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Effect of the habitat fragmentation on the Grevys zebra population genetic structure

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Abstract

The exponential growth of the human population is limiting the wildlife habitat all around the word. In recent years habitat loss and fragmentation is one of the main reasons that threats the wild life species. The Grévy's zebra (*Equus grevyi*) is the most endangered member of Zebras. Their historical range was previously from north Ethiopia to southwest Somalia and to northern Kenya. Currently they are distributed only in fragmented habitats in central and eastern part of Ethiopia and in the north of Kenya. They are listed as endangered in the IUCN red list, as their population has declined 68% in 27 years. There are very few studies on genetic structure of this species, and investigating the genetic connection between different populations is needed.

Molecular markers are one of the best tools to understand the level of fragmentation, population bottlenecks or potential inbreeding. In this study, the population structure of Ethiopian zebra population from Alledeghi Wildlife Reserve (WR) and Sarite area was studied using non-invasively obtained fecal samples collected during 2001-2011. This study analyzes genetic variation at 10 microsatellite loci and a 350-bp fragment of the mitochondrial DNA control region. The results showed that the genetic diversity is very low between the populations (π=0.00116 for Alledeghi WR and π=0 for Sarite population). The population of Alledeghi WR is probably isolated from the population of Sarite, as they don't share any haplotypes. As the population of Alledeghi WR is separated from the ones from Sarite and Kenya, applying more conservational programs in this area is needed to protect the genetic diversity of the Grévy's zebras in this area.

Contents

INTRODUCTION

The exponential growth of the human population is limiting the wildlife habitats all around the world. Humans affect the natural habitats by transforming them to agricultural farms and human settlements in addition to over grazing and hunting (Ceballos and Ehrlich 2002). These changes can result in reducing the population of a species to the extent of extinction (Diamond et al. 1989).

Humans are also one of the main reasons of climate change in the recent century (Ellis et al. 2010). These changes, together with the human interventions can result in fragmentation of species habitats. The combination of climate change and habitat fragmentation can threat wildlife in different ways, such as shifting their range, population decline, losing genetic diversity and local extinction in small patches (Travis 2003; Opdam and Wascher 2004).

Molecular markers have been widely used in recent years for studying genetics. They are one of the best tools to understand how the gene flow is affected by migration or dispersal and in which direction (Ellegren 2004; Selkoe and Toonen 2006).

Molecular markers can determine several population diversity parameters such as expected and observed heterozygosity, demography related estimators like genetic differentiation between and within populations (e.g., F-statistics parameters), effective population size (Ne), gene flow, population bottlenecks, potential inbreeding, and hybridization.

It is also possible to estimate the geographical distribution patterns of genetic diversity by using landscape genetics tools. Molecular markers are also being used in conservation, by indicating conservation units and assist the decision maker in the process of managing the endangered species and protected areas (Allendorf et al. 2010; Hedrick et al. 1996).

Moreover, these markers are able to transfer across related species. They can be efficient and effective tools in conservation studies. This has been previously studied in another member of the Equidae family, African wild ass (*Equus africanus*). Published horse microsatellites were tested on the samples from this species. From totally 25 microsatellites, 15 of them amplified well and gave significant results (Rosenbom et al. 2011). In this study the same 15 microsatellites were tested on the Grévy's zebra samples.

Molecular markers can also be used to amplify DNA of low quality samples originating from fecal, hair, and skin. Non-invasive sampling (i.g. collection of faeces) is a great opportunity to sample DNA from endangered species without disturbing them (Taberlet et al. 1999).

Among different groups of endangered species, mammals are more vulnerable to the extinction than others (Turvey and Fritz 2011) and Grévy's zebra (*Equus grevyi*) is one of them, it is the most endangered member of Zebras. Grévy's zebra are distributed in central and eastern part of Ethiopia and north of Kenya. They are listed as endangered in the IUCN red list as the population has declined 68% during 27 years (from 1980 to 2007).

Conservation management of the species can be more efficient if there is sufficient data available, not only about the ecology of the species but also on the genetic structure and diversity of species. There are many studies on physiology, habitat, ecology, behavior, and social structure of the *E. Grévy's* along with their conflict with local people on the recourses but their genetic diversity has never been investigated before. There are some local nongovernmental organizations, especially in Kenya that are involved in conservation of this species based on its ecological needs, but there is no particular conservation program for conservation of their genetic diversity.

