CZECH UNIVERSITY OF LIFE SCIENCE PRAGUE

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Phylogeny and population characteristics of Derby eland (*Taurotragus derbianus*)

Master's thesis

Prague 2016

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Declaration:

I declare that I have made my diploma thesis "Phylogeny and population characteristics of Derby eland (*Taurotragus derbianus*)" personally and I have used the literature sources mentioned in references.

In Prague, 20th April 2016.

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Signature

Acknowledgement**:**

I would like to thank my supervisor Mgr. Barbora Černá Bolfíková, Ph.D. who led me and trained me to work in genetic laboratory. I would like to thank Ing. Karolína Brandlová, Ph.D. for her help and advices. I would like to thank to my colleagues, namely Ing. Anna Kubátová and also to my family for endless support during my whole studies.

My appreciation also belongs to WildGenes laboratory in Edinburgh for cooperation.

This study could be realized due to financial support of Internal Grant Agency under the number 20145027.

Abstract:

Derby eland (*Taurotragus derbianus*) occurs in two lineages, usually recognized as subspecies, Eastern Giant eland (*T. d. gigas*) and Western Derby eland (*T. d. derbianus*). Eastern subspecies is currently listed as Least Concern (LC) and population seem to be stable. Nevertheless, the western subspecies is claimed as Critically Endangered. We assessed 11 new polymorphic microsatellite markers and presented their usage in particular population genetic analyses. Using this panel, it was possible to categorize each individual according to its origin into respective population. Despite low genetic variance, internal structure of Western Derby eland was detected, probably correlating with maternal lineages. It was confirmed that inbreeding coefficient is increasing with each generation in captive population of Western Derby eland. Comparison of last generation of Western Derby eland and populations from the Zoological gardens provided evidence of strong effect of genetic drift. Each of these populations is also highly influenced by founder effect. Our results confirmed the potential of genetic approach, which is essential for effective long term conservation and management. We also tried to estimate mothers of several individuals where the information was missing in the pedigree.

Key words: *Taurotragus derbianus*, genetic parameters, inbreeding coefficient, microsatellites, parentage analysis

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List of abbreviations

Ar	Allelic richness
Ban	Samples from last sampled offspring of from Bandia reserve
CEO	Chief Executive Officer
EGE	Eastern Giant eland
F1	first filial generation
F2	second filial generation
F_{IS}	coefficient of inbreeding
F_{ST}	fixation index
GEN	generation
He	expected heterozygosity
Ho	observed heterozygosity
IAE	International Animal Exchange
ISIS	International Species Information System
IUCN	The International Union for Conservation of Nature
K	number of clusters
LA	Los Angeles zoo
MCMC	Marcov Chain Monte Carlo
Na	number of allele
PCR	polymerase chain reaction
WDE	Western Derby eland
WO	Samples from White Oak Conservation Center
ZIMS	Zoological Information Management System
Zoo	Samples from White Oak Conservation Center and Los Angeles Zoo

1 Introduction

Derby eland or Giant eland (*Taurotragus derbianus*) is one of the largest antelopes in the world. Together with Common eland (*Taurotragus oryx*) belongs to the genus *Taurotragus*. One of the differences between Common eland and Derby eland is in the population size of the species. In the case of Derby eland, the number of individuals is continuously decreasing in the wild, whereas Common eland has a stable population trend (IUCN, 2008). Derby eland can be divided into the two subspecies, Eastern Derby eland (*Taurotragus derbianus gigas*) and Western Derby eland (*Taurotragus derbianus derbianus*). Both subspecies have different categorization according to The International Union for Conservation of Nature (IUCN) Red List of Threatened Species. Eastern Derby eland, more numerous one of the two subspecies, has the status "Least Concern" and Western Derby eland is declared as "Critically Endangered".

The genetic analysis, used in this study, contributed to reveal relationships between the two subspecies of Derby eland. The microsatellite markers provided exact information about population structure and actual genetic variability among populations which bred in different conditions. The factors which affected genetic variability of the populations were detected. The parentage analysis also completed the missing data in pedigree.

The samples used for this study which consisted of blood, hairs and tissues, were obtained from individuals of Eastern Derby eland kept in zoological garden in Los Angeles, USA; White Oak Conservation, USA and national parks in Cameroon and South African Republic. The samples from Western Derby elands were obtained from the population bred in semi-captivity in two natural reserves in Senegal – Bandia and Fathala reserves, which are managed under the auspices of the Western Derby Eland Conservation Programme in Senegal (Brandlová *et al.*, 2013). The Western Derby eland is not kept at any zoo (IUCN, 2008; ZIMS, 2014).

Populations of the sampled Eastern Derby eland have a different origin of individuals. The first semi-captive population of Western Derby eland has been established in Bandia with only 6 founders – 5 females and only 1 male captured from Niokolo Koba National Park in 2000 (Nežerková *et al.*, 2004). There was no possibility to reinforce the population with the individuals from the wild till now.

Nowadays, the whole population comprises about 90 living individuals (Brandlová *et al.*, 2015).

All captive populations live in relatively small groups and moreover they have a limited genetic contribution due to low number of founding individuals and restricted gene flow. Thus, the high risk of inbreeding arises with each generation (Frankham *et al*., 2002; Zemanová *et al.,* 2015). To minimize inbreeding in critically endangered Western Derby eland, the breeding management is applied. The kinship relations are observed and determined by maternity-young interactions (especially suckling behaviour) and all potential sires in the herds are registered. These kinship relations serve as input data for the studbook and arrangements of herd are managed with a regard on these relations. The animals are divided into breeding (5) or bachelor (1) herds (Brandlová *et al.*, 2015). The determination by observing can be inaccurate. The identification of dam is carried out with offspring registration during calving period. The whole process, which takes place in the field, is really difficult (Nežerková *et al.*, 2004). Paternity determination is more complicated after filial males come to the breeding maturity. The genetic tests can provide final parentage determination in undetected individuals and confirm the results obtained by observations and thereby help in proper genetic management.

In this study, the application of microsatellite markers with cross-species amplification approach was chosen. The advantage of microsatellites is their high mutation rate and thus high variability of alleles. The microsatellites of related bovids, namely species as cattle, goat, sheep, deer and gazelle, were tested for ability to amplify in elands, because microsatellites for Derby eland have not been developed till present. The same method was already used in the past (Zemanová *et al.*, 2015). Five microsatellite loci with suitable reaction settings were found but it has been recommended to use at least 7 verified polymorphic microsatellites to reconstruct genealogies (Blouin, 2003).

In this study, 17 new and 5 validated markers have been tested by polymerase chain reaction (PCR) through various PCR conditions to determine the most suitable multilocus markers. The level of polymorphism was determined in all loci for both subspecies. Subsequently, the population characteristics (Ho, He, F_{IS}, Ar) were assessed for both subspecies of Derby eland. The genetic data were used for intraspecific comparison.

2 Bibliographic research

2.1 Taxonomy

The elands (*Taurotragus* spp.) belong to the tribe *Tragelaphini,* which are also known as spiral-horned antelopes. In general, tribe Tragelaphini includes genera *Taurotragus* and *Tragelaphus* (Grubb, 2005) but occasionally we can find inclusion into the genus *Tragelaphus* (Van Gelder, 1977; IUCN, 2008) where has been previously classified. There is a suggestion that genus *Taurotragus* may be a branch of the kudu line (Estes, 1991) and could be classified with *Boocercus* as subgenus into genus *Tragelaphus* due to possible hybridization between Common eland and Greater kudu as Van Gelder (1977) suggested. This hybridization in San Diego Wild Animal Park is closely described by Jorge *et al*. (1976).

Recent taxonomical work commonly uses *Taurotragus* as full genus with two living species included – Common eland *(Taurotragus oryx*) and Derby eland (*Taurotragus derbianus*) (Groves and Grubbs, 2011). The one extinct species *T. arkelli* (Leakey 1965) is mentioned by Pappas (2002). However, *Taurotragus* was historically classified as conspecific (Haltenorth, 1963). Actually, the split between the species *Taurotragus oryx* and *Taurotragus derbianus* was estimated by Fernández and Vrba (2005) about of 1.6 Ma.

Species *Taurotragus derbianus* is divided in two lineages, generally described as subspecies, *Taurotragus derbianus gigas* and *Taurotragus derbianus derbianus*. Other synonyms as *colini, typicus*, *cameroonensis*, *congolanus* and *derbii* for subspecies of Derby eland can occur in the literature. Those synonyms Grubb (2005) and Kingdon *et al.* (2013) mentioned in their publication. The taxonomical status of Derby eland can be subject of controversy. Groves and Grubb (2011) consider species *Taurotragus derbianus* as monotypic. This opinion is based on lack of morphological differences, albeit on very limited sample size. In the contrary Lutovská (2012) confirmed morphometric differences in cranial parameters.

Common names used in publications can be a bit of misnomer. In case of *Taurotragus derbianus* many different common names are widely used, such as Lord Derby's eland (Kingdon, 2015) or Giant sable (Estes, 1991). In the Integrated Taxonomic Information System (ITIS, 2016) Derby eland or Giant eland are published as valid names for the species, especially the western subspecies as "western eland" but eastern subspecies is not specified common name. The IUCN (IUCN, 2008) defines

the subspecies as Eastern Giant eland for *T. d. gigas* and Western Giant eland for *T. d. derbianus*. By Grubb (2005), "Giant eland" refers only to the subspecies *T. d. gigas*. Therefore, the names Eastern Giant eland (bellow EGE) for *T. d. gigas* and Western Derby eland (bellow WDE) for *T. d. derbianus* were used in this study.

2.1.1 Description

Derby elands and Common elands are very similar in weight and height (Dollman, 1936; Dorst and Dandelot, 1970; Groves and Grubb, 2011). Elands have short and robust neck, also their legs are shorter. The adult males of Derby eland weigh up to one tonne (Kingdon, 1984). Derby elands are not characterised by the great body size, as much as the massive horns (Dollman, 1936; Estes, 1991; Groves and Grubb, 2011). This is probably the reason why they received the name "Giant eland" (Estes, 1991; Groves and Grubb, 2011).

Horns are typical for sexual dimorphism in Tragelaphini. There is an exception in eland and bongo species where horns are presented in both sexes (Sclater and Thomas, 1899; Kingdon, 1984; Estes, 1991). The shape, length and sharpness depend on age of individual (Hillman, 1975). The horns are much longer in Derby eland than in Common eland (Dorst and Dandelot, 1970; Groves and Grubbs, 2011) and increase the total height of animal.

They have a flap of loose skin or dewlap between the chin and chest (Sclater and Thomas, 1899) which begins on the chin instead of at the throat like in Common eland (Estes, 1991). They have short and smooth coat (Haltenorth and Diller, 1980). Both sexes have a short brown to black mane from the neck to the middle of the back (Wilson and Mittermeier, 2011).

The Derby eland is richer in coloration of body than the Common eland and has more pronounced markings (Estes, 1991). Adult males have a black neck (Kingdon *et al*., 2013) and they tend to turn grey as they age (Wilson and Mittermeier, 2011). The body of Derby eland is marked by varying number of white transverse stripes (Haltenorth, 1963). The stripe pattern can be different on each side of the body and it is unique for each animal (Hillman, 1975). In eastern subspecies (Figure 1) the hair coat has sandy ground colour with in average 12 vertical stripes on the flank (Kingdon *et al*., 2013). The western subspecies has more stripes than the eastern one (Wilson and Mittermeier, 2011; Kingdon *et al*., 2013). The western subspecies has bright rufous

ground colour with in average 15 vertical stripes on the flank (Kingdon *et al*., 2013) (Figure 2). According to Akakpo *et al*. (2004) the number and shape of white stripes on the flanks do not change during the life in WDE. The unique stripe pattern, black and white marks and coloration enable individual identification (Nežerková *et al.* 2004). This finding serves as a base for identification cards in the studbook of WDE (Antonínová *et al.*, 2004). It is possible that the same principle can be found also in the eastern subspecies, but it has not been proved yet.

Figure 1 Eastern Giant eland in Cincinnati Zoo, USA © 2006 Jeff Whitlock. Source: www.theonlinezoo.com

Figure 2 Western Derby eland in Bandia reserve, Senegal © 2011 Pavel Brandl.

2.1.2 Social systems

The elands are one of the most mobile antelopes (Hillman, 1975). They have large home ranges (Owen-Smith, 1976) with the exception of adult males. Adult males often travel alone over comparatively small areas (Hillman, 1975). The elands are gregarious animals (Estes, 1991; Castelló, 2016). They tend to live in larger groups as other species from open habitats (Owen-Smith, 1976). The herds containing up to 60 animals of both sexes have been reported, though groups of 15 to 25 individuals are more usual (Kingdon, 1984). The number of individuals in herd is affected by local conditions, density of population (Dorst and Dandelot, 1970) habitat and season (Estes, 1991). Free ranging eland populations tend to form smaller groups in dry season and cluster in larger groups during the wet season (Estes, 1991; Castelló, 2016) that may be reflected abundance of food and greater numbers of mothers with calves (Pappas, 2002). Smaller groups often consist of a few females, one dominant male and young (Kingdon *et al.*, 2013). Largest groups of elands always contain calves and juveniles (Hillman, 1975), which aggregate in subgroups, known as crèches or nursery (Estes, 1991). Non-breeding males wander solitary or in bachelor group (Kingdon *et al.*, 2013). There is no evidence of territoriality. Males rarely display aggressive tendencies, even during the breeding season (Castelló, 2016).

