CZECH UNIVERSITY OF LIFE SCIENCES PRAGUE

FACULTY OF TROPICAL AGRISCIENCES

Genetic Diversity of Semi-captive Population of Western Derby Eland (*Taurotragus derbianus derbianus*) in Senegal and Phylogenetical Relationships between Western Derby Eland (*T. d. derbianus*) and Eastern Giant Eland (*T. d. gigas*)

(Ph. D. thesis)

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Prague 2015

Declaration

I declare that I have written the Ph. D. thesis **Genetic Diversity of Semi-captive Population of Western Derby Eland (*Taurotragus derbianus derbianus*) in Senegal and Phylogenetical Relationships between Western Derby Eland (*T. d. derbianus*) and Eastern Giant Eland (*T. d. gigas*)** on my own and I have used the literature sources mentioned in references.

Prague the 9th September 2015

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Acknowledgement

Above all I would like to thank Mgr. Barbora Černá Bolfíková, Ph.D., who helps me a lot with the methodology, explains me the right procedures and patiently answers my questions. Without her I cannot do my thesis.

Further I want to thank Mgr. Richard Polícht, Ph.D., who has endeavoured to motivate me in the moments, when I was exhausted and without any motivation to continue my work, and my colleagues, who have given me the hope, when I felt helpless.

Last but not least I would like to thank a lot my supervisor prof. MVDr. Daniela Lukešová, CSc. for her support during my studies.

My thanks belong to "The antelope team" from the Czech University of Life Sciences Prague and "Derbianus Conservation, z. s." (NGO), especially Ing. Karolína Brandlová, Ph.D., and prof. RNDr. Pavla Hejcmanová, Ph.D., and the Society for the Protection of Environment and Fauna in Senegal and Directorate of National Parks of Senegal, which enabled me to obtain the study material from the critically endangered antelope within the conservation programme. Furthermore, I cannot forget the elands, without their existence my work did not make any sense.

The research was supported by the grant of the foundation "Nadání Josefa, Marie a Zdeňky Hlávkových".

I thank my family, which has supported me by all possible ways and all my friends, who help me when I was really feeling hopeless of my work.

I would like to devote my work to my grandfather, who has not regrettably lived to see its results, but who has taught me getting to know the nature around me all the time.

ANNOTATION

Subject: Genetic Diversity of Semi-captive Population of Western Derby Eland (*Taurotragus derbianus derbianus*) in Senegal and Phylogenetical Relationships between Western Derby Eland (*T. d. derbianus*) and Eastern Giant Eland (*T. d. gigas*)

Abstract:

Representatives of family Bovidae are subjects of many studies concerning with their phylogeny, phylogeography, time of divergence or genetic diversity. Taxonomy is solved by comparison of morphological characteristics or by genetic approaches, genetic diversity could be solved by pedigree or by genetic analyses too.

Tragelaphinae number nine species of two genera, *Tragelaphus sp.* and *Taurotragus sp*. The antelopes of the genus *Taurotragus* (*T. derbianus* and *T. oryx*) belong to the largest antelopes of the world. Derby eland (*Taurotragus derbianus*) has two subspecies, Western Derby eland (*T. d. derbianus*) and Eastern Giant eland (*T. d. gigas*), which are distinguished on the basis of morphological characteristics.

Western subspecies (*T. d. derbianus*) is classified as critically endangered. There lives the only population in Niokolo Koba National Park in Senegal, which numbers fewer than 200 individuals. For the conservation, the semi-captive breeding programme has been established in 2000. It was created by six founders (one male and five females), which are presumed to be non-related. The population within this programme had 95 living individuals in 2013, living in seven herds in Bandia and Fathala reserves in Senegal. The population is under breeding management, which efforts to minimize kinship of the individuals. Studbook was established for the Western Derby eland (*T. d. derbianus*) in 2008 and is published annually.

It acts about small population with low number of founders and no gene flow, which is threatened by inbreeding and genetic drift. Genetic diversity of the population was evaluated by means of microsatellite markers and the results were compared with the results of pedigree analysis.

Pedigree analysis showed the highest genetic diversity in the generation of founders (FOUNDERS). It decreased in the generation of founders´ offspring (OFFSPRING 1; born in season 2007/2008), due to the fact, that the only male took part in the reproduction. And it increased again in the generation of offspring of founders´ offspring (OFFSPRING 2; born in the season 2009/2010), because more individuals were included into the reproduction.

Fifteen individuals and five polymorphic microsatellite loci (from the total number of 13 tested loci) were chosen for the genetic study. The parameters of genetic diversity (H_E and H_O, Ar and deviations from Hardy-Weinberg equilibrium, and F_{IS} and F_{ST}) were evaluated.

Not any deviations from Hardy-Weinberg equilibrium were found out. The results of genetic analysis confirmed the highest genetic diversity in the population of founders (Ar = 2.79; H_E = 0.664; H_O = 0.750; F_{IS} = –0.154). In both generations of offspring values of allelic richness and observed and expected heterozygosity decreased (Ar = 2.15; H_O = 0.580; HE = 0.586 in OFFSPRING 1 and Ar = 2.14; H_O = 0.370; H_E = 0.480 in OFFSPRING 2). Contrary to the results of pedigree analysis, there was not been observed any improvement in OFFSPRING 2. The resultant values of genetic diversity parameters were quite satisfactory, despite of the low number of founders and mating of related individuals.

Keywords: Bovidae, captivity, conservation, microsatellite, small population, Tragelaphinae, Western Derby eland

ANOTACE

Téma: Genetická diverzita v populaci západního poddruhu antilopy Derbyho (*Taurotragus derbianus derbianus*) chované v polozajetí v Senegalu a fylogenetické vztahy mezi západním (*T. d. derbianus*) a východním poddruhem antilopy Derbyho (*T. d. gigas*)

Abstrakt:

Zástupci čeledi Bovidae jsou předmětem mnoha výzkumů, které se zabývají jejich fylogenezí, taxonomií, časem divergence, nebo genetickou diverzitou. Taxonomie se řeší pomocí srovnání morfologických znaků nebo genetickými metodami, genetická diverzita může být zjištěna analýzou rodokmenu nebo taktéž genetickými analýzami.

Tragelaphinae čítají devět druhů v rámci dvou rodů *Tragelaphus sp.* a *Taurotragus sp*. Antilopy rodu *Taurotragus* (*T. derbianus* and *T. oryx*) patří mezi největší antilopy světa. Antilopa Derbyho (*Taurotragus derbianus*) má dva poddruhy, západní (*T. d. derbianus*) a východní (*T. d. gigas*), které se rozlišují na základě morfologických znaků.

Západní poddruh antilopy Derbyho (*T. d. derbianus*) patří ke kriticky ohroženým živočichům. Jediná populace tohoto druhu, čítající méně než 200 jedinců, žije národním parku Niokolo Koba v Senegalu. Pro záchranu tohoto poddruhu byl v roce 2000 založen chov v polozajetí. Vznikl za účasti šesti zakladatelů (jednoho samce a pěti samic), u kterých předpokládáme, že nejsou příbuzní. Populace v roce 2013 čítá 95 jedinců, žijících v sedmi stádech v rezervacích Bandia a Fathala v Senegalu. Populace je spravována managementem, který se snaží minimalizovat příbuznost jedinců. V roce 2008 byla vytvořena plemenná kniha antilopy Derbyho (*T. d. derbianus*), která je každoročně publikována.

Jedná se o malou populaci s nízkým počtem zakladatelů a bez genového toku, ohroženou inbreedingem a genetickým driftem. Genetická diverzita této populace byla zhodnocena pomocí mikrosatelitních markerů a výsledky této analýzy byly porovnány s výsledky analýzy rodokmenu.

Analýza rodokmenu ukázala největší genetickou diverzitu v generaci zakladatelů (FOUNDERS).

V generaci potomků zakladatelů (OFFSPRING 1; narozených v sezóně 2007/2008) genetická diverzita klesla díky tomu, že reprodukce se účastnil jediný samec. V další generaci potomků, tj. mláďat potomků zakladatelů (OFFSPRING 2; narozených v sezóně 2009/2010) diverzita vzrostla díky zapojení více jedinců do reprodukce.

Pro genetickou analýzu bylo vybráno patnáct jedinců a pět polymorfních mikrosatelitních lokusů (z celkového počtu třinácti testovaných). Byly hodnoceny parametry genetické diverzity (H_E a H_O, Ar a odchylky od Hardy-Weinbergovy rovnováhy, a F_{IS} a F_{ST}).

Nebyly zjištěny žádné odchylky od Hardy-Weinbergovy rovnováhy. Výsledky genetické analýzy potvrdily nejvyšší genetickou diverzitu u zakladatelů (FOUNDERS: Ar = 2.79; H_E = 0.664; H_O = 0.750; F_{IS} = –0.154). V obou generacích potomků hodnoty alelické bohatosti a pozorované a očekávané heterozygotnosti klesly (Ar = 2.15; H_O = 0.580; H_E = 0.586 u OFFSPRING 1 a Ar = 2.14; H_O = 0.370; H_E = 0.480 u OFFSPRING 2). Oproti výsledkům analýzy rodokmenu nebylo pozorováno žádné zlepšení v generaci OFFSPRING 2. Výsledné hodnoty parametrů genetické diverzity byly celkem uspokojivé, navzdory nízkému počtu zakladatelů a páření příbuzných jedinců.

Klíčová slova: antilopa Derbyho, lesoňovití (Tragelaphinae), malé populace, mikrosatelity, ochrana, turovití, zajetí

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1. INTRODUCTION

Bovids live in diverse environments from high mountains to tropical areas. They occur on four continents, namely Europe, Asia, Africa and America. A lot of representatives have economic importance for us. They provide us a lot of products – meat, milk and milky products, horny tissue, leather, wool, and they also create the important part of various ecosystems (Teyrovský, 1957; Danell *et al.,* 2006*;* Bibi and Vrba, 2010).

There has been published a lot of studies concerning with the phylogeny of bovids, with their adaptations, time of their divergence, phylogeography, genetic diversity and others (for example Matthee and Robinson, 1999; Hassanin and Ropiquet, 2004; Willows-Munro *et al.,* 2005; Lorenzen *et al.* 2010). The further scientists solved the phylogenetic relationships by comparison of morphological characters. The modern recent studies are based on the genetics, which is very informative in solving evolutionary relationships, genetic variability etc. thanks to its almost unlimited number of data (for example Hassanin and Douzery, 2003; Rubes *et al.,* 2008 etc.).

In my study, I have focused on the Western Derby eland (*Taurotragus derbianus derbianus*), which is classified as "critically endangered" (IUCN, 2012). There live the only population in the wild and the conservation programme of Western Derby eland (*T. d. derbianus*) has been established. The population in semi-captivity reproduces very well, but it has arisen from only 6 founders and it is too closed – the inbreeding occurs in the population (Bro-Jørgensen, 1997; Nežerková *et al*., 2004; Koláčková *et al.,* 2011a, 2012).

The small isolated populations are threatened by inbreeding consequences – inbreeding depression, decrease of the genetic diversity, loss of rare alleles or accumulation of the deleterious alleles in the population. These consequences can lead to decrease of individual fitness and adaptability to possible environment changes (Lande, 1988; Lacy, 1997).

The management of the captive population of Western Derby eland (*T. d. derbianus*) is based on the pedigree construction (by means of direct observation and identification of the individuals) and on the selection of as few

as possible related individuals for further breeding. The inclusion of some “new” individual with different alleles is not very probable (Antonínová *et al.,* 2004, 2006; Koláčková *et al.,* 2011a, 2012).

The cases of the Arabian oryx (*Oryx leucoryx*), Przewalski's horse (*Equus caballus przewalskii*) or addax (*Addax nasomaculatus*) demonstrate the possible way of conservation management. The threats of these species are very similar – for example hunting, loss of the natural habitat or competition with domestic livestock. Contrary to the Western Derby eland (*T. d. derbianus*), the Arabian oryx (*O. leucoryx*) and Przewalski's horse (*E. c. przewalskii*) have been eradicated in the wild, but due to the well timed intervention of conservationists they have survived and been reintroduced to the wild. The conservation programmes of both, the Arabian oryx (*O. leucoryx*) and Przewalski's horse (*E. c. przewalskii*), have been established by more founders (11 in Arabian oryx (*O. leucoryx*) and 13 in Przewalski's horse (*E. c. przewalskii*)), contrary to the Western Derby eland (*T. d. derbianus*) – only 6 founders) (Asmodé and Khoja, 1989; Ostrowski *et al.,* 1998; Marshall *et al.,* 1999; Wakefield *et al.*, 2002; Nežerková *et al*., 2004; Walzer *et al.*, 2012; IUCN, 2011, 2013).

Genetic diversity in the population of Western Derby eland (*T. d. derbianus*) has been evaluated by using 13 microsatellite markers developed for cattle (*Bos taurus*), goats (*Capra hircus*), roe deer (*Capreolus capreolus*) or gazelles (*Gazella granti* and *G. dorcas*). The results were compared with the results of pedigree analysis of Koláčková *et al.* (2011a). For this comparison the investigated individuals were divided into three groups. First group was created by the generation of founders, which came from the wild and which are presumed to be unrelated. The second group consisted of founders´ offspring born in the season 2007/2008. All of these offspring were sired by the only one founding male, in comparison with the last group, which was created by offspring of founders´ offspring, born in the season 2010/2011, because since 2009 up to five other males participated in the reproduction due to the breeding management applications.

The problem was that we do not have samples of all founders. Samples of two founder females are missing because it was not possible to obtain them – the blood samples were obtained by transport of animals among the reserves or enclosures, when the animals are narcotized, and the tissue samples originate from dead animals. A few samples were obtained due to biopsy darts too. The two female founders still live and were not transported neither was used biopsy, so there was no possibility to obtain the samples.

The evaluation of the phylogenetic relationships between the subspecies of the Derby eland (*Taurotragus derbianus*) was studied using the mitochondrial DNA.

2. AIM OF THE THESIS

The aim of the thesis was to determinate basic parameters of genetic diversity (number of alleles per locus, expected and observed heterozygosity (H_E and H_O), inbreeding coefficient (F_{IS}), fixation index (F_{ST}) ...) in the population of the Western Derby eland (*T. d. derbianus*) bred in semi-captivity within the conservation programme of the Western Derby eland (*T. d. derbianus*) in Senegal by means of the microsatellite markers. According to the pedigree analysis of Koláčková *et al.* (2011a) we suppose that the highest level of the genetic diversity will be by founders, which are presumed to be unrelated, the parameters of genetic diversity will be lowest in the generation of founders´ offspring (born in the season 2007/2008) and it will increase in the generation of offspring of founders´ offspring (born in the season 2010/2011), after implementation of actions of genetic management.

Furthermore, the phylogenetic relationship between the two subspecies of the Derby eland (*T. derbianus*) will be investigated by means of mitochondrial DNA markers.

3. LITERATURE OVERVIEW

3.1 Taxonomy and phylogeny of *Taurotragus spp.*

The antelopes of the genus *Taurotragus*, *Taurotragus oryx* (Eland or Common eland) and *T. derbianus* (Derby eland) are considered to be the largest antelopes in the world. They belong to the tribe Tragelaphini, subfamily Bovinae (or Tragelaphinae), family Bovidae, suborder Ruminantia, order Cetartiodactyla, superorder Laurasiatheria, class Mammalia (Estes, 1991; Wilson and Reeder, 2005).

The Tragelaphini are also known as spiral-horned antelopes. Except the elands belong to this tribe also the bushbuck (*Tragelaphus scriptus*), nyala and mountain nyala (*T. angasii* and *T. buxtoni*), greater and lesser kudu (*T. strepsiceros* and *T. imberbis*), bongo (*T. eurycerus*) and sitatunga (*T. spekei*) (Kingdon, 1982; Grzimek, 1990; Estes, 1991).

3.1.1 Phylogeny of Cetartiodactyla and Ruminantia

Cetartiodactyla are the monophyletic group, including the cetaceans, which are closely related to the hippos. Hippopotamidae and Cetacea create the sister group to the Ruminantia (Janis and Scott, 1987; Estes, 1991; Madsen *et al.,* 2001; Murphy *et al.,* 2001a, b; Nikaido *et al.,* 2003; Price *et al.*, 2005; Wilson and Reeder, 2005; Gatesy, 2009; Bibi, 2013; Figure 1).