By using non-invasive samples, we can collect small amounts of DNA left behind by the animals without disturbing the target species. These samples may have degraded to low quality and quantity DNA, but by using the Polymerase Chain Reaction (PCR), we can use nanogram to microgram amounts of DNA to make several copies of a specific part of it (Beja-Pereira et al. 2009).

In this study, we investigate the population structure of the Ethiopian population of Grévy's zebra from Alledeghi Wildlife Reserve (WR) and Sarite area. The distance between these two areas is approximately 700 km and they have been separated from each other for about 50 years. We used fecal samples collected during 2001-2011 to determine whether these two populations are separated from each other and if yes, to which extend. We used an mtDNA control region fragment to investigate levels of genetic diversity in two populations. 10 Microsatellite markers were also used to calculate expected (HE) and observed (HO) heterozygosity.

Study species

Grévy's zebra

Among all the zebras living in Africa, Mountains zebra (*Equus zebra* (Linnaeus, 1758)), plains zebra (*Equus quagga* (Boddaert, 1785)) and the Grévy's zebra (*Equus grevyi* (Oustalet, 1882)), Grévy's zebra is the largest one. Their unique characteristics, the stripes that are evenly divided and does not cover the belly, their big and round ears and a brown spot on the nose make it possible to separate it from the other zebras (Rubenstein 2004). Their length is about 250–275 cm and they have a shoulder height of about 140–160 cm. Males are larger than females (380-450 kg compared to 350-400 kg). Compared to other zebra species their social structure is much opener. They can be found in different type of groups, young males without territory, females with or without foals, males and females in one group (Hostens 2009).

Distribution

The historical distribution range of the Grévy's zebra used to be from Danakil desert in Eritrea to north east of Lake Turkana in Ethiopia. In the south they were distributed from north of Tana River in north Kenya to western Somalia. Currently their population is decreased in many parts of their distribution range. From 1970 until now, the Grévy's zebra has suffered from one of the most substantial reductions in terms of distribution range and population size. At the end of the 1970s, there were 15000 individuals of Grévy's zebra, which currently have declined to 3000 – 3500 individuals. This is one of the most rapidly decline in numbers among the African mammals (Moehlman et al. 2013). Their current distribution range consists of discontinuous habitats in Kenya and Ethiopia (Figure 1). In Ethiopia the Grévy's zebra's habitats are limited to two isolated populations, northern population in Alledaghi Plain and southern population which extend from Sarite/Chew Bahir into northern Kenya to Laikipia near Mt. Kenya. Chew Bahir Wildlife Reserve has the largest number of Grévy's zebra in early 1970s –around 1500 individuals- but today there are less than 30 individuals left. The number of individuals in Alledaghi WR today are at least 143 individuals which has declined from about 175 in 1992, less than 300 in 1978 and around 600 in 1970 (Kebede et al. 2012, Williams 2002).