The elands interact little among herd members except for mating and mother-calf behaviour (Hillman, 1987). Mother and calf bond very quickly and stays as only stable association (Underwood, 1979; Estes, 1991). The most eland cows will not interact with any calf other than their own (Underwood, 1979). The essential role for mother-calf recognition is naso-anal contact before suckling (Wronski *et al.*, 2006). The most calves are weaned by 6 months of age (Underwood 1979) but they accompany their mother for about one year (Kingdon *et al.*, 2013). The strong mother-calf bond serves for determination of WDE kinship in semi-captivity. That is based on observing of interactions among calves and adult females; focused on nursing behaviour (Antonínová *et al.*, 2004). Determination has been performed every calving season with an exception in 2003, when identification of new-borns was dropped (Nežerková *et al.*, 2004). In order to confirm kinship relations, the DNA analysis was recommended for individuals calved in season 2002–2003 (Antonínová *et al.*, 2004).

2.1.3 Distribution and population status

Tragelaphini can be found exclusively in Africa in these days (Estes, 1991; Kingdon, 2015). However, there is an opinion of Asian origin based on extant geographical distribution and their fossil record (Hassanin and Ropiquet, 2004). An ancestral spiral horn bovine presumably came to the African continent with the second wave of Eurasian bovine immigrants (about 15–18 Ma) (Kingdon *et al.*, 2013).

Rapid ecological specialization in the Late Miocene gave rise to species adapted to more specific environment in Tragelaphini spp. (Willows-Munro *et al*., 2005; Ropiquet, 2006; Rubeš *et al*., 2008). There are two opinions on the process of specialization of this tribe. The first one assumes that development of open savannah specialists preceded the one in more tropical/wet environment (Willows-Munro *et al*., 2005). In contrary, Rubeš *et al*. (2008) declared that cladogenesis confined to moist forest environment at first and then more recently to *T. oryx* and *T. derbianus* whose diversification supervened due to adaptation on arid savannah environment (Willows-Munro *et al*., 2005; Rubeš *et al*., 2008). This theory corresponds with expanded rainforests in early Pliocene (5–3.5 Ma) which have been replaced by dry and cool climate during late Pliocene (3.5–1.6 Ma). Increase of aridity was caused by climatic oscillations at higher latitudes in Africa (Gasse, 2006). African continent has been less affected by climatic changes in comparison to the other continents. Nevertheless, the climate was still influenced due to the fluctuation of glacial and interglacial periods during whole Pleistocene (Steele, 2007). Forests were continuously replaced by savannas in Africa during the glacial periods (Gasse, 2006). This resulted in varied geographic expansion of individual population (Bishop and Turner, 2007).

Derby elands have been widespread through West and Central Africa from Senegal to the Nile (Kingdon, 1984; East, 1999). Dollman (1936) specified range of distribution from Lado in the Sudan (East) to Senegambia (West). The species recently occurred at all of mentioned ranges, but subsequently they were reduced to limited distribution at two separated ranges (Gentry, 1971) (Figure 3).

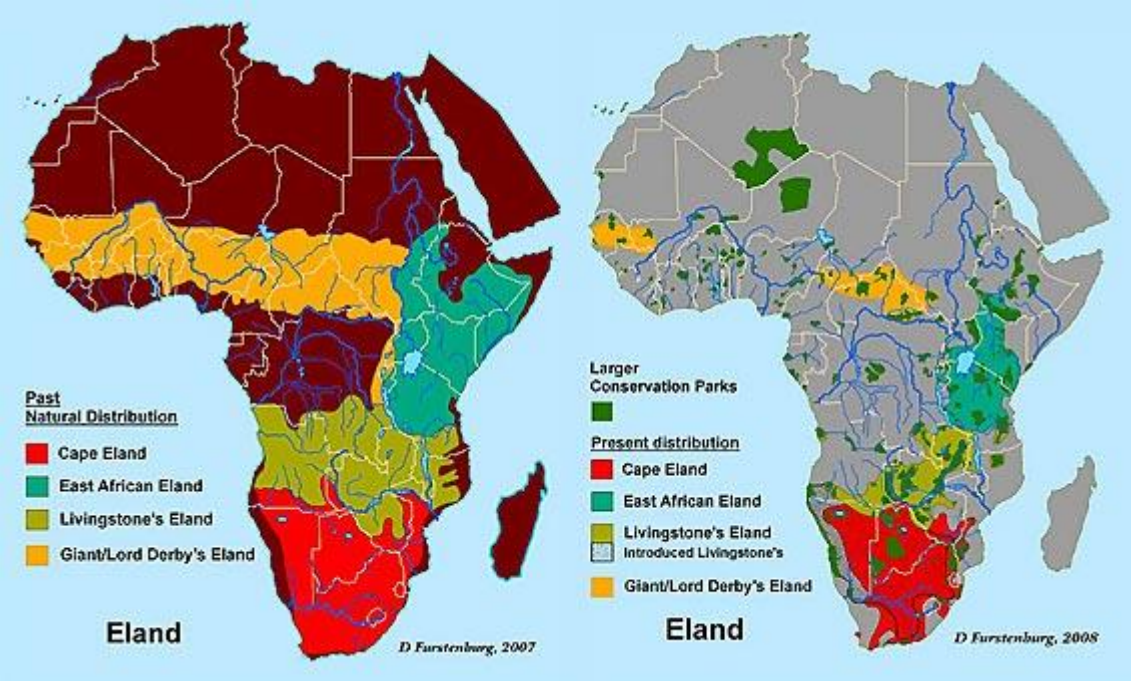

Figure 3 Distribution of *Taurotragus* spp. in the past (left) and recent (right) (Wildlife ranching, 2009)

Other threats were crucial in formation of current population. Derby elands have been eliminated from more than half of their former range (Estes, 1991; East, 1999; Kingdon, 2015). The main problem started with expansion of human population which brought together habitat destruction, poaching (East, 1999; Renaud *et al.* 2006; Brandlová *et al*., 2013) and cattle competitors (East, 1999). Elands suffered heavy mortality during rinderpest epizootic during 1980s, which revealed their high susceptibility to this disease (Kingdon, 1984; Estes, 1991; East, 1999). Derby eland is hunted for meat, sport and primarily for trophy. East (1999) considers mature bulls of Derby elands as one of the world's most prized big game trophies. Thus, trophy hunting quotas have been established in parts of the species range (East, 1999).

Nowadays, EGE´s range (Figure 4) includes Cameroon, Central African Republic, Sudan and Democratic Republic of the Congo – the Garamba National Park (Bouché *etal*., 2009). Total population numbers in the wild are estimated at 15,000–20,000 (IUCN, 2008) The EGE's overall long-term population trend is probably gradually

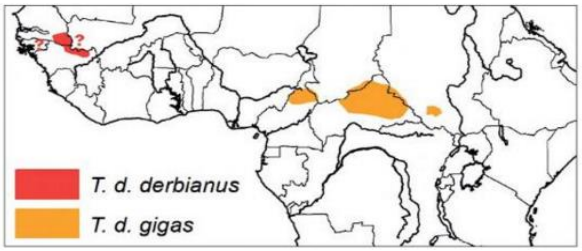

Figure 4 Distribution of Derby eland subspecies (Kingdon, 2015)

downwards (East, 1999). However, Bouché *et al*. (2009) mentioned that population seems to be stable or increasing over last 20 years.

The natural habitat of WDE is very limited in the contemporary West African landscape and solely found in national parks or nature reserves (Brandlová *et al*., 2013). Presumably the only viable population is located in Niokolo Koba National Park (NKNP) in Senegal (Antonínová *et al.*, 2006; IUCN, 2008). Currently, the total number estimate is less than 200 animals living in the wild (IUCN, 2008).

However, Derby eland go through strong population decline, they are currently listed as Least Concern (LC) by the IUCN. Numbers of eastern subspecies are presumably more stable but the western subspecies is claimed as Critically Endangered (CE) with still decreasing trend (IUCN, 2008).

2.2 Conservation management

Antelopes are valuable natural resource. They provide an important source of protein for human consumption and other valuable products such as skins and trophies in many African countries. They are also significant component of fauna which attracts game-viewing tourists to better-known national parks and reserves in Africa (East,1999). The best strategy for long-term protection is preserving the population in its natural habitat (Primack, 2000). Continued effective protection and management of national parks and reserves will be essential to maintain satisfactory antelope conservation status (East, 1990).

The future of elands and other large mammals depends on the approach of Africa's permanent residents (East, 1999). Wildlife conservation has the potential to bring benefits to local communities, operators and states; but there is need to improve awareness and communication on the benefits of such projects (Tsi *et al*., 2008). East (1990) also mentions the need to increase public awareness of the wildlife conservation and promote the rational exploitation to ensure continued availability of natural resources.

Most of African natural parks were gazetted for the purposes of conservation, education and tourism (African parks, 2005), *e. g.* Niokolo Koba or Bandiar National Park (East, 1990) being among them.

Tourism is one of the non-consumptive ways of utilization of nature reserves. Especially, ecotourism has become the fastest growing sub-sector of the tourist industry (Ijeomah *et al*., 2007).

Trophy or sport hunting may bring major economic values to the region economy (East, 1999; Tsi *et al*., 2008). The areas where wildlife receives effective protection are often within hunting zones (East, 1999). The sport hunting program should be implemented with caution because of its impact on animal population stakeholders and the parks themselves (Tsi *et al*., 2008). An effective management will ensure the sustainability of conservation projects and the protection of endangered species in parks (Tsi *et al*., 2008).

Improved anti-poaching activity in national parks will increase the benefits for the park by conserving rare and valuable species (East, 1999). EGE are one of the most sought antelope trophies (IUCN, 2008). EGE occurs in several game reserves and hunting concessions, thus supporting conservation activities for the animal, its natural habitat and other species (Brandlová *et al*., 2013). The WDE has also some potential future value, similar as the eastern subspecies. The potential value of the WDE in trophy hunting tourism could be exposed within a certain timeframe, provided that strictly enforced protection leads to a significant increase in the current population (Brandlová *et al*., 2013).

Conservation management of wild populations generally includes recovery of small inbred populations; genetic management of the fragmented populations and efforts to increase their population size. For recovery of the population size, several methods are used; *e. g*. prohibited or controlled hunting; protection and translocation or captive breeding and release programs (Frankham *et al*., 2002).

Sustaining of healthy population of some species in captivity requires careful assessment and manipulation of genetic and demographic features (Wilcken and Lees, 2012). The strategy consists of securing population by establishing a sufficient number of breeding animals at several suitable sites, to shelter the population against uncontrolled illegal hunting and against various eventual catastrophes or disease outbreaks (Frankham *et al*., 2002; Brandlová *et al*., 2013). Another aim is to manage the population to retain its genetic diversity as high as possible (*e. g.* through genealogical or genetic approaches). To accomplish genealogical data effectively, it is necessary to have access to accurate up to date information on populations in a standard format for

analyses (Wilcken and Lees, 2012). The studbooks are well designed to perform these data (Wilcken and Lees, 2012). When information about genealogy cannot be provided, the genetic parameters can be inferred from the analysis by molecular markers (Armstrong *et al*., 2011). Managing of the conservation breeding should reflect the paradigmatic framework of the conservation genetic in small population (Ebenhard, 1995). Combined data from genealogical and genetic analyses can increase the probability of designing a successful breeding management strategy (Armstrong *et al*., 2011; Koláčková *et al.*, 2011). Conservation program is declared as successful only if the effective population size raises above the minimum viable population (Harmon and Braude, 2010).

2.2.1 Eastern Derby eland

The Eastern Derby eland is the only subspecies bred in captivity in facilities listed in ZIMS (Zoological Information Management System) by ISIS (International Species Information System). EGE captive population comprises around 30 individuals in several zoos in the USA, United Arab Emirates and the Republic of South Africa (ZIMS, 2014).

The first documented captive Giant elands (IAE, 2011) were captured in Chad and transported to Europe to the Antwerp Zoo in 1967. No eland from this line remained (IAE, 2011).

The current captive population of Giant eland in North America are descendants of a group of eland imported by Brian Hunt, CEO of International Animal Exchange Inc. (IAE), in 1986 (IAE, 2011). This population was established from nine wild-caught animals imported from the Central African Republic (East, 1999). These animals were placed at the Cincinnati Zoo and the Los Angeles Zoo. Recent North American populations are descendants of eight founder animals (Romo, 2000). Their offspring were transferred to the African Safari Wildlife Park, Houston Zoo, Miami Metrozoo, San Diego Zoo, and White Oak Conservation Center (IAE, 2011). Several descendants of this lineage born at White Oak have been sent to international zoo partners to initiate breeding programs in Costa Rica and South Africa (White Oak, 2013).

According to East (1999) the safari hunting is the most likely justification for the long-term preservation of the substantial areas of unmodified savannah woodland which this antelope requires, and sustainable trophy hunting is a key to the

Giant eland's future. However, political instability and armed conflict are major barriers to the implementation of effective protection and management over large parts of the eastern subspecies' remaining range (East, 1999). Alternatively, effective long-term management of national parks and hunting zones in regions such as Cameroon's North Province and northern and eastern Central Africa would ensure this subspecies' survival (East, 1999); *e. g.* Chinko Project in the Central African Republic which is a member of the African Parks since 2014 (African Parks, 2005).