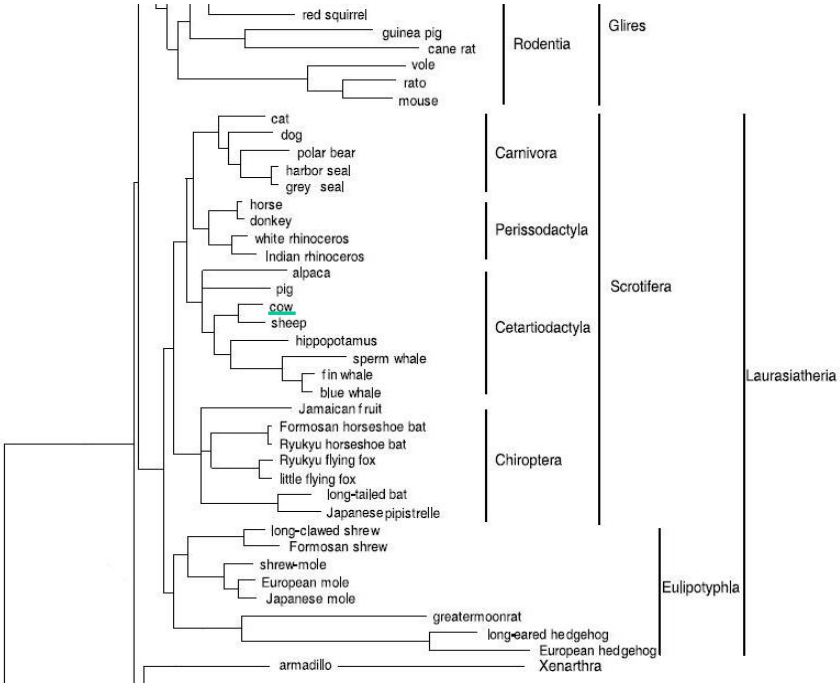

Figure 1: Part of the phylogenetic tree displaying Laurasiatheria, based on the mitochondrial protein analysis. The bovids in order Cetartiodactyla are highlighted (Nikaido *et al.*, 2003 – modified).

Artiodactyla (which means Cetartiodactyla without the cetaceans) contain three morphologically diverse suborders, the Suiformes (including *Hippopotamus sp.*), Tylopoda, and Ruminantia (Matthee *et al.,* 2001).

In most of examples of Ruminantia they miss the upper incisors and at least the males possess paired bony structures (horns, antlers, or ossicones) on their skulls (Eisenberg, 1981; Nowak, 1991). Six extant families of Ruminantia (Giraffidae (giraffes and okapis), Tragulidae (chevrotains), Moschidae (musk deer), Cervidae (deer), Antilocapridae (pronghorns) and Bovidae (cattle, sheep, and antelopes)) are traditionally recognized on the basis of their morphological characters like cranial appendages, skull characters, limbs, and dentition (for example Janis and Scott, 1987; Wilson and Reeder, 2005).

3.1.2 Phylogeny of Bovidae

Bovidae are characteristic by incomplete findings for the period, in which a great number of bovid subfamilies evolved (Matthee and Robinson, 1999), and by a basal division which separates the Bovinae (branched to the three living groups - cattle (Bovini), the spiral-horned antelopes (Tragelaphini) and the Boselaphini that includes Indian nilghai, *Boselaphus sp.* and chousingha, *Tetracerus sp.*) from the other bovid taxa (for example Cephalophinae, Antilopinae, Caprinae and further) (Kingdon, 1982; Matthee and Davis, 2001).

Hassanin and Douzery (2003) have dealt with the phylogeny of Pecora (group including the “higher ruminants” - Antilocapridae, Giraffidae, Bovidae, Moschidae and Cervidae). They have analyzed forty-eight morphological characters to deduce the phylogenetic relationships among the five pecoran families in comparison with the molecular analyses (7 mitochondrial and nuclear markers). The results of molecular and morphological analyses were congruent. The Antilocapridae and Giraffidae are basal groups, separated approximately 29 MYA (million years ago), and Cervidae, Moschidae, and Bovidae are closely related to them. Moschidae are sister group of Bovidae (separated around 26 MYA; Figure 2) (Hassanin and Douzery, 2003; Wang and Yang, 2013).
Contrary to these results, Bibi (2013) suggests younger times for the separations of the particular groups of bovids. He used both fossil and molecular (mitochondrial genome) data to obtain the results. According to this study, Bovidae and Moschidae diverged between 19.3 and 16.6 MYA.

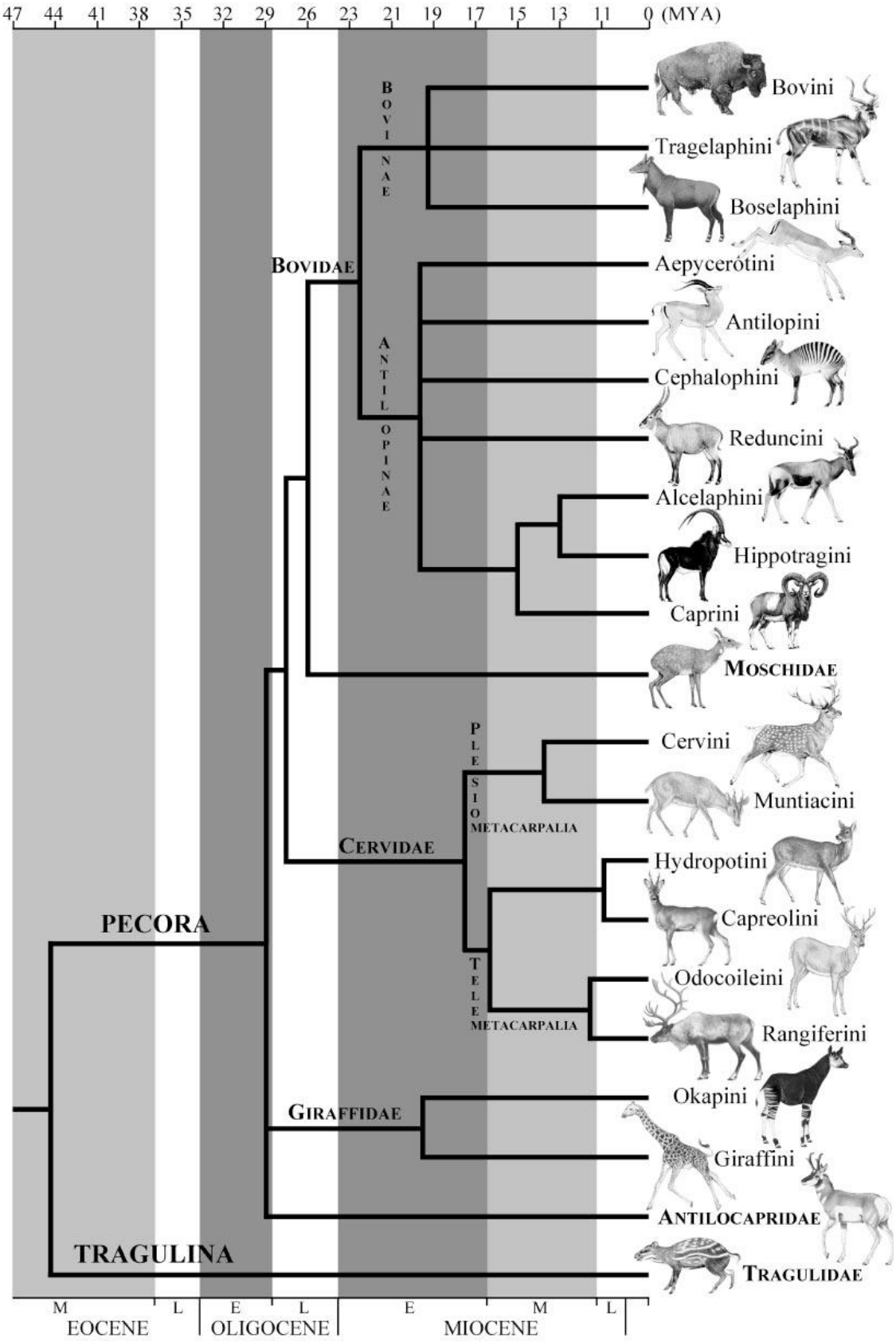

Figure 2: Phylogenetic tree (established on the basis of maximum parsimony, maximum likelihood and Bayesian analyses) with the time-scale for evolution of ruminants (derived from Bayesian relaxed molecular clock approach) constructed by the analyses of 7 mitochondrial and nuclear markers (Hassanin and Douzery, 2003).

The results of studies of Allard *et al.* (1992) and Rubes *et al.* (2008) showed the monophyly of Bovidae. Rubes *et al.* (2008) reached to this conclusion by studying of chromosomal homologies and Allard *et al.* (1992) tested the nucleotide sequences for the mitochondrial rRNA gene complex by parsimony analysis. This study also claims there are two clades within the family Bovidae – (1) including the tribes Boselaphini, Bovini, and Tragelaphini and (2) including Antilopini, Neotragini and other tribes (Allard *et al.,* 1992; Figure 3).

Monophyly of Bovidae (and also the families Moschidae, Giraffidae and Cervidae) also confirms the study of Wang and Yang (2013).

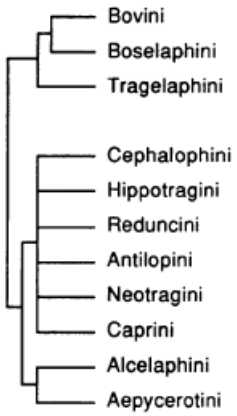

Figure 3: Tree displaying the relationships among the Bovidae (Allard *et al.,* 1992).

3.1.3 Phylogeny of Tragelaphinae

The taxonomy and phylogenetic relationships within Tragelaphinae were solved in the range of the studies, for example Matthee and Robinson (1999), Matthee and Davis (2001), Willows-Munro *et al.* (2005) and others.

Tragelaphinae (or Bovinae) are the monophyletic group according to the studies of Matthee and Davis (2001) and Hassanin and Ropiquet (2004). They came to this solution by very similar approaches - Matthee and Davis (2001) solved the evolution within the family Bovidae by means of 4 independent nuclear DNA markers, located in the protein-coding region (B-Spectrin nonerythrocytic 1 (SPTBN1), Protein-Kinase C1 (PRKC1), Kappa-casein (Kap-cas), and Thyrotropin (Thy) (Matthee *et al.,* 2001) and 3 mitochondrial DNA genes (cytochrome *b*, 12S rRNA and 16S rRNA). The parsimony,

maximum-likelihood and neighbour-joining analyses have been done to show the monophyly of the Bovinae as a sister lineage to the other bovid subfamilies.

3.1.4 Phylogeny of Tragelaphini

Hassanin and Ropiquet (2004) suggest the monophyly of the tribes Tragelaphini, Boselaphini and Bovini, and sort the kouprey (*Bos sauveli*) into the Bovini too. In their research they used promotor of the lactoferrin and two mitochondrial genes (cytochrome *b* and subunit II of the cytochrome *c* oxidase). The maximum parsimony method and Bayesian approach were used for the phylogenetic analysis.

According to the more recent research the subfamily Bovinae includes three distinct lineages - (1) Buffalo clade, (2) Banteng, Gaur and Mithan and (3) domestic cattle clades that have arisen after the Bovini split from the Boselaphini and the Tragelaphini tribes. Within the Bovini tribe there are distinguished two subtribes, the Bubalina and Bovina. It was found out by autosomal gene sequences analysed by neighbour joining algorithm with Kimura´s two parameter method (MacEachern *et al.,* 2009).

The divergence of the Tragelaphini from the other bovid tribes was estimated at approximately 14.08 MYA (Willows-Munro *et al.,* 2005) (contrary to this, Bibi (2013) suggests the divergence time between 10.1 and 5.4 MYA). This was followed by a period of rapid ecological specialization which divided the species living in moist forest environments (*Tragelaphus buxtoni*, *T. eurycerus*, *T. spekei*, and *T. scriptus*), and those adapted to a more arid savannah environment (*Taurotragus derbianus*, *T.oryx*, and *Tragelaphus strepsiceros*). The times of divergence among species are in accordance with hypotheses proposing that climatic oscillations and their impact on habitats were the major forces driving speciation in the tribe Tragelaphini (Willows-Munro *et al.,* 2005).

Pagáčová (2009) underlines the connection of the phylogenetic evolution and the environment, where the species live. She affirms, due to the study of the chromosomal homologies, that the basal species of the Tragelaphini are lesser kudu (*Tragelaphus imberbis*) and nyala (*T. angasii*). The nyala (*T. angasii*) and lesser kudu (*T. imberbis*) live in the bush, while the other

Tragelaphini prefer predominantly woodier environment (Willows-Munro *et al.,* 2005). This theory is in contrast with the results of the study of Rubes *et al.* (2008) that suggests the arid adaptation of *T. oryx* and *T. derbianus* is recent. The results of Pagáčová (2009) and Rubes *et al.* (2008) show that the cross-species chromosome paintings offer a novel approach to phylogeny determination within the Tragelaphinae. The investigation of chromosomal homologies using FISH (fluorescence *in-situ* hybridization) markers analysed by maximum parsimony method and the karyotypic change within the Tragelaphini was examined by using conventional and molecular cytogenetic techniques. These techniques rely on whole-chromosome and subchromosomal painting probes developed from cattle (Rubes *et al.,* 2008; Pagáčová, 2009).

The analysis (*in-situ* hybridization) of two different types of satellites (1.714 and 1.715) on the autosomal chromosomes and chromosome X indicates that the Tragelaphini and Bovini (cattle) are evolutionary older than the Reduncini, Hippotragini, Alcelaphini, Aepycerotini and Caprini (Chaves *et al.,* 2005; Figure 4).

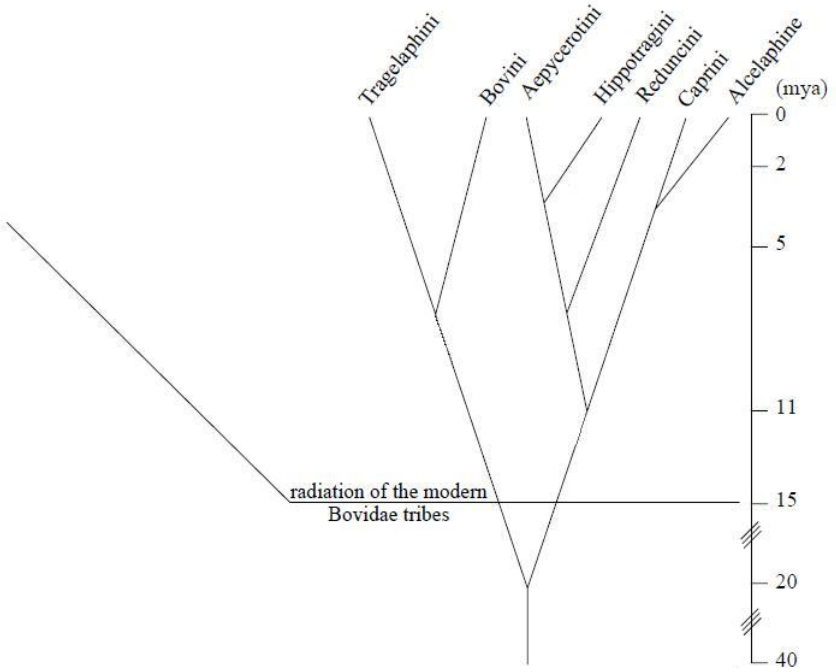

Figure 4: Evolutionary diagram of Bovidae based on the presence or absence of the 1.714 and 1.715 satellite sequences on the autosomal chromosomes and chromosome X. The axis y shows divergence times (Chaves *et al.,* 2005 – modified).

3.1.5 Question of inclusion of *Taurotragus spp.* into the genus *Tragelaphus*

The elands – the Common (*T. oryx*) and Derby eland (*T. derbianus*) belongs to the genus *Taurotragus*. Some researchers suggest inclusion of the genus *Taurotragus* into the genus *Tragelaphus* (Hassanin and Douzery, 1999; Matthee and Robinson, 1999; Matthee and Davis, 2001). This question has been solved by means of cytochrome *b* by maximum parsimony, maximum likelihood and neighbour-joining methods (Hassanin and Douzery, 1999; Matthee and Robinson, 1999). In addition, Matthee and Robinson (1999) say there is very little support for recognition of the *Booceros sp.* (bongo) as the separate genus. According to some authors bongo (*T. eurycerus*) is ranged into the genus *Tragelaphus* (Grzimek, 1990; Murphy *et al.,* 2001a; Wilson and Reeder, 2005 and others), another authors range bongo into the genus *Booceros* (for example Dorst and Dandelot, 1970).

3.2 Natural history of Tragelaphini

The most of Tragelaphini live in wooded habitats in the sub-Saharan Africa, *Taurotragus oryx* prefers the open habitats. Except the elands (*Taurotragus sp.*) and kudus (*Tragelaphus strepsiceros* and *T. imberbis*) that are adapted to the arid conditions, the antelopes of this group depend on water (Kingdon, 1982; Estes, 1991).

The tragelaphine antelopes feed on soft nutritious vegetation and fruit, to which their teeth and digestive system are adapted. The way of food picking up could be called a “gleaners” strategy. The antelopes belong to the “true ruminants”, they have four-chambered stomach and ruminate, usually they have the gall bladder too (Walker, 1964; Kingdon, 1982, 1997).

The Tragelaphini have variable number of chromosomes from 2n = 30 by sitatunga (*Tragelaphus spekei)* to 2n = 56 by nyala (*T. angasii*). For comparison the cattle (*Bos taurus*) has 2n = 60 (Rubes *et al.,* 2008).

3.2.1 Typical traits of Tragelaphini

The spiral-horned antelopes have typical spiral horns, present by males, only by elands (*Taurotragus sp.*) and bongos (*Tragelaphus eurycerus*) also by females. The study of Stankowich and Caro (2009) deals with the presence of the horns by females, mainly with the reasons, why the females of bovids have the weaponry, which are often smaller and shaped differently to male horns, suggesting a different function. The authors infer, that the large bovids living predominantly in open habitat has the weaponry as the protection against predators (fight is more probable than flight or caching), the smaller and territorially species (like duikers, *Cephalophus sp.*) can profit from the weaponry in intrasexual competition. The Derby eland (*Taurotragus derbianus*) is very special case, because the animals have cryptic body coloration but the females bear the horns too. The reason of the horns by this species is the object of speculations.

