Eur J Wildl Res
DOI 10.1007/s10344-014-0880-6

SHORT COMMUNICATION

First estimates of genetic diversity for the highly endangered giant sable antelope using a set of 57 microsatellites

Pedro Vaz Pinto · Susana Lopes · Sofia Mourão · Sendi Baptista · Hans R. Siegismund · Bettine Jansen van Vuuren · Pedro Beja · Nuno Ferrand · Raquel Godinho

Received: 10 September 2014 / Revised: 21 November 2014 / Accepted: 25 November 2014

Abstract Confined to a small region in central Angola, the giant sable antelope (*Hippotragus niger variani*) experienced a dramatic decline in numbers and is currently one of the most endangered African mammals. In spite of its iconic status, conservation efforts have been hindered by unsustainable hunting and lack of adequate tools to promote its recovery. In this work, we developed a set of 57 microsatellites specific for the giant sable, which revealed depleted levels of genetic diversity and an allele frequency spectrum consistent with a recent evolutionary history characterized by severe population crashes. In contrast, the high number of private alleles exhibited by other *H. niger* populations from Zimbabwe and Tanzania may suggest the occurrence of reduced levels of gene flow among sable populations. Our microsatellite panel was successfully tested on the roan antelope, *Hippotragus equinus*, and will prove highly applicable on the characterization of different *Hippotragus* populations, but in particular for the conservation of the Angolan giant sable antelope.

Keywords Nuclear markers · Bottleneck · Conservation genetics · Angola · *Hippotragus niger variani* · *Hippotragus equinus*

Communicated by C. Gortázar

Electronic supplementary material The online version of this article (doi:10.1007/s10344-014-0880-6) contains supplementary material, which is available to authorized users.

P. Vaz Pinto (✉) · S. Lopes · S. Mourão · P. Beja · N. Ferrand · R. Godinho (✉)
CIBIO/InBio—Centro de Investigação em Biodiversidade e Recursos Genéticos, Universidade do Porto, Campus Agrário de Vairão, 4485-661 Vairão, Portugal
e-mail: pedrovazpinto@gmail.com
e-mail: rgodinho@cibio.up.pt

P. Vaz Pinto · P. Beja · N. Ferrand · R. Godinho
Departamento de Biologia, Faculdade de Ciências, Universidade do Porto, Rua do Campo Alegre s/n, 4169-007 Porto, Portugal

P. Vaz Pinto · P. Beja · N. Ferrand · R. Godinho
ISCED—Instituto Superior de Ciências da Educação da Huíla, Rua Sarmento Rodrigues, Lubango, Angola

P. Vaz Pinto · S. Baptista
The Kissama Foundation, Rua Joaquim Capango n°49, 1°D, Luanda, Angola

H. R. Siegismund
Department of Biology, University of Copenhagen, Ole Maaløes Vej 5, 2200 Copenhagen N, Denmark

P. Vaz Pinto · B. Jansen van Vuuren · N. Ferrand · R. Godinho
Department of Zoology, Faculty of Sciences, University of Johannesburg, Auckland Park 2006, South Africa

Introduction

The giant sable *Hippotragus niger variani* is an emblematic antelope restricted to an isolated population in central Angola and critically endangered (IUCN 2008). It is characterized by a dark facial mask, retention of brown hocks in bulls, and much longer sweeping horns that can grow on average ca. 30 cm longer than in other sable populations (Estes 2013). The giant sable was feared extinct until a combined effort with camera traps and molecular tools led to its rediscovery (Pitra et al. 2006) but remains on the brink of extinction and reduced to a few dozen individuals.

The sable species *H. niger* ranges from coastal Kenya to southern Africa and is often sympatric with the congeneric roan antelope *Hippotragus equinus*, although the latter has a wider distribution extending to the savannas of western Africa (East 1999). Naturally occurring in low densities, both species are rare outside managed areas (East 1999). In recent years, the sable in particular has become one of the most highly prized trophy hunting species, boosting its commercial value

Published online: 08 January 2015

Table 1 Genetic diversity measures for three populations of *Hippotragus niger* and one of *H. equinus* based on 57 and 54 microsatellite loci, respectively (three loci did not amplify for *H. equinus*)

