CZECH UNIVERSITY OF LIFE SCIENCES PRAGUE

FACULTY OF TROPICAL AGRISCIENCES

Department of Animal Science and Food Processing

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 summary)

Ing. Hana Zemanová

Prague 2015

This Ph.D. thessis has been carried out during the study of the branch Agriculture in Tropics amd Subtropics (4106V022) at Department of Animal Science and Food Processing, Faculty of Tropical AgriSciences Prague.

Applicant:	Ing. Hana Zemanová
Supervisor:	prof. MVDr. Daniela Lukešová, CSc.
Consultant:	Mgr. Barbora Černá Bolfíková, Ph.D.
Opponents:	prof. Ing. Jaroslav Červený, CSc. Mgr. Jarmila Krojerová-Prokešová, Ph.D. doc. RNDr. František Sedláček, CSc.

The Ph.D. thesis defence will take place on the 3. 12. 2015 in front of the committee for defence of dissertation thesis in the study branch Agriculture in Tropics and Subtropics at .... o´clock in the room number .... in the pavilion T of the FTA CULS Prague.

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* a *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 (HE a HO, Ar a odchylky od Hardy-Weinbergovy rovnováhy, a FIS a FST).

Nebyly zjištěny žádné odchylky od Hardy-Weinbergovy rovnováhy. Výsledky genetické analýzy potvrdily nejvyšší genetickou diverzitu u zakladatelů (FOUNDERS: Ar = 2.79; HE = 0.664; HO = 0.750; FIS = –0.154). V obou generacích potomků hodnoty alelické bohatosti a pozorované a očekávané heterozygotnosti klesly (Ar = 2.15; HO = 0.580; HE = 0.586 u OFFSPRING 1 a Ar = 2.14; HO = 0.370; HE = 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

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 (HE and HO, Ar and deviations from Hardy-Weinberg equilibrium, and FIS and FST) 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; HE = 0.664; HO = 0.750; FIS = –0.154). In both generations of offspring values of allelic richness and observed and expected heterozygosity decreased (Ar = 2.15; HO = 0.580; HE = 0.586 in OFFSPRING 1 and Ar = 2.14; HO = 0.370; HE = 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

CONTENTS

1. INTRODUCTION 6
2. AIM OF THE THESIS 8
3. LITERATURE OVERVIEW 9
    3.1 Taxonomy and phylogeny of *Taurotragus spp.* 9
        3.1.1 Phylogeny of Cetartiodactyla and Ruminantia 9
        3.1.2 Phylogeny of Bovidae 9
        3.1.3 Phylogeny of Tragelaphinae 10
        3.1.4 Phylogeny of Tragelaphini 10
        3.1.5 Question of inclusion of *Taurotragus spp.* into the genus *Tragelaphus* 10
    3.2 Natural history of Tragelaphini 10
        3.2.1 Typical traits of Tragelaphini 11
    3.3 Genetic studies 11
    3.4 Pedigree analysis 11
    3.5 Issues of small populations 12
    3.6 Western Derby eland (*Taurotragus derbianus derbianus*) 13
4. METHODOLOGY 14
    4.1 Material 14
    4.2 Methods 14
        4.2.1 DNA isolation 14
        4.2.2 PCR (Polymerase chain reaction) 15
        4.2.3 Electrophoresis 15
        4.2.4 Fragmentation analysis 15
        4.2.5 Data analysis 16
        4.2.6 Comparison of data obtained by pedigree analysis and genetic analyses 16
        4.2.7 Phylogenetical relationships between Eastern (*T. d. gigas*) and Western subspecies (*T. d. derbianus*) of the Derby eland (*T. derbianus*) 17
5. RESULTS 18
6. CONCLUSION 21
7. CURRICULUM VITAE OF THE AUTHOR 22

1. INTRODUCTION

Bovidae live in various environments on four continets. A lot of representatives have economical importance for us, they provide us meat, milk and milky products, horny tissue, leather or wool (Teyrovský, 1957; Danell *et al.,* 2006*;* Bibi and Vrba, 2010).

A lot of studies concerns with bovid´s phylogeny, adaptations, time of their divergence, phylogeography, genetic diversity, morphology and others (for example Matthee and Robinson, 1999; Hassanin and Ropiquet, 2004; Willows-Munro *et al.,* 2005; Lorenzen *et al.* 2010).

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 – only 6 founders) (Asmodé and Khoja, 1989; Ostrowski *et al.,* 1998; Marshall *et al.,* 1999; Wakefield *et al.*, 2002; Walzer, 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 (HE and HO), inbreeding coefficient (FIS), fixation index (FST) ...) 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 the 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) belong to the tribe Tragelaphini, subfamily Bovinae (or Tragelaphinae), family Bovidae, suborder Ruminantia, order Cetartiodactyla, superorder Laurasiatheria, class Mammalia. Cetartiodactyla are the monophyletic group, including the cetaceans (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).