Kingdon (1982) mentions, that the development of horns in the females of bongos (*Tragelaphus eurycerus*) and elands (*Taurotragus sp.*) is their evolutionary response to the challenge of predators, while the crypsis is ineffective with regard to their body size. The females and youngs form larger groups while the smaller tragelaphines and Greater kudu (*Tragelaphus strepsiceros*) live dispersedly or directly solitary, their females and youngs rely on their crypsis to precede predation in their dense habitat.

The horns by elands (*Taurotragus sp.*) and bongos (*Tragelaphus eurycerus*) females became the instruments of a social hierarchy too and may also serve as tools to break branches (Kingdon, 1982; Estes, 1991). Furthermore, Kingdon (1982) suggests that hornlessness may be the product of female strategy or of the male competition.

Further typical trait are white vertical strips and the scent glands, located in front of the teats (by bushbuck (*T. scriptus*), sitatunga (*T. spekei*), lesser kudu (*T. imberbis*) and mountain nyala (*T. buxtoni*)) and glands around false hooves in hindfeet (these glands are absent in bushbuck (*T. scriptus*), sitatunga (*T. spekei*) and bongo (*T. eurycerus*). The females have four teats (Grzimek, 1972; Kingdon, 1982; Grzimek, 1990; Estes, 1991).

The sexual dimorphism is characteristic for the Tragelaphini - the horns by the males, as described above, the darker coloration of the males and also the size dimorphism (the females are usually smaller than the males) (Kingdon, 1982; Estes, 1991).

The cranial similarity was observed between sitatunga (*T. spekei*) and nyala (*T. angasii*) and between the greater (*T. strepsiceros*) and lesser kudu (*T. imberbis*) (Kingdon, 1982). According to Ruggiero (1990) the various skull characteristics place the Derby eland (*Taurotragus derbianus*) directly between the Common eland (*Taurotragus oryx*) and bongo (*Tragelaphus eurycerus)*.

3.3 Genetic studies

Genetic studies concerning with phylogeny (for example Hassanin and Ropiquet, 2004; Chaves *et al.,* 2005; Pagáčová, 2009 and others) are mentioned in the chapter "Taxonomy and phylogeny of T*aurotragus spp.*"

3.3.1 Genome maps

For some members of the family Bovidae there has been created the map of their genome, for example for cattle (Bishop *et al.,* 1994; Samson *et al.,* 2008a) or goats (Samson *et al.,* 2008b). It can be very helpful when searching for some "new" primers by the species whose primers are not known yet. For example when we were choosing the suitable primers for Western Derby eland (*Taurotragus derbianus derbianus*), we utilized these sources too.

3.3.2 Phylogeography

The phylogenetic split between the regions of East and southern Africa (where the Pleistocene refugia occurred) has already been identified in the species adapted to the arid environment, for example in hartebeest (*Alcelaphus buselaphus*), wildebeest (*Connochaetes taurinus*), sable antelope (*Hippotragus niger*) and others. It has shown varying degrees of mitochondrial lineage differentiation between east and south (Arctander *et al.,* 1999; Birungi and Arctander, 2000; Flagstad *et al.,* 2001; Pitra *et al.,* 2002).

Phylogeographic analysis of the Common eland (*Taurotragus oryx*) was performed to judge the hypotheses of Pleistocene refugial areas in East and southern Africa and the existence of genetic traces of Pleistocene climate change. The analysis was performed by means of mitochondrial DNA control-region fragment. The phylogeographic split among major genetic lineages was dated using Bayesian coalescent-based methods (Lorenzen *et al.,* 2010).

Two major phylogeographic lineages including East and southern African localities were separated. The stable population in the south, absence of isolation-by-distance among populations in the region and supposed gene flow designs that the southern mitochondrial lineage in the Common eland (*T. oryx*) fused earlier than the eastern lineage. The southern region showed few haplotype structuring among localities, and higher genetic diversity than in the east (Lorenzen *et al.,* 2010).

Study of Lorenzen *et al.* (2012), using molecular data of 19 ungulate taxa (including *Taurotragus oryx* and *Tragelaphus strepsiceros*), determined as Pleistocene savannah refugia localities in West, Southern and South-West Africa and mosaic of temporal and spatial refugia in East Africa.

3.4 Pedigree analysis

Pedigree analysis is one of important tools how to describe genetic variability and its evolution across generations. It is also the simplest method to inbreeding determination and prevention. It helps to choose the most suitable individuals for the reproduction. Pedigree analyses are mostly used for captive populations, because the data often lack for the wild populations (Gutiérrez *et al.,* 2003; Ralls and Ballou, 2004).

Pedigree analysis takes into consideration several parameters – generation interval (average age of parents, when their grandchildren are born), completeness of the pedigree, inbreeding coefficient (F, used for measuring of homozygosity level) related with average relatedness (shows proportion of representation of each individual in the whole pedigree).

Further parameter is effective population size (N_e), which means the number of breeding individuals in an idealized population, that shows the qualities of genetic parameters as the population of interest. N_e is calculated according to this equation: $N_e = 1 / 2\Delta F$, ΔF means relative increase in inbreeding by generation (or relative increase of homozygosity between two generations, or decrease of heterozygosity between two generations). There is possible to use also effective number of founders and effective number of ancestors, which describes the probable origin of the genes. Effective number of founders expresses the number of equally contributing founders and effective number of ancestors is the minimum number of ancestors that are responsible for the complete diversity of the whole population. Founders are defined as the individuals that have no relatives in the pedigree excluding their own offspring as well as they are the animals their parents are not known, so they are presumed to be unrelated (Lacy *et al.*, 1995; Gutiérrez *et al.,* 2003).

Another quantity, which have to be mentioned in connection with pedigree analyses are founder equivalent (f_e) and founder genome equivalent (f_g). Founder equivalent expresses the number of equally contributing founders, it decreases when the contribution of founders is unequal. It is created by $f_e = 1 / \Sigma(p_i^2)$, where p_i means the number of genes in the population of offspring, established by founder i. Living founders are excluded from the founder representations. Unequal contribution of founders results in fewer founder equivalents (Lacy, 1989).

The founder genome equivalent is the number of equally contributing founders with no random loss of founder alleles in offspring, so the genetic diversity of founders stays preserved. It defined by the equation $f_g = 1 / \Sigma(p_i^2 / r_i)$, where r_i means the expected number of alleles of founder i that occur in the population of offspring (p_i was explained above) (Lacy, 1989).

Founder equivalent and founder genome equivalent are related according to $f_g = r * f_e$ where r (allele retention or founder allele survival) expresses the constant proportion of genes of each founder, which are retained in the population of offspring. Number of founder equivalent is higher than the number of founder genome equivalents (Lacy, 1989; Ralls and Ballou, 2004).

The amount of family relationships in the population is expressed by mean kinship (MK), that describes the loss of genetic diversity in the population of offspring related with founders. It is defined by means of the kinship coefficient, which shows the probability that the two alleles are identical by descent. Low mean kinship indicates, that the individual has few relatives in the population and so that its genetic potential is important for the next reproduction (Ballou and Lacy, 1995; Grueber and Jamieson, 2008).

Gene diversity (GD) shows the proportion of heterozygotes expected in the population of offspring that is in Hardy-Weinberg equlibrium, it describes the variation in frequencies of alleles at a genetic locus. It is counted according the equation GD = 1- $\Sigma(p_i^2)$, p_i means the frequency of allele i (Grueber and Jamieson, 2008; Koláčková *et al.*, 2011a).

3.4.1 Tools for pedigree analysis

There exist several tools for pedigree analysis. The first tool is usable, when we know the whole pedigree of the population, in detail it means to known the relations between all pairs of individuals in the population. Then it is possible to calculate the genotype probabilities. When there is little information about the population and individuals of the population, the management follows common principles of population genetics. Animals, especially founders, with unknown parents are presumed to be unrelated (Lacy *et al.*, 1995; Gutiérrez *et al.,* 2003).

When the population structure is known (for example sex ratio, number of animals, rates of fecundity and mortality, and social structure), but the pedigree is not complete, there is possible to use the second tool and simulate the possible pedigrees. The last tool enables to create the equations for description of genetic processes in population (Lacy *et al.*, 1995).

3.5 Issues of small populations

Population size is one of the IUCN criteria, which ranges the species into the red list categories. Captive populations are often small and fragmented, because the institution, where they are bred, like zoos, have limited capacity for breeding of large populations. They serve as the source of individuals for supplement or restoration of the wild population, or as the prevention against the extinction of the species (Ballou and Lacy, 1995; Frankham *et al.,* 2003; Lacy *et al.*, 2009).

Small populations are more predisposed to the extinction because they have higher tendency to the progress of inbreeding and loss or fixation of some alleles as a consequence of genetic drift. Inbreeding and loss of genetic diversity is influenced by effective population size (N_e). N_e can be determined according to the demographic data or by genetic methods based on the changes of allele frequencies and allelic diversity, rate of heterozygotes and homozygotes across the generations or rate of increase in pedigree inbreeding coefficient (Frankham *et al.,* 2003).

Breeding of small populations in captivity can lead to the loss of genetic diversity and displays of inbreeding depression, particularly when such population comes from small number of founders (Lacy *et al.*, 1995; Primack, 2000; Thévenon and Couvet, 2002; Frankham *et al.*, 2003). Inbreeding depression can manifest if any deleterious genes (or alleles) occur in the population. Loss of genetic diversity and accumulation of such deleterious alleles may result in reduction of adaptability of the population in the face of sudden environmental changes (Frankham *et al.,* 2003).

Inbreeding depression can display in higher mortality, lower fertility, reduced mating ability, slower growth, more developmental defects and other. It is also important to know, that inbreeding can have different effects on wild populations living in natural habitats and on experimental species – like mice or domesticated livestock living in modified environment. The results of genetic drift occurrence in small population are loss of polymorphism and accumulation of maladaptive traits and changes in allele frequencies. Inbreeding also causes

the decrease of homozygosity of an individual at a locus (Lande, 1988; Lacy, 1997; Frankham *et al.*, 2003; Lacy *et al.*, 2009).

These processes progress very slowly, so they are very hard to be observed. They could be recognized through their consequences – lower ability of survival in the present environment and more difficult adaptation in new environment. They are also connected with non-genetic factors influencing the population. Exchange of the individuals between two different populations can help to increase genetic variability but can also lead to the loss of alleles, which are unique in certain population (Lacy, 1997).

Situation of low number of founders in the population could be similar to the settlement of the island, or of some new environment. Founders colonize the environment, and then the population grows. But the genetic drift and inbreeding also occur in the population. Rate of the threat is influenced by size of the area and by the population size (that depends on the area size and carrying capacity of the environment). Smaller populations are more susceptible to disturbances, because they can lose some beneficial alleles due to genetic drift and the deleterious alleles can accumulate due to the inbreeding. Such population may have reduced ability to adapt on the changing environment conditions, and is more threatened by extinction (Frankham, 1997).

Populations of endangered species bred in captivity, for example in zoos or reserves, are small too, so they are threatened by inbreeding and its consequences. Frankham *et al.*, (2003) recommend moving of individuals among the groups to kinship minimization. Also he recommends dividing the population into several smaller not quite isolated subpopulations, because they have possibility to maintain more genetic variability than one larger population. The total inbreeding coefficient of these subpopulations should be lower than in one population of the same total size (Lacy, 1987; Frankham *et al.*, 2003).

3.5.1 Examples of breeding programmes of small populations

3.5.1.1 Arabian oryx (*Oryx leucoryx*)

In 2011 Arabian oryx (*O. leucoryx*) belonged into the category "Endangered D" (this category means that the population has fewer than 250 mature individuals), in spite of this, there lived more than 250 mature individuals in the wild - in Oman, Saudi Arabia and Israel. The IUCN suggested that the category "Vulnerable" D1 (population size numbers fewer than 1000 mature individuals) would be more appropriate and now, in 2013, Arabian oryx belongs to this category (IUCN, 2011, 2012).

At the beginning of 20th century the Arabian oryx (*O. leucoryx*) lived through most of the Arabian Peninsula, but it has progressively declining, at first in the north, but later in the south too, due to hunting. The species became extinct in the wild in 1972 (Asmodé and Khoja, 1989; Ostrowski *et al.,* 1998; Marshall *et al.,* 1999; IUCN, 2012), contrary to the Western Derby eland (*Taurotragus derbianus derbianus*), which still occurs in the wild (Bro-Jørgensen, 1997; Kingdon, 1997; Nežerková *et al*., 2004).

There has been established the captive breeding programme in the USA in 1960. Two breeding groups were founded by five males and six females. Management used the identification cards for the individuals (Asmodé and Khoja, 1989). Breeding was very successful, the number of population increased and the animals were sent to the reintroduction programmes to Oman and Saudi Arabia and to the European and Far East zoos too (Ostrowski *et al,.* 1998; Marshall *et al.,* 1999).

Nowadays, the reintroduced populations exist (in Oman, Saudi Arabia and Israel). They number around 1100 animals. The threats are represented by capturing and sale to private ownership and also the habitat destruction by overgrazing and drought (IUCN, 2011).

International studbook is managed and regional Arabian Oryx conservation strategy was developed in 2007 (Marshall *et al.* 1999; IUCN, 2011).

There is a similarity of study of Marshall *et al.* (1999) to our study of genetic diversity of the Western Derby eland (*T. d. derbianus*). The genetic analysis of the individuals of the Arabian oryx (*O. leucoryx*) originating from different populations (from USA or Arabian Peninsula) was performed by microsatellite markers. From total sixty-six tested loci, originally develop for cattle (*Bos taurus*), sheep (*Ovis aries*), red deer (*Cervus elaphus*) and gazelle (*Gazella gazella*), nineteen were polymorphic (2 – 7 alleles, only 7 loci had more than 2 alleles). The allele frequencies, deviations from Hardy-Weinberg equilibrium and expected heterozygosity and other parameters were calculated by using the software Cervus 1.0 (Marshall *et al.,* 1998). Allelic diversity across the population was not very clear, but some alleles, that were rare in some population, were missing in others – there were lost possibly due to the genetic drift (Marshall *et al.*, 1999).

3.5.1.2 Przewalski's horse (*Equus caballus przewalskii*)

Przewalski's horse (*E. c. przewalskii*) is now classified as “Endangered D” (Walzer *et al.*, 2012; IUCN, 2013).

Originally Przewalski's horse (*E. c. przewalskii*) lived in Europe and Asia, from Germany across Russia, Kazakhstan and Mongolia to northern China. It became extinct in the wild, last individuals were seen in Dzungarian Gobi desert in Mongolia in 1969 (Wakefield *et al.*, 2002; IUCN, 2013).

Captive breeding programme was established by 12 individuals and one domestic mare. Studbook was created in 1959, now it is handled by Prague Zoo (Wakefield *et al.*, 2002; Walzer *et al.*, 2012; IUCN, 2013).

First harem group was released into the wild in 1997 in Great Gobi-B national park and international biosphere reserve (western section of Gobi national park) – in Takhin Tal. The reintroduction was successful, and except of the decline of the population caused by severe winter in 2009, the population does well (Wakefield *et al.*, 2002; Walzer *et al.*, 2012; ITG International Takhi Group, 2013; IUCN, 2013).

Reintroduced populations are threatened by hybridization with domestic horses and infection of diseases (for example *Babesia equi*, *B. caballi* or strangles - infection by *Streptococcus equi*), and also by competition with domestic livestock and horses. Another important threat is caused by habitat destruction and predation of wolves, mainly on foals. On the other hand, the wolves serve as important selection factor too. Problems can be caused also by stochastic factors like severe winter (ITG International Takhi Group, 2013; IUCN, 2013).

3.5.1.3 Addax (*Addax nasomaculatus*)

Addax (*A. nasomaculatus*) is classified as "Critically endangered C1+2a(ii)" (the population has fewer than 250 mature individuals and the estimated decrease of the population is at least 25% in three years or one generation and there are at least 90% of mature animals on one subpopulation). The last remaining population numbers less than 300 individuals, it occurs in the Termit/Tin Toumma region of Niger. Originally addax (*A. nasomaculatus*) used to live in the Sahelo-Saharan region of Africa. It was native in Mauritania, Chad and Niger, but it became extinct in Algeria, Egypt, Libya, Sudan and Western Sahara too. The population decreased due to hunting and habitat loss too (IUCN, 2013).

First reintroduction was realized in Jebil National Park, in Tunisia. At least 1 600 individuals live in European and African zoos or in North American, Japan and Australian breeding programmes and also in private collections in USA and Middle East (IUCN, 2013).

Addaxes (*A. nasomaculatus*) are protected by law in Morocco, Tunisia, and Algeria (IUCN, 2013).

As further examples of species, whose populations in the wild decreased and they were reintroduced thanks to captive breeding programmes, could be mentioned black-footed ferret (*Mustela nigripes*) or California condor (*Gymnogyps californianus*) (Frankham *et al.,* 2003; IUCN, 2013).