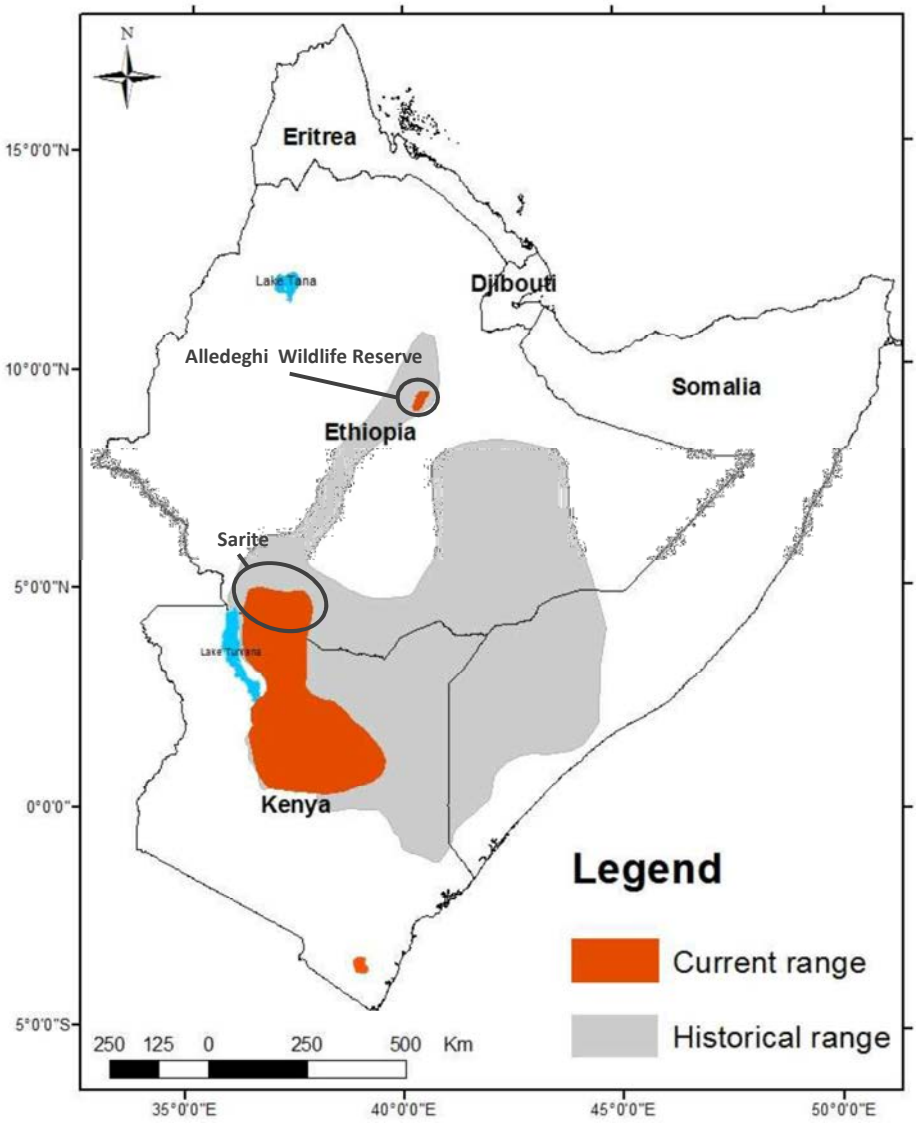

Figure 1: Historical and current distribution of the Grévy's zebra (Kebede et al, unpublished data)

Threats

Like many large mammals, the main threats to Grévy's zebra are caused by expansion of human populations, settlements and agriculture all along their distribution range. This has caused habitat loss, fragmentation, increased competition with livestock for water, grazing sources and increased transmission of diseases. Hunting for food or medical uses is also another threat, especially in Ethiopia (Williams 2002).

In Alledeghi WR in Ethiopia, competition with local pastoral people and their livestock over resources such as grazing areas and water, has shifted the Grévy's zebra to less suitable areas (Kebede et al. 2012). In Kenya, where the Grévy's and plain zebra live close together,

hybridization might be a threat. However this in particular will affect more on the plain zebras since the hybrid offspring are from male Grévy's and female plain's, and the offspring integrate themselves to plain zebra's society (Cordingley et al. 2009). As there is no plain zebra in the range of north population of Ethiopia, Alledeghi WR, it is safe to conclude that they are probably not threatened by hybridization in this area.

In Kenya, local people believe that Grévy's zebras are threatened by drought and diseases, mainly anthrax (Lelenguyah 2012). Also sharing the same limited water sources between humans, their livestock and wild animals such as Grévy's zebras, caused gastro-intestinal parasites like *Trichostrongylus* and *Haemonchus contortus* in Grévy's zebra (Muoria et al. 2005).

In south Ethiopia, Chew Bahir and Sarite, the human caused problems are the hunting of the zebras for cultural ceremonies and medical uses (Limenh 2007), and also there is a great competition between zebras and livestock on natural resources, especially water which is the main reported threat (Limenh 2007).

Conservation

Grévy's zebra are listed as endangered in the IUCN red list and on Appendix I of Convention on International Trade in Endangered Species of Wild Fauna and Flora (CITES) CITES Appendix I (Moehlman et al. 2013). They are legally protected in Ethiopia, although official protection has been limited. Benefiting from being in CITES Appendix I, there is no poaching for skins, but local hunters kill the animals for food or local medical uses (Kebede et al. 2012). In Kenya, they have been protected by a hunting ban since 1977. Nowadays, protected areas form less than 0.5% of the distribution range of Grévy's zebra. In Ethiopia, the protected areas are nominal (Alledeghi WR, Yabello Sanctuary, Borana Controlled Hunting Area and Chalbi Sanctuary) (Williams 2002; Moehlman et al. 2013).