The Tragelaphini are also known as spiral-horned antelopes. They numbers nine species in two genera.

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).

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 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 from the other bovid taxa (for example Cephalophinae, Antilopinae, Caprinae and further) (Kingdon, 1982; Matthee and Davis, 2001). Bovidae are monophyletic group (Allard *et al.,* 1992; Rubes *et al.*, 2008; Wang and Yang, 2013).

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).

3.1.4 Phylogeny of Tragelaphini

Hassanin and Ropiquet (2004) suggest the monophyly of the tribes Tragelaphini, Boselaphini and Bovini. 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 (MacEachern *et al.,* 2009).

The divergence of the Tragelaphini from the other bovid tribes was estimated at approximately 14.08 MYA (Bibi (2013) suggests the divergence time between 10.1 and 5.4 MYA) .

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

Some researchers suggest inclusion of the genus *Taurotragus* into the genus *Tragelaphus* (Hassanin and Douzery, 1999; Matthee and Robinson, 1999; Matthee and Davis, 2001), but they still belong to the genus *Taurotragus sp.* (Wilson and Reeder, 2005).

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 belong to the “true ruminants”, they have four-chambered stomach, they feed on soft nutritious vegetation and fruit (Walker, 1964; Kingdon, 1982; Kingdon, 1997).

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. Further typical trait are white vertical strips and the scent glands, located in front of the teats and around false hooves in hindfeet. 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, the darker coloration of the males and also the size dimorphism (the females are usually smaller than the males) (Kingdon, 1982; Estes, 1991).

3.3 Genetic studies

Genetic studies dealt with a range of topics, for example with taxonomy and phylogenetic relationships (Matthee and Robinson, 1999; Matthee and Davis, 2001; Willows-Munro *et al.*, 2005), phylogeography (Arctander *et al.,* 1999; Birungi and Arctander, 2000; Flagstad *et al.,* 2001; Pitra *et al.,* 2002), creation of genome maps (Bishop *et al.,* 1994; Samson *et al.,* 2008a, b) or genetic diversity (Marshall *et al.*, 1999; Eblate *et al.*, 2011). The studies are based on nuclear and mitochondrial markers (Hassanin and Douzery, 1999; Matthee and Robinson, 1999; Matthee *et al.,* 2001) or chromosomal homologies studies (Rubes *et al.,* 2008; Pagáčová, 2009).

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, completeness of the pedigree, inbreeding coefficient (F) related with average relatedness, then effective population size (N_e). There is possible to use also effective number of founders and effective number of ancestors. 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). Important in the pedigree analysis are founder equivalent (f_e) and founder genome equivalent (f_g) too. Founder equivalent expresses the number of equally contributing founders, the founder genome equivalent is the number of equally contributing founders with no random loss of founder alleles in offspring (Lacy, 1989).

The amount of family relationships in the population is expressed by mean kinship (MK) and the proportion of heterozygotes expected in the population of offspring that is in Hardy-Weinberg equlibrium describes gene diversity (GD) (Ballou and Lacy, 1995; Grueber and Jamieson, 2008; Koláčková *et al.*, 2011a).

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 (Frankham *et al.,* 2003).

Arabian oryx (*Oryx leucoryx*) and Przewalski's horse (*Equus caballus przewalskii*) belong to the species that became extinct in the wild. Their breeding programmes were established with low number of founders, but they reproduced successfully and they were reintroduced back to the wild. The main threat represents now the competition with domestic livestock and habitat destruction (Asmodé and Khoja, 1989; Ostrowski *et al.,* 1998; Marshall *et al.* 1999; Wakefield *et al.*, 2002; Walzer, 2012; ITG International Takhi Group, 2013; IUCN, 2011, 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).

Western Derby eland (*T. d. derbianus*) is classified as “Critically Endangered”, the last population lives in Niokolo Koba National Park in Senegal (Bro-Jørgensen, 1997; Kingdon, 1997; Nežerková *et al*., 2004). The population numbers fewer than 200 individuals and it is sharply decreasing due to poaching, habitat loss and grazing competition with livestock (Bro-Jørgensen, 1997; Renaud *et al.,* 2006; Koláčková *et al.,* 2011b).

