Vial L, Maudet C, Luikart G (2003). Thirty-four microsatellites for European roe deer. Mol. Ecol. Notes, 3: 523-527.

Voeten MM, Van de Vijver CADM, Olff H, Van Langevelde F (2009). Possible causes of decreasing migratory ungulate populations in an East African savannah after restrictions in their seasonal movements. Afr. J. Ecol., 48: 169-179.