3.6 Western Derby eland (*Taurotragus derbianus derbianus*)

Usually there are recognized two subspecies of the Derby eland (*Taurotragus derbianus*), Western Derby eland (*T. d. derbianus*) and Eastern giant eland (*T. d. gigas*) (Dorst and Dandelot, 1970; East, 1998; Wilson and Reeder, 2005). The difference between the subspecies has been determined only on the basis of the morphological description till today (Koláčková *et al.,* 2009).

3.6.1 Distribution

The Eastern giant eland (*T. d. gigas*) lives in Cameroon, Central African Republic and Sudan (Bro-Jørgensen, 1997; IUCN, 2010; Figure 5). The total population estimates vary between less than 15 000 individuals and less than 35 000 individuals. The population decline is attributed to poaching and rinderpest epizootic (in 1982 – 1983) in Central Africa (Bro-Jørgensen, 1997).

In the past the Western Derby eland (*T. d. derbianus*) was found in the West of Africa (Senegal, Gambia, Guinea, Guinea-Bissau, Mali, Sierra Leone, Ivory Coast, Togo and Ghana), but now the only sure distribution of the Western subspecies is in Senegal in Niokolo Koba National Park (Bro-Jørgensen, 1997; Kingdon, 1997; Nežerková *et al*., 2004; Figure 5). The population of Western Derby eland (*T. d. derbianus*) in the wild has fewer than 200 individuals (Renaud *et al.,* 2006). The number of individuals is sharply decreasing, probably due to poaching, habitat loss and grazing competition with livestock (Bro-Jørgensen, 1997; Koláčková *et al.,* 2011b).

Derby elands (*T. derbianus*) prefer mostly the flats or gentle slopes and densely wooded savannas, they are adapted to *Isoberlinia* woodlands, contrary to the Common elands (*T. oryx*), which prefer more open habitats (Dorst and Dandelot, 1970; Kingdon, 1982; Ruggiero, 1990; Estes, 1991; Kingdon, 1997).

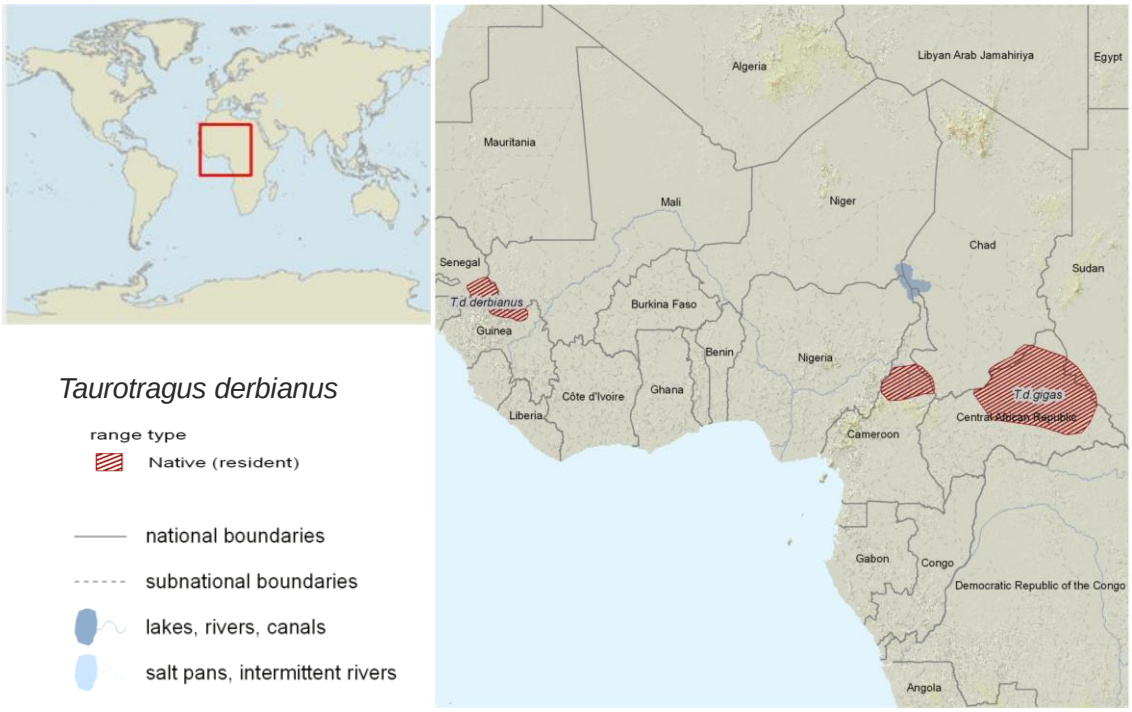

Figure 5: Distribution of the Derby eland (*Taurotragus derbianus*) (IUCN, 2010 - modified).

3.6.2 Description

The Derby eland (*Taurotragus derbianus*) is considered to be the largest antelope in the world, but it is very disputable, because its weight and size is comparable with the Common eland's (*T. oryx*) measurements (Bigalke, 1968; Dorst and Dandelot, 1970; Kingdon, 1982; Estes, 1991).

The body length of the Derby eland (*T. derbianus*) is 290 cm in the bulls and 220 cm in the cows, the height at the withers is between 150 and 176 cm in the bulls and 150 cm in the cows. The tail measures 55 to 78 cm. The weight of the male can reach up to 1000 kg, the female can weigh up to 440 kg. Both sexes have large and massive spiral horns up to 1 – 1.2 m long, which are intermediate between those of the kudu (*T. strepsiceros*) and the eland (*T. oryx*) and show much variation in shape. The horns of the males are longer, more widely splayed and have a looser spiral than in the Common eland (*T. oryx*) (Dorst and Dandelot, 1970; Kingdon, 1982; Estes, 1991; Koláčková *et al.,* 2010).

With the development of the horns by the Western Derby eland (*T. d. derbianus*) dealt Antonínová *et al.* (2008). She describes the differences in the horns development of males and females during their first 3 years of the age.

The general colour of the Derby elands (*T. derbianus*) is ruddy fawn or chestnut, in adult bulls may pass to bluish grey. This depends on the age of the animal and on the climatic period. The blackness of the neck may reflect the androgen status of the male, with the maximum in mature bulls during the rut (Bro-Jørgensen, 1997; Koláčková *et al.,* 2010). The animals have nine to seventeen white stripes on the flanks, the number and pattern of the stripes differs on each side, is unique for each animal and does not change during the animal´s life (Dorst and Dandelot, 1970; Hillman, 1975; Akakpo *et al.*, 2004; Nežerková *et al.,* 2004; Koláčková *et al.,* 2010).

The adult bulls have a tuft of the brown hairs on its forehead, a black mane on the neck, continuing along the back like a black stripe, and a dewlap growing from their chin to the chest (Koláčková *et al.,* 2010).

The Derby elands (*T. derbianus*) have two white cheek spots, a white stripe in front of the eye on both sides, wide rounded ears, marked by white and black and the white and black spots on their hocks (Koláčková *et al.,* 2010; Figure 6).

The animals have false hoof glands and maybe the apocrine glands under the tuft on the forehead too. The hooves are narrower than by the Common eland (*T. oryx*), the false hooves are large. The cow has four teats (Bro-Jørgensen, 1997).

Figure 6: The male of Western Derby eland (*Taurotragus derbianus derbianus*) Niokolo (Photo by author).

3.6.3 Differences between Eastern (*T. d. gigas*) and Western subspecies (*T. d. derbianus*)

The Western Derby eland (*T. d. derbianus*) is smaller than the Eastern Giant eland (*T. d. gigas*), it has bright rufous ground colour and from eleven to fifteen body stripes. It has elongated white cheek spots. The Eastern subspecies (*T. d. gigas*) is characterised by larger body size, sandy colour and from ten to fourteen body stripes. Its white cheek spots have round shape. It has longer horns than the Western subspecies (Lydekker, 1914; Dollman, 1936; Haltenorth, 1963; Dorst and Dandelot, 1970; Kingdon, 1982; Bro-Jørgensen, 1997; Kingdon, 1997; Lutovská, 2012; Böhmová, 2013).

Lutovská (2012) dealt with the comparison between the subspecies of Derby eland. Her study says that it is not possible to distinguish the subspecies by coat colour or number of body stripes, but they differ in one parameter of horn and another parameter, the length of teeth row, is very close to conventional subspecies boundary (Lutovská, 2012). Also the subspecies differ in the number of white strips on the left flank – the Eastern Giant elands (*T. d. gigas*) have less strips (13 strips on average) than the Western Derby elands (*T. d. derbianus*) (14 strips on average) (Böhmová, 2013).

3.6.4 Nutrition

The elands (*Taurotragus sp.*) belong to intermediate (or mixed) feeders. They are more adaptable to seasonal changes in diet (Hofmann, 1973). The Derby elands (*T. derbianus*) are browsers contrary to the Common elands (*T. oryx*). The main part of their food creates the shoots, leaves of various trees and shrubs, branches and fruits (Kingdon, 1982; Ruggiero, 1990; Bro-Jørgensen, 1997; Mares, 1999; Hejcmanová *et al.,* 2010). The Derby elands (*T. derbianus*) are generalists, their diet is very variable. They also use their horns to break branches in order to get the leaves (Ruggiero, 1990; Kingdon, 1997).

Generally the elands are water independent, water obtained from their diet (plants with high water content) is sufficient for them, but they drink when they have the possibility. To conserve the water the animals excrete dry feces and concentrated urine (Mares, 1999).

Hejcmanová *et al.* (2010) has studied the diet composition of Western Derby eland (*T. d. derbianus*) in the dry season by faecal analysis. The results confirm the Western Derby eland (*T. d. derbianus*) is predominantly a browser consuming the grass in insignificant amounts in the dry season.

3.6.5 Status of the threat

The Western Derby eland (*T. d. derbianus*) is on the IUCN Red list of threatened species with status “Critically Endangered” (CR C2a (ii)) (contrary to the Eastern subspecies (*T. d. gigas*), which has the status “Least Concern”). This classification include taxa whose population size is estimated at less than 250 mature individuals with continuing decline, observed, projected, or inferred, in numbers of mature individuals and at least 90% of mature individuals in one subpopulation (Frankham *et al.,* 2003; IUCN, 2012).

The decrease of the population size has been caused by overhunting for meat and habitat destruction due to the expansion of human and livestock populations. The Derby eland (*T. derbianus*) has also suffered heavy mortality from rinderpest, to which is probably more susceptible than the other antelopes. The populations in Gambia, Mali and Central African region have been affected by this disease (Kingdon, 1982; Ruggiero, 1990; Bro-Jørgensen, 1997; Kingdon, 1997; IUCN, 2010).

3.6.6 Western Derby eland conservation programme

In 2000 the *ex-situ* conservation breeding programme was established. Six individuals, one male and five females, which were caught in the Niokolo Koba National Park, became the founders of a semi-captive breeding programme in Senegal. They were placed to the separated enclosure in Bandia Reserve (Nežerková *et al*., 2004).

In June 2012, the Western Derby eland (*T. d. derbianus*) in semi-captivity formed a population of 83 living individuals. The population was divided in 5 breeding herds: 3 in Bandia Reserve and 2 in Fathala Reserve, and 2 bachelor herds, one in each reserve (Figure 7), that aggregates the young males, which were not selected for the breeding herds. The animals are identified shortly after their birth by means of direct observations and individuals for breeding are selected on the basis of their kinship relations, age and sex (Antonínová *et al.,* 2004, 2006; Koláčková *et al.,* 2009, 2012; Figure 8).

Today (June 2013) the population has 95 individuals (Brandlová, 2013, personal communication). It is the only population of the Western Derby eland (*T. d. derbianus*) in captivity (or more exactly in semi-captivity). The database ISIS (ISIS, 2010) mentions only the individuals of the Eastern subspecies (*T. d. gigas*) in captivity.

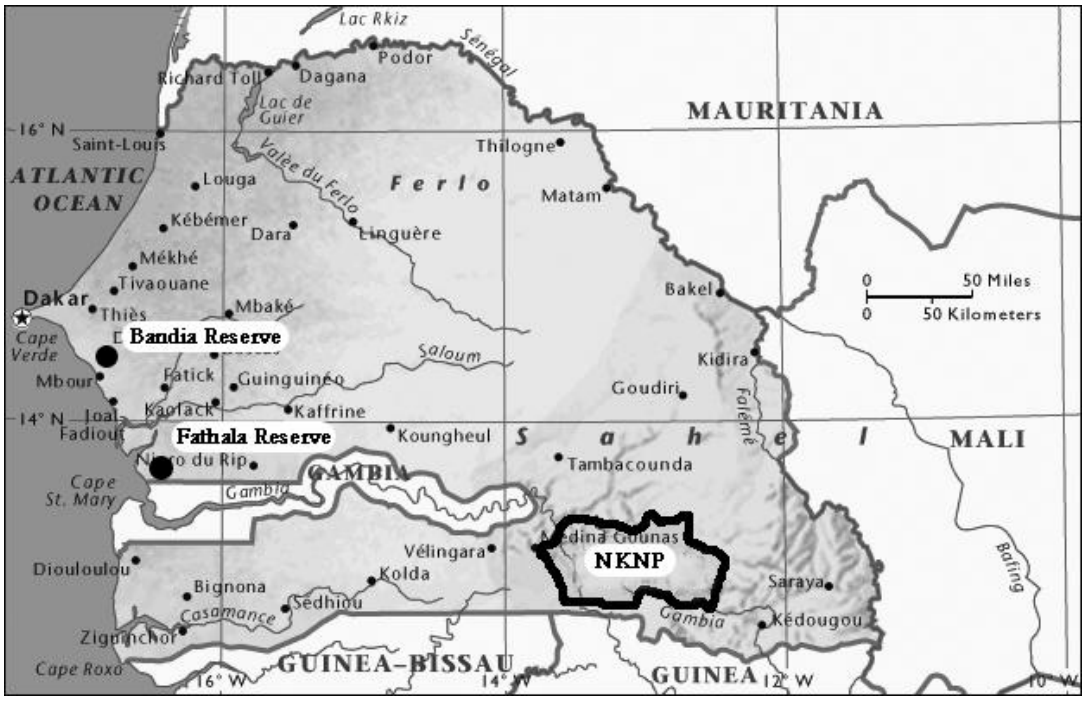

Figure 7: Locations of the Bandia and Fathala reserves and Niokolo Koba National Park (NKNP) (Koláčková *et al.,* 2011a).

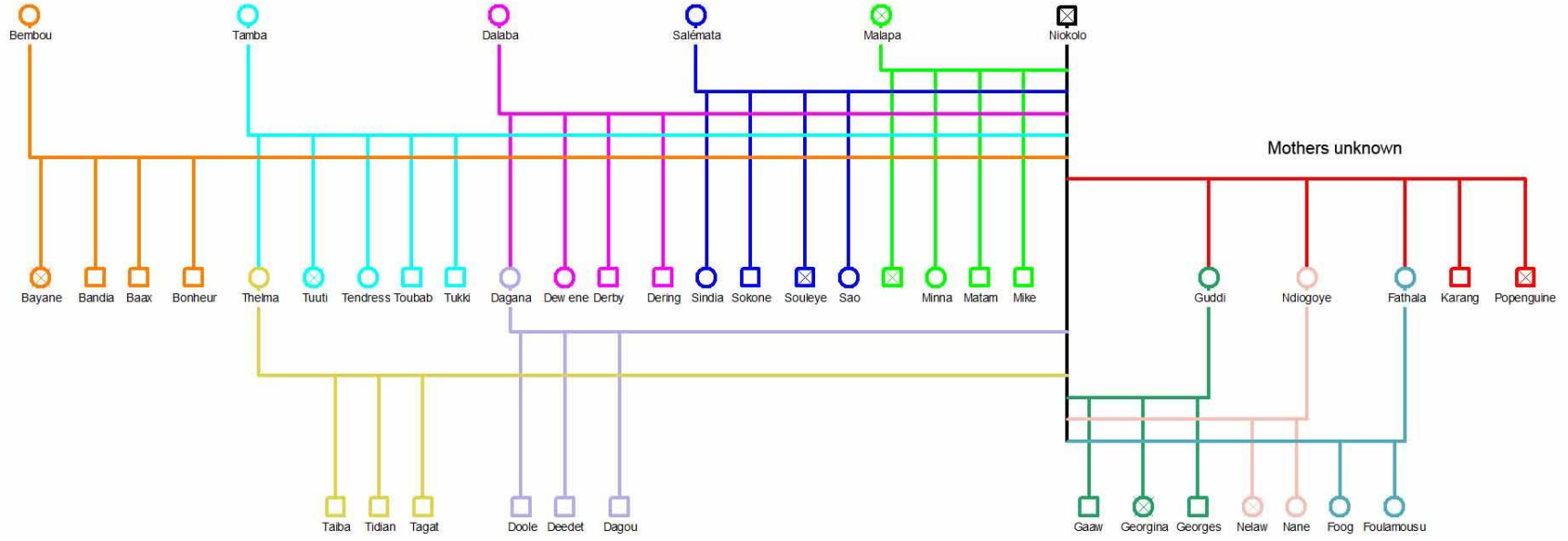

Figure 8: The part of the family tree of the Western Derby elands (*Taurotragus derbianus derbianus*) bred in semi-captivity in Bandia and Fathala reserves (includes the offspring born till the end of January 2007).
☐ = male, ○ = female, × = dead individual, colours = related individuals (families) (Created in the programme GenoPro 2007)

The estimated mean level of inbreeding in the population was 0.124 in 2012, that is lower than in 2008 (0.136). This value is quite low (inbreeding coefficient reaches the values from 0 to 1, higher value means higher level of inbreeding and higher probability that the two alleles are identical by descent – gathered from the common ancestor of the both parents of an individual) (Beebee and Rowe, 2008; Koláčková *et al.,* 2009, 2010, 2011a, 2012). This value of inbreeding coefficient has been found out from the pedigree of the antelopes, it is not supported by any genetic analysis (Koláčková, 2011a).

