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FULL LENGTH RESEARCH PAPER

Genetic diversity of the Ethiopian Grevy's zebra (*Equus grevyi)* populations that includes a unique population of the Alledeghi Plain

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Abstract

The endangered Grevy's Zebra (*Equus grevyi*) is confined to the Horn of Africa, specifically Ethiopia and Kenya. It is threatened by habitat loss and fragmentation due to human encroachment of historic range. Knowledge of population genetics is essential for the development of appropriate conservation actions and management. The focus of this study was to assess the heterogeneity and genetic distinctiveness of the two Grevy's zebra populations in Ethiopia. Non-invasive fecal samples ($N = 120$) were collected during 2009–2010 from Grevy's zebra populations in the Alledeghi Wildlife Reserve and the Sarite area, Ethiopia. Analyses of a 329 bp of the mtDNA control region of 47 sequences, revealed the existence of two unreported haplotypes in the northern population of Alledeghi, that were not shared with the southern population of Sarite. The Sarite population is contiguous with the Grevy's zebra population in Kenya. The nucleotide diversity levels found in both the populations are extremely low.

Keywords

Conservation, Ethiopia, genetic diversity, Grevy's zebra

History

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Introduction

Historically, the Grevy's zebra ranged from the Alledeghi Plain in northern Ethiopia, through the Awash River valley to the Ogaden to southwestern Somalia, to the northeast of Lake Turkana and south into northern Kenya (Bauer et al., 1994). Currently the Grevy's zebra has a discontinuous range (Figure 1). There is a small isolated population in the Alledeghi Plain in northern Ethiopia. In southern Ethiopia (~700 km from Alledeghi), there is a population that extends from Sarite/Chew Bahir into northern Kenya to Laikipia near Mt. Kenya (Figure 1). From 1980 to 2007, the global population of Grevy's Zebra declined by 68%. The current population is estimated to be approximately 2700 individuals (April 2012 Kenya Wildlife Service workshop), and this species is listed as Endangered by the IUCN Red List (Moehlman et al., 2008). In Ethiopia, the population decline was severe and went from an estimated 1900 in 1980 to approximately 128 in 2006 (Moehlman et al., 2008). The available suitable habitat has been dramatically reduced in Ethiopia due to increasing human populations, development of rangelands for agriculture and increasing competition for resources, particularly water, with local pastoral people and their livestock (Kebede et al., 2012).

Grevy's zebra is listed as Endangered on the IUCN Red List and is CITES Appendix I. However, poaching for meat and medicine continues to be a threat to Grevy's zebra survival (Kebede et al., 2012; Williams, 2002). In recent years, the global population has spread farther south in Kenya to the Laikipia Plateau which was not part of the historic range. This range shift resulted in a larger overlap in populations of Grevy's and Plains zebra and adds one more source of concern, the increased potential for hybridization in the Kenyan portion of the Grevy's zebra population. Hybridization between these species could compromise their genetic integrity (Cordingley et al., 2009). The isolated population of Grevy's zebra in the Alledeghi Plain of Ethiopia is sheltered from this problem, but still faces challenges such as inbreeding and genetic drift.

Basic knowledge of levels of genetic diversity in endangered species and their disparate populations is an essential step for determining conservation actions that are sensitive to units of conservation and appropriate management plans for the entire range of Grevy's zebra (Frankham et al., 2002; Moritz, 1994).

Collection of samples from natural populations using a non-invasive method is a great advantage when studying elusive, rare and/or endangered species (e.g. Beja-Pereira et al., 2009). However, there can be problems with analyzing such samples, i.e. fecals, due to the poor quality/quantity of the obtained DNA (Broquet et al., 2006; Taberlet et al., 1996).

We have investigated levels of genetic diversity in two populations of Grevy's zebra in Ethiopia using sequence analyses of a mtDNA control region fragment. We discuss the results in light of previous studies reporting levels and patterns of nucleotide (π) and haplotype (h) diversity in populations of Plains zebra (*Equus quagga*, Lorenzen et al., 2008) and Mountain zebra (*Equus zebra*, Moodley & Harley, 2005) and provide important first insights into genetic diversity of this endangered African equid.