As Williams (2002) proposed, conservation actions on wild populations should focus on, management and protection of water supplies and protected areas, monitoring of numbers in the wild and conservation by the indigenous people.

In Ethiopia Community-based conservation has been more effective compare to other types of conservation.

In addition in a study conducted by Low et al. (2009) it is proposed that providing direct benefit for local people through tourism will reduce the number of livestock and the conflicts between local people and wildlife.

Involving local scouts may result in improving the conservation knowledge in these communities. Plus that, direct conservation of the areas preferred by females with foals, will lead to a higher successful reproduction. Raising the community awareness and achieving sustainable conservation through increasing local capacity can lead to monitoring and understanding the wildlife better (Low et al. 2009). Subsequently, all this can help conserve the Grévy's zebra.

MATERIAL AND METHODS

Study area

Fecal samples were collected in the Alledeghi WR and Sarite (Yabelo wildlife Sanctuary) in Ethiopia. Alledeghi WR has the northernmost distribution range of the Grevyi's zebras. The two study populations in Ethiopia are separated as a result of increasing human population and density during the last decades. Developing human settlements, agriculture fields, rangelands and increasing number of livestock results to reducing and fragmenting suitable habitats for wildlife and increasing the competition on the limited water and food recourse.

Sampling

Before the polymerase chain reaction (PCR) was discovered, scientists needed to kill the animals to take the tissue sample for genetic analysis, or in the less aggressive method, animals should have been captured in order to take a biopsy or blood sample. Combination of non-invasive sampling and the PCR technique, now allows us to use a small amount of DNA which is left behind by the animal to do the analysis. The DNA can be found in hair, feces, urine, feather, egg shells and skin. One of the noninvasive materials often used by researchers is feces, as it is relatively easy to collect and it cause little disturbance for the species. Also it can provide other information about the animal such as its diet, hormones and parasite infection (Beja-Pereira et al. 2009).

From March 2001 to August 2010, a total number of 211 fecal samples were collected from two areas of Alledeghi WR and Sarite in Ethiopia. From all the obtained samples, 34 were from Sarite and the rest were gathered from Alledeghi WR. Samples were collected randomly during the field work in the area where only Grévy's zebra graze, to ensure that they belong to *E. grevyi* and not the other zebra species. The samples were preserved in separate plastic bags. From all the obtained samples, some of them were eliminated in different steps due to limitations in the laboratory or the quality of the samples (Supplementary material – Table S1).

DNA extraction

All 34 samples from Sarite and 61 samples from Alledeghi WR were used for DNA extraction. From 177 samples from Alledeghi WR, 116 samples that were collected between 2001 and 2008 were eliminated and only the ones from 2009 and 2010 were used for extraction to ensure that we can obtain a high quality DNA. Extractions was carried out in a laminar flow chamber and processed in batches with a maximum of 20 samples per set. A negative control was always included in the samples sets to detect contamination. This negative control is processed in the same way as the samples. All the material used for the extraction process was sterilized prior to use and were always discarded between samples.

The DNA was extracted using an adapted protocol recommended by the JETQUICK Tissue DNA Spin Kit manufacture (GENOMED GmbH Lohne, Germany) (Costa et al. unpublished data). The outer layer of the scats, which contains the intestinal epithelial cells, were removed and transferred to 15 ml Falcon tubes. Samples were digested in lysis buffer and proteinase K and incubated overnight at 56°C. After digestion, the samples were centrifuged and the supernatant was transferred to new tubes. InhibitEX tablets were added to remove the PCR inhibitors such as complex polysaccharides and products from food degradation (secondary plant compounds) (Rådström et al. 2004). After the tablets were dissolved the tubes were vortexed and centrifuged in order to residue the remnants. All the solid components were discarded and for the second time the samples supernatant were transferred to new Eppendorf tubes. The following steps to the DNA elution were carefully processed following the JETQUICK extraction kit manufacturer's instructions.