Risk of occurrence of inbreeding depression in the population is increased by the fact, that there is no gene flow. The population is closed, the breeding groups have been established in different environments, but there exists no change of the individuals and so no gene flow among the breeding groups.

The World Conservation Union (IUCN) recommends establishing the captive population earlier than the number of wild population drops under 1000 individuals and simultaneously the captive population should be established with at least 20 wild founders to maintain appropriate genetic level (Lacy, 1989; Frankham *et al.,* 2003).

A general aim in population management is to maintain 90% of the original genetic diversity at the end of a 100 year period (Primack, 2000; Frankham *et al.,* 2003). The actual genetic diversity of the population of Western Derby eland (*T. d. derbianus*) based on the model data from the pedigree (counted in Population Management 2000 software (Lacy and Ballou, 2002; Pollak *et al.,* 2002)) was determinated 78%, lower than required 90%. The population size needful for maintaining 90% of genetic diversity is 958 individuals, with simultaneously introduction of at least 40 founders. It is not feasible to achieve this aim, so the alternative aim has been established – to maintain 80% of genetic diversity at the end of a 100 year period. This alternative aim can be realized by more ways, for example by simple introduction of 15 founders, by simultaneous population size of 379 individuals, or by introduction of 5 founders every 45 years, by simultaneous population size of 364 individuals and likewise. The choice of the optimal way depends mainly on financial possibilities, eventually on political situation (Koláčková *et al.,* 2010, 2011a).

The population of Western Derby eland (*T. d. derbianus*) is small, but it reproduces successfully. The trouble can be the low number of founders and no gene flow. There occurs the inbreeding in the population and potentially there could occur the genetic drift too (it has not been proved yet). The both phenomena can flow into the reduced genetic variability, which predicts lower individual fitness and lower population adaptability to the changing environment.

It is possible that the low level of the genetic variability occurs in the population of the Western Derby eland (*T. d. derbianus*) in the wild too. It could be the consequence of the bottleneck, which has not been described. But it is very controversial topic. For example Matocq and Villablanca (2001) analysed, if the low genetic diversity is caused by bottleneck or not. They tested the control region and cytochrome *b* gene of the post-bottleneck Morro Bay kangaroo rat (*Dipodomys heermanni morroensis*) and pre-bottleneck samples of the closely related subspecies Lompoc kangaroo rat (*D. h. arenae*). The maximum likelihood estimate was evaluated, the estimate of nucleotide diversity and the selection on mitochondrial haplotypes were tested. The results suggest that the low genetic diversity need not to be caused by the population decline, but it could be historical pattern of the population. The authors advert that it is very important to choose suitable reference groups to the evaluation of the genetic diversity in endangered species. Further if the genetic diversity is used as a tool for the conservation management, they recommend analyzing of the archival specimens.

4. METHODOLOGY

4.1 Material

The collection of samples proceeded in the years 2006 to 2012. The samples of blood and hairs were collected annually during the transports of animals among the reserves (Bandia and Fathala) or herds by the experienced veterinarian (Antonínová *et al.*, 2006; Koláčková *et al.*, 2011a).

The tissue samples were obtained in September 2006 by biopsy darts. Other samples were obtained from the dead animals (mostly the ear, or part of the inner tissue).

All samples were collected in Bandia reserve, in Senegal. The blood samples were heparinized and stored in the freezer by –18°C. The tissue samples were stored in the 96% ethanol in room temperature and after in the freezer too.

There were obtained 66 samples of DNA in total. The list of the samples presents the Table 1.

Table 1: Samples of the Western Derby eland (*Taurotragus derbianus derbianus*).

DNA Sample no.	Individual	Date of birth	Type of the sample	Date of collection	Collected by	Locality of collection
1	Bandia	14.12.2004	blood	3 / 2006	MVDr. Jiří Váhala	Bandia reserve
2	Taiba	5.1.2005	blood	3 / 2006	MVDr. Jiří Váhala	Bandia reserve
3	Derby	2.12.2004	blood	3 / 2006	MVDr. Jiří Váhala	Bandia reserve
4	Doole	11.1.2005	blood	3 / 2006	MVDr. Jiří Váhala	Bandia reserve
5	Gaaw	25.1.2005	blood	3 / 2006	MVDr. Jiří Váhala	Bandia reserve
6	Popengiune	23.2.2003	blood	3 / 2006	MVDr. Jiří Váhala	Bandia reserve
7	Karang	3.1.2003	blood	3 / 2006	MVDr. Jiří Váhala	Bandia reserve
8	Sokone	29.11.2003	blood	3 / 2006	MVDr. Jiří Váhala	Bandia reserve
9	Matam	23.11.2003	blood	3 / 2006	MVDr. Jiří Váhala	Bandia reserve
10	Sindia	22.11.2004	blood	3 / 2006	MVDr. Jiří Váhala	Bandia reserve
11	Minna	10.12.2004	blood	3 / 2006	MVDr. Jiří Váhala	Bandia reserve
12	Toubab	31.12.2003	blood	3 / 2006	MVDr. Jiří Váhala	Bandia reserve
13	Bayane	10.12.2003	blood	3 / 2006	MVDr. Jiří Váhala	Bandia reserve
14	Bembou	unknown	blood	3 / 2006	MVDr. Jiří Váhala	Bandia reserve
15 A	young of Malapa	18.12.2005	tissue	12 / 2005	Ing. Markéta Antonínová, Ph.D.	Bandia reserve
15 B	young of Malapa	18.12.2005	tissue	12 / 2005	Ing. Markéta Antonínová, Ph.D.	Bandia reserve
16	Bayane	10.12.2003	tissue	12 / 2006	Ing. Markéta Antonínová, Ph.D.	Bandia reserve
17	Bayane	10.12.2003	tissue	12 / 2006	Ing. Markéta Antonínová, Ph.D.	Bandia reserve
18	Niokolo	unknown	tissue	9 / 2006	Ing. Markéta Antonínová, Ph.D., Ing. Daniel Bada	Bandia reserve
19	Salémata	unknown	tissue	9 / 2006	Ing. Markéta Antonínová, Ph.D., Ing. Daniel Bada	Bandia reserve
20	Malapa	unknown	tissue	9 / 2006	Ing. Markéta Antonínová, Ph.D., Ing. Daniel Bada	Bandia reserve
21	Dagana	1.3.2002	tissue	9 / 2006	Ing. Markéta Antonínová, Ph.D., Ing. Daniel Bada	Bandia reserve
22	Thelma	1.4.2002	tissue	9 / 2006	Ing. Markéta Antonínová, Ph.D., Ing. Daniel Bada	Bandia reserve
23	Ndiogoye	1.1.2003	tissue	9 / 2006	Ing. Markéta Antonínová, Ph.D., Ing. Daniel Bada	Bandia reserve
24	Fathala	12.2.2003	tissue	9 / 2006	Ing. Markéta Antonínová, Ph.D., Ing. Daniel Bada	Bandia reserve
25	Tuuti	4.12.2004	tissue	11 / 2007	Ing. Markéta Antonínová, Ph.D.	Bandia reserve
26	young of Tuuti	25.11.2007	tissue	11 / 2007	Ing. Markéta Antonínová, Ph.D.	Bandia reserve
27	Deedet	22.12.2005	blood	6. 2. 2008	MVDr. Jiří Váhala	Bandia reserve
28	Souleye	4.12.2005	blood	6. 2. 2008	MVDr. Jiří Váhala	Bandia reserve
29	Tukki	23.12.2005	blood	7. 2. 2008	MVDr. Jiří Váhala	Bandia reserve
30	Tidian	28.12.2005	blood	7. 2. 2008	MVDr. Jiří Váhala	Bandia reserve
31	Georgina	7.2.2006	blood	9. 2. 2008	MVDr. Jiří Váhala	Bandia reserve
32	Nelaw	12.12.2005	blood	9. 2. 2008	MVDr. Jiří Váhala	Bandia reserve
33	Foog	19.12.2005	blood	11. 2. 2008	MVDr. Jiří Váhala	Bandia reserve

Table 1 – continued.

DNA Sample no.	Individual	Date of birth	Type of the sample	Date of collection	Collected by	Locality of collection
34	Foulamousu	9.1.2007	blood	11. 2. 2008	MVDr. Jiří Váhala	Bandia reserve
35	Nane	20.1.2007	blood	11. 2. 2008	MVDr. Jiří Váhala	Bandia reserve
36	death young 02/08	3.12.2007	tissue	2 / 2008	Ing. Markéta Antonínová, Ph.D.	Bandia reserve
37	Mike	16.12.2006	blood	3 / 2009	MVDr. Jiří Váhala	Bandia reserve
38	Dewene	8.1.2007	blood	2 / 2009	MVDr. Jiří Váhala	Bandia reserve
39	Dagou	29.12.2006	blood	2 / 2009	MVDr. Jiří Váhala	Bandia reserve
40	Bandiagara	21.12.2007	blood	2 / 2009	MVDr. Jiří Váhala	Bandia reserve
41	Dering	21.12.2005	blood	2 / 2009	MVDr. Jiří Váhala	Bandia reserve
42	Tagat	24.12.2006	blood	2 / 2009	MVDr. Jiří Váhala	Bandia reserve
43	Georges	22.12.2006	blood	3 / 2009	MVDr. Jiří Váhala	Bandia reserve
44	Galago	15.2.2008	blood	2 / 2009	MVDr. Jiří Váhala	Bandia reserve
45	Nature	11.12.2007	blood	2 / 2009	MVDr. Jiří Váhala	Bandia reserve
46	Toubacouta	16.2.2008	blood	2 / 2009	MVDr. Jiří Váhala	Bandia reserve
47	Tendresse	26.12.2006	blood	2 / 2009	MVDr. Jiří Váhala	Bandia reserve
48	Fatou	18.2.2008	blood	2 / 2009	MVDr. Jiří Váhala	Bandia reserve
49	Didi	18.12.2007	blood	2 / 2009	MVDr. Jiří Váhala	Bandia reserve
50	Mansarinku	4.12.2007	blood	2 / 2009	MVDr. Jiří Váhala	Bandia reserve
51	Sao	20.12.2006	blood	2 / 2009	MVDr. Jiří Váhala	Bandia reserve
52	Saroudia	19.12.2007	blood	2 / 2009	MVDr. Jiří Váhala	Bandia reserve
53	Teranga	3.1.2009	hairs	2 / 2011	MVDr. Jiří Váhala	Bandia reserve
54	Nanuk	10.12.2008	hairs	2 / 2011	MVDr. Jiří Váhala	Bandia reserve
55	Mbalax	10. 1. 2009	hairs	2 / 2011	MVDr. Jiří Váhala	Bandia reserve
56	Soleil	21.12.2008	hairs	2 / 2011	MVDr. Jiří Váhala	Bandia reserve
57	Mirabelle T.	17.12.2009	hairs	2 / 2011	MVDr. Jiří Váhala	Bandia reserve
58	Mango T.	4.12.2008	hairs	2 / 2011	MVDr. Jiří Váhala	Bandia reserve
59	Sabar T.	12.12.2008	hairs	2 / 2011	MVDr. Jiří Váhala	Bandia reserve
60	Dara	8.12.2008	hairs	2 / 2011	MVDr. Jiří Váhala	Bandia reserve
61	Gaanga	5.1.2009	hairs	2 / 2011	MVDr. Jiří Váhala	Bandia reserve
62	Sindibad T.	26.12.2010	blood	3 / 2012	MVDr. Jiří Váhala	Bandia reserve
63	Tamtam D.	7.11.2010	blood	3 / 2012	MVDr. Jiří Váhala	Bandia reserve
64	Tamarin D.	25.11.2010	blood	3 / 2012	MVDr. Jiří Váhala	Bandia reserve
65	Dodo	24.12.2010	blood	3 / 2012	MVDr. Jiří Váhala	Bandia reserve
66	Destin T.	7.12.2010	blood	3 / 2012	MVDr. Jiří Váhala	Bandia reserve

4.1.1 Choice of individuals for comparison with the results of pedigree analysis

Fifteen individuals from three generations were chosen on the basis of pedigree analysis for comparison of results of genetic and pedigree analyses. The first group consisted of four founders (marked as FOUNDERS) because from two remaining founders there was not possible to obtain the samples. The kinship of founders is not known, but they are assumed to be non-related (Koláčková *et al.*, 2011a). The second group, marked as OFFSPRING 1, was formed by five offspring of the founders, sired with the only founding male and born in the season 2007/2008. The last group, OFFSPRING 2, was formed by six offspring of the founders´ offspring. The five individuals were born in the season 2010/2011, except one, born in the season 2009/2010. In this generation (more exactly since 2009) there were up to five males included into the reproduction (Table 2).

Table 2: Individuals selected for comparison of results of genetic and pedigree analyses (Season of the birth – year of the birth in bold).

Tested set	Individual	Sex	Season of the birth	Sire / Dam		Note
FOUNDERS	Niokolo	♂	1999	unknown - founder		
	Bembou	♀	1999	unknown - founder		
	Salémata	♀	1997	unknown - founder		
	Malapa	♀	1999	unknown - founder		
not tested	Tamba	♀	1999	unknown - founder		no sample
not tested	Dalaba	♀	1997	unknown - founder		no sample
OFFSPRING 1	Bandiagara	♀	**2007** / 2008	Niokolo		
				Bembou		
	Saroudia	♀	**2007** / 2008	Niokolo		
				Salémata		
	Mansarinku	♂	**2007** / 2008	Niokolo		
				Malapa		
	Toubacouta	♀	2007 / **2008**	Niokolo		
				Tamba		
	Didi	♀	**2007** / 2008	Niokolo		
				Dalaba		
OFFSPRING 2	Mirabelle T.	♀	**2009** / 2010	Toubab	Niokolo	
					Tamba	
				Minna	Niokolo	
					Malapa	
	Sindibad T.	♂	**2010** / 2011	Toubab	Niokolo	
					Tamba	
				Sindia	Niokolo	
					Salémata	
	Tamtam D.	♂	**2010** / 2011	Dering	Niokolo	
					Dalaba	
				Tendresse	Niokolo	
					Tamba	
	Tamarin D.	♂	**2010** / 2011	Dering	Niokolo	
					Dalaba	
				Toubacouta	Niokolo	
					Tamba	
	Dodo	♂	**2010** / 2011	Niokolo / Baax / Bonheur	Niokolo	
					Bembou	
				Dagana	Niokolo	
					Dalaba	
	Destin T.	♂	**2010** / 2011	Toubab	Niokolo	
					Tamba	
				Dewene	Niokolo	
					Dalaba	

4.2 Methods

All steps, except measuring of DNA concentration and fragmentation analysis, were performed in laboratories of Czech University of Life Sciences Prague. The part of the samples, until 2009, was processed in the laboratory of the Department of Genetics and Breeding of the Faculty of Agrobiology, Food and Natural Resources, the rest of procedures proceeded in the Laboratory of Molecular Biology of the Faculty of Tropical AgriSciences.

Measuring of DNA concentration and fragmentation analysis were performed in the Sequencing laboratory of the Faculty of Science of Charles University in Prague.

4.2.1 DNA isolation

The DNA was isolated from the blood, tissues or hairs using DNeasy Blood and Tissue kit by Qiagen. The procedure was performed according to the protocol enclosed in the kit. The obtained DNA was stored in the freezer by –18°C.

The concentration of isolated DNA was measured on the Nanodrop® ND-1000 (Thermo Scientific) in the Sequencing laboratory of the Faculty of Science of the Charles University in Prague. Too concentrated samples (more than 20 ng/μl) were diluted to the resultant concentration to approximately 5 ng/μl.

4.2.2 PCR (Polymerase chain reaction)

From 41 tested primers in total, thirteen primers – microsatellites were chosen (5 pairs and 4 separate primers, 1 primer used twice) for PCR and following fragmentation analysis. Five pairs of primers were mixed according to their annealing temperatures and fluorescently marking, one primer (AF533518) was used in two mixtures – at first tested in one mixture and then used to the second mixture. The separate primers were used because there was not possible to incorporate them in the mixture, due to their different annealing temperatures and fluorescently marking. The method of

cross-species amplification was used for primer testing. The primers were originally developed to related species – *Bos taurus*, *Capra hircus*, *Ovis aries*, *Capreolus capreolus*, *Nanger granti* and *Gazella dorcas*. The characteristics of the primers are showed in the Table 3.