	Number of samples	H_O	H_E	NA	NPA	F_{IS}
H. niger						
Angola	20	0.315 (0.038)	0.306 (0.035)	2.3 (0.2)	9	−0.023 (0.024)
Tanzania	20	0.485 (0.038)	0.524 (0.033)	4.4 (0.3)	54	0.081 (0.035)
Zimbabwe	20	0.474 (0.036)	0.489 (0.035)	4.2 (0.3)	53	0.023 (0.028)
H. equinus						
Namibia/S. Africa	20	0.359 (0.041)	0.407 (0.044)	4.5 (0.5)	145	0.106 (0.026)

Standard error values are given in parenthesis

H_O observed heterozygosity, H_E expected heterozygosity, *NA* mean number of alleles per locus, *NPA* number of private alleles, F_{IS} inbreeding coefficient

and leading to widespread introductions on private land in southern Africa (East 1999; Bothma and Van Royen 2005).

In spite of the conservation importance and significant investment channeled for intensive conservation programs dedicated to both species, including breeding efforts, genetic research has, until now, mainly relied on mitochondrial DNA (mtDNA) analysis. Matthee and Robinson (1999) provided a first comprehensive mtDNA phylogeography of sable and roan antelopes, and subsequently the genetic structuring of *H. niger* was further refined and introgression events were inferred from populations in eastern Africa (Pitra et al. 2002). More recent mtDNA studies have focused specifically on the giant sable antelope and have established its monophyletic status (Pitra et al. 2006; Jansen van Vuuren et al. 2010).

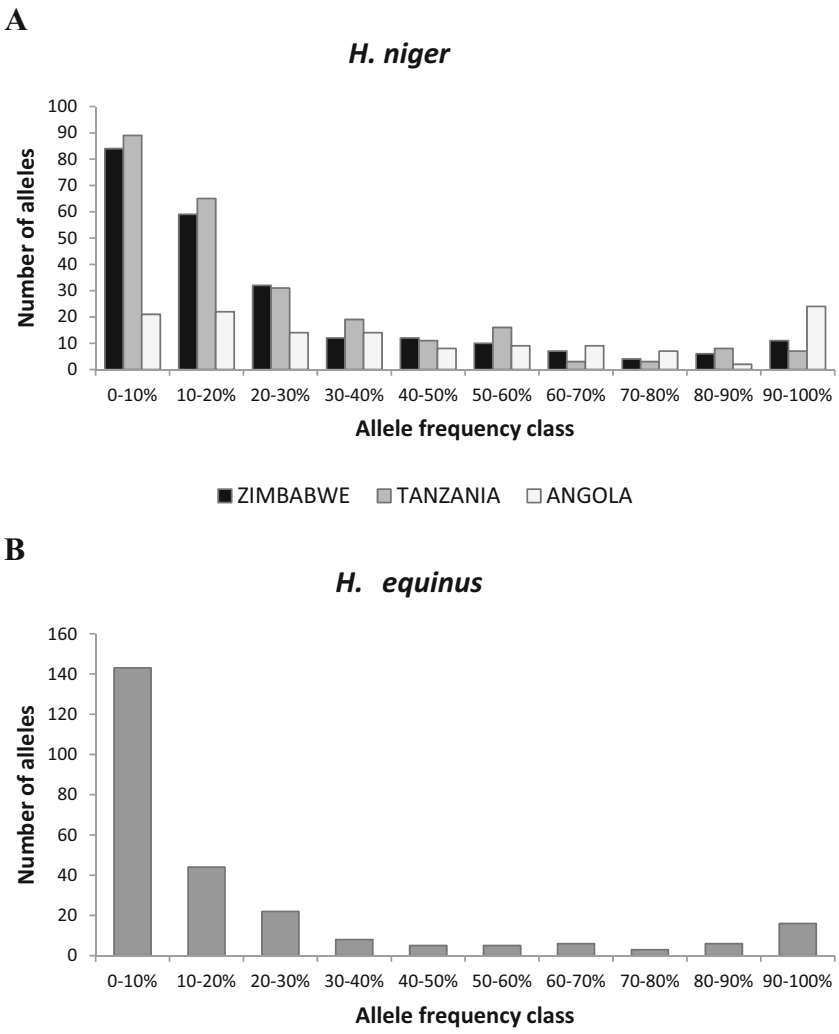

Fig. 1 Allele frequency spectrum (AFS) obtained from 57 and 54 microsatellite loci, respectively, for **a** the three *Hippotragus niger* populations and **b** the *H. equinus* sample

Notwithstanding this, the use of specific nuclear markers could be critical to understand intraspecific evolutionary relationships (Avise 1994; Godinho et al. 2008), to assist ongoing conservation initiatives (Frankham 2008) and to the implementation of breeding programs (Robert 2009) for both sable and roan antelope (Chardonnet and Crosmary 2013; Estes 2013). In particular, the giant sable antelope is currently being subjected to an intensive management program in situ (Vaz Pinto 2009; Estes 2013), and its conservation could benefit directly from the development of such novel molecular tools. Here, using next-generation sequencing, we developed and characterized 57 microsatellite markers for sable, and further tested them on roan antelope. In addition, we provide a first assessment of nuclear genetic diversity within the giant sable of Angola and compared it with two other conspecific populations, evaluating preliminary gene flow and demographic patterns.