The elution of the DNA was done in two steps, in different Eppendorf tubes. The first tube contains more DNA and more inhibitors and the second one has higher quality DNA although, not as much. Both DNA extracts were visualized in a 0.8% agarose gel to test the amount of DNA in each tube. DNA samples with high concentration -according to the testing results on the 0.8% agarose gel- were diluted in elusion buffer (10m µl tricl (ph 8.5)), and stored in the fridge.

Mitochondrial DNA

A 350 bp fragment of the mitochondrial (mtDNA) control region was amplified from 48 samples from Alledaghi WR and 34 samples from Sarite. Due to the laboratory limitations, only 48 out of 61 samples from Alledaghi WR were used for mitochondrial analysis.

The PCR cycling was performed in a Dual 96-Well GeneAmp® PCR System 9700 thermocycler (Applied Biosystems, Foster City, CA, USA) or in an ABI Verity (Applied Biosystems, Foster City, CA, USA) according to the following conditions, an initial denaturation step at 95°C for 15 min, followed by 45 cycles of denaturation at 95°C for 30 s, annealing at 54°C for 60 s and elongation at 72°C for 45 s and a final elongation step at 72°C for 15 min. Each PCR had a total volume of 18 µl of the PCR mix containing 49.7% H_2O (9.94 µl), 10% Buffer (2.00 µl), 6% $MgCl_2$ (1.2 µl), 6% dNTPs mix (0.60 µl), 0.3% taq polymeraraze (0.06 µl), 1% BSA (0. 3 µl) and 10% of primers Eq-CR-1F (CCTCATGTACTATGTCAGTA) and Eq-Cr-534R (CCTGAAGAAAGAACCAGATGCC) (2.00 µl) plus 10% of samples (2 µl) per each tube.

The mtDNA control region sequences from four samples from Grevys zebra collected in Kenya [GQ176428, 2009; GQ176429, 2009; GQ176430, 2009; GQ176432, 2009] (Cordingley et al. 2009) and two samples from zebras in the San Diego Zoo [AF220928, 2002; AF220930, 2002] (Oakenfull, Lim, and Ryder 2000) were downloaded from Genbank to be compared with the samples from Ethiopia (Supplementary material – Table S2).

The PCR amplicons were sequenced using primer Eq-CR-1F and Eq-Cr-534R (in both directions) using Bigdye reaction sequencing (Applied Biosystem, CA, USA).

Sequences were edited by DNASTAR 5.0 package (DNASTAR Inc., Madison, WI, USA) to spot any misreading and possible gaps. MtDNA sequences from the Kenyan individuals were used as a reference to adjust the sequences. The same length sequences were aligned using Mega version 5 (Arizona, USA) (Tamura et al. 2011) software. The amplified sequences were

supposed to have 350 bp length but after editing and aligning them with references, the final length was 329 bp. The DnaSP 5.10 software (Barcelona, Spain) (Librado and Rozas 2009) was used to calculate haplotype and nucleotide diversity. Finally a median joining network was drawn using the software Network 4.6 (www.fluxus-engineering.com (Bandelt et al. 1999).

Microsatellites

30 samples from Alledaghi and 17 samples from Sarite were genotyped for 10 horse microsatellite markers (Table 1). The DNA samples were diluted in accordance with the concentrations obtained from the visualization in the 0.8% agarose gel and arranged in sample plates with two replicates plus a negative control. The multiple tubes approach was used to avoid allele dropouts and/or amplifications of false alleles (Taberlet et al. 1996).

Samples were amplified in a Dual 96-Well GeneAmp® PCR System 9700 thermocycler (Applied Biosystems) using the following cycling, an initial denaturation step at 94°C for 10 min, followed by 45 cycles of denaturation at 94°C for 30 s, annealing at temperatures between 52-60°C (variable with the locus) for 40 s and elongation at 72°C for 35 s, followed by 15 cycles of denaturation at 94°C for 30 s, annealing temperature at 53°C for 30 s and elongation at 72°C for 30 s, the elongation step was held at 72°C for 20 min.