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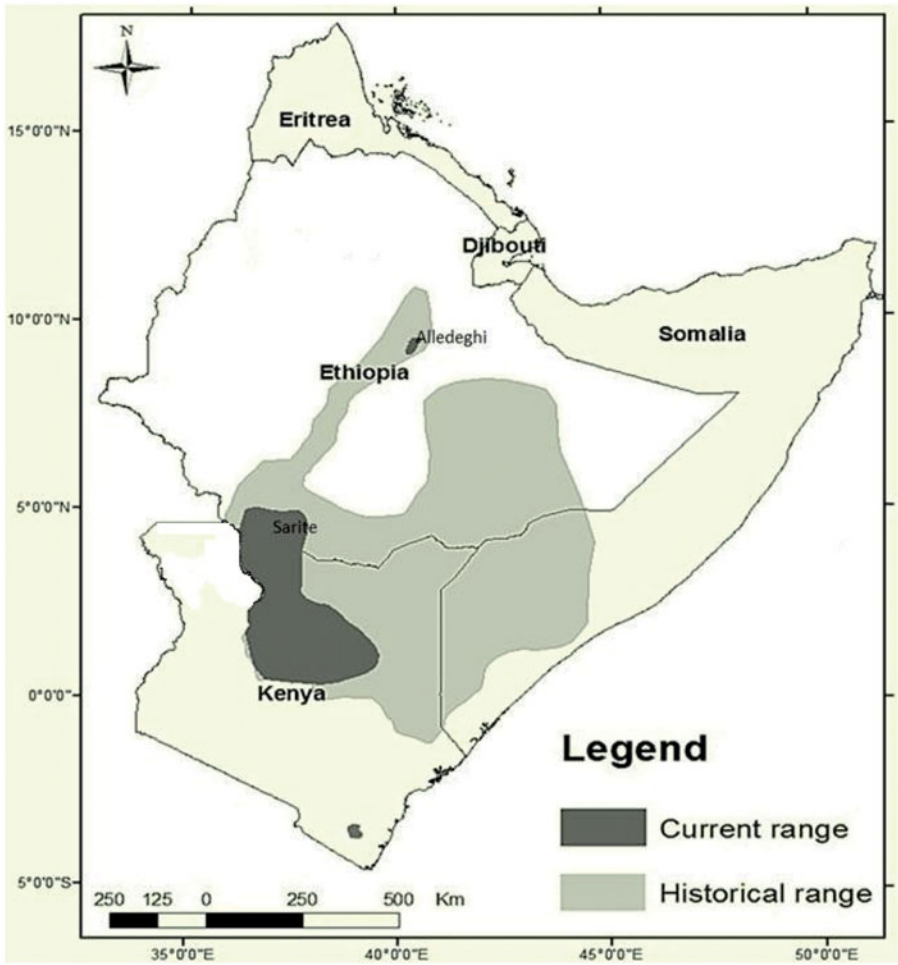


Figure 1. Historic and current distribution of Grevy's zebra (based on Bauer et al., 1994).

Materials and methods

A total of 120 non-invasive samples were collected from two sites in Ethiopia, i.e. Alledeghi ($n = 86$) and Sarite ($n = 34$) during the 2009 and 2010 wildlife surveys. Fecal samples were collected in the field and stored in individual brown paper bags with details on the collection site, time and date. The samples were dried naturally and stored at room temperature until further processing. A total of 82 individuals were selected for DNA extraction. In order to minimize potential contamination issues inherent to non-invasive samples, DNA extraction was carried on a laminar flux chamber, physically separated from the PCR room. The samples were processed in batches with a maximum of 16 samples per set. All the materials used for the extraction process was disinfected between samples processing and between all groups of samples as well. In each batch of samples, a negative control containing all reagents but not the sample was included to detect contaminations. The fecal samples were processed for DNA extraction using JETquick Tissue DNA Spin Column (Genomed; Bad Oeynhausen, Germany). For each sample, the external part, containing the intestinal epithelial cells, was removed to a falcon tube. Standard lysis buffer and proteinase K were added and the samples were digested overnight at 56 °C. After digestion, the samples were centrifuged and the supernatant was removed. All the solid components were discarded and the samples supernatant were processed as blood following the extraction kit manufacturer's instructions until the elution step. In this step, two elutions were carried out, each one for a different tube resulting in two replicates at the end. After the DNA extraction, both replicates of all samples were tested on 0.8% agarose gel and visualized in order to test the success of the extraction. According to the amount of DNA visible on the gel, the samples were diluted in buffer and stored at −20 °C until the PCR.