Material and methods

We analyzed a total of 80 tissue samples, 20 from each of three presumably different populations of *H. niger*, collected in Angola, Tanzania, and Zimbabwe, plus 20 from *H. equinus* collected in Namibia and South Africa.

Total genomic DNA was isolated from a pool of ten *H. niger* individuals with different geographic origins using the QIAGEN DNeasy Blood & Tissue Kit and sent to Genoscreen, France, for microsatellite development through 454 GS-FLX Titanium pyrosequencing of enriched DNA libraries (Malausa et al. 2011). Total DNA was enriched for AG, AC, AAC, AAG, AGG, ACG, ACAT, and ATCT repeat motifs. Briefly, GS-FLX libraries were constructed following manufacturer's protocols (Roche Diagnostics) and sequenced on a GsFLX-PTP. The bioinformatics program QDD (Meglécz et al. 2010) was used to filter for redundancy, resulting in a final set of 8224 sequences from which 652 primers pairs were designed. Fifty-seven out of 80 tested loci had specific and reliable amplifications and were genotyped for the 80 samples in nine multiplex reactions using M13-primer genotyping protocol (Schuelke 2000) with four different dye-labeled tails and forward primer concentration of 1/10 of reverse and tail primers (Online Resource Table S1). PCR amplifications were conducted using the Multiplex PCR Kit (QIAGEN) following the manufacturer's instructions in a final 10-μl volume, always in the presence of a negative control. Annealing temperatures were adjusted to each multiplex (Table S1). Amplicons were separated by size on an ABI3130xl Genetic Analyser. Allele sizes were scored against the GeneScan500 LIZ Size Standard, using the GENEMAPPER 4.0 (Applied Biosystems) and manually checked twice independently.

We used GENALEX 6.501 (Peakall and Smouse 2012), to test for Hardy-Weinberg (HW) proportions, to estimate observed and expected heterozygosities (H_O and H_E) for all loci in each population and to calculate the mean number of alleles (NA) and the number of private alleles (NPA) within populations. GENEPOP 4.2 (Raymond and Rousset 1995) was used to test for genotypic linkage disequilibrium (GLD) among loci within populations. The presence of null alleles was tested using MICROCHECKER 2.2.3 (van Oosterhout et al. 2004). The distribution of allele frequencies at all loci was determined and visualized in a histogram with ten frequency classes as an allele frequency spectrum (AFS; Chakraborty et al. 1988). The shape of the AFS is influenced by long-term demography, being a recent population expansion detected by an excess of rare alleles, while a recent population collapse results in a deficit of rare alleles and an excess of common alleles (Maruyama and Fuerst 1985). This analysis was conducted to determine whether current levels of genetic diversity in the giant sable antelope may reflect a demographic history different from other sable populations.

Results

All 57 loci proved polymorphic for *H. niger*, but populations exhibited very different diversity patterns. While 51 and 52 polymorphic loci were observed in Zimbabwe and Tanzania, respectively, only 37 polymorphic loci were scored for the Angolan giant sable. The total number of alleles detected for all loci ranged from 2 to 16, and the expected heterozygosity ranged from 0.05 to 0.87 (Online Resource Table S2). We found no significant deviations from HW proportions after Bonferroni corrections, except at loci HN60 and HN101 in Tanzania, with an excess of detected homozygotes most likely due to the presence of null alleles (Online Resource Table S2). Only two significant GLD tests were observed after Bonferroni corrections in *H. niger* populations (Online Resource Table S3). As expected, the Angolan giant sable exhibited the lowest genetic diversity parameters (H_E=0.306; mean NA=2.3; NPA=9), compared to Tanzania (H_E=0.524; mean NA=4.4; NPA=54) and Zimbabwe (H_E=0.489; mean NA=4.2; NPA=53), which did not experience significant demographic events (Table 1).