2.2.2 Western Derby eland

Probably the only wild viable population of Western Derby eland is concentrated in the Niokolo Koba National Park (IUCN, 2008). The NKNP has been recognised for long time as one of the most important wildlife refuges in the West Africa (Sournia and Dupuy, 1990) and it was accepted as a Biosphere Reserve and World Heritage Site by UNESCO in 1981 (Brandlová *et al*., 2015). NKNP continues to support a savannah antelope community of major international importance, including WDE (East, 1999). Nevertheless, the habitat destruction and poaching are still responsible for the population decline of elands (Renaud *et al*., 2006; Brandlová *et al*., 2013). The survival of the western subspecies in the wild depends on continued protection of the Niokolo Koba population in Senegal (Estes, 1991).

The WDE is not kept in any zoo or other captive facility listed in ZIMS by ISIS (ZIMS, 2014).

In 1978, there was an effort to establish the first *ex situ* herd of WDE by San Diego Animal Park (USA). The operation of capture was not successful (all animals died in the boma after the capture) and no other attempts were initiated for many years (Brandlová *et al*., 2013).

In April 2000, another capture of WDE was carried out. In order to establish a viable population, 6 adult animals and 3 yearlings (1 adult male and 8 females) were translocated from NKNP into Bandia reserve in Senegal (Akakpo *et al.*, 2004; Nežerková *et al.*, 2004). They were immediately placed into quarantine boma. Unfortunatelly 3 adult females did not adapt to the change of conditions and died. In August 2000, remaining 6 animals were released into enclosure and created unique worldwide breeding herd of that subspecies in semi-captivity (Nežerková *et al*., 2004).

Thereby, a unique conservation programme was launched and it has been running till present due to close coordinated cooperation of the partners (Brandlová *et al*., 2015).

Nežerková *et al.* (2004) mentioned the need of separation of sub-adults before they reach reproductive age and establishment of new breeding herd. Therefore, the new enclosures were built in the Fathala reserve in Senegal. Consequently, four animals were selected for the second breeding herd and nine sub adult males for formation of bachelor group in Fathala. The first transport of animals to Fathala reserve was performed in March 2006 (Antonínová *et al.*, 2006). The reproductive programme is assessed and managed according to the pedigree (Antonínová *et al.*, 2004; Brandlová *et al*., 2013). In June 2015, WDE formed a population of 89 living registered individuals; unregistered offspring (up to 1 year) from last calving season were not included (Brandlová *et al*., 2015). Three reproductive herds and one bachelor herd in Bandia and 2 reproductive herds in Fathala are available in 2015 (Brandlová *et al*., 2015). All herds are separated by fences. Thereby, free migration of these ungulates among the reservations and the national park is not possible and because of this there is no natural gene flow among the populations (Brandlová *et al.*, 2015.).

2.3 Small populations

In evaluation of conservation priorities, the population size is very important for a species. The populations are affected by events which has greater influence in small population, *e. g* genetic drift, Allee effect and inbreeding, which can eventually lead to inbreeding depression (Primack, 2000; Courchamp *et al.*, 2008). The rapid decline of population size can be caused by natural (formation of natural barrier, disease outbreak) or artificial (establishing of breeding herd, poaching, development pressures) ways (East, 1999; Frankham *et al*., 2002). In that case the population have to deal with limited gene pool due to founder or bottleneck effects (Primack, 2000; Frankham *et al*., 2002).

Genetic drift

The stochastic fluctuations in allele frequency are termed as a genetic drift. Genetic drift can approach to the fixation or elimination of the allele. It based on number of offspring and from each subsequent generation (Frankham *et al*., 2002; Courchamp *et al.*, 2008; Harmon and Braude, 2010). The gene flow is the key solution

how to maintain genetic diversity. Even moderate gene flow reduces the effect of genetic drift (Harmon and Braude, 2010).

Inbreeding

The inbreeding means mating with genetically relative individuals. In the wild, the probability of inbreeding is higher in case of species which tend to live in family groups or near proximity with close relatives of the opposite sex (Koenig and Haydock, 2004). In spite of this, many species have evolved inbreeding-avoidance strategy (Frankham *et al*.,2002; Koenig and Haydock, 2004). Inbreeding may be tolerated under certain circumstances where benefits of inbreeding outweigh the costs of inbreeding depression (Waser *et al.*, 1986). In very large random mating population, the inbreeding probability is close to zero (Frankham *et al*., 2002). In small closed populations, even random mating will inevitably lead to mating among genetically relatives (Frankham *et al*., 2002; Harmon and Braude, 2010). The inbreeding occurs when an offspring inherits two copies of the same allele at locus from the common ancestor (Harmon and Braude, 2010). The level of inbreeding can be assessed by the probability that homozygote is identical by descent at particular locus (Frankham *et al*.,2002). The inbreeding leads to a loss of heterozygosity across all alleles in the population (Harmon and Braude, 2010). The accumulation of homozygosity can cause a decline of adaptability to environmental changes and reduced reproductive output. This trend is known as inbreeding depression (Lynch and Walsh, 1998; Frankham, 2008; Harmon and Braude, 2010).

Inbreeding depression is an expression of increased frequency of deleterious alleles (Frankham *et al*., 2002; Nielsen *et al*., 2012). These harmful alleles are usually rare and occur in recessive form (Nielsen *et al*., 2012). Empirical data show that inbreeding depression produces strong effects on fitness (Keller and Waller, 2002; Nielsen *et al*., 2012; Brommer *et al*., 2015)

Bottleneck and founder effect

If the population go through short-term but great reduction of population size, we speak about bottleneck. Small size can be only temporary phenomenon at the expense of greater decrease of heterozygosity and low population growth rate. That results in loss of genetic diversity, loss of rare alleles and higher effect of genetic drift (Frankham *et al*., 2002; Keller and Waller, 2002). Populations experiencing bottlenecks should expose deleterious recessive mutations to selection, reducing inbreeding

depression (Keller and Waller, 2002). Special example of recent bottleneck is a founder effect. Small size of founder population leads to change in the genetic composition of a population through single generation bottleneck (Frankham *et al*., 2002).

The result of these effects is usually loss of genetic diversity. Accumulation of these genetic changes can lead to harmful expression, *e. g.* inbreeding depression, accumulation of new mildly deleterious mutations (Frankham *et al*., 2002). These changes cause a reduction in reproduction and survival in the short term. They also diminish the capacity of populations to evolve in response to environmental change in the long term (Primack, 2000). Small populations are more susceptible to extinction because of demographic stochasticity and genetic drift and environmental variation (Caughley, 1994; Harmon and Braude, 2010). This is called an extinction vortex; the negative consequences of lower effective population size make the population smaller, causing stronger negative effects and leading to an even smaller population size (Gilpin and Soule, 1986). However, there are many empirical studies which proves that populations are able to cope with low diversity, even though they had experience with the most of these effects and went through strong reduction of genetic variability. Moreover, some successful breeding, *e. g.* European bison (*Bison bonasus*) (Tokarska *et al.*, 2009) or Arabian oryx (*Oryx leucoryx*) (Marshall *et al.*, 1999), which recover the population, increase the population size and enable reintroduction. Frankham *et al*. (2002) mentioned genetic adaptation as a difference in adaptation among wild and captive population.

Captive populations

Captive populations (especially endangered species) are usually kept in small population size and they are usually dispersed among many institutions (Frankham, 2008). The key limiting factor is breeding capacities of each institution (namely costs for establishment of the captive population and lack of space) and problem to obtain many animals (particularly unrelated individuals) from the wild due to low number of natural population. Therefore, the breeding programs are usually based on small number of initial animals (bellow as "founders") (Frankham *et al*., 2002); subsequently, their descendants are more susceptible to mating among relatives resp. inbreeding (Frankham *et al*., 2002; Harmon and Braude, 2010).

The conservation in captivity may minimize the impact of catastrophes (*e. g.* fires, extreme weather and diseases) (Frankham *et al*., 2002). Nevertheless, breeding in captivity inevitably brings similar risks in small isolated population as we mention above. To avoid of potential decline of genetic diversity, an appropriate genetic management has to be specified (Thévenon and Couvet, 2002); *e. g.* management of valuable species is maintained as a single random mating in population, implemented by regular translocation of animals among institutions (Frankham *et al*., 2002). The management of single population strategy is very expensive and the risk of spreading the pathogens is increased (Woodford and Rossiter, 1994).

The both subspecies of Derby eland have a representative small population which is highly inbred. That can be understood by considering low number of founders, they are affected by founder event. In case of EDE bred in captivity, the current populations have eight ancestors (Romo, 2000). In case of WDE bred in semi-captive condition in Senegal, the initial herd has 6 founders; the five females and one male (Nežerková *et al*., 2004). Moreover, there was no possibility to enrich the reproductive herd by new breeding male, so even no gene flow occurs until now (Brandlová *et al.*, 2015). Thus, the only one male sired all offspring; as well as the same male is the only donor of chromosome Y. This is a situation similar to breeding of the Lowland European bison, which had only one founding bull (Tokarska *et al*., 2009).

Population characteristic of WDE

In 2015, the pedigree of WDE had 72% of certain ancestry genotypes in the current population. The 93% ancestry is known in the population, although not certainly due to multiple sires (Brandlová *et al*. 2015). Zemanová *et al.* (2015) proved the loss of genetic diversity and increasing of inbreeding with each generation by molecular analysis.

Zemanová *et al.* (2015) compared the genealogical and genetic approach in this population until 2011. Their study demonstrates that the pedigree results are somewhat more optimistic than the empirical data from the microsatellites. For example, the rate of inbreeding derived from microsatellite loci increased very rapidly from founder generation (F_{IS}=−0.154) to the F2 (F=0.369) and observed heterozygosity declines in the same duration from first generation (Ho=0.750) to the third generation (Ho=0.366). The pedigree analysis shows that the population in 2015 has retained 78.99 % of GD from the founders, which is more or less stable since 2008. However, the

mean of inbreeding (F) increased from 0.1364 in 2008 up to 0.1788 in 2015 (Brandlová *et al*. 2015). The result from pedigree have been probably influenced by presumption that founder was not related. In that fact, the molecular analysis using microsatellite markers is more corresponding to the reality.

2.4 Genetic methods

2.4.1 Microsatellites

Microsatellite markers are also known as Short Tandem Repeats (STR) or Simple Sequence Repeats (SSR) and Simple Sequence Tandem Repeats (SSTR) (Hussain, 2013).

This stretch of DNA usually contains 1–6 nucleotide tandem repeats (bp); accordingly, we talk about mono, di, tri, tetra, penta or hexa-nucleotide unit (Tóth *et al*., 2000). The length of microsatellite is determined by number of repetition; *e. g*. $(CA)_n$, is one of the most common dinucleotide motif found in mammals, where n is the number of repeats (Weber and Wong, 1993; Rohrer *et al*., 1994).

Microsatellites are classified according to repetitive sequence as a perfect, imperfect, interrupted or compound (Oliveira *et al*., 2006). The perfect type has the only repeated motif without any modification (…CACACACACA…); the imperfect type has disrupted repetition by on base pair (…CTCTGTCTCT…). The interrupted type has inserted different short sequence inside repeated motif (…GAGACGTGGAGA…). The compound microsatellite consists two or more short tandem repeat; *e. g.* $(CA)_n(GA)_m$.

Other changes in microsatellite structure can be caused by mutations. A several mutation mechanisms have been described. It includes errors during recombination, unequal crossing-over and polymerase slippage during DNA replication or repair (Strand *et al*., 1993).

The microsatellites are bordered (“at the beginning” and “at the end”) by sequence of basis in exact order, so called flanking regions. These flanking regions are unique for each locus. They cover the sequence of chain which the primer anneals. Therefore, it is possible to invent microsatellite markers resp. primers for target locus. In the order detection of alleles, two oligonucleotide primers (forward and reward) are used in amplification method PCR (polymerase chain reaction) (Oliveira *et al*., 2006).

The most effective genetic marker has to be stable, highly polymorphic, easily detectable, accessible and easily multiplied, regularly distributed throughout whole genome (Hajeer *et al*., 2000). It is extremely difficult to find marker which meets all the criteria. Therefore, the marker can be used, if it meets at least one of the mentioned requirements (Joshi *et al*., 1999). Microsatellites meet most of them, therefore the microsatellites markers are currently the most widespread and preferred group of molecular markers, classified by DNA amplification methodology (Hussain, 2013).

Microsatellites are the most common form of repetitive sequence in DNA; they represent 0.5% of the genome (Hajeer *et al*., 2000). They are interspersed in whole genome, even though the distribution is not regular, including both coding as well as non-coding regions of eukaryotes and also in prokaryotes (Tóth *et al*., 2000; Li *et al.*, 2004). However, the biological significance of this repetition is still unknown.

Microsatellite loci have co-dominant inheritance (the length of each microsatellite is inherited by Mendelian law), which allowing them to be comparatively easy to score directly (Hussain, 2013). The microsatellites are *a priori* assumed as a selectively neutral, however, some loci may be adjacent to important functional genes, and may also be a subject to selection pressure (Li *et al*. 2004; Hussain, 2013). Microsatellites can be also characterized by fast mutation rate, which provide insight into recent population structure. Mutation rate of microsatellites is generally much higher than the rest of the genome: ranges 10^{-2} do 10^{-6} nucleotides per locus for generation (Sia *et al*., 2000), but usually remains within 10^{-3} to 10^{-4} per generation (Whittaker *et al*., 2003). The fast mutation rate is also responsible for the high polymorphism of microsatellites (Sia *et al*., 2000). The high polymorphism is often accompanied by high heterozygosity, which may reach up to 90% (Baker *et al*., 1999). In homozygous individuals, the microsatellite has the same number of repeats on both homologous chromosomes, while the number of repeats differs in each allele in heterozygous individuals (Oliveira *et al*., 2006).