The PCR products were tested on a 0.8% agarose gel and visualized by ultraviolet imagination (Molecular Imager® Gel Doc™ trans-illuminator (BIO-RAD Laboratories, Milan, Italy) to determine the amount of DNA in each tube. According to the quality of the amplification, samples were diluted in water, mixed with formamide and LIZ® 500 bp internal size standard (Applied Biosystems, Foster City, CA) and detected by capillary electrophoresis using a 3100 Genetic Analyzer® 108 (Applied Biosystems, Foster City, CA). Results were visualized using the GeneMapper® v4.0 software (Applied Biosystems, Foster City, CA). Allele scoring should repeat three times separately to increase the accuracy of the dataset. Obtaining three identical allele scores for heterozygous samples and two identical allele scores for homozygous samples can confirm the genotyping. The genotypes was analyzed using the software GenALEx 6.5 (Peakall and Smouse 2012). By this software the expected (HE) and observed (HO) heterozygosity, the effective number of alleles per locus and deviation from the Hardy-Weinberg proportions (HWE) can be calculated.

Table 1: Genotyped microsatellites, their specific dyes, optimal $MgCl_2$ concentrations and temperatures

Marker	Fluorescent dye	$MgCl_2$ conc. (mM)	Annealing temperature (°C)
AHT4	FAM	3.2	54
AHT5	VIC	3.2	56
COR20	NED	2.7	58
COR90	NED	2.2	58
HMS6	VIC	2.7	56
HMS7	FAM	2.2	52
HTG6	VIC	2.2	52
NVHEQ18	VIC	2.2	52
UM11	NED	2.7	54
VHL20	FAM	2.5	60

RESULTS

Haplotype diversity

Good quality mtDNA fragment sequences were obtained from 22 samples and 26 samples from Alledeghi WR and Sarite populations, respectively. These sequences were compared with the four sequences from Kenya, Laikipia [GQ176428, 2009; GQ176429, 2009; GQ176430, 2009; GQ176432, 2009] and the sequences from the captive individuals from San Diego Zoo, [AF220928, 2002 and AF220930, 2002] and three haplotypes were identified (H1, H2 and H3) (Table 2.) All of the polymorphic sites are the result of a transition mutation based on table 2.

Table 2: Variable sites of the three mtDNA control region haplotypes found in Grevyi's zebra

		Basepair positions		
Haplotype	**Locality**	**69**	**189**	**251**
H1	San Diego Zoo, Laikipia (Kenya), Sarite (Ethiopia)	T	A	G
H2	Alledeghi WR	C	G	G
H3	Alledeghi WR	C	G	A

Haplotype H1 was found in the individuals from San Diego Zoo, Laikipia (Kenya) and Sarite (Ethiopia). Two other haplotypes (H2 and H3) found only in Alledeghi WR samples (north

Ethiopia) and not in Sarite (south Ethiopia) or Kenya. Haplotype diversity (h=0.381) and nucleotide diversity (π=0.00116) for the Alledeghi WR samples, were higher than those found in the Sarite samples (h=0 and π=0) where only one haplotype was found.

Median joining network of the three haplotypes shows that the Alledaghi WR population is different and is apart from the Sarite population (Figure 2).

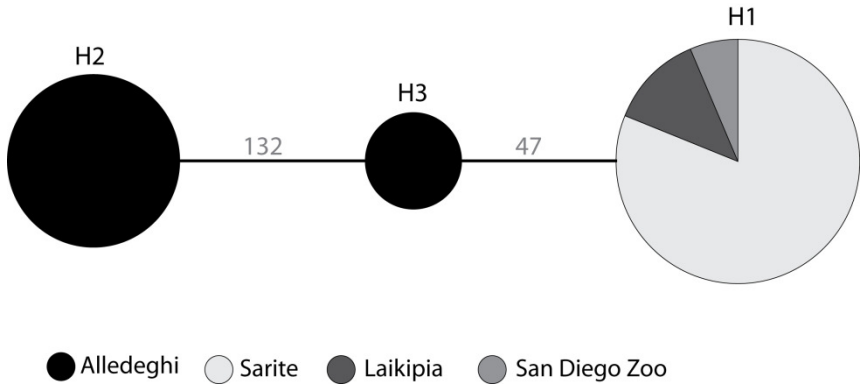

Figure 2: Median-joining network of three Grévy's zebra haplotypes

Population structure

After the first set of running of the microsatellite markers, I genotyped them but reliable results didn't obtained. I repeat the PCRs, sequenced and genotyped them again but still the genotyping data are not the same. As the quality of the samples was low, I repeat the extractions and repeat the PCRs with the new extracted DNA, unfortunately again it was not possible to obtain reliable results from the sequencings.