The AFS chart produced typical L-shaped distributions for populations from Tanzania and Zimbabwe, consistent with populations in mutation-drift equilibrium (Fig. 1a). In contrast, the giant sable exhibits a rugged allelic distribution profile predictable in populations that have undergone recent bottlenecks (Fig. 1a).

For *H. equinus*, all but three loci (HN89, HN110, and HN116) were successfully amplified, and 41 loci proved polymorphic, with a total number of alleles ranging from 2 to 17 and expected heterozygosity between 0.05 and 0.91.

Loci HN11 and HN17 deviated from HW proportions, with an excess of homozygotes most likely due to the presence of null alleles (Online Resource Table S2). Significant GLD after Bonferroni corrections was detected for 17 pairs of loci (Online Resource Table S3). Average heterozygosity for the *H. equinus* population was 0.407, and the average number of alleles per locus was 4.5 (Table 1). The AFS for this species is a typical L-shaped distribution (Fig. 1b).

Discussion

Since 2009, the giant sable antelope is under a conservation program that includes breeding efforts aimed to rescue the remnant population in Angola. Our results clearly show a depletion of genetic diversity in this population relative to Tanzanian and Zimbabwean populations and highlight the utility of the large number of loci developed in this work for the implementation of more effective conservation measures. As expected, patterns of genetic diversity are consistent with documented observations of recent and severe bottlenecks and further reflected on the very distinct AFS exhibited by the giant sable. Our results are in line with the low levels of genetic diversity observed in other remnant and bottlenecked populations of African ungulates such as the black rhino (Harley et al. 2005), the dorcas gazelle (Lerp et al. 2011; Godinho et al. 2012), the dama gazelle (Senn et al. 2014), or the addax (Armstrong et al. 2011).

In contrast, much higher albeit similar genetic diversity values were found for the two other *H. niger* populations included in this study (Tanzania and Zimbabwe). Additionally, the number of private alleles found in these two populations is remarkably high, suggesting reduced levels of gene flow on a larger scale. This pattern is consistent with both the geographical distance between Tanzania and Zimbabwe and the fact that the two populations exhibit distinct mitochondrial clades (Jansen van Vuuren et al. 2010).

As no genus-specific nuclear markers were available to date for population genetic analyses within *Hippotragus* species (but see Alpers et al. 2004 and Eblate et al. 2011 for cross-genus amplification of microsatellites on roan antelope), this new panel can provide a decisive tool to be applied in evolutionary, conservation, and management practices. In particular, it may prove to be particularly useful to address relatedness and parentage analyses with direct application in breeding programs for these species. Furthermore, the fact that some of the surviving giant sables are being closely managed in semi-captivity (Vaz Pinto 2009; Estes 2013) offers a unique opportunity to apply these findings directly on the conservation of one of the most endangered and iconic African mammals.

Acknowledgments The Giant Sable Conservation Project is coordinated by the Ministry of Environment of the Republic of Angola, in collaboration with the Kissama Foundation. We thank Cardoso Bebeca, Joaquim Manuel, and General João Traguedo for support during the animal capture and handling, and the Angolan Air Force for heavy logistics made available. We also thank Bruce Fivaz for providing additional samples from Zimbabwe. We thank two anonymous reviewers for helpful comments on a previous version of this manuscript. The research was partly funded by the ExxonMobil Foundation. RG is supported by a IF2012 Research contract from FCT (Portuguese Science Foundation, IF/564/2012). This is scientific paper no. 1 from the Portuguese-Angolan TwinLab established between CIBIO/InBIO and ISCED/Huíla, Lubango.

Conflict of interest The authors declare that there are no conflicts of interest.

References

Alpers DL, Jansen van Vuuren B, Arctander P, Robinson TJ (2004) Population genetics of the roan antelope (*Hippotragus equinus*) with suggestions for conservation. Mol Ecol 13(7):1771–1784

Armstrong E, Leizagoyen C, Martinez AM, Gonzalez S, Delgado JV, Postiglioni A (2011) Genetic structure analysis of a highly inbred captive population of the African antelope *Addax nasomaculatus*. Conservation and management implications. Zoo Biol 30:399–411

Avise JC (1994) Molecular markers, natural history and evolution. Springer

Bothma JDP, Van Royen N (2005) Intensive wildlife production in Southern Africa. Van Schaik Publishers, Pretoria