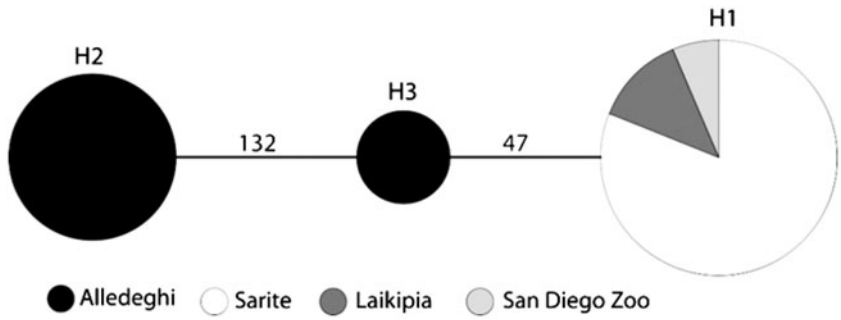
Approximately 2–5 ng of genomic DNA was used as template to amplify. A 350 bp fragment of the mitochondrial control region was amplified using the primers Eq-CR-1F (CCTCATGTACTAT GTCAGTA) and Eq-Cr-534R (CCTGAAGAAAGAACCAGAT GCC). Samples were amplified in a Dual 96-Well GeneAmp® PCR System 9700 thermocycler (Applied Biosystems™) according to the following conditions: initial denaturation at 95 °C for 15 min, followed by 45 cycles of 30 s at 95 °C, 60 s at 54 °C and 45 s at 72 °C. A final elongation step was held at 72 °C for 15 min. Successfully amplified PCR products were purified and sequenced for both strands at the High-Throughput Genomics Unit, Department of Genome Sciences, University of Washington (http://www.htseq.org/). Sequence trace files were checked and aligned by DNASTAR 5.0 package (DNASTAR Inc., Madison, WI) and aligned by software Mega version 5 (www.megasoft.net; Tempe, AZ) (Tamura et al., 2011). All sequences generated in this work are available at GenBank (accession numbers KJ399477–KJ399523).

Available mtDNA control region sequences for this species were downloaded from GenBank and added to the obtained dataset (Table 1). Diversity measures, namely, haplotype and nucleotide diversities were calculated by DnaSP 5.10 software (Barcelona, Spain) (Librado & Rozas, 2009) and software Network 4.6 (www.fluxus-engineering.com) (Bandelt et al., 1999) was used to draw a median-joining network of haplotypes.

Table 1. Haplotypes and sequenced base pairs (bp) obtained from DNA analysis of fecal samples of Grevy's zebra (*Equus grevyi*) collected from Ethiopia.

Sample	Country	Location	Haplotype	Sequence length (bp)	Reference Paper
EG002, EG006, EG007, EG008,EG009, EG010, EG013, EG015, EG016, EG017, EG018, EG028, EG032, EG036, EG041, EG043, EG047	Ethiopia	Alledeghi	H_2	329	Current
EG012, EG014, EG037, EG048	Ethiopia	Alledeghi	H_3	329	Current
EG179, EG180, EG181, EG182, EG183, EG186, EG190, EG192, EG193, EG194, EG196, EG197, EG198, EG200, EG201, EG202, EG203, EG204, EG205, EG206, EG207, EG208, EG209, EG210, EG211	Ethiopia	Sarite	H_1	329	
GQ176428, GQ176429, GQ176430, GQ176432	Kenya	Laikipia	H_1	329	Cordingley et al., 2009
AF220928, AF220930	Unknown	S.Diego zoo	H_1	329	Oakenfull et al., 2000

Figure 2. Median-joining network of three Grevy's zebra (*Equus grevyi*) haplotypes.



Results and discussion

Twenty-five sequences were obtained for the Sarite population and 21 for the Alledeghi population. Four sequences were published in a study by Cordingley et al. in 2009 and belonged to individuals sampled in the Laikipia region of Kenya. The remaining two sequences belonged to captive individuals and there was no information about their geographical origin. Three haplotypes were defined by two mutational steps and named H1, H2 and H3 (Figure 2). Haplotype H1 was the most frequent and it was shared by the population in southern Ethiopia (Sarite) and Kenya reflecting their geographical proximity. Two previously unpublished haplotypes were identified in the northern population of Alledeghi which were not shared with individuals that belonged to either Kenya or Sarite. Obtained results for haplotype and nucleotide diversities were low (Table 2). Values for the northern population of Alledeghi ($h = 0.381$ and $\pi = 0.00116$) were higher than those found in the Sarite population ($h = 0$ and $\pi = 0$) where only one haplotype was found.