From the ten microsatellite, one (VHL20) has not amplified, although it was optimized and tested before. From all of 47 samples, we could analyze only 27 (15 from Alledeghi WR and 12 from Sarite). It was not possible to use the others as the number of amplified alleles was low. AHT4 and Cor20 were monomorphic in Alledeghi WR population and HMS7, UM11 and Cor20 were monomorphic only in Sarite population, all of the other loci were polymorphic and the number of alleles ranged from 0 to 5 (table 3).

We should repeat the sequencings for obtaining more accurate data to be able to analyze them in the future.

Table 3: Levels of genetic diversity in nine microsatellite loci observed in Grevyi's zebra

	Total number of alleles		Number of private alleles		Observed heterozygosity		Expected heterozygosity		Hardy–Weinberg equilibrium	
	Alledeghi WR	Sarite	Alledeghi WR	Sarite	Alledeghi WR	Sarite	Alledeghi WR	Sarite	Alledeghi WR	Sarite
AHT4	1	0	1	0	0.00	0.00	0.00	0.00	M*	M
AHT5	4	5	1	2	0.51	0.76	0.44	0.00	0.68	0.00
HMS6	2	4	0	2	0.47	0.68	0.31	0.30	0.21	0.01
HMS7	2	1	2	1	0.22	0.00	0.25	0.00	0.62	M
UM11	3	1	2	0	0.42	0.00	0.36	0.00	0.85	M
COR90	1	3	0	2	0.00	0.56	0.00	0.00	M	0.02
COR20	2	1	2	1	0.44	0.00	0.33	0.00	0.54	M
HTG6	4	6	2	3	0.54	0.76	0.37	1.00	0.01	0.82
NVHEQ18	3	4	1	2	0.52	0.72	0.71	0.67	0.23	0.42

*M refers to the loci that were monomorphic and the HW equilibrium could not be tested.

DISCUSSION

The population of the Grévy's zebras has declined extremely fast during the last decades. Their habitat has also decreased and became fragmented during these years. Additionally Alledeghi WR in northern Ethiopia has become isolated from other habitats in southern Ethiopia and Kenya because of human settlements developing during last decades.

The most frequent mtDNA haplotype was H1, present in the Sarite (south Ethiopia) and reference individuals (Kenya), 60% of the total samples carry this haplotype. Haplotype H2 was carried by 32% of the total samples while haplotype H3 was the less common one that carries by 8% of samples. This can be as a result of their geographical whereabouts. The mtDNA analysis indicates that Alledeghi WR population has unique haplotype, which does not appear in Sarite or Kenyan population. Haplotype and nucleotide diversities were overall low comparing to other zebra species. In Plain zebras (*Equus quagga*) which have the widest distribution range of zebras, the average nucleotide and haplotype diversity was 0.029±0.001 and 0.98 respectively (Lorenzen et al. 2008). On the other hand Moodley & Harley 2006 has reported that in two subspecies of Mountain zebra (*Equus zebra*), *E. z. hartmannae* (Namibia) and *E. z. zebra* (South Africa) who have more limited distribution range, haplotype and nucleotide

diversity was lower (h=0.936, π=0.018 and h= 0.561, π=0.006 respectively) but it is still more than *E. grevyi* Given that these two subspecies have overcome a severe bottleneck, we can conclude that the *E. grevyi* has the lowest diversity and needs urgent action in terms of conservation.

Continuing current conservation programs and probably community base conservation might be a solution to preserve this unique diversity. Although the populations of Sarite and Kenya shared the same haplotype, I believe more population genetic studies should be carried out to indicate whether any gene flow exists between the populations and/or to indicate if the populations are connected. To gain this goal, there is need for systematic sampling from fresh samples of both populations. Failing of the microsatellite amplifications, especially from Sarite can be a result of different diet of *E.grevyi* in this habitat. It is possible to overcome this problem with more efficient sampling.

Studying the possibility of habitat reclamation, on the corridors between Alledeghi WR - Sarite and Sarite - Kenya can also be a solution to increase the genetic diversity among the Grévy's zebras.

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Table S1 – Number of the samples in sampling, extraction, mtDNA amplification and microsattelite amplification.