Chakraborty R, Smouse PE, Neel JV (1988) Population amalgamation and genetic variation: observations on artificially agglomerated tribal populations of Central and South America. Am J Hum Genet 43:709–725

Chardonnet P, Crosmary W (2013) *Hippotragus equinus* Roan Antelope. In: Kingdon J, Happold D, Butynski T, Hoffmann M, Happold M, Kalina J (eds) Mammals of Africa. Bloomsbury, London, pp 548–556, Vol 6

East R (1999) African antelope database 1998. IUCN/SSC antelope specialist group. IUCN, Gland

Eblate EM, Lughano KJ, Sebastian CD, Peter ML, Knut RH (2011) Polymorphic microsatellite markers for genetic studies of African antelope species. Afr J Biotechnol 10:11817–11820

Estes RD (2013) *Hippotragus niger* sable antelope. In: Kingdon J, Happold D, Butynski T, Hoffmann M, Happold M, Kalina J (eds) Mammals of Africa. Bloomsbury, London, pp 556–565, Vol 6

Frankham R (2008) Genetic adaptation to captivity in species conservation programs. Mol Ecol 17:325–333

Godinho R, Abáigar T, Lopes S, Essalhi A, Ouragh L, Cano M, Ferrand N (2012) Conservation genetics of the endangered Dorcas gazelle (*Gazella dorcas* spp.) in Northwestern Africa. Conserv Genet 13:1003–1015

Godinho R, Crespo EG, Ferrand N (2008) The limits of mtDNA phylogeography: complex patterns of population history in a highly structured Iberian lizard are only revealed by the use of nuclear markers. Mol Ecol 17:4670–4683

Harley EH, Baumgarten I, Cunningham J, O'Ryan C (2005) Genetic variation and population structure in remnant populations of black rhinoceros, *Diceros bicornis*, in Africa. Mol Ecol 14:2981–2990

IUCN (2008). *Hippotragus niger ssp. variani*. SSC Antelope Specialist Group, The IUCN Red List of Threatened Species. Version 2014.2

Jansen van Vuuren B, Robinson TJ, Vaz Pinto P, Estes R, Matthee C (2010) Western Zambian sable: are they a geographic extension of the giant sable? S Afr J Wildl Res 40:35–42

Lerp H, Wronski T, Pfenninger M, Plath M (2011) A phylogeographic framework for the conservation of Saharan and Arabian Dorcas gazelles (Artiodactyla: Bovidae). Org Divers Evol 11:317–329

Malausa T, Gilles A, Meglécz E et al (2011) High-throughput microsatellite isolation through 454 GS-FLX Titanium pyrosequencing of enriched DNA libraries. Mol Ecol Resour 11:638–644

Maruyama T, Fuerst PA (1985) Population bottlenecks and nonequilibrium models in population genetics. II. Number of alleles in a small population that was formed by a recent bottleneck. Genetics 111: 675–689

Matthee CA, Robinson TJ (1999) Mitochondrial DNA population structure of roan and sable antelope: implications for the translocation and conservation of the species. Mol Ecol 8: 227–238

Meglécz E, Costedoat C, Dubut V et al (2010) QDD: a user-friendly program to select microsatellite markers and design primers from large sequencing projects. Bioinformatics 26:403–404

Peakall R, Smouse PE (2012) GenAlEx 6.5: genetic analysis in Excel. Population genetic software for teaching and research—an update. Bioinformatics 28:2537–2539

Pitra C, Hansen AJ, Lieckfeldt D, Arctander P (2002) An exceptional case of historical outbreeding in African sable antelope populations. Mol Ecol 11:1197–1208

Pitra C, Vaz Pinto P, O'Keeffe BW, Willows-Munro S, van Vuuren BJ, Robinson TJ (2006) DNA-led rediscovery of the giant sable antelope in Angola. Eur J Wildl Res 52:145–152

Raymond M, Rousset F (1995) GENEPOP (version 1.2): population genetics software for exact tests and ecumenicism. J Hered 86: 248–249

Robert A (2009) Captive breeding genetics and reintroduction success. Biol Conserv 142:2915–2922

Schuelke M (2000) An economic method for the fluorescent labelling of PCR fragments. Nat Biotechnol 18:233–234

Senn H, Banfield L, Wacher T et al (2014) Splitting or lumping? A conservation dilemma exemplified by the critically endangered Dama Gazelle (*Nanger dama*). PLoS One 9(6):e98693

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

Vaz Pinto P (2009) Giant sable rescue and translocation. Gnusletter 28(1): 8–10