Zebra species (*Equus quagga*, *Equus zebra* and *Equus grevyi*) have mostly allopatric distribution in the African continent that reflects their physiology, feeding ecology and distinct ecological requirements (Bauer et al., 1994). Plain zebras (*Equus quagga*) are widely distributed from Ethiopia to southern Africa and are dominant members of savannah ecosystems (Lorenzen et al., 2008). Lorenzen et al. (2008) analyses of the mitochondrial DNA control region determined that the morphological variation of Plains zebra was consistent with higher levels of genetic variation (Table 2). Genetic diversity parameters were obtained from mtDNA control region sequencing for several zebra species ((π) nucleotide diversity and (h) haplotype diversity; Table 2).

In southern Africa, the Mountain zebra (*Equus zebra*) inhabits more rocky terrain and may have a comparatively lower dispersal potential. Hartmann's Mountain zebra (*E. z. hartmannae*) mainly occurs in Namibia and the Cape Mountain zebra persists in South Africa (*E. z. zebra*). Both subspecies have experienced population fluctuations/reductions. The Hartmann mountain zebra, although it may have experienced an ephemeral bottleneck, has recovered to an estimated 25,000 plus individuals (Moehlman et al., 2008) and this subspecies has a reasonable level of genetic diversity (Table 2) (Moodley & Harley, 2005). By contrast, during the past 300 years, the Cape mountain zebra populations had a very severe and prolonged bottleneck and by the 1950's the population was less than 100 individuals (Novellie, 2008). In the 1960's and 1970's conservation actions and reintroductions led to a recovery of the subspecies such that the population is now over 1500 individuals. The small number of founders appears to have had consequences, and the overall diversity level is low (Table 2). Some Cape Mountain zebra populations i.e. Cradock, Kammanassie, and Gamka, have only one haplotype and low genetic diversity.

Comparison of the genetic diversity values for these three species of zebra (Table 1) observed at the same genetic marker (mtDNA control region) indicate that, given the current information, some Grevy's zebra populations exhibit very low levels of nucleotide diversity. This raises concerns as to their genetic resilience for their long-term survival.

The Alledeghi Grevy's zebra population is at the northern extreme of the species' range and is geographically isolated. It retains what appears to be unique mitochondrial DNA and haplotypes.

The Grevy's zebra in Ethiopia is an important flagship species for the Alledeghi Plains grassland. In addition, this population appears to have a unique genetic makeup and strong support is needed for a community based conservation programme in this multiple use area. The current analyses are based only on mtDNA

Table 2. Genetic diversity parameters obtained from mtDNA control region sequencing for several zebra species.

Species	Population	Location	*N*	*h*	π	Ref. paper
Equus grevyi	Ethiopia	Alledeghi	21	0.381	0.001	Current study
		Sarite	25	0	0	Current study
	Kenya	Laikipia	4	0	0	Cordingley, 2009
	San Diego Zoo	*	2	0	0	Oakenfull et al., 2000
Equus zebra	Namibia	Gamsberg	11	0.894	0.017	Moodley & Harley, 2005†
		Kamanjab	13	0.962	0.019	‘‘
	South Africa	Cradock	10	0	0	‘‘
		Kammanassie	11	0.545	0.006	‘‘
Equus quagga	Kenya	Masai Mara	19	0.98	0.030	Lorenzen et al., 2008†
	Tanzania	Maswa	20	0.98	0.032	‘‘
		Burko	20	0.88	0.026	‘‘
		Ikiri-Rungwa	23	0.92	0.016	‘‘
	Zambia	Kasama	10	0.96	0.027	‘‘
		Lochnivar South	11	1.00	0.016	‘‘
	Namibia	Etosha	24	0.96	0.025	‘‘

π; Nucleotide, *h*; haplotype diversity.
*Unknown source population.
†Only information from populations with sample sizes $\geq$10 were choosen.

control region sequences and this information needs to be combined with microsatellite data as a next step in understanding the population genetics of the Alledeghi Grevy's zebra population. Additional population genetic studies are ongoing to determine whether the Alledeghi and Sarite populations are still connected (via male dispersal) and, if the southern Sarite population currently has genetic exchange with the northern Kenya populations. Further work is needed on Grevy's zebra population genetics throughout its range in order to understand its genetic diversity distribution and dynamics.

Declaration of interest

The authors report no conflicts of interest. The authors alone are responsible for the content and writing of the paper.

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