The main disadvantage of microsatellite markers is their species specificity. Disadvantages may be also the apparent neutrality, which is difficult to detect, some of the loci may be linked with loci under selection; fast rate of mutation, which may lead to convergence (*e. g.* the same allele have not same ancestry) and at least, this approach is very expensive and long lasting.

2.4.2 Cross-species amplification

Using species-specific primers for genetic studies may be the most accurate technique (Cosse *et al*., 2007), especially, for species which are examined for the first time, there is the need of *de novo* isolation. However, development of specific primers is relatively laborious and very expensive (Oliveira *et al*., 2006). The alternative is utilization of cross-amplification (Frankham *et al*., 2002).

Cross-species amplification or transferability is an attribute of the microsatellite markers (Oliveira *et al*., 2006). It enables to use microsatellite markers, which were developed to particular species, in PCR and amplify selected loci of another species. These species should be closely related (Frankham *et al*., 2002; Eblate *et al*., 2011). The probability of successful amplification rate declines as genetic divergence increases between them (Scribner and Pearce, 2000; Primmer and Merilä, 2002). Cross-amplification is possible due to flanking regions. These flanking sequences appear to have been conserved with the microsatellites together within closely related species (Oliveira *et al*., 2006) and even across families (Lorenzini, 2005). For testing applicability of cross-amplified primers, the reaction of PCR has to be optimized. Optimization is a process of selecting and testing of the most appropriate parameters which affect the reaction, *i. e.* composition of reaction mixture, temperature and time course of the reaction (Lorenzini, 2005). Consequently, the level of polymorphism has to be determined (Lorenzini, 2005; Eblate *et al.*, 2011).

This method can be very useful especially when working on taxa with low microsatellite frequencies or from which microsatellites are difficult to isolate (Oliveira *et al*., 2006). It also provides two advantages, it is less expensive and time consuming.

The cattle microsatellites are one of the most used cross-species amplification for ungulates; for example, they were applied to Apennine chamois (*Rupicapra pyrenaica ornata*) (Lorenzini, 2005); European bison (Gralak *et al.*, 2004) or Roe deer (*Capreolus capreolus*) (Galan *et al.*, 2003).

3 Aim of the thesis

The aim of this study was to prepare a panel of polymorphic microsatellites, tested by the cross-species amplification, which can be used to study the genetic characteristics of Derby eland (*Taurotragus derbianus*). Another goal was the assessment of population structure differences between the two subspecies of Derby eland - Eastern Giant eland (*T. d. gigas*) and Western Derby eland (*T. d. derbianus*), as well as analysis of population characteristics derived from microsatellites markers, *i.e.* analyse observed and expected heterozygosity, allelic richness and inbreeding rate and discuss observed patterns in respect to history and pedigree of the populations. We also aimed to assign the maternal lineage of western subspecies through parentage testing.

Hypotheses**:**

i. Because of long time of captive breeding and small population number, the rate of inbreeding will be higher in captive population of eastern subspecies than in captive population of western subspecies.

ii. The results obtained by genetic analyses of newly developed microsatellite panel will correspond to the results of genetic analyses obtained from five microsatellite markers published by Zemanová *et al.* (2015).

4 Materials

4.1 Samples

The Eastern Giant eland's samples of tissue and hairs were obtained from zoos in USA and Africa reserves. Exactly 2 samples of living individuals were obtained from Los Angeles zoo; 6 individuals from White Oak Conservation Center in Florida; 6 individuals from Cameroon; 4 individuals from South African Republic. The samples from Berlin museum and Prague museum were acquired in 2014. Total number of examined samples from eastern subspecies was 20 (Table 1).

Blood, tissue and hair samples of Western Derby eland were obtained from 111 individuals living in Bandia and Fathala natural reserves in Senegal. The sample types are listed in Table 2. The blood samples were collected during transports of individuals between herds in Bandia and Fathala reserves. Blood samples were treated with anticoagulants (heparin or EDTA). The heparinized blood samples were stored at temperature -18 or -20°C in the freezer.

Tissues were sampled by biopsy darts (biopsy darts by Pneu-Dart Inc.) or from dead animals. The sample collection was completed under the control of experienced veterinarian. The tissue samples were treated with 96% ethanol in room temperature and then stored in the freezer for possible further use.

All samples are dated from 2006–2015.

Table 1 The list of samples of the Eastern Derby eland (*Taurotragus derbianus gigas*)					
ID	**origin**	**source of samples**	**Sex**	**Type of the sample**	**Note**
G1	no data	Museum Berlin	U	skin	excluded
G2	no data	Praha Museum	U	skin	excluded
G3	Central African Republic	Zoo LA	U	hairs	used
G4	Central African Republic	Zoo LA	U	hairs	used
G5	Central African Republic	Zoo White Oak	U	hairs	used
G6	Central African Republic	Zoo White Oak	U	hairs	used
G7	Central African Republic	Zoo White Oak	U	hairs	used
G8	Central African Republic	Zoo White Oak	U	hairs	used
G9	Central African Republic	Zoo White Oak	U	hairs	used
G10	Central African Republic	Zoo White Oak	U	hairs	used
G11	Cameroon	Cameroon	U	muscle	used
G12	Cameroon	Cameroon	U	muscle	used
G13	Cameroon	Cameroon	U	muscle	used
G14	Cameroon	Cameroon	U	muscle	used
G15	Cameroon	Cameroon	U	muscle	used
G16	Cameroon	Cameroon	U	muscle	used
G17	no data	South African Republic	U	tissue	used
G18	no data	South African Republic	U	tissue	used
G19	no data	South African Republic	U	tissue	excluded
G20	no data	South African Republic	U	tissue	used

Table 2 The list of samples of the Western Derby eland (*Taurotragus derbianus derbianus*)					
ID	**Name of animal**	**Sex**	**Year of collection**	**Type of the sample**	**Note**
1	Bandia	M	2006	blood	used
2	Taiba	M	2006	blood	used
3	Derby	M	2006	blood	used
4	Doole	M	2006	blood	used
5	Gaaw	M	2006	blood	used
6	Popengiune	M	2006	blood	used
7	Karang	M	2006	blood	used
8	Sokone	M	2006	blood	used
9	Matam	M	2006	blood	used
10	Sindia	F	2006	blood	used
11	Minna	F	2006	blood	used
12	Toubab	M	2006	blood	used
13	Bayane	F	2006	blood	duplicity with 17; excluded
14	Bembou	F	2006	blood	used
15	young of Malapa	M	2005	tissue	used
16	Bayane	F	2006	tissue	duplicity with 17; excluded
17	Bayane	F	2006	tissue	used
18	Niokolo	M	2008	tissue	used
19	Salémata	F	2008	tissue	used
20	Malapa	F	2008	tissue	used
21	Dagana	F	2008	tissue	used
22	Thelma	F	2008	tissue	used
23	Ndiogoye	F	2008	tissue	used
24	Fathala	F	2008	tissue	used
25	Tuuti	F	2008	tissue	used
26	ml. Tuuti	F	2008	tissue	Noname2; used
27	Deedet	M	2008	blood	used
28	Souleye	M	2008	blood	used
29	Tukki	M	2008	blood	used
30	Tidian	M	2008	blood	used
31	Georgina	F	2008	blood	used
32	Nelaw	F	2008	blood	used
33	Foog	F	2008	blood	used
34	Foulamousou	F	2008	blood	used
35	Nane	F	2008	blood	used
36	x ml. 02/2008	M	2008	blood	Noname3; used
37	Mike	M	2009	blood	used
38	Dewene	F	2009	blood	used
39	Dagou	F	2009	blood	used
40	Bandiagara	F	2009	blood	duplicity with 98; excluded
41	Dering	M	2009	blood	used
42	Tagat	F	2009	blood	used

Western Derby eland (*Taurotragus derbianus derbianus*) continued.					
ID	**Name of animal**	**Sex**	**Year of collection**	**Type of the sample**	**Note**
43	Georges	M	2009	blood	used
44	Galago	M	2009	blood	used
45	Nature	F	2009	blood	used
46	Toubacouta	F	2009	blood	used
47	Tendresse	F	2009	blood	used
48	Fatou	F	2009	blood	used
49	Didi	F	2009	blood	used
50	Mansarinku	M	2009	blood	used
51	Sao	F	2009	blood	used
52	Saroudia	F	2009	blood	used
53	Teranga	M	2011	hairs	used
54	Nanuk	M	2011	hairs	used
55	Mbalax	F	2011	hairs	used
56	Soleil	M	2011	hairs	used
57	Mirabelle T.	F	2011	hairs	used
58	Mango T.	M	2011	hairs	used
59	Sabar T.	M	2011	hairs	used
60	Dara	F	2011	hairs	used
61	Gaanga	F	2011	hairs	used
62	Sindibad T.	M	2012	blood	used
63	Tamtam D.	M	2012	blood	used
64	Tamarin D.	M	2012	blood	used
65	Dodo	M	2012	blood	used
66	Destin T.	M	2012	blood	used
67	Dada T.	M	2012	blood	used
68	Demba T.	M	2012	blood	used
69	Droit	M	2012	blood	used
70	Fort	M	2012	blood	used
71	Marabout	M	2012	blood	used
72	Nemo	M	2012	blood	used
73	Nguekokh	M	2012	blood	used
74	Salut T.	M	2012	blood	used
75	Souhel	M	2012	blood	used
76	Timbre D.	M	2012	blood	used
77	Titi	M	2012	blood	used
78	Triomphe D.	M	2012	blood	used
79	vz. z ledna 2014	U	2014	tissue	Noname9 or 10; used
80	Bonheur	M	2013	tissue	used
81	Not Mike	M	2013	tissue	used
82	Minna	F	2013	tissue	duplicity with 11; excluded
83	Tuur	M	2014	blood	used
84	Tembo	M	2014	blood	used

Western Derby eland (*Taurotragus derbianus derbianus*) continued.					
ID	**Name of animal**	**Sex**	**Year of collection**	**Type of the sample**	**Note**
85	Ted	M	2014	blood	used
86	Salut T.	M	2014	blood	duplicity with 74; excluded
87	Sultana	F	2014	blood	used
88	Diego	M	2014	blood	used
89	Dawal	M	2014	blood	used
90	Saanga	F	2014	blood	used
91	Seraphine	M	2014	blood	used
92	Nigella	F	2014	blood	used
93	Dine	F	2014	blood	used
94	Daphne	F	2014	blood	used
95	Daraja	F	2014	blood	used
96	Farata	F	2014	blood	duplicity with 110; excluded
97	Donja	F	2014	blood	used
98	Bandiagara	F	2014	blood	used
99	Bouba	F	2015	tissue	used
100	David	M	2015	tissue	used
101	Marketa	F	2015	tissue	used
102	Driankee	F	2015	tissue	used
103	Docteur	M	2015	tissue	used
104	Mario	M	2015	tissue	used
105	Safira	F	2015	tissue	used
106	Felicia	F	2015	tissue	used
107	Mammouth	M	2015	tissue	used
108	Fanfan	M	2015	tissue	used
109	Soukeina	F	2015	tissue	used
110	Farata	F	2014	tissue	used
111	11 / 2014	U	2014	tissue	used

5 Methods

The most of laboratory analyses were processed at the Faculty of Tropical AgriSciences (FTA) laboratory of molecular genetics at Czech University of Life Sciences Prague (CULS). These processes comprise DNA extraction; verification of concentration, electrophoresis and PCR. Fragmentation analyses were run at sequencing machine at Faculty of Environmental Sciences (FES) laboratory of molecular genetics at CULS.

5.1 Extraction of DNA

Genomic DNA from samples was extracted using DNeasy Blood and Tissue Kit (Qiagen). The procedure was performed according to enclosed protocol. The chemical volumes were modified in order to raise the concentration (Černá Bolfíková pers. com., 2014). The followed instructions for tissue and non-nucleated blood slightly diverges in the way of preparation of the sample.

At first step of the tissue extraction, the small piece of the tissue (up to 25 mg) is put into 1.5 ml microcentrifuge tube, 180 µl buffer ATL and 20 µl proteinase K for tissue lysis are added. The microcentrifuge tube is mixed by vortexing and then incubated at temperature 56°C until the tissue is completely lysed. AccuBlock™ Digital Dry Bath D1200 from Labnet International, Inc was used for incubation. Occasionally vortex during incubation is recommended. Tissue lysis takes a few hours, so we did the tissue incubation usually through the night. Before proceeding to the next step, vortexing is needed. In case of mammal's blood (with non-nucleated erythrocytes), the advanced preparation is not necessary. The anticoagulant-treated blood (100 µl) is pipeted into microcentrifuge tube with 15 µl proteinase K added. At the end, 105 µl PBS is used to adjust to volume 220 µl. Mixing of blood sample in this step should be done gently, slow inversion of sample tube is recommended.

The Second step serves for other cell lysis by adding 200 µl of the buffer AL and vortexing. Blood samples should be incubated at 56°C for 10 minutes. Additional mixing by inversion can be done during incubation. Then 96% ethanol in volume of 200 µl is added and mixed thoroughly by vortex.

The next steps consist of binding DNA in filter of DNeasy Mini spin column and repeated washing procedure. For binding DNA, the mixture is transferred

at membrane of DNeasy Mini spin column placed in collection tube and centrifuged. The Centrifuge 5424 by Eppendorf was used. Washing process is similar only addition of washing buffer AW1 and AW2 in column and centrifugation.

The last step is elution of the DNA. The DNeasy Mini spin column is placed into new microcentrifuge tube and 100 µl of the elution buffer AE is added to the spin column membrane. After 1 minute of incubation under room temperature and subsequent centrifugation we obtain final product in the form of pure DNA solution.