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. The both subspecies differ in the shape of the white cheek spots, in one parameter of horn, and in the length of teeth row, which parameter is very close to conventional subspecies boundary (Lydekker, 1914; Dollman, 1936; Haltenorth, 1963; Dorst and Dandelot, 1970; Kingdon, 1982; Bro-Jørgensen, 1997; Kingdon, 1997; Lutovská, 2012; Böhmová, 2013).

The elands (*Taurotragus sp.*) belong to intermediate (or mixed) feeders, they are water independent. Derby elands (*T. derbianus*) are browsers, they feed on shoots, leaves, branches and fruits (Hofmann, 1973; Kingdon, 1982; Ruggiero, 1990; Bro-Jørgensen, 1997; Mares, 1999; Hejcmanová *et al.,* 2010).

In 2000 the *ex-situ* conservation breeding programme was established by 6 founders. Today the population has 95 individuals (Brandlová, 2013, personal communication) that are divided in 5 breeding herds and 2 bachelor herds in the reserves in Bandia and Fathala in Senegal. 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. Studbook for Western Derby eland was established and it is published annually (Antonínová *et al.,* 2004, 2006; Nežerková *et al*., 2004; Koláčková *et al.,* 2009, 2012).

The estimated mean level of inbreeding in the population was 0.124 in 2012, that is lower than in 2008 (0.136) (Koláčková *et al.,* 2009, 2010, 2011a, 2012).

4. METHODOLOGY

4.1 Material

Samples were collected in the years 2006 to 2012 in Bandia reserve. 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 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.

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 born in the season 2010/2011 (except one, born in the season 2009/2010). In this generation there were up to five males included into the reproduction.

4.2 Methods

All steps, except measuring of DNA concentration and fragmentation analysis, were performed in laboratories of Czech University of Life Sciences Prague. 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) and 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)

Thirteen microsatellite markes were used for PCR and following fragmentation analysis. 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 PCR Master Mix by Fermentas was used for the PCR preparation. The PCR proceeded in the thermocycler QB-96 (Quanta Biotech Ltd.). The composition of the reaction mixture and the PCR conditions are stated in the Ph. D. thesis (Table 4 and 5).

4.2.3 Electrophoresis

The functionality of PCR was verified on the 1% agarosis gel in TBE buffer. 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). 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 the Hardy-Weinberg equilibrium and allelic richness (Ar) were determined using the 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).

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; Table 6). The pedigree is based on direct observation of suckling young and their mothers.

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 values of gene diversity (GD) of pedigree analysis were compared with the values of expected and observed heterozygosity and level of inbreeding (F) with inbreeding coefficient (F_{IS}).

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 (AF 533 518, BM 4505, ETH 225, Oar FCB 304 and X 80 214), 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 populations were in Hardy-Weinberg equilibrium in all loci, because the heterozygote deficit or excess was not proved.

The results of all analyses are in tables 7, 8 and 9.

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 in the calculation. The differentiation increased with the distance of the generations (Table 10).

Table 7, 8, 9: Results of genetic analysis- summary.

GENETIC ANALYSIS	Number of alleles per locus	F_{IS}	H_O	H_E	Ar
FOUNDERS	3.00	-0.154	0.750	0.664	2.79
OFFSPRING 1	2.20	0.090	0.580	0.586	2.15
OFFSPRING 2	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

Comparison of the results of pedigree and genetic analysis are shown on the Figure 9 and 10.

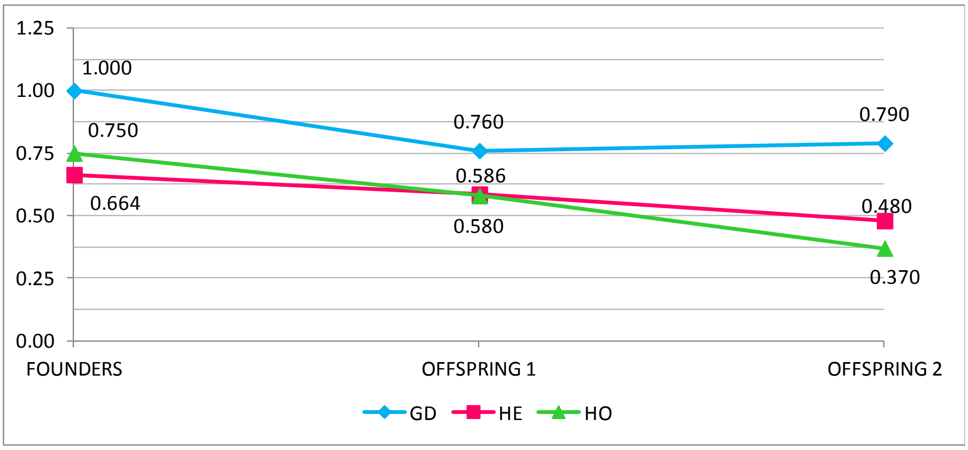


Figure 9: Comparison of gene diversity (GD) obtained from pedigree analysis with expected heterozygosity (H_E) and observed heterozygosity (H_O).