Process step	Alledeghi WR	Sarite	Total
Sampling	177	34	211
Extraction	61	34	95
MtDNA amplification	48	34	82
MtDNA analysis	22	26	48
Microsatellites amplification	30	17	47
Microsatellites analysis	15	12	27

Table S2 – Sample code, country and location of origin, haplotype, sequence length and reference paper (if applicable) of used mt DNA sequences for Grevy´s zebra (*Equus grevyi*).

Sample	Country	Location	Haplotype	Seq. lenght (bp)	Ref. paper
EG002	Ethiopia	Alledeghi WR	H2	329	Unpublished
EG006	Ethiopia	Alledeghi WR	H2	329	Unpublished
EG007	Ethiopia	Alledeghi WR	H2	329	Unpublished
EG008	Ethiopia	Alledeghi WR	H2	329	Unpublished
EG009	Ethiopia	Alledeghi WR	H2	329	Unpublished
EG010	Ethiopia	Alledeghi WR	H2	329	Unpublished
EG012	Ethiopia	Alledeghi WR	H3	329	Unpublished
EG013	Ethiopia	Alledeghi WR	H2	329	Unpublished
EG014	Ethiopia	Alledeghi WR	H3	329	Unpublished
EG015	Ethiopia	Alledeghi WR	H2	329	Unpublished
EG016	Ethiopia	Alledeghi WR	H2	329	Unpublished
EG017	Ethiopia	Alledeghi WR	H2	329	Unpublished
EG018	Ethiopia	Alledeghi WR	H2	329	Unpublished
EG028	Ethiopia	Alledeghi WR	H2	329	Unpublished
EG032	Ethiopia	Alledeghi WR	H2	329	Unpublished
EG36	Ethiopia	Alledeghi WR	H2	329	Unpublished
EG037	Ethiopia	Alledeghi WR	H3	329	Unpublished
EG041	Ethiopia	Alledeghi WR	H2	329	Unpublished
EG043	Ethiopia	Alledeghi WR	H2	329	Unpublished
EG047	Ethiopia	Alledeghi WR	H2	329	Unpublished
EG048	Ethiopia	Alledeghi WR	H3	329	Unpublished
EG179	Ethiopia	Sarite	H1	329	Unpublished
EG180	Ethiopia	Sarite	H1	329	Unpublished
EG181	Ethiopia	Sarite	H1	329	Unpublished
EG182	Ethiopia	Sarite	H1	329	Unpublished
EG182	Ethiopia	Sarite	H1	329	Unpublished
EG183	Ethiopia	Sarite	H1	329	Unpublished
EG186	Ethiopia	Sarite	H1	329	Unpublished

EG190	Ethiopia	Sarite	H1	329	Unpublished
EG192	Ethiopia	Sarite	H1	329	Unpublished
EG193	Ethiopia	Sarite	H1	329	Unpublished
EG194	Ethiopia	Sarite	H1	329	Unpublished
EG196	Ethiopia	Sarite	H1	329	Unpublished
EG197	Ethiopia	Sarite	H1	329	Unpublished
EG198	Ethiopia	Sarite	H1	329	Unpublished
EG200	Ethiopia	Sarite	H1	329	Unpublished
EG201	Ethiopia	Sarite	H1	329	Unpublished
EG202	Ethiopia	Sarite	H1	329	Unpublished
EG203	Ethiopia	Sarite	H1	329	Unpublished
EG204	Ethiopia	Sarite	H1	329	Unpublished
EG205	Ethiopia	Sarite	H1	329	Unpublished
EG206	Ethiopia	Sarite	H1	329	Unpublished
EG207	Ethiopia	Sarite	H1	329	Unpublished
EG208	Ethiopia	Sarite	H1	329	Unpublished
EG209	Ethiopia	Sarite	H1	329	Unpublished
EG210	Ethiopia	Sarite	H1	329	Unpublished
EG211	Ethiopia	Sarite	H1	329	Unpublished
GQ176428	Kenya	Laikipia	H1	329	Cordingley et al, 2009
GQ176429	Kenya	Laikipia	H1	329	Cordingley et al, 2009
GQ176430	Kenya	Laikipia	H1	329	Cordingley et al, 2009
GQ176432	Kenya	Laikipia	H1	329	Cordingley et al, 2009
AF220928	Unknown	S.Diego Zoo	H1	329	Oakenfull et al, 2000
AF220930	Unknown	S.Diego Zoo	H1	329	Oakenfull et al, 2000