Resulting DNA products were marked properly and stored in freezer at -20°C.

5.2 Control of DNA concentration

Concentration and purity of extracted nucleic acids were measured by NanoDrop 2000 Spectrophotometer from Thermo Scientific with original software included. The calibration was carried out through elution buffer as a blank solution. The samples which had low concentration (≥0.5 ng/µl) were excluded. DNA extraction was repeated in those samples until the concentration suitable for further examination was proved in all measured samples.

5.3 Polymerase chain reaction (PCR)

In the present study, the microsatellite analysis was chosen for evaluation of population characteristic. It is essential for the microsatellite examination to indicate specific locus and proliferate it into million copies by polymerase chain reaction. It is necessary to use suitable primers for accurate performance of PCR. The microsatellites for our studied species, which are elands, have not been developed yet. Inventing of new primers would be expensive and very time consuming, therefore cross-species amplification was used. The chosen microsatellite primers were published for closely related species (Bishop *et al.*, 1994; Eblate *et al.*, 2011) and suggested by specialists from WildGenes laboratory in Edinburgh (17 microsatellites). These suggested microsatellites were completed by 5 microsatellites already tested on Derby eland (Zemanová *et al.*, 2015). In total 17 microsatellite primers were tested for the ability of amplification (Annex I).

The initial PCR was designed in the volume of 25 µl reaction mixture containing 12.5 µl PCR Master mix (Qiagen), 2 µl of the primer (1 µl of the forward and 1 µl of the reverse primer), 2 µl of the extracted DNA and adjusted 8.5 µl of the nuclease

free water. The PCR conditions were set according to the literature in which the primers were used before and optimized to the most suitable condition. The ability of amplification of 17 microsatellite primers for was tested in different conditions. The "Touch Up" condition was used for gradual increasing of annealing temperature +0.2°C per cycle. "Touch Down" condition was used for gradual decreasing of annealing temperature - 0.2°C per cycle. Both protocols varied in the range 54–60°C for the annealing temperature (Table 3).

The PCR were proceeded in Thermocycler – T100^{TM} Thermal Cycler (BIORAD). The final products were stored in freezer at -20°C or directly used for electrophoresis.

5.4 Electrophoresis

The verification of successful amplification of selected marker in PCR product was visualized by horizontal electrophoresis. Every PCR product (2 μl) was coloured by 6x Loading Dye Solution – Fermentas (1 μl). The coloured samples were placed separately in 1% agarose gel with TBE buffer. For electrophoresis PowerPac^{TM} Basic (BIORAD) was utilized; reactions were run for 40 minutes by 120 V.

The length of microsatellites fragments was estimated by comparison with ladder (GeneRuler^{TM} 100bp DNA Ladder Plus, Fermentas) under the UV light produced by Electronic UV Transilluminator ECX-24-MX and Cleaver Scientific Ltd. (MicroDoe). The photos of the gels were taken by Canon PowerShot G5 camera. The analysis confirmed successful amplification in 14 microsatellite primers.

Table 3 PCR Condition

MSTZK – initial amplification; volume 25 µl

1)	95°C	5 min	
2)			
	95°C	30 sec	
	56°C	30 sec	30 cycles
	72°C	1 min	
3)	72°C	10 min	

MST52; volume 25 µl

1)	95°C	3 min	
2)			
	95°C	1 min	
	52°C	1 min	35 cycles
	72°C	1 min	
3)	72°C	5 min	

MST60; volume 25 µl

1)	95°C	3 min	
2)			
	95°C	1 min	
	60°C	1 min	35 cycles
	72°C	1 min	
3)	72°C	5 min	

Touch Up; Touch Down

1)	95°C	10 min	
2)			
	95°C	30 sec	
	anneal. t°	1 min	30 cycles
	72°C	1 min	
3)	72°C	10 min	

MST58 – for labelled primers; volume 10 µl

1)	95°C	3 min	
2)			
	95°C	1 min	
	58°C	1 min	35 cycles
	72°C	1 min	
3)	72°C	5 min	

5.5 Multiplex and Fragmentation analysis

Non-functional microsatellites (those that did not generate sufficient amount of PCR product) in electrophoresis were excluded from testing. Remained 14 primers were ordered in labelled form. The forward primer of each locus was labelled with a fluorescent dye on 5´–end. These primers were further divided by colour and length of microsatellites into multiplex mixes. PCR was repeated in different condition. The mixtures were tested for potential polymorphism determination by frequenting analysis. Polymorphism in the case of Derby eland was detected in 6 new microsatellites.

The 7 new microsatellites and 5 microsatellites validated by Zemanová *et al*. (2015) were divided by colour and length of microsatellites into 3 final multiplex mixes as described in Table 4. The primer CSSM42 was included in the Multiplex 6 even though monomorphic locus was confirmed due to ongoing testing on Common elands (CE). In the case of Common elands this primer has polymorphic character. The primer multiplex contains 5 μl of the reverse primer, 5 μl of the labelled forward primer from chosen primers. Mixture was adjusted by TE buffer to the final volume 250 μl.

Repeated PCR were performed in thermocycler including 5 μl Master mix (Qiagen Multiplex PCR Master Mix, 2x), 3 μl of the water, 1 μl of the extracted DNA and 1 μl of the primer mix. The reaction was optimized by protocol MST58 (Table 3) in a final volume of 10 μl. The PCR program comprised an initial denaturation step of 95°C for 3 min, the cycling parameters included 35 cycles at 95°C for 1 min, an annealing temperature of 58°C for 1 min, 72°C for 1 min and a final extension step of 72°C for 5 min.

The fragmentation analysis was done in specialized sequencing laboratory FES. The initial testing was based on the capillary spectrophotometry. The sequencer Gene Analyser 3500 (Applied Biosystems) was used. The resulting dataset was assessed by standard process in program GeneMarker version 2.2.0 (SoftGenetics) in comparison with corresponding template.

Table 4 Multiplex composition					
Multiplex	**Primer**	**Fluorescent dye**	**Size range detected**	**Size range**	**References**
MULTIPLEX 5	BL42	FAM	283-299	229-238	Bishop *et al*. (1994)
	BRR	NED	244-257	240-260	Flyn (2009)
	CSRM60	FAM	91-100	79-115	Moore *et al*. (1994)
	ETH10	FAM	206-211	198-234	Toldo *et al*. (1993); Flyn (2009)
	ETH225	FAM	141-154	141-159	Stephen *et al.* (1994); Beja-Pereira *et al*. (2004)
	X80214	HEX	213-241	228-243	Pépin *et al.* (1995)
MULTIPLEX 6	BM4505	FAM	247-262	154-282	Beja-Pereira *et al.* (2004)
	CSSM42	PET	180	175-219	Moore *et al*. (1994)
	INRA107	FAM	159-179	160-166	Vaiman *et al.* (1994)
	SPS113	PET	138-151	136-142	Moore *et al*. (1993)
MULTI-PLEX 9	AF533518	HEX	211-249	286	Huebinger *et al.* (2006)
	OarFCB304	FAM	147-163	158-177	Buchanan and Crawford (1993)

5.6 Data analysis

The obtained dataset from fragmentation analysis, performed on 11 microsatellite markers, were manually assessed by standard process in program GeneMarker version 2.2.0 (SoftGenetics, StateCollege, PA, USA) (Figure 5) and tabulated in Microsoft Excel.

The raw data were rounded by programme AutoBin (www.bordeaux-aquitaine.infra.fr/biogeco/Ressources/Logiciels/Autobin) to contribute for next analysis.

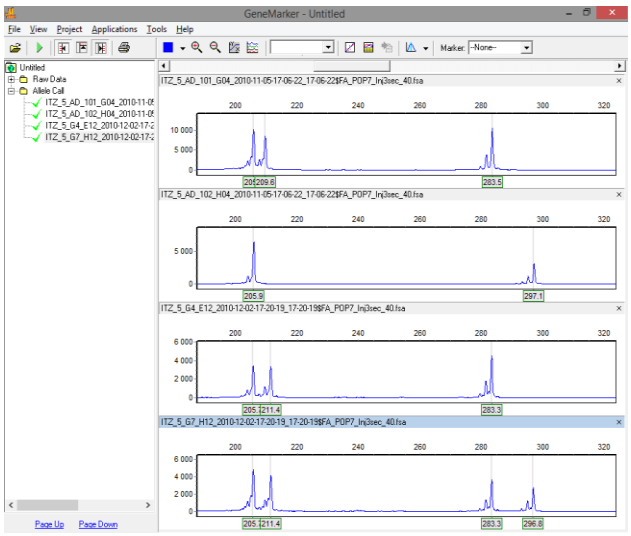

Figure 5 Output from GeneMarker 2.2.0

The percentage of the missing data were indicated in the initial test. The samples with ≥20% of the missing data and duplicated samples were excluded from future analyses. Three samples of EGE had to be discarded. Two samples were excluded because of poor DNA concentration and one sample due to misidentified sample (probably substitution of Common eland). Therefore, the 105 samples of the WDE and 17 samples of the EGE were subjects of the following analyses.

Errors at genotyping due to the occurrence of artefacts in vitro amplification such as stuttering, large allele dropout or presence of null allele were tested in programme Micro-Checker 2.2.3 (Van Oosterhout *et al*., 2004). The methods which evaluated null allele frequency were those of Oosterhout, Chakraborty, Brookfield. There was no evidence of null alleles at all loci across all samples. Hardy Weinberg equilibrium was checked in GenePop 4.2 (Rousset, 2008) for all tested population. The comparison of EGE (Zoo) from both American Zoo toward WDE (Ban) population and also comparison of EGE (Cam) from Cameroon toward WDE (Ban) was omitted from analysis because of significant deviation from Hardy Weinberg equilibrium.

For basic visualization of the relations between individuals and populations, the 2D factorial correspondence analysis on the base of microsatellites was done in programme Genetix 4.0 (Belkhir *et al.* 2004). Individuals were sorted to groups due to the subspecies. Total number of the all tested individuals of Derby eland was 122.

For detection of genetic structure of populations, the Bayesian clustering method implemented in programme Structure 2.3.4 (Pritchard *et al*., 2000) was used Marcov Chain Monte Carlo (MCMC) repetition number was 1 000 000 steps after 100 000 steps long burn-in period. Number of clusters (K) was set from K=1 to K=5. The analysis was run for each K in 5 iterations. The likelihood scores for delineating the most likely level of population subdivision was visualized in Structure Harvester (Earl and von Holdt, 2012). Individuals for this analysis were sorted to groups due to the subspecies resp. species (WDE, EGE, CE). In the first control analysis of structure between species was used samples of WDE (N=105), EGE (N=17), CE (N=19). For second Bayesian clustering analysis was the sample size equalized; WDE (N=18), EGE (N=17), CE (N=19). The next analysis should reveal the efficiency of microsatellite loci in determination of subspecies, thus only WDE (N=105) and EGE (N=17) were used.

Basic statistics as expected (He) and observed (Ho) heterozygosity was evaluated in Genetix 4.0, F-statistic (F_{IS}, F_{ST}) were calculated in programme GenePop 4.2. F_{IS} (coefficient of inbreeding) measures heterozygosity decrease due to mating between genetically related individuals. The values of F_{IS} occurs in range -1 (no occurrence of homozygotes) to +1 (no occurrence of heterozygotes). F_{ST} (fixation index) shows decrease of heterozygosity of subpopulation in proportion to the total population. because of genetic drift in subpopulations. This decrease occurs due to genetic drift in subpopulations with reduced gene flow. Values of F_{ST} are between 0 (no differentiation) to 1 (fixation of different alleles). Values in range of $0 – 0.05$ are considered as a little genetic differentiation between $0.05 – 0.15$ for medium and $0.15 – 0.25$ for a large genetic differentiation. For determination of allelic richness (Ar) the programme FSTAT 2.9.3.2 (Goudet, 2002) was used. This statistical method compares genetic variation regardless of the number of samples in each population.

For concrete analysis, the populations had to be designate to specific groups according to required condition (without deviations from Hardy Weinberg equilibrium). The basic population statistics were analysed and compared among populations according to breeding management. The first analysis comparing both subspecies, the individuals of WDE from last breeding season (N=6) in Bandia Reserve (Ban) was chosen for comparing with individuals of EGE from captive condition (N=6) in White Oak (WO). This analyses compare both subspecies and influence of their *ex situ* breeding management. The second analysis comprises population of EGE from captive

condition (N=8) in American zoos (Los Angeles and White Oak - Zoo) and wild condition (N=6) in Cameroon (Cam). The last analysis was performed on 14 individuals from three generations of WDE according to Zemanová *et al.* (2015). The first group (GEN 0) includes only four founders because the samples from all six founders were not available. These wild born individuals were presumed to be unrelated to each other. The second group (GEN 1) consists five F1 offspring born in the 2007/2008 season (direct descendants of founders). The last group (GEN 2) was selected from F2 offspring born in the 2009/2010 and 2010/2011 season that all founding lineages were included (Table 5).

The parentage analysis according to most likely candidate mother was done in Cervus 3.0.7 (Kalinowski *et al*., 2007) for western subspecies. The maternity test was done for all sampled individuals which were born in 2003 (unknown mother-offspring kinship). During this year, a calf was born to each of five founding mothers. It was not possible to obtain all samples of stakeholders. Therefore, determination of parentity was more difficult. For testing were used the data of known sire, three sampled mother and four sampled offspring. The maternal analysis detects most likely mother with multiple level of confidence according to number of mismatching loci. Subsequently, the remaining individuals were manually assigned to the most likely mother-calf pair. Then the missing parts of the pedigree were reconstructed.