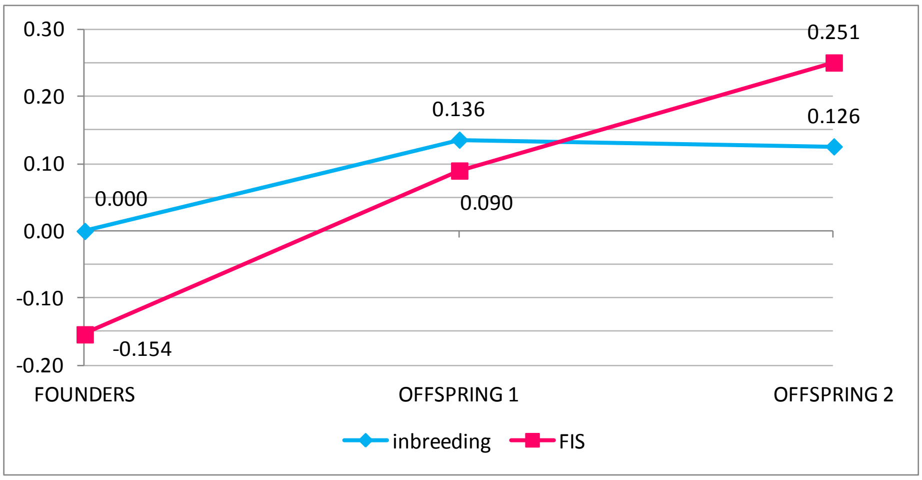


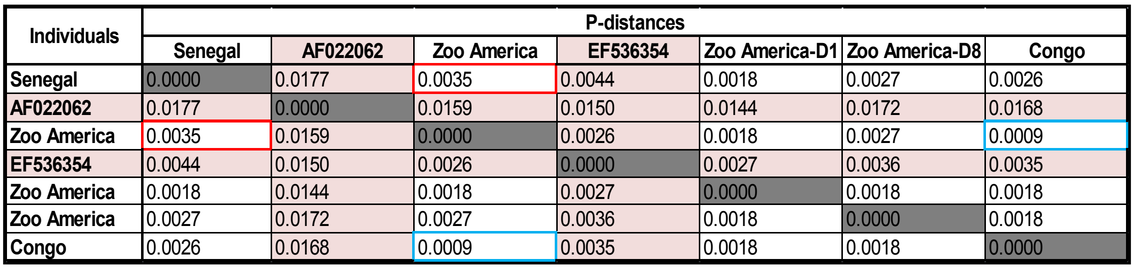
Figure 10: Comparison of inbreeding (F, value obtained from pedigree analysis) and inbreeding coefficient (F_{IS}) found out by genetic analysis.

The genetic diversity from the pedigree analysis showed the decrease in 2008 (OFFSPRING 1) and slight increase in 2011 (OFFSPRING 2), 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.

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).

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. 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 and Eastern subspecies maximally 0.35%.

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

7. CURRICULUM VITAE OF THE AUTHOR

Curriculum vitae

Personal information

Name: Hana Zemanová, Ing.
Date and place of birth: 28. 4. 1983, Městec Králové
Address: Křejpského 1512 / 21
Praha 4, 149 00
Czech Republic
Telephone: +420 604 689 399
E-mail: desir@atlas.cz
Nationality: Czech

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.

Zemanová H, Bolfíková B, Hejcmanová P, Brandlová K. 2012. Genetická diverzita a fylogeneze Západního poddruhu antilopy Derbyho (*Taurotragus derbianus derbianus*). In: Bryja J, Albrechtová J, Tkadlec E. (Eds.). Zoologické dny Olomouc 2012, s. 216.

Zemanová H, Bolfíková B, Hejcmanová P, Koláčková K. 2011. Genetic diversity and phylogeny of the Western Derby eland (*Taurotragus derbianus derbianus*). In: ECM 2011 – VIth European Congress of Mammalogy, Abstract volume. Paříž. s. 108.

Zemanová H, Antonínová M, Bryja J, Martínková N. 2008. Polymorfní mikrosatelitní markery pro určování příbuzenských vztahů u antilopy losí (Taurotragus oryx) a antilopy Derbyho (Taurotragus derbianus). In: Harabiš F, Suvorov P. (Eds.). Kostelecké inspirování 2008. Kostelec nad Černými lesy. FŽP ČZU, s. 52 – 53

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, 11th September 2015