The mix of 2 fluorescently marked primers contained 5 μl (c = 100 μM) of the first fluorescently marked forward primer (HEX), 5 μl (c = 100 μM) of the first non-marked reverse primer, 5 μl (c = 100 μM) of the second fluorescently marked forward primer (FAM), 5 μl (c = 100 μM) of the second non-marked reverse primer and the volume was filled up to the 250 μl by the buffer TE.

The PCR Master Mix by Fermentas was used for the PCR preparation. The composition of the reaction mixture is presented in the Table 4 and the PCR conditions in the Table 5. The PCR proceeded in the thermocycler QB-96 (Quanta Biotech Ltd.).

Table 3: Primers.

	Primer	Fluorescent dye	Primer sequence	Ann. t / °C	Allele length (size range) / bp	References
MIX 1	AF533518	HEX	CAG GAA GAC CTG TAT GGA AAT CTA TGC CTG GGA GGA	50	286 (*Nanger granti*)	Huebinger *et al.* (2006)
	AF533521	FAM	TCC AGA TGG TAT TTT CCT CA CCA GTG TTT TAC CGA GCA	50	231-247 (*N. granti*)	Huebinger *et al.* (2006)
MIX 2	INRA005	HEX	CAA TCT GCA TGA AGT ATA AAT AT CTT CAG GCA TAC CCT ACA CC	45	120-180 (*Ovis aries*)	Beja-Pereira *et al.* (2004)
	MHCII-DR	FAM	GGA CAC GTT CTT GCA GAT ACA ACT AC GAA CTC TCC TTA AGC ATA CTT GCT C	45	197-229 (*Capra hircus*)	Luikart *et al.* (1999)
MIX 3	L37208	HEX	AGT CTG AAG GCC TGA GAA CC CTT ACA GTC CTT GGG GTT GC	55	186-202 (*C. hircus*)	Kemp *et al.* (1995)
	INRABERN192	FAM	AGA CCT TTA CAG CCA CCT CTT C GTC CCA GAA ACT GAC CAT TTT A	55	152-208 (*C. hircus*)	Schibler *et al.* (1998)
MIX 4	X80214	HEX	CGA GTT TCT TTC CTC GTG GTA GGC GCT CGG CAC ATC TTC CTT AGC AAC T	50	223 (*C. hircus*)	Pépin *et al.* (1995)
	CSSM39	FAM	AAT CGG AAC CTA GAA TAT TTT GAG AGA TAA AAT GTG AGT GTG GTC TCC	50	177-183 (*Capreolus capreolus*)	Galan *et al.* (2003)
MIX 5	AF533518	HEX	CAG GAA GAC CTG TAT GGA AAT CTA TGC CTG GGA GGA	50	286 (*N. granti*)	Huebinger *et al.* (2006)
	BM4505	FAM	TTA TCT TGG CTT CTG GGT GC ATC TTC ACT TGG GAT GCA GG	50	154-282 (*Gazella dorcas*)	Beja-Pereira *et al.* (2004)
	AF252500	FAM	AGG AGT TGC TGA TGG ACA TCT GTT CAG CTT GGG TGA	50	144 (*Bos taurus*)	Reed *et al.* (2001)
	BM1818	FAM	AGC TGG GAA TAT AAC CAA AGG AGT GCT TTC AAG GTC CAT GC	50	253-272 (*B. taurus*)	Beja-Pereira *et al.* (2004)
	ETH225	FAM	GAT CAC CTT GCC ACT ATT TCC T ACA TGA CAG CCA GCT GCT ACT	45	141-159 (*B. taurus*)	Beja-Pereira *et al.* (2004)
	OarFCB304	FAM	CCC TAG GAG CTT TCA ATA AAG AAT CGG CGC TGC TGT CAA CTG GGT CAG GG	55	158-177 (*C. capreolus*)	Galan *et al.* (2003)

Table 4: The composition of the PCR reaction mixture and mixture of the primers.

	c	V / µl
H_2O		2.5
PCR Master Mix	2x	5
primer MIX	0.2 µM	1.5
DNA	5ng / µl	1
Total		10

Primer MIX:	c / µM	V / µl
primer 1 - HEX	100	5
- R	100	5
primer 2 - FAM	100	5
- R	100	5
H_2O		230
Total		250

Table 5: PCR conditions.

1)	95°C	1 min	
2)	95°C	1 min	30 cycles
	Ann. t	1.5 min	
	72°C	1.5 min	
3)	72°C	15 min	
4)	4°C	for ever	

4.2.3 Electrophoresis

The functionality of PCR was verified on the 1% agarosis gel in TBE buffer in the beginning. The electrophoresis ran 20 – 30 minutes by 120V. The ladder GeneRuler™ 100bp DNA Ladder Plus and 6x Loading Dye Solution by Fermentas were used. For the transillumination the UV Transilluminator ECX – 26.MX by Vilber Lourmat was used and the photos of the gel were made by the conventional camera.

4.2.4 Fragmentation analysis

The mixture for the fragmentation analysis was prepared by 0.5 μl of the PCR product, 9 μl of the formamide and 0.5 μl of the standard - GeneScan™ 500 LIZ™ Size Standard by Applied Biosystems.

This mixture was 5 min denaturated by 95°C and than chilled to 4°C (or stored in –18°C).

The fragmentation analysis was performed in the Sequencing laboratory of the Faculty of Science of the Charles University in Prague in the Sequencing machine – 4-capillary 3130 Genetic Analyzer or 16-capillary 3130xl Genetic Analyzer (Applied Biosystems) on the 50 cm capillaries with the polymer POP-7 and standard DS-30 (or DS-33).

4.2.5 Data analysis

Results of the fragmentation analysis were visualized in the program GeneMarker V1.95 Demo (Softgenetics, 2010). The lengths of the alleles were scored manually using the GeneMarker.

At first, the presence of null alleles was estimated by the software Microchecker version 2.2.3 (Van Oosterhout *et al.*, 2004). Null alleles may falsely increase homozygosity of the studied populations, when in the heterozygotic individual one of alleles does not amplified during the PCR, so the false homozygote can be detected.

Parameters of genetic diversity were computed in the following software. Software Cervus 3.0.3 (Kalinowski *et al.*, 2007) was used for calculation of expected (H_E) and observed (H_O) heterozygosity, deviations from Hardy-Weinberg equilibrium and allelic richness (Ar) were determined using FSTAT 2.9.3.2 (Goudet, 1995). Inbreeding coefficient (F_{IS}) and fixation index (F_{ST}) were computed in the Genepop 4.0.10. (Rousset, 2008) according to Weir and Cockerham (1984).

Expected heterozygosity (H_E) describes the proportion of heterozygous loci computed from the allele frequencies in the sample population, assuming that this population is in Hardy-Weinberg equilibrium. It is computed according to the equation $H_E = 1 - \sum p_i^2$, where p_i means the frequency of the i^{th} allele. Observed heterozygosity (H_O) expresses the mean proportion of individuals, which are heterozygous through the set of loci or the mean proportion of heterozygous loci by an individual (Beebee and Rowe, 2008).

Hardy-Weinberg equilibrium describes the ratio of the homozygotes and heterozygotes in the population. It says that the proportion of the genotypes does not change among the generations, when selection, migration and mutation do not occur in the population. According to the Hardy-Weinberg equilibrium, there is possible to predict the frequency of the genotypes, when the gene frequencies are known (Crow, 1988).

For a single locus with two alleles the Hardy-Weinberg equilibrium is characterized by the equation $p^2 + 2pq + q^2 = 1$, where p and q represent the alleles, so p^2 and q^2 are the frequencies of the homozygotes and 2pq means the proportion of heterozygotes. The frequency of the alleles is formulated by the equation $p + q = 1$. The population shows the deviations from Hardy-Weinberg equilibrium, when there is heterozygote excess or deficit in the population (Beebee and Rowe, 2008; Rousset, 2008).

Allelic richness (Ar) shows the mean number of alleles per locus, this number is averaged over the number of loci and balanced for the sample size. It is strongly influenced by effective population size, and sensitive to uneven sample size (Widmer and Lexer, 2001).

Inbreeding coefficient (F_{IS}) is defined by the equation $F_{IS} = (H_S - H_i) / H_S$, where H_S means the average expected heterozygosity across subpopulations and H_i means the average observed heterozygosity across subpopulations. It reaches the values from -1 to 1, higher values point to higher number of homozygotes in the population and higher risk of inbreeding. The negative values note the heterozygote excess (Wright, 1922; Frankham *et al.* 2003; Beebee and Rowe, 2008).

Fixation index (F_{ST}) considers the degree of inbreeding of subpopulation to the total population. It is defined by the equation $F_{ST} = (H_t – H_S) / H_t$. The H_S has been described above, the H_t means the expected heterozygosity of the total population. F_{ST} reaches the values from 0, signifying no structure of the population, to 1, which means that the populations are fully separated. Values higher than 0.2 show strong structuring of the populations. F_{ST} can also reach negative values, due to a sampling bias correction in the calculation when the sample size is too low (Beebee and Rowe, 2008; Bird *et al.*, 2011).

4.2.6 Comparison of data obtained by pedigree analysis and genetic analyses

Results of the genetic analyses were compared with results obtained from the pedigree analyses made by Koláčková *et al.* (2011a, 2012).

Pedigree of the population of Western Derby eland (*Taurotragus derbianus derbianus*) is known for all generation except one, born in 2003. In this generation mothers of offspring were not been determined. Pedigree is kept in SPARKS - Single Population Animal Record Keeping System (ISIS, 2010) in the cooperation with Prague Zoo. Pedigree data from SPARKS were processed in Population Management – PM2000 software (Pollak *et al.*, 2002). The results of demographic and "genetic" (based on the pedigree) analyses are published in the studbook for Western Derby eland, which has been established in 2008 and is published annually (Koláčková *et al.*, 2012).

The pedigree is based on direct observation of suckling young and their mothers. Main period of calving is in November and December, at the beginning of the dry season (Koláčková *et al.*, 2011a). The breeding herds were assembled by using the minimal kinship strategy, according to the pedigree. They are periodically restored, the subadult animals are transferred among breeding herds or bachelor herds (Antonínová *et al.*, 2006; Koláčková *et al.*, 2012).

For the first tested group, formed by founders, individuals that came from the wild, which are presumed to be unrelated, the gene diversity was 100 (GD = 1), mean inbreeding and mean kinship were 0 (F = MK = 0). The second group, formed by the founders´ offspring, which were sired by the only founding male and born in the season 2007/2008, maintained 76% of the gene diversity (GD = 0.759) of the founders, while the level of inbreeding (F) was 0.136 and mean kinship (MK) 0.241 (Table 6). The offspring of founders´ offspring, born in the season 2010/2011, created the last group. In this generation, more than the only male was included in the reproduction. Maintained gene diversity in this generation was 79% (GD = 0.79), level of inbreeding was 0.126 and mean kinship 0.212 (Koláčková *et al.*, 2011a, 2012; Table 6).

We compared the gene diversity of pedigree analysis with the values of expected and observed heterozygosity and level of inbreeding with inbreeding coefficient.

Table 6: Results of pedigree analysis (Koláčková *et al.* 2009, 2010, 2011a, b, 2012).

PEDIGREE ANALYSIS	Gene diversity (GD)	Mean inbreeding (F)	Mean kinship (MK)
FOUNDERS	1.000	0.000	0.000
Year 2008 (OFFSPRING 1)	0.759	0.136	0.241
Year 2009	0.774	0.119	0.226
Year 2010	0.784	0.116	0.216
Year 2011 (OFFSPRING 2)	0.788	0.126	0.212
Year 2012	0.792	0.124	0.208

4.2.7 Phylogenetical relationships between Eastern (*T. d. gigas*) and Western subspecies (*T. d. derbianus*) of the Derby eland (*T. derbianus*)

There were used two sequences of cytochrome *b* (part of the mitochondrial DNA) for evaluation of the phylogeny between the subspecies. These sequences (AF022062 and EF536354) were obtained from the GenBank (GenBank, 2013), origin of both is unknown.

Into the analyses were included the four representatives of the Western subspecies (*T. d. derbianus*) – the founders of the breeding programme in Senegal, and eight representatives of the Eastern subspecies (*T. d. gigas*) – two individuals coming from Los Angeles Zoo and six individuals from Congo.

All laboratory proceedings were done according to Kocher *et al.* (1989). Amplified genes were sequenced by forward and reverse primers. All sequences were edited in BioEdit (Hall, 1999) to final length of products 1140 bp. P-distances were computed among all sequence.

5. RESULTS

From thirteen tested microsatellite loci only five were polymorphic (AF533518, BM4505, ETH225, OarFCB304 and X80214), six were monomorphic and by remaining two the amplification was not successful. The analysis in Microchecker version 2.2.3 did not proved presence of null alleles or other genotyping errors in our data set.

The values of observed and expected heterozygosity differed except the one case – locus ETH225 in the population of FOUNDERS ($H_E = H_O = 0.750$; Table 7). Mean H_O ($H_O = 0.750$) was higher than mean H_E ($H_E = 0.664$; Table 7) in FOUNDERS, in OFFSPRING 1 ($H_O = 0.580$; $H_E = 0.586$; Table 8) and OFFSPRING 2 ($H_O = 0.370$; $H_E = 0.480$; Table 9) mean values of H_O were lower than mean values of H_E. FOUNDERS had higher both heterozygosities than the OFFSPRING 1 and 2.

The populations were in Hardy-Weinberg equilibrium in all loci, because the heterozygote deficit or excess was not proved.

Mean allelic richness across all generation was 2.53, maximum was 4 alleles at locus OarFCB304 (Table 7, 8 and 9). Mean allelic richness was the highest by FOUNDERS (Ar = 2.79; Table 7), by OFFSPRING 1 (Ar = 2.15; Table 8) and OFFSPRING 2 (Ar = 2.14; Table 9) the value decreased. The OFFSPRING 2 had in one case (locus X 80 214, Ar = 2.50) higher allelic richness than OFFSPRING 1 (Ar = 2.00), the other values were lower in OFFSPRING 2 (Table 9) than in OFFSPRING 1 (Table 8).

Mean value of F_{IS} was lowest in the FOUNDERS ($F_{IS} = –0.154$), than in the generations of OFFSPRING 1 ($F_{IS} = 0.090$) and OFFSPRING 2 ($F_{IS} = 0.251$; Table 7, 8 and 9). These results may indicate higher risk of inbreeding depression in populations of OFFSPRING than in FOUNDERS.

Fixation index (F_{ST}) was lower for the FOUNDERS and OFFSPRING 1 generations ($F_{ST} = 0.036$) than for the FOUNDERS and OFFSPRING 2 ($F_{ST} = 0.133$). For the OFFSPRING 1 and OFFSPRING 2 populations the value was negative ($F_{ST} = –0.091$), probably due to a sampling bias correction i n the calculation. The differentiation increased with the distance of the generations (Table 10).

Table 7: Results of the analyses for the founders´generation.

FOUNDERS	Number of alleles per locus	Allele length (allele range) / bp	F_{IS}	H_O	H_E	Ar
AF533518	3	212 - 224	-0.600	1.000	0.679	2.75
BM4505	3	249 - 262	-0.125	0.750	0.679	2.75
ETH225	3	140 - 153	0.000	0.750	0.750	2.96
OarFCB304	4	148 - 161	0.053	0.750	0.786	3.50
X80214	2	228 - 240	-0.200	0.500	0.429	1.96
Mean	3		-0.154	0.750	0.664	2.79

F_{IS} - Inbreeding coefficient, H_O - observed heterozygosity, H_E - expected heterozygosity, Ar - allelic richness

Table 8: Results of the analyses for the founders´ offspring.

OFFSPRING 1	Number of alleles per locus	Allele length (allele range) / bp	F_{IS}	H_O	H_E	Ar
AF533518	2	212 - 224	-0.500	0.750	0.536	2.00
BM4505	2	249 - 256	0.571	0.250	0.536	2.00
ETH225	3	140 - 153	-0.333	0.800	0.622	2.73
OarFCB304	2	148 - 161	-0.091	0.600	0.556	2.00
X80214	2	228 - 240	1.000	0.500	0.679	2.00
Mean	2.20		0.090	0.580	0.586	2.15

F_{IS} - Inbreeding coefficient, H_O - observed heterozygosity, H_E - expected heterozygosity, Ar - allelic richness

Table 9: Results of the analyses for the offspring of founders´ offspring.

OFFSPRING 2	Number of alleles per locus	Allele length (allele range) / bp	F_{IS}	H_O	H_E	Ar
AF533518	2	212 - 224	-0.333	0.600	0.467	1.97
BM4505	2	249 - 256	0.412	0.333	0.545	1.99
ETH225	3	142 - 153	0.259	0.333	0.439	2.27
OarFCB304	2	148 - 161	0.333	0.333	0.485	1.97
X80214	3	228 - 243	0.500	0.250	0.464	2.50
Mean	2.40		0.251	0.370	0.480	2.14

F_{IS} - Inbreeding coefficient, H_O - observed heterozygosity, H_E - expected heterozygosity, Ar - allelic richness

Table 10: Fixation index (F_{ST}) among populations.