Table 5 Generation assignment (Zemanová *et al.*, 2015)

Tested set	Individual	Sex	Season of the birth	Sire Dam
GEN 0 (founders)	Niokolo	♂	1999	unknown – founder
	Bembou	♀	1999	unknown – founder
	Salémata	♀	1997	unknown – founder
	Malapa	♀	1999	unknown – founder
GEN 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
GEN 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
	Destin T.	♂	**2010** / 2011	Toubab Niokolo Tamba Dewene Niokolo Dalaba

Season of the birth – the year of the birth is noted in bold.

6 Results

From all 22 microsatellite markers that were tested for amplification and polymorphism, 12 loci showed to be usable in *Taurotragus* spp. However, only 11 microsatellite markers that were successfully amplified in 122 samples in two subspecies of Derby eland were assessed as polymorphic (Annex I.). The level of polymorphism was detected for both subspecies (Annex II.); the 10 microsatellites were polymorphic in both subspecies and the additional locus CSRM60 was polymorphic only in Western Derby eland. 105 samples of WDE and 17 samples of EGE were used for this purpose (Table 1, Table 2). The average number of alleles per locus was higher in eastern subspecies (4.1667) with range 1–8 number of allele per locus (Na). Western subspecies has lower average of Na (2.6667) with range 2–5 allele per locus.

The program STRUCTURE can be used division of individuals into certain classes (clusters – K). For this analysis, 12 microsatellites were tested (Table 4) for all 122 samples of Derby eland and 17 samples of Common eland for control. The highest likelihood was detected for two clusters (K=2) in Structure Harvester (Figure 6).

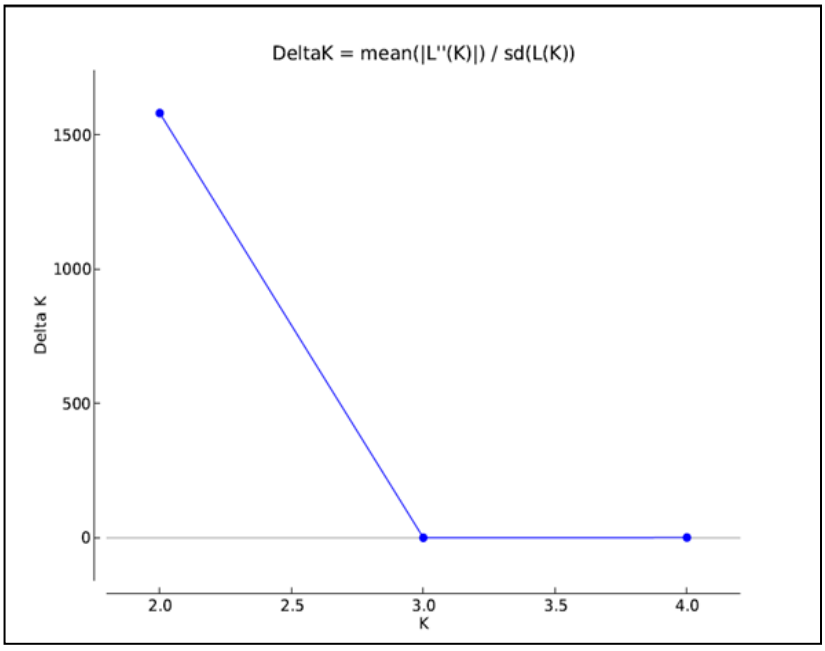

Figure 6 Highest likelihood for clustering of all samples of *Taurotragus* spp.; output from Structure Harvester

This structure is visualized in bar graph (Figure 7).

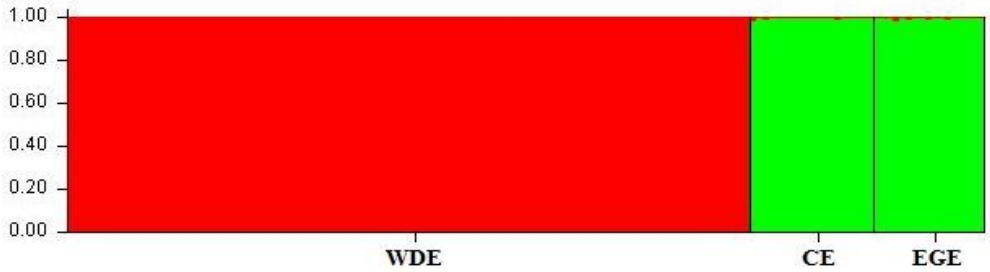

Figure 7 Population structure of *Taurotragus* spp.; output of Bayesian clustering method (Structure 2.3.4)
Western Derby eland (WDE), Common eland (CE), Eastern Giant eland (EGE)

The same analysis found highest likelihood (Figure 8) for three clusters (K=3) when the number of tested representatives was approximately the same (mean N=18). The bar graph (Figure 9) indicates the separation between all three populations with no gene admixture among respective populations.

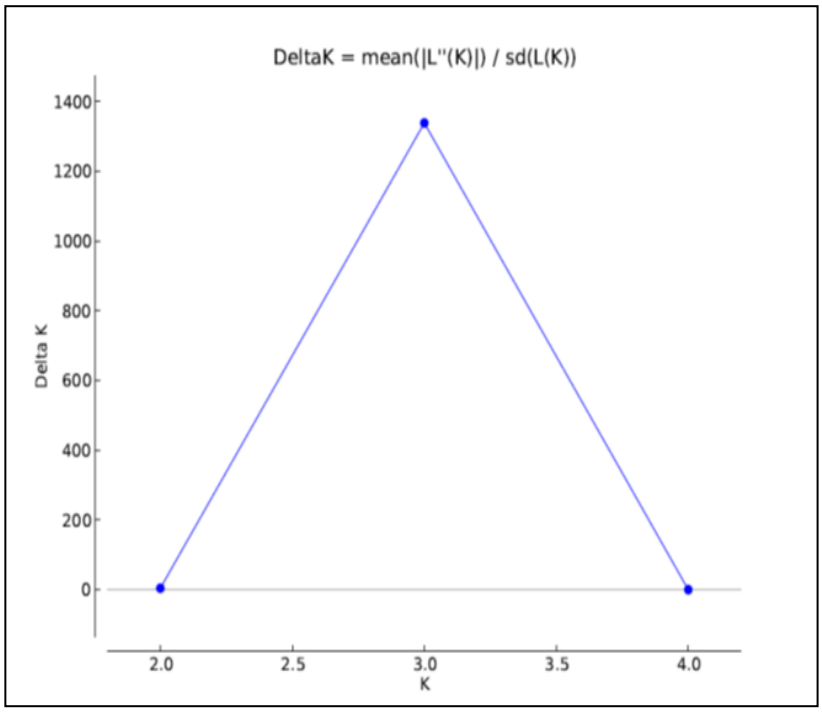

Figure 8 Highest likelihood for equalized sample size of *Taurotragus* spp.; output from Structure Harvester

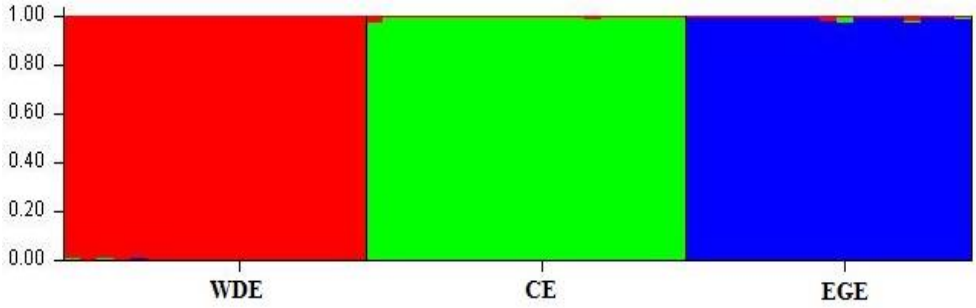

Figure 9 *Taurotragus* spp. division according to equalized sample size;
output of Bayesian clustering method (Structure 2.3.4)
Western Derby eland (WDE), Common eland (CE), Eastern Giant eland (EGE)

All sampled individuals of the two subspecies of Derby eland were analysed separately to see detail differentiation between them. The highest likelihood was detected for two clusters (K=2), but also the separation into four clusters (K=4) had some support (Figure 10). The graphical visualization in bar graph (Figure 11) shows structure, although the populations are still very well recognizable.

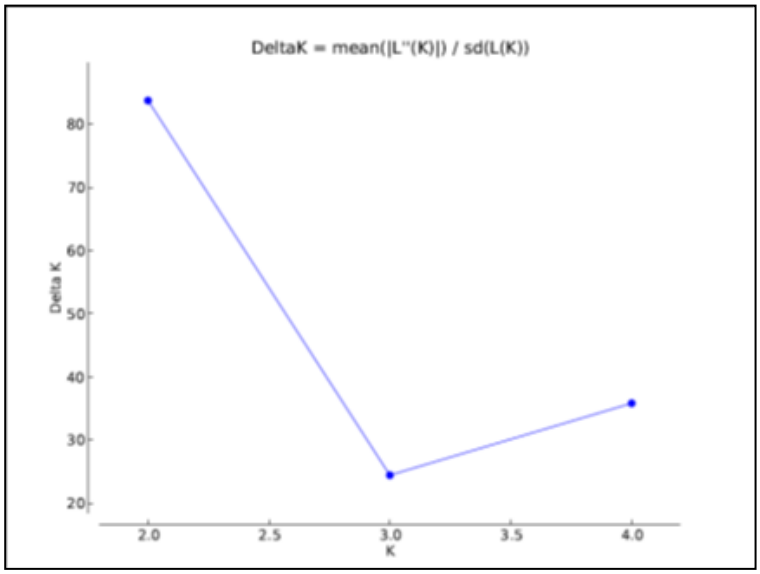

Figure 10 Highest likelihood for clustering of Derby Eland subspecies;
output from Structure Harvester

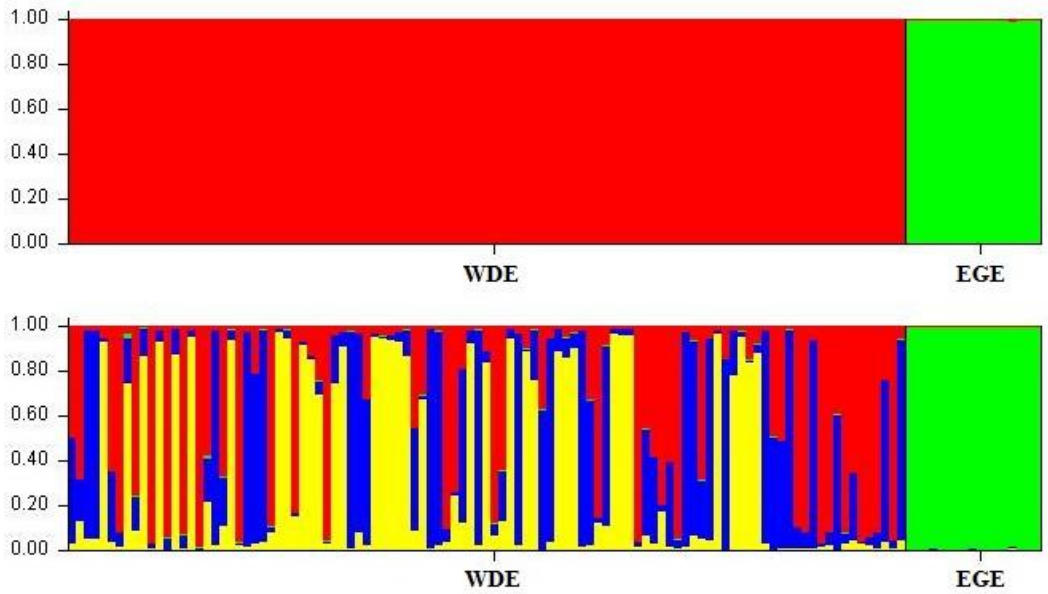

Figure 11 Population structure of Derby eland subspecies; division to K=2 (top) and K=4 (bottom); output of Bayesian clustering method (Structure 2.3.4)
Western Derby eland (WDE), Eastern Giant eland (EGE)

Factorial correspondence analysis was done in programme Genetix. This approach is providing very useful visualization of relations between individuals and populations. The genetic distances are visible among all sampled individuals of both subspecies on Figure 12. Western subspecies has much lower variability compared to eastern subspecies. Population of Estern Giant eland show accumulation into few groups with closer distances. This trend was expected due to sampling among different institution

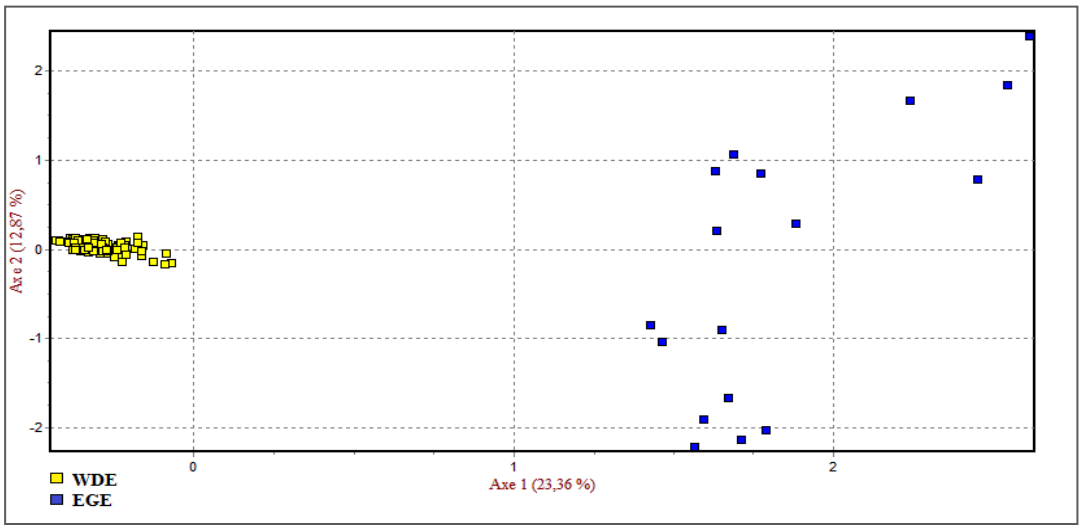

Figure 12 The 2D factorial correspondence analysis; output from Genetix 4.0;
Western Derby eland (WDE), Eastern Giant eland (EGE)

and localities.

In Table 6 are listed values of F_{ST} according to pairwise analysis. All obtained values are generally considered as very high, which propose very large differentiation among analysed groups. The greatest difference is between WDE (Ban) and Zoo population of EGE.

Table 6 The fixation index (F_{ST}) in pairwise analysis

Population	WDE (Ban)	EGE (Cam)
EGE (WO)	0.3935	0.2637
EGE (Zoo)	0.4011	0.2662
EGE (Cam)	0.3778	

WDE – offspring of western subspecies from last breeding season, (Ban) – Bandia
EGE – eastern subspecies, (WO) – White Oak, (Zoo) – White Oak and Los Angeles, (Cam) – Cameroon

The basic population statistics were analysed and compared among populations according to breeding management. Genetic characteristics for all compared populations are showed in Table 7.

Table 7 Basic F-statistic

	N samples	F_{IS}	Ho	He	Ar
WDE (Ban)	6	-0.175	0.5606	0.4444	2.5455
EGE (WO)	6	-0.153	0.5152	0.4154	2.5455
EGE (Zoo)	8	-0.205	0.5455	0.4291	1.9078
EGE (Cam)	6	-0.076	0.6212	0.5253	2.3026

Number of samples, breeding coefficient (F_{IS}), observed (Ho) and expected heterozygosity (He), allelic richness (Ar) among selected populations.
WDE (Ban) – Last breeding season in Bandia, EGE (WO) – White Oak, EGE (Zoo) – White Oak and Los Angeles, EGE (Cam) – Cameroon

Total amount of alleles in this analysis was 46, in average 4.2 per locus. The allele variability ranged between one to nine alleles per locus. Tested individuals of EGE are monomorphic in locus CSRM60. Furthermore, the lowest variability was detected in locus BL42; which has only two alleles in both subspecies. The highest variability was detected in locus X800214; which has nine alleles but no one shared between Ban and WO population.

The population characteristics were analysed in three generations of WDE and compared with published results by Zemanová *et al*. (2015). The pattern of F_{IS} changes between generations is shown at Figure 13. The results correspond with increasing trend of coefficient of inbreeding which was confirmed by Zemanová *et al*. (2015).

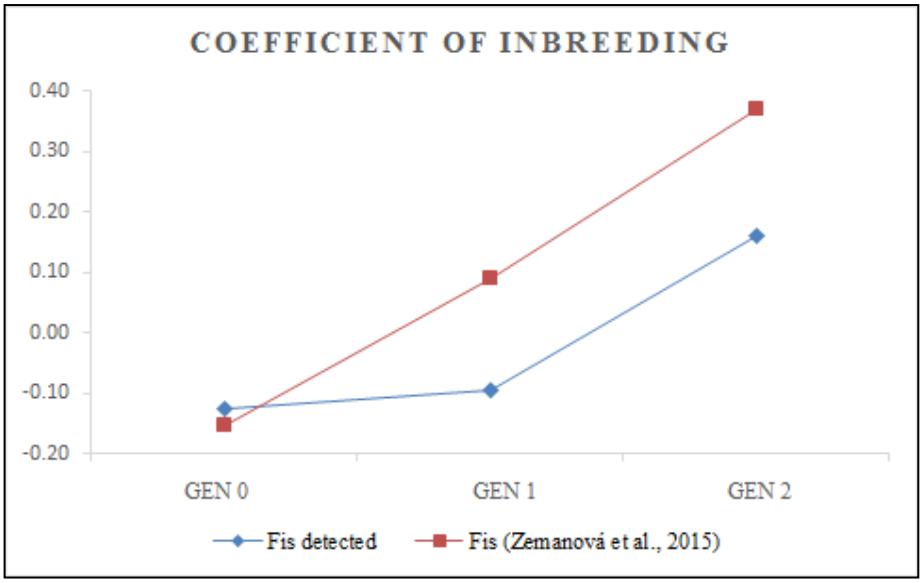

Figure 13 Comparison of F_{IS} values between 3 generations of Western Derby eland with published results (Zemanová *et al.*,2015)

The comparison of observed and expected heterozygosity and allelic richness is shown at Figure 14. The heterozygosity observed in populations decrease with each generation. In GEN 0 the value of Ho=0.6136, in GEN 1 the Ho decrease to 0.5455. In the third generation the value of Ho was reduced to 0.4227 and drops below level of He=0.4426.

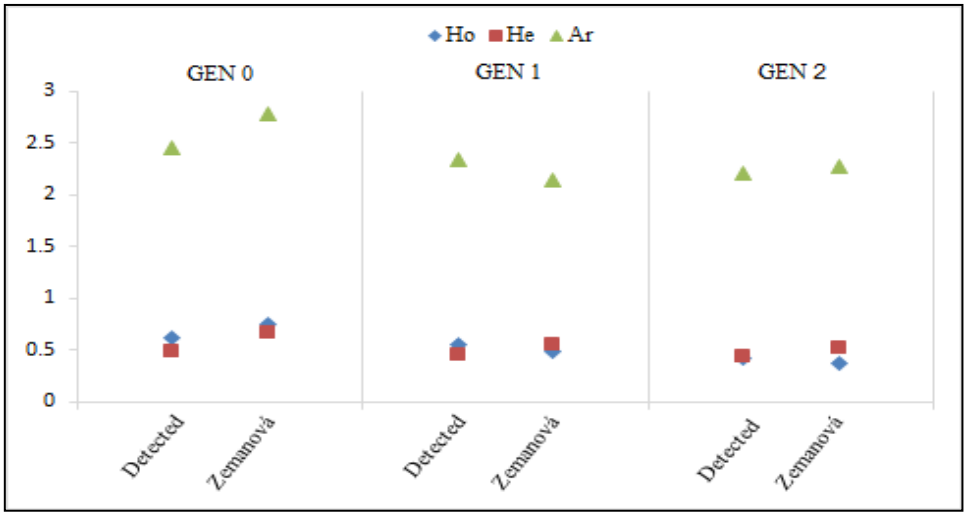

Figure 14 Comparison of Ho, He, Ar values for 3 generations of Western Derby eland with published results (Zemanová *et al.*,2015)

The parentage analysis was done in Cervus programme. Two sampled mother were detected with no mismatching locus (Table 8). The maternity of third sampled mother is assigned to non-sampled young Guddi. The non-sampled candidate mothers are probably mothers of remaining sampled offspring.

Table 8 Parentage analysis and maternity assignment

ID	Name	ID Mother	Name mother	Trio confidence
AD006	Popengiune	AD014		1.58217816525807E+0000
AD007	Karang	AD014	Bembou	5.25052190556677E+0000
AD023	Ndiogoye	AD020		0.00000000000000E+0000
AD024	Fathala	AD019	Salémata	4.07568648815698E+0000
NoSample	Guddi	AD020	Malapa	

7 Discussion

The panel of 12 loci that were successfully amplified and showed to be polymorphic for both *Taurotragus* spp. enables to provide genetic identification of eland species and distinguish their potential hybridization. From all 11 microsatellite markers that were tested in Derby elands, 10 microsatellite loci were polymorphic in both subspecies and the additional locus CSRM60 was polymorphic in Western Derby eland. The polymorphic loci within particular species can provide subspecies determination, genetic identification of single animals, paternity tests, and assessment of relatedness. Similar analyses are involved also in other current conservation projects focused on ungulates (Lorenzini, 2005; Tokarska *et al.*, 2009; Eblate *et al.*, 2011). Despite the relatively low sample size, our results showed relatively high genetic variability in EGE but low genetic variability within the whole population of WDE. This is an evidence that low level of genetic variability in WDE might be a result of reduced population size rather than the lack of allelic variation because of use of cross-species primers.

Population genetic pattern

The utilization of highly polymorphic microsatellites loci has also great potential for understanding to population structure. The new set of primers exposed a sufficient level of polymorphism and it provided the insight into population structure of Derby eland subspecies.

The Bayesian clustering results showed the highest support for separation into two the clusters (K=2) which separated the population of WDE from EGE + CE that created one cluster together. This was probably caused by the influence of unproportional sample size which is one of the factors affecting model-based clustering method (Pritchard *et al.*, 2000). Using all the individuals from WDE means that we included highly related animals and thus increased the uniformity of the population. EGE which was sampled within different localities and thus showed higher genetic diversity across individuals, clustered together with different species (CE) rather than with WDE. Results did change after random equalization of sample size. Using the same proportion of individuals from different populations division into the three clusters was the most likely and subsequently, the three tested populations were distinguished in their own

cluster. In this run of the analysis WDE + EGE clustered together and CE was separated.

The nested analysis which included just samples of Derby eland provided well recognizable separation in all clusters (K=1–5) with highest likelihood for two clusters, as was expected. In separation into 4 clusters with partial likelihood, the sub structuralizing pattern was revealed in case of WDE. This pattern may expose pedigree lines, *i. e.* excess of shared allele among closely relative individuals due to breeding management. In the fact, the reproductive herds of WDE were managed in separate enclosures according to the parent lineages without possibility to interbreed. This fact could not be correlated in our analysis but it should be implemented in other analysis which would include also additional variables.

Structures analyses are well applicable for free-ranging population, where immigration (Lorenzen *et al.*, 2006) or possible hybridization occurs (Randi, 2008).

The factorial analysis determined relations among individuals. The analysis exposed limited variation within WDE population. On the contrary, higher genetic variability was visible in case of EGE. This higher variability is probably just relative because sampled animals were from differrent institutions and localities. Each of the sampled populations is rather closed population without gene flow, thus the genetic drift changed allele frequencies in different ways in each of these population. Higher genetic variability seen when we analyse all these samples together may be an artefact of several uniform populations which are drifted from the ancestral population.

The genetic diversity can be generally reinforced by merging of wild living animals and animals bred in captivity (Olech and Perzanowski, 2002). The closer distances among individuals of EGE correspond with division into related population according to location, where the samples were taken.

F_{ST} is probably one of the most reported statistics in population and evolutionary genetics. Our results confirmed rather high F_{ST} values in pairwise analysis of all tested populations. Although F_{ST} between EGE populations are lower compared to WDE (Ban) population. The differentiation between free ranging population and populations bred in captivity seem to be also significant (F_{ST}=0.2662), in case of eastern subspecies. It is probably the result of different origin or long term separation between Cameroon and Central African population, from where the American captive population originated

(Fernández and Vrba, 2005; Willows-Munro *et al.*, 2005). However, the F_{ST} might be criticized for inaccurately estimating population differentiation when genetic variation is high (Balloux and Lugon-Moulin, 2002; Jost, 2008), which is not the case of this study.

Population characteristic

Analysis showed the same average number of alleles in both subspecies, even though it revealed differences between subspecies in individual loci. The monomorphism was detected in one locus in EGE, but generally they had higher allele variability among remaining loci.

For evaluation of inbreeding, comparison between two facilities with WDE (Bandia Reserve – Ban) and EGE (White Oak – WO) was done. The population from White Oak had higher F_{IS} (- 0.153) in comparison to WDE from Bandia Reserve (- 0.175). However, level of inbreeding did not differ too much as we presumed. By this, the hypothesis that eastern subspecies have been exposed to high inbreeding was confirmed. Nevertheless, the higher level of inbreeding in White Oak could be caused by testing individuals with unknown pedigree and the analysis could contain highly related individuals. In spite of this, in case of Bandia Reserve, individuals born in the last genetically monitored breeding season (2015) were included in analysis.

Even that the hypothesis was confirmed, the cause of higher level of inbreeding was different than we supposed. Despite the long-lasting breeding of EGE across American facilities, the inbreeding calculated for whole EGE zoo kept population is maintained at low level. The possible explanation is that all founding animals were taken from different herds or regions (Romo, 2000). Thus, the founders were most probably unrelated. This strategy could not be performed with such efficiency in WDE, due to the limited distribution (Antonínová *et al.*, 2006). The founders of WDE were presumed to be unrelated, but this is probably not true. Population of EGE in whole USA were established by 3 initial males and 5 females in two facilities; concretely zoos in Cincinnati and Los Angeles (Romo, 2000). It means that founder effect was not as strong as it was in the case of WDE (Brandlová *et al.*, 2015). The other conservation projects with strong founder effect were implemented on addax (*Addax nasomaculatus*) (Armstrong *et al.*, 2011) or European bison, which both had only reproductive bull (Tokarska *et al.*, 2009). The EGE may be comparable to Australian population of

Scimitar oryx (*Oryx dammah*), which was established by low number of initial animals, but it had two subsequent supplementations by incoming animals (Frankham *et al.*, 2013).