F_{ST}	**FOUNDERS**	**OFFSPRING 1**
OFFSPRING 1	0.036	
OFFSPRING 2	0.133	-0.091

The analysis of cytochrome *b* for evaluation of phylogenetic relationship between the Eastern (*T. d. gigas*) and Western subspecies (*T. d. derbianus*) showed the difference between the locality/population, but no difference was found among individuals of the same population/locality. P-distances among localities varied from 0.09% to 1.77% (Table 11). The highest value of p-distance was between Senegalese and GenBank sequences, but because we were not able to verify the origin of the Genbank sequences, it was excluded from the results. So the highest value of p-distance was between the samples from Senegal and from American Zoo (0.35%) and lowest between the Congo and American Zoo (0.09%).

Table 11: P-distances among localities/populations.

Individuals	P-distances						
	Senegal	AF022062	Zoo America	EF536354	Zoo America-D1	Zoo America-D8	Congo
Senegal	0.0000	0.0177	0.0035	0.0044	0.0018	0.0027	0.0026
AF022062	0.0177	0.0000	0.0159	0.0150	0.0144	0.0172	0.0168
Zoo America	0.0035	0.0159	0.0000	0.0026	0.0018	0.0027	0.0009
EF536354	0.0044	0.0150	0.0026	0.0000	0.0027	0.0036	0.0035
Zoo America	0.0018	0.0144	0.0018	0.0027	0.0000	0.0018	0.0018
Zoo America	0.0027	0.0172	0.0027	0.0036	0.0018	0.0000	0.0018
Congo	0.0026	0.0168	0.0009	0.0035	0.0018	0.0018	0.0000

light violet colour – GenBank sequences, red boundaries – highest values, blue boundaries – lowest values

6. DISCUSSION

Bovidae are important component of several bioms – for example taiga, savannah or steppe. Their taxonomy and phylogeny is a subject of many studies (for example Janis and Scott, 1987; Estes, 1991; Price *et al.*, 2005; Wilson and Reeder, 2005; Gatesy, 2009; Bibi, 2013 and others). The studies agree with ranging of Cetacea within Artiodactyla creating the order Cetartiodactyla and confirm their monophyly.

Further taxonomic studies occupied with the question of ranging of the genus *Taurotragus* into *Tragelaphus* (Hassanin and Douzery, 1999; Matthee and Robinson, 1999) and also ranging of genus *Booceros* (bongo) into *Tragelaphus* (Matthee and Robinson, 1999). The results showed the genus *Tarotragus* as separate genus, but the *Booceros* was ranged into *Tragelaphus* (Grzimek, 1990; Matthee and Robinson, 1999; Murphy et al., 2001a; Wilson and Reeder, 2005 and others). The ranging of bongo into the separate genus *Booceros* can be found particularly in the older publications like Dorst and Dandelot (1970).

The taxonomy was solved by means of the study of morphological characters (for example Janis and Scott, 1987; Wilson and Reeder, 2005) and by molecular approaches too (for example Hassanin and Douzery, 2003). Results are congruent in the taxonomy, but they differ in the times of divergence of the groups of bovids – for example Hassanin and Douzery (2003) determined the separation of Bovidae and Moschidae 26 MYA and the study of Bibi (2013) suggests the time of separation between 19.3 and 16.6 MYA, where Bibi (2013) claims, that his study is more complex, because he took account of both fossil and molecular data. He used 16 fossil calibration points, while the previous studies (according to his opinion) use fewer fossil calibration points.

Tragelaphini (or spiral-horned antelopes), numbering 9 species, are monophyletic group. Their phylogeny is confirmed range of genetic studies (for example Allard *et al.*, 1992; Hassanin and Ropiquet, 2004; Rubes *et al.*, 2008; Wang and Yang, 2013). The studies differ again in the time of divergence of Tragelaphini and the other bovid tribes. The study of Willows-Munro *et al.* (2005) presents 14.08 MYA, compared to the study of Bibi (2013) that suggest the divergence time between 10.1 and 5.4 MYA.

For evaluation of genetic diversity in the population, for example for the conservation purposes, there are often used genetic methods. If we have enough suitable samples, there is possible to determine the parameters of genetic diversity exactly and really. Except this approach, there is possible to use also the pedigree analysis. It is useful especially when we do not have the samples for genetic analysis, but the pedigree of the population is well known. In the best case there is known the complete pedigree (for example from studbook), origin of all individuals in the population is obvious.

Of course, there is possible to use the both approaches and compare the results, as in the study of the genetic diversity of Western Derby eland (*T. d. derbianus*).

Western Derby eland (*T. d. derbianus*) belong to the species endangered by extinction. The only wild population lives in Niokolo Koba National Park in Senegal. The breeding programme for conservation of the Western Derby eland (*T. d. derbianus*) has been also established by the group of scientists of the Faculty of AgriSciences of Czech University of Life Sciences Prague. The whole population of Western Derby eland (*T. d. derbianus*) bred in semi-captivity is divided into 7 subpopulations (5 breeding and 2 bachelor herds) in two reserves, Bandia and Fathala (Figure 7), in the recent time it numbers 95 animals.

The breeding programme has been established by only six founders – five females and only one male. This number of founders is quite low, because Lacy (1987) recommends at least 20 – 30 wild born founders for establishing a viable population with sufficient gene pool. The real numbers of individuals founding the breeding programmes of endangered species strongly differs from this recommendation, because obtain more animals from the wild could be very difficult (for both ecologically and economically reasons), especially when goes for so large species like Western Derby eland (*T. d. derbianus*), or for example Arabian oryx (*Oryx leucoryx*), which became extinct in the wild in 1972.

The breeding programme of Arabian oryx (*O. leucoryx*) was found in USA with only 11 founders, five males and six females. Balanced proportion of the genders of founders was advantage for this breeding programme, contrary to the breeding programme of Western Derby eland (*T. d. derbianus*). The breeding programme of Arabian oryx (*O. leucoryx*) was successful and it was reintroduced back to its natural environment, to Saudi Arabia and Oman. Nowadays, the population in the wild has more than 1000 individuals (Ostrowski *et al.*, 1998; Marshall *et al.*, 1999; Price, 2011).

Other successful reintroduction programmes started with comparable number of founders, for example European bison (*Bison bonasus*) with 12 founders (Olech and Perzanowski, 2002) or Przewalski´s horse (*Equus caballus przewalskii*) with 13 founders (Bouman, 1979). The low number of founders is represented also in current population of Eastern Giant Eland (*Taurotragus derbianus gigas*) in North American Zoos, where only eight animals (three males and five females) contributed to the reproduction (McCaffree, 2011).

Small populations are threatened by mating of related individuals and genetic drift. It is possible to face the potential genetic drift by introduction of the new immigrants to the population. Lacy (1987) suggests that the help for the genetic diversity preservation is to divide the original population to several smaller subpopulations. In polygynous species, as in the Derby eland (*Taurotragus derbianus*), this division enables to involve less represented males into the reproduction with selected females. Selection of individuals for the reproduction that minimize the kinship, belongs to the appropriate and highly recommended genetic management strategy (Montgomery *et al.*, 1997; Kleiman *et al.*, 2010).

The results of genetic analyses of the Western Derby eland (*T. d. derbianus*) population could be comparable with the data of the population of various ungulates kept in captivity (or semi-captivity). Mean value of expected heterozygosity in FOUNDERS was similar as in the study of Arabian oryx (*Oryx leucoryx*) (Marshall *et al.*, 1999), or in the study genetic diversity of five species (topi *Damaliscus lunatus*, eland *Taurotragus oryx*, hartebeest *Alcelaphus buselaphus*, Grant´s gazelle *Nanger granti* and impala *Aepyceros melampus*) living in Serengeti National Park (Eblate *et al.*, 2011).

Low values of F_{ST} (lower than 0.1) showed that the structure among the 4 tested populations of Arabian oryx (Marshall *et al.*, 1999) was lower than between FOUNDERS and OFFSPRING 2 (F_{ST} = 0.133; Table 10). The values between FOUNDERS and OFFSPRING 1 and the two generations of offspring were lower than 0.1 (Table 10). These results proved that genetic differences were higher among generations that were more distant.

The parameters of genetic diversity (values of Ar and heterozygosity) were the highest for the generation of FOUNDERS, similarly the inbreeding coefficient was lowest for the FOUNDERS. In both generations of offspring the genetic variability slightly decreased and inbreeding coefficient increased (Table 7, 8 and 9).

According to the pedigree analysis, the population of Western Derby eland (*T. d. derbianus*) has lost 21% of genetic diversity from the founders till 2012. This loss was lower than in 2008 (24%), because more individuals were involved in the reproduction in 2012 than in 2008, when only the one male sired all the offspring (Koláčková *et al.*, 2012; Table 6).

The genetic diversity from the pedigree analysis showed the decrease in 2008 and slight increase in 2011, contrary to the results of microsatellite analyses (values of expected and observed heterozygosity and allelic richness too), which showed the decreasing tendency of the genetic diversity from generation to generation. This disagreement may be given by different way of calculation of the quantities – while the expected heterozygosity comes out the frequency of the alleles, the genetic diversity is calculated from the pedigree by using effective population size (N_e) (Figure 9).

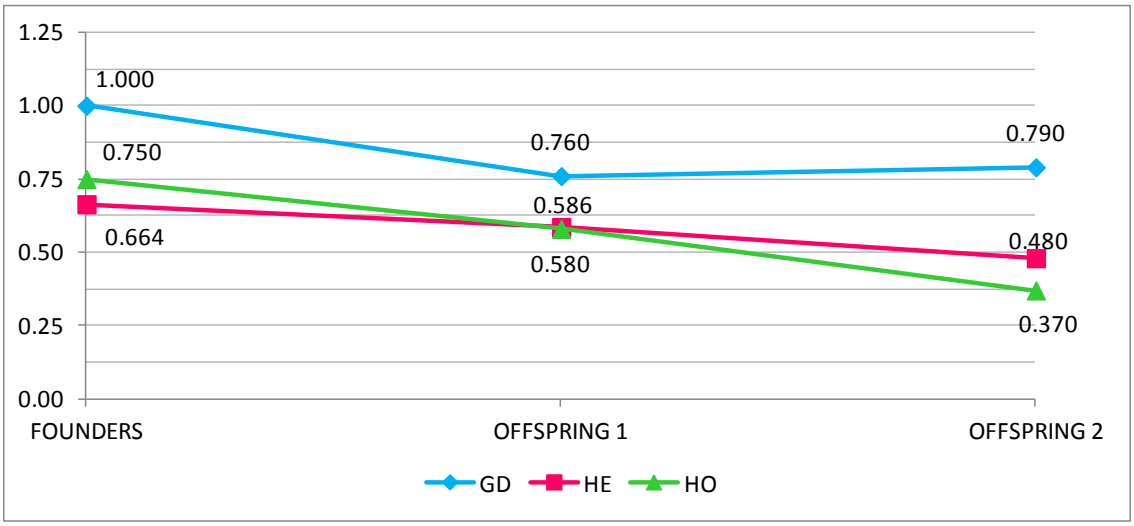

Figure 9: Comparison of gene diversity (GD) obtained from pedigree analysis with expected heterozygosity (H_E) and observed heterozygosity (H_O).

The estimated mean level of inbreeding in the population of Western Derby eland (*T. d. derbianus*) was 0.137 in 2008 and 0.126 in 2012 (Koláčková *et al.*, 2012). In comparison with the molecular analyses data, the mean inbreeding coefficient, F_{IS}, across all polymorphic loci was 0.090 for OFFSPRING 1 and 0.251 for OFFSPRING 2.

Results of microsatellite analyses did not support the presumption, that the level of inbreeding would be higher by OFFSPRING 1 than by OFFSPRING 2 (Koláčková *et al.*, 2012) (Figure 10).

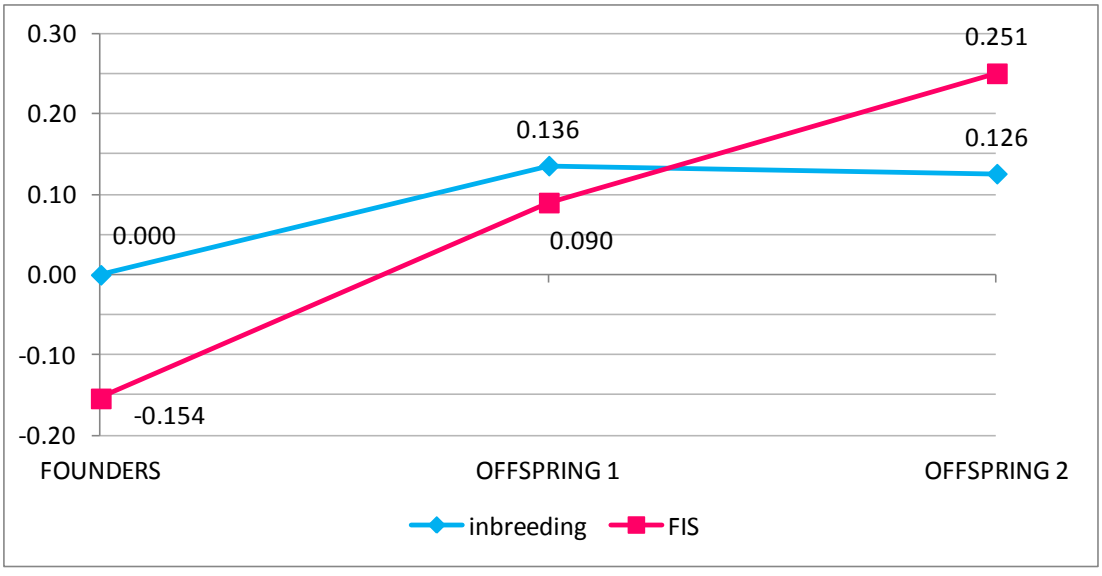

Figure 10: Comparison of inbreeding (F, value obtained from pedigree analysis) and inbreeding coefficient (F_{IS}) found out by genetic analysis.

The genetic analysis confirmed the presumption, that the generation of FOUNDERS had the highest genetic diversity and the lowest F_{IS}. The genetic diversity decreased and the inbreeding coefficient increased in the next generations, so the improvement of the values of genetic diversity, presumed according to the pedigree analysis, after the application of breeding management based on minimizing kinship was not confirmed by microsatellites.

All three studied groups were in Hardy-Weinberg equilibrium, they did not show heterozygote excess or deficit.

Despite of the mating of related individuals in the population of Western Derby eland (*T. d. derbianus*) and the influence of genetic drift, the analyses did not show low observed heterozygosity not even conspicuously high inbreeding coefficient.

Role of inbreeding and inbreeding depression was discussed a lot in recent studies (Charlesworth and Willis, 2009; Townsend and Jamieson, 2013). Inbreeding changes the frequency of genotypes in the population and increases homozygosity, which leads to the risk of expression of recessive deleterious alleles (Keller and Waller, 2002) and fitness reduction (Cassinello, 2005). The correlation between inbreeding and fitness is still the topic of many studies (Chapman *et al.*, 2009; Zeng *et al.*, 2013).

The decreasing genetic variation during the generations in the population of Western Derby eland (*T. d. derbianus*) may lead to the risk of inbreeding depression in next generations. Till 2013, there were not been observed any signs of reduced fitness (Brandlová, 2013 – personal observation), but the breeding management based on the pedigree and genetic data analyses should be applied for maintaining of the population.

For the replenishment of new alleles into the population, it could be recommended introduction of some new individuals from the wild. Koláčková *et al.* (2011a) recommended adding at least 15 individuals. It would bring the long-term improvement for the Western Derby eland (*T. d. derbianus*) population in captivity. But it is not so easy, because of high financial costs and because of the attitude of representatives of Senegalese conservation organizations.

Good genetic management in the breeding programme can lead to the survival of the species. The examples of some species were described above – Arabian oryx (*Oryx leucoryx*), Przewalski's Horse (*Equus caballus przewalskii*), Addax (*Addax nasomaculatus*) and other, which would become extinct without establishment of breeding programme. Populations of these species declined due to the influence of human – they were hunted, their natural environment was degradated and reduced by herdsmen of domestic livestock. But they were saved despite of the low number of founders in the breeding programme and reintroduced. Such programmes give hope for the Western Derby eland (*Taurotragus derbianus derbianus*) included into the conservation programme too, that they will return in the wild. Now they are the subject of many studies, because it is very important to know them very well to apply the best possible management for their breeding and, if we will think about reintroduction, to choose the best possible way, how to reintroduce them back to the nature.

The analysis of mitochondrial DNA, evaluating the differences between the subspecies of the Derby eland, showed that the difference between the Western and Eastern subspecies is between 0.09% and 0.35%. It is congruent with the study of Lutovská (2012) that says, there is very low difference between the subspecies. It is possible to distinguish the both subspecies according to their body size (the Eastern subspecies, *T. d. gigas*, is larger) and some details in coloration – for example the shape of the cheek spots. Further they differ in one parameter of horn and the length of teeth row, is very close to conventional subspecies boundary.

According to the analysis of cytochrome *b* the differences among populations are very small, but it seems not to be caused recently by the human influence, but the divergence of the population could be older than thousand or hundred thousand years (Brown *et al.*, 1979; Wilson *et al.*, 1985). There is assumed the slower evolution and mutation in bovids than by mammals in general (Hassanin and Douzery, 1999).

7. CONCLUSION

The discovered parameters of genetic diversity show, that the situation in the population of Western Derby eland (*T. d. derbianus*) is quite satisfactory, due to the breeding management and its effort to minimize the kinship.

The assumption that the highest level of genetic diversity is in the generation of founders has been confirmed. The second assumption, that in the generation of founders´s offspring the genetic diversity decreased, because there was only one male included into the reproduction, and then in the generation of the offspring of founders´ offspring the diversity increased because of involvement of more males (and females too) in the reproduction, was not confirmed. According to the microsatellite analysis, the genetic diversity decreased and the inbreeding coefficient increased across the generations.

The phylogenetic analysis made by means of the mitochondrial marker – cytochrome *b* showed the differentiation between the Western (*T. d. derbianus*) and Eastern subspecies (*T. d. gigas*) maximally 0.35%.

For the next management of the breeding programme it could be recommended to continue with kinship minimizing in the breeding herds and make an effort to obtain some new individuals from the wild.

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9. ANNEXES

LIST OF ANNEXES

Annexe 1: Poster for Kostelecké inspirování, 2008.

Polymorfní mikrosatelitní markery pro určování příbuzenských vztahů u antilopy losí (*Taurotragus oryx*) a antilopy Derbyho (*Taurotragus derbianus*)

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Úvod

Antilopa losí (*Taurotragus oryx*) a antilopa Derbyho (*Taurotragus derbianus*) (Obr. 1 a 2) patří mezi největší antilopy na světě. Jejich počet ve volné přírodě klesá, několik jedinců obou druhů je chováno v zajetí. U populací chovaných v zajetí je nutné znát příbuzenské vztahy, zejména kvůli prevenci příbuzenské plemenitby (inbreedingu). Tyto vztahy jsou založeny na přímém pozorování mateřského chování zvířat a pro jejich ověření jsme testovali možnost použití genetických markerů. Byla použita metoda cross-amplifikace s markery původně vyvinutými pro jiné příbuzné druhy.

Obr. 1. Antilopa losí (nahoře) a antilopa Derbyho - samice (vlevo) a samec.

Cíl práce

- ověřit schopnost amplifikace mikrosatelitních markerů od skotu, ovcí, koz a gazely Grantovy u antilop losích a antilop Derbyho
- nalézt polymorfní markery, které budou vhodné pro určování příbuzenských vztahů u těchto antilop

Metody

- Izolace DNA ze vzorků krve nebo tkání pomocí DNeasy Blood and Tissue kit od Qiagenu
- PCR za použití mikrosatelitních markerů od turovitých (skotu, ovcí, koz a gazely Grantovy)
- PCR probíhala v 20μl reakční směsi v Peltier Thermal Cykleru–200 od MJ Research (Obr. 3)
- Podmínky PCR převzaty z odborných článků (Tab. 1)
- Amplifikace prováděna několikrát s různými podmínkami, za účelem nalezení těch nejoptimálnějších
- Úspěšnost amplifikace a případný polymorfismus ověřen elektroforézou (Obr. 4)

Obr. 2. Mláďata antilop Derbyho patří k zalehávacímu typu (hidder).

Výsledky

44 mikrosatelitních markerů bylo testováno na 22 antilopách losích a 33 antilopách Derbyho pro zjištění polymorfismu a možné použitelnosti pro určování příbuznosti. Bylo nalezeno deset polymorfních markerů u antilopy losí a šest u antilopy Derbyho (Tab 2). Tyto markery mohou být použity pro určování příbuzenských vztahů, jejich polymorfismus bude v budoucnu ověřen ještě sekvenací.

Tab. 1: Testované podmínky PCR
Hybr. t = hybridizační teplota, specifická pro každý primer.
Touchdown = modifikace klasické PCR, pro dosažení lepších výsledků amplifikace. Při prvním cyklu je hybridizační teplota vyšší o 10°C, proběhne 10 cyklů, při každém se hybridizační teplota snižuje o 1°C, po 10ti cyklech dosáhne specifické hodnoty a poté proběhne uvedený počet cyklů při nichž už se hybridizační teplota neměni.

Antonínová (2007, osobní sdělení):

1)	96°C	2 min	
2)	96°C	45 s	40 cyklů
	Hybr. t	30 s	
	72°C	1 min	
3)	72°C	2 min	

Beja-Pereira et al. (2004) - touchdown:

1)	96°C	10 min	
2)	96°C	30 s	40 cyklů
	Hybr. t	30 s	
	72°C	30 s	
3)	72°C	10 min	

Galan et al. (2003):

1)	94°C	12 min	
2)	94°C	1 min	35 cyklů
	58°C nebo hybr. t	1 min	
	72°C	1 min	
3)	72°C	10 min	

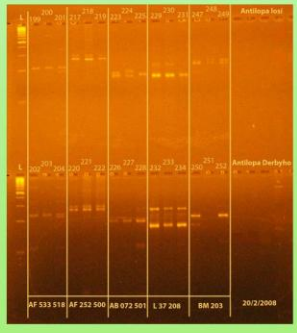
Přizpůsobené podmínky - touchdown:

1)	95°C	10 min	
2)	95°C	30 s	35 cyklů
	Hybr. t	30 s	
	72°C	30 s	
3)	72°C	10 min	

Tab. 2: Polymorfní markery u antilopy losí a antilopy Derbyho.
Jako podmínky PCR jsou uvedeny podmínky, při jejichž použití byly získány nejlepší výsledky amplifikace.

Antilopa losí			
Název	**Hybridizační teplota (°C)**	**Původ**	**Podmínky PCR**
AB 072 501	55	*Bos taurus*	Přizpůsobené podmínky
AF 252 500	50	*Bos taurus*	Přizpůsobené podmínky
AF 533 521	50	*Gazella granti*	Přizpůsobené podmínky
BM 203	50	*Capra hircus*	Beja-Pereira et al. (2004)
BM 1443	50	*Ovis aries*	Beja-Pereira et al. (2004)
BM 1818	50	*Capra hircus*	Beja-Pereira et al. (2004)
CSSM 39	50	*Bos taurus*	Galan et al. (2003)
ETH 10	50	*Capra hircus*	Beja-Pereira et al. (2004)
INRABERN 192	55	*Capra hircus*	Antonínová (2007, osobní sdělení)
L 37 208	55	*Bos taurus*	Přizpůsobené podmínky
Antilopa Derbyho			
Název	**Hybridizační teplota (°C)**	**Původ**	**Podmínky PCR**
AF 252 500	50	*Bos taurus*	Přizpůsobené podmínky
AF 533 518	50	*Gazella granti*	Přizpůsobené podmínky
BM 4505	50	*Ovis aries*	Beja-Pereira et al. (2004)
IDVGA 8	55	*Bos taurus*	Galan et al. (2003)
L 37 208	55	*Bos taurus*	Přizpůsobené podmínky
SRCRSP 24	50	*Capra hircus*	Beja-Pereira et al. (2004)

Obr. 3. Thermocykler (Peltier Thermal Cykler–200 od MJ Research).



Obr. 4. Gel prosvícený UV světlem
L = ladder (žebřík), obsahuje fragmenty DNA o známé délce, slouží k odhadu délky amplifikovaných fragmentů, čísla = vzorky; Světlé proužky na gelu značí přítomnost PCR produktu a tudíž úspěšnost amplifikace.

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Výzkum byl podpořen grantem IAA 6093404 Akademie věd České republiky.

Annexe 2: Poster for VI[th] European Congress of Mammalogy, 2011.

Genetic diversity and phylogeny of the Western Derby eland (*Taurotragus derbianus derbianus*)

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Figure 1: The male of the Western Derby eland (*T. d. derbianus*)

Introduction

The Western Derby eland (Fig. 1) is considered to be one of the largest antelopes in the world. It is classified as „critically endangered" (IUCN 2010 [online]). The last wild population of approximately 170 individuals (in 2006) was discovered in Niokolo Koba National Park in Senegal (Fig. 2).

Since 2000 there exists the semi-captive breeding programme in Senegal. It was established by only 6 founders (1 male, 5 females). Nowadays it numbers 76 individuals divided into 5 breeding herds (and one bachelor herd) in Bandia and Fathala reserves (Fig. 3). Inbreeding occurs in the population, so the genetic diversity evaluation is necessary.

The kinship relations in the population are determined by means of direct observation of sucking calves and their mothers. Some relation are uncertain, the microsatellite analysis should aid to solve them (Fig. 4).

The differences between subspecies were specified on the basis of morphological characters, the genetic differences are not known.

Aim of the study

The aim of this study was to evaluate the genetic diversity in the population of the Western Derby eland bred in semi-captivity, to determinate the number of alleles per locus, observed and expected heterosygosity etc.

Another aims were to revise the uncertain pedigree data discovered by direct observation and to identify the phylogenetic relationship between Western (*T. d. derbianus*) and Eastern (*T. d. gigas*) subspecies.

Figure 2: Distribution of the Giant eland (*Taurotragus derbianus*) (IUCN 2010 [online] - modified).

Figure 3: Locations of the Bandia and Fathala reserves and Niokolo Koba National Park (NKNP) (Koláčková *et al.* 2011, *accepted*).

Material

Sixty-one samples were obtained in the Bandia reserve from 2006 to 2011. The blood samples were obtained by the tranquillization during the transport of the animals between the breeding herds, the tissue samples were obtained from dead animals or by means of the biopsy darts.

Methods

DNA isolation was performed by DNeasy Blood and Tissue kit by Qiagen.

For the evaluation of genetic diversity and determination of kinship relations the 13 microsatellite markers of cattle, goats, sheep and other related species were used. 8 were polymorphic, 5 monomorphic (Tab. 1). The markers were tested on 10 individuals (including the 4 founders). The results of fragmentation analysis were visualised and the lengths of the alleles were estimated using the GeneMarker V1.95 Demo (Softgenetics 2010 [online]).

The phylogeny has been identified by means of the mitochondrial DNA analysis. We have sequenced 1140 bp of cytochrome *b* of the Western subspecies (*T. d. derbianus*) from Senegal and of the Eastern subspecies (*T. d. gigas*) from Zoo Los Angeles.

Preliminary results

The preliminary results indicate low number of alleles in the population. There were analysed 13 microsatellite loci, 8 are polymorphic, but only 5 have more than 2 alleles. Maximum of alleles is 4 alleles per locus (Tab. 1).

By studying the phylogeny we observed very low differencies between the populations of Western (*T. d. derbianus*) and Eastern (*T. d. gigas*) subspecies - p-distance was 0.004.

Primer	Fluorescent dye	Primer sequence	Ann. t /°C	Origin	Number of alleles per locus By T. d. derbianus	Allele lenght (size range) - for the T. d. derbianus
AF 252 588	FAM	AGG AGT TGC TGA TGG AGA TCT GTT CAG CTT GGG TGA	50	*Bos taurus*	2	259 - 262
AF 533 518	HEX	CAG GAA GAC CTG TAT GGA AAT CTA TGC CTG GGA GGA	50	*Gazella gazella*	3	212 - 224
BM 4505	FAM	TTA TCT TGG CTT CTG GGT GC ATC TTC ACT TGG GAT GCA GG	50	*Gazella dorcas*	3	248 - 252
ETH 225	FAM	GAT CAC CTT GCC ACT ATT TCC T ACA TGA CAG CCA GCT GCT ACT	46	*B. taurus*	3	140 - 150
INRA MERN 192	FAM	AGA CCT TTA CAG CCA CCT CTT C GTC CCA GAA ACT GAC CAT TTT A	55	*Capra hircus*	2	111 - 165
L 07 206	HEX	AGT CTG AAG GCC TGA GAA CC CTT ACA GTC CTT GGG GTT GC	55	*O. hircus*	2	170 - 222
OarFCB 304	FAM	CCC TAG GAG CTT TCA ATA AAG AAT CGG CGC TGC TGT CAA CTG GGT CAG GG	55	*Capreolus capreolus*	4	148 - 161
X 80 214	HEX	CGA GTT TCT TTC CTC GTG GTA GGC GCT CGG CAC ATC TTC CTT AGC AAC T	50	*C. hircus*	3	226 - 240

Table 1: Polymorphic loci and their alleles

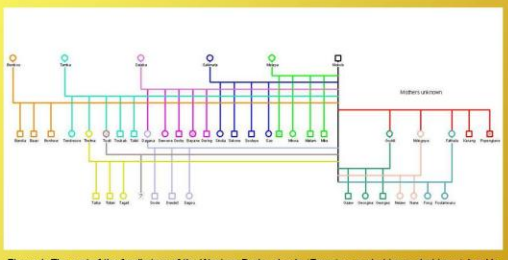

Figure 4: The part of the family tree of the Western Derby elands (*Taurotragus derbianus derbianus*) bred in semi-captivity in Bandia and Fathala reserves (actual in November 2007).
□ = male, O = female, ? = gender unknown, × = dead individual, colours = related individuals (families)
(Created in the programme GenoPro 2007)

Conclusion

According the preliminary results we can assume the low genetic diversity in the population, probably due to the low number of founders and inbreeding occurrence in the population.

Likewise, there is very low support for the genetical separation of the two subspecies.

For the management of the breeding would be good to add some „new" individuals. The benefit of this step could be also disputable, because the founders show the low polymorphism too.

This research was supported by "Derbianus Czech Society for African Wildlife" (NGO) and by the foundation "Nadání Josefa, Marie a Zdeňky Hlávkových".

Thanks belong to the Society for the Protection of Environment and Fauna in Senegal and Directorate of National Parks of Senegal too.

Annexe 3: Scientific paper.

Conservation genetics of the Western Derby eland (Taurotragus derbianus derbianus) in Senegal: Integration of pedigree and microsatellite data

Hana Zemanová, Barbora Černá Bolfíková, Karolína Brandlová, Pavla Hejcmanová, Pavel Hulva

Mammalian Biology 80 (2015) 328-332

Abstract

Less than 200 wild individuals of the critically endangered Western Derby eland (*Taurotragus derbianus derbianus*) live in the Niokolo Koba National Park (NKNP) in Senegal. A semi-captive breeding programme was established in 2000 with six founding individuals (one male, five females) transferred from the NKNP. In 2013, the population consisted of 92 individuals living in seven separate herds in the two fenced reserves of Bandia and Fathala in Senegal. Because of the low number of founding individuals in the breeding programme and the resulting high kinship, we compared the results from genealogical and genetic approaches to assess the level of genetic diversity. We used the data from the founder, F1 and F2 generations. In F1, the founder contribution was highly biased towards the only founding male, which sired all the offspring. In F2, the founder contributions were more balanced, as the male descendants of founding females entered the reproduction. This resulted in higher genetic diversity and lower inbreeding (based on pedigree data) in F2 than in F1. Results of molecular analysis using microsatellite loci confirmed the highest level of heterozygosity and lowest level of inbreeding in the founder generation; however, the implementation of a management strategy was not reflected in the empirical results. The results differed for F2, where empirical values of heterozygosity continued to decrease and inbreeding continued to increase. However, the allelic richness corresponded with the results of pedigree analyses, reflecting the more equalized founder contributions. We conclude that the overall results for genetic parameters were comparable with other breeding programmes for endangered ungulates. Nevertheless, we suggest the use of comprehensive molecular data to refine the studbook and to correct relatedness of founders and assign the missing paternities. Our suggestions correspond with the Western Derby Eland Conservation Strategy and confirm the need to introduce new founders into the semi-captive population, in order to minimize the risk of inbreeding depression and improve genetic diversity and suitability for potential reintroduction.

Key words: breeding management, conservation, inbreeding, Senegal, small population, antelope

Annexe 4: Curriculum vitae of the author.

Curriculum vitae

Personal information

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	Praha 4, 149 00
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Education

2008 – till now	Czech University of Life Sciences Prague Faculty of Tropical AgriSciences Course: Agriculture in Tropics and Subtropics
2010 – 2012	Czech University of Life Sciences Prague Institute of Education and Communication Course: Teaching of Vocational Subjects (Bc.)
2010	Montpellier SupAgro, France Study under the Erasmus programme
2006 – 2008	Czech University of Life Sciences Prague Institute of Tropics and Subtropics Course: Wildlife Management (Ing.)
2003 – 2006	Czech University of Life Sciences Prague Institute of Tropics and Subtropics Course: Tropical and Subtropical Agriculture (Bc.)
1995 – 2003	Gymnázium Jiřího z Poděbrad, Poděbrady

Occupation

January 2015 – till now	Státní fond rozvoje bydlení position: legal officer activities: communication with clients, simple legal acts, administration
2012 – 2013	Dynamic Future s. r. o. position: consultant activities: data collection and analysis
2005 – 2009	Zoo Prague position: guide activities: communication with visitors, guiding of groups and educational programmes, sale of souvenirs

Skills and abilities

Languages:

English – advanced level

French – advanced level

Driving licence B

PC:

MS Windows, MS Word, Excel, PowerPoint – extraordinary user knowledge

Adobe Photoshop – user knowledge

Publication activity

Zemanová H, Černá Bolfíková B, Brandlová K, Hejcmanová P, Hulva P. 2015. Conservation genetics of the Western Derby eland (*Taurotragus derbianus derbianus*) in Senegal: Integration of pedigree and microsatellite data. Mammalian Biology 80, 328 – 332.

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Interests and hobbies

Nature and nature conservation, zoology, ecology, ethology, nutrition and feeding of animals, genetics, working with children, travelling, literature, foreign languages, handwork, square dance, round dance

Prague, 9th September 2015