The differentiation across captive herds can be lowered by breeding strategy of genetic mixing among populations as it was described in Arabian oryx (Marshall *et al*., 1999). Also inbreeding could be delayed or reduced by the good genetic management of the population (Arif *et al.*, 2010). For maintaining of efficient genetic diversity, it is suggested to keep another unrelated populations as a genetic reservoir in order to provide gradual gene flow (Olech and Perzanowski, 2002; Nežerková *et al.*, 2004).

From all tested populations (Table 7), EGE individuals from American zoo population (WO, LA) had the lowest coefficient of inbreeding. The high difference between White Oak and Zoo population was caused by adding of two individuals from Los Angeles. This zoo was one of the two first founding institutions and thus experienced with one founder effect. Because of this, individuals from Los Angeles might have lower inbreeding and higher variability than individuals from recent herd in White Oak, which went through at least double founder effect (bottleneck). Examples of the species that also suffered by several bottlenecks are *e. g.* Alpine ibex (*Capra ibex*) (Biebach and Keller, 2009) and Apennine chamois (Lorenzini, 2005). The study of Biebach and Keller (2009) determined changes in genetic parameters connected with number of bottlenecks. The first bottleneck was the most crucial in loss of average number of allele per locus (Na) but expected heterozygosity (He) decreased about a similar degree with each bottleneck repetition. Our results correspond with this statement, which means that no difference in Na was found between White Oak and Zoo population but lower He was detected between them.

Surprisingly, the wild population of EGE from Cameroon has the highest coefficient of inbreeding from all tested populations. This could be a sampling bias if closely related individuals were sampled and taken for analyses. In comparison to Zoo populations, the genetic variability is relatively high according to the high values of heterozygosity (He=0.5253). This wild population also disposes higher values of allelic richness (Ar=2.3) than captive Zoo populations of EGE. Cameroon population had probably no experience with strong bottleneck effect and migration contributes to the natural gene flow. These results correspond to results in other non-endangered

antelopes living in the wild (Lorenzen *et al.*, 2006; Eblate *et al.*, 2011). In the contrary, the diversity does not reach the diversity of the most widely distributed European cervid, Roe deer (Galan *et al.*, 2003). Cameroon population was probably influenced mainly by natural selection, genetic drift and partially population decrease due to trophy hunting. The reduced genetic variability of captive Zoo population in comparison to wild Cameroon population might be a consequence of severe population bottlenecks and founder effect as it was in population of Apennine chamois or Alpine ibex (Lorenzini, 2005; Biebach and Keller, 2009); eventually strong founder effect and prolonged permanence at low numbers as it was in case of European bison (Tokarska *et al.*, 2009). The inbreeding in captivity is preserved on the lowest level as it is possible through appropriate breeding management (Antonínová *et al.*, 2004). However, during the breeding with no exchange of individuals between zoos, there will be noticeable effect of genetic drift which will randomly change allele frequencies and will increase differentiation between zoo populations.

The second hypothesis was confirmed. According to our expectations, we found that genetic diversity of WDE is decreasing in the captive population and inbreeding is increasing in each generation. Our results confirmed the results of Zemanová *et al.* (2015), who used only limited amount of loci (5 microsatellites). Authors were aware of limited explanatory power of such a few markers used but these new results provide similar pattern using twice more markers. Values of observed parameters differ but it didn't change the conclusions made by Zemanová *et al.* (2015). The increase of inbreeding still prevails, but the progress is not as rapid as it seemed. Despite the high level of inbreeding, no phenotypic signs of inbreeding depression have been observed, as well as in European bison which disposes much higher rate of inbreeding (Tokarska *et al.*, 2009). The low heterozygosity may affect fitness of animals; *e. g.* the genetic adaptability may decrease with reduced allele variability. Decreased heterozygosity of WDE can also negatively influence its reproductive rate. The population size of WDE gradually rose until the first animals grew old and started dying (Brandlová *et al.*, 2015). Current population size seems to be stable now but there is no more increase detected. The next years will reveal if the population size will decrease or will stay balanced. The similar trend was noticed in Arabian oryx bred in National Wildlife Research Center in Saudi Arabia (Ostrowski *et al.*, 1998).

Parentage analysis

Season in 2003 was the only one without data of maternal relationships and offspring identifications in WDE. During this year, a calf was born to each of five founding mothers. To avoid wrong estimation of paternity, new maternal lineage was founded. These animals had unknown pedigree and their position in the pedigree was uncertain. Through molecular markers, it is possible to set allele equipment and count probability of paternity due to their ancestors.

Missing samples from two of five founding females and several other individuals from ongoing generations are responsible for decreased success in discovering of missing maternities. Samples were usually collected during the transportations, treatments and in case of death of animals (if the carcass was found), so only part of population was sampled. In generation born in 2003, one from five newly born individuals was not sampled. The parentage analysis detects highest support for two candidate mothers from three sampled, with no mismatching loci. The last sampled candidate mother is presumed as a mother to non-sampled offspring and two remaining offspring were assigned to non-sampled mothers.

The parentage and identity analysis are widely used for species which are bred under conservation programmes (Marshall *et al.*, 1999; Tokarska *et al.*, 2009). It is highly recommended to confirm kinship relations by DNA analysis for each generation or calving season individually. The kinship was estimated by observation until now (Nežerková *et al*., 2004). For accurate reconstruction of large and deep pedigrees, it is recommended to use 10–15 polymorphic microsatellite loci (Riester *et al*., 2008). The results suggested 11 microsatellites markers that can be regularly use for paternity determinations for studbook information and breeding purposes. Also the polymorphism of these markers enables to detect the most unrelated individuals that can be selected for compositions of new reproductive herds.

8 Conclusion

The new polymorphic microsatellite panel was developed for Derby eland species. We were able to distinguish both *Taurotragus* species and also both subspecies of Derby eland from themselves. Such a panel of polymorphic loci may have various applications in respect to the breeding program. In the future, we will be able to estimate changes in population structure and see the development of genetic parameters in this species. Obvious differences between species will allow us to monitor possible hybridization between them.

The Derby eland disposed of relatively high genetic variability but variability with each generation significantly declines. High fixation index between the populations is indication that each population is developing without gene flow and is affected by strong genetic drift, especially animals from captive conditions. Regarding the low number of founding individuals and thus strong founder effect it is possible that all the populations are at risk of inbreeding depression. Until now, there haven't been observed any signals of decreased fitness, so continuous genetic management lowering the kinship of individuals is highly recommended in these populations.

Through the genetic testing the missing maternities of western Derby eland were completed. Missing individuals from several generations decreased probability of assessed kinship. For proper parentage analysis, it is necessary to genotype all possible sires and dams, but also missing offspring may affect the probability of the results.

The polymorphic markers can be efficiently used in molecular analyses in order to improve the conservation management.

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Appendices

Annex I. Results of cross-species amplification tests and level of polymorphism

Annex II. Polymorphism of Eastern Giant eland (EGE) and Western Derby eland (WDE)

Annex I. Results of cross-species amplification tests and level of polymorphism

N°	Primer	Fluorescent dye	Primer sequence	Ann. t / °C	Amplification	Allele length (size range) / Origin	References
1	BL42	FAM	CAAGGTCAAGTCCAAATGCC GCATTTTTGTGTTAATTTCATGC	53	polymorphic	229-238 (*Ovis aries*)	Bishop *et al*. (1994)
2	BM5004	FAM	TCTGGAGTGAATGTTTCTGAGG TTGTGATGACACCTGAAGG	58	no amplification	120-154 (*B. taurus*)	Bishop *et al*. (1994)
3	BRR	NED	TGCTCTTACCTGCCACACCCG CCCTCTTCTCACCCCCCAAAAC	61	polymorphic	240-260 (*B. taurus*)	Flyn (2009)
4	CSRM60	FAM	AAGATGTGATCCAAGAGAGAGGCA AGGACCAGATCGTGAAAGGCATAG	54	polymorphic	79-115 (*B. taurus*)	Moore *et al*. (1994)
5	CSSM42	PET	GGGAAGGTCCTAACTATGGTTGAG ACCCTCACTTCTAACTGCATTGGA	55-60	monomorphic	175-219 (*B. taurus*)	Moore *et al*. (1994)
6	CYP21	NED	GGTTACAGTCCATGAGTTTGCAAAAG GCTCGCGATCCAACTCCTCCTGAAG	61	monomorphic	188-224 *(B. taurus*)	Fries (1993); Flyn (2009)
7	ETH3	NED	GAACCTGCCTCTCCTGCATTGG ACTCTGCCTGTGGCCAAGTAGG	60	poor amplification	89-131 (*B. taurus*)	Toldo *et al.* (1993); Bishop *et al*. (1994)
8	ETH10	FAM	GTTCAGGACTGGCCCTGCTAACA CCTCCAGCCCACTTTCTCTTCTC	60	polymorphic	198-234 (*Capra hircus*)	Toldo *et al*. (1993); Flyn (2009)
9	ILSTS006	PET	TGTCTGTATTTCTGCTGTGG ACACGGAAGCGATCTAAACG	55	no amplification	281-304 (*B. taurus*)	Brezinski *et al.* (1993)
10	INRA23	HEX	GAGTAGAGCTACAAGATAAAC TAACTACAGGGTGTTAGATGAACTCA	60	no amplification	194-236 (*B. taurus*)	Vaiman *et al.* (1994)
11	INRA107	FAM	TCCCAGATACAGATGCAACAG GGAGAGCCGAGGGCTTCAGC	61	polymorphic	160-166 (*B. taurus)*	Vaiman *et al.* (1994)
12	RM067	HEX	TGAGTAATGCAATAGATACAGTATT GCTTTGGCCATATGAAGAGCTTT	60	monomorphic	90-102 (*B. taurus*)	Kossarek *et al*. (1993); Bishop *et al*. (1994)
13	SPS113	PET	CCTCCACACAGGCTTCTCTGACTT CCTAACTTGCTTGAGTTATTGCCC	52	polymorphic	136-142 (*B. taurus*)	Moore *et al*. (1993)

Annex I. Results of cross-species amplification tests and level of polymorphism (continued)							
14	TGLA53	NED	GCTTTCAGAAATAGTTTGCATTCA ATCTTCACATGATATTACAGCAGA	60	monomorphic	147-197 (*B. taurus*)	Georges and Massey (1992); Flyn (2009)
15	TGLA122	PET	CCCTCCTCCAGGTAAATCAGC AATCACATGGCAAATAAGTACATAC	60	poor amplification	133-193 (*B. taurus*)	Georges and Massey (1992); Flyn (2009)
16	TGLA126	HEX	CTAATTTAGAATGAGAGAGGCTTCT CTAATTTAGAATGAGAGAGGCTTCT	60	poor amplification	104-132 (*B. taurus*)	Georges and Massey (1992); Flyn (2009)
17	TGLA227	PET	CGAATTCCAAATCTGTTAATTTGCT ACAGACAGAAACTCAATGAAAGCA	60	monomorphic	63-115 (*Bison bison*)	Georges and Massey (1992); Mommens *et al*. (1998)
Primers assessed by Zemanová (2008)							
18	AF533518	HEX	CAGGAAGACCTGTATGGA AATCTATGCCTGGGAGGA	50	polymorphic	286 (*Nanger granti*)	Huebinger *et al*. (2006)
19	BM4505	FAM	TTATCTTGGCTTCTGGGTGC ATCTTCACTTGGGATGCAGG	50	polymorphic	154-282 (*Gazella dorcas*)	Beja-Pereira *et al.* (2004)
20	ETH225	FAM	GATCACCTTGCCACTATTTCCT ACATGACAGCCAGCTGCTACT	45	polymorphic	141-159 (*B. taurus*)	Stephen *et al.* (1994); Beja-Pereira *et al.* (2004)
21	OarFCB304	FAM	CCCTAGGAGCTTTCAATAAAGAATCGG CGCTGCTGTCAACTGGGTCAGGG	55	polymorphic	158-177 (*Capreolus. capreolus*)	Buchanan and Crawford (1993)
22	X80214	HEX	CGAGTTTCTTTCCTCGTGGTAGGC GCTCGGCACATCTTCCTTAGCAACT	50	polymorphic	223 (*C. hircus*)	Pépin *et al*. (1995)

Annex II. Polymorphism of Eastern Giant eland (EGE) and Western Derby eland (WDE)								
Primer	**Na (EGE)**	**Na (WDE)**	**Allele variability**	**Ho (EGE)**	**He (EGE)**	**Ho (WDE)**	**He (WDE)**	**Size range detected (bp)**
BL42	3	2	3	0.5882	0.4931	0.3810	0.3421	283-299
BRR	4	2	5	0.6471	0.7249	0.5333	0.4310	244-257
CSRM60	1	3	3	0.0000	0.0000	0.7429	0.6507	91-100
ETH10	3	2	3	0.6471	0.5381	0.1714	0.1567	206-211
ETH225	4	4	6	0.7059	0.6107	0.6571	0.5552	141-154
X80214	6	5	10	0.6471	0.8045	0.7553	0.6952	213-241
BM4505	6	3	8	0.6471	0.7543	0.4952	0.5185	247-262
INRA107	5	2	5	0.7059	0.7301	0.3143	0.2778	159-179
SPS113	4	2	5	0.6471	0.7249	0.5333	0.4310	138-151
AF533518	5	2	6	0.6000	0.7400	0.4419	0.4989	211-249
OarFCB304	8	4	8	0.7059	0.6522	0.557	0.5433	147-163
Mean	4.4545	2.8182	5.64	0.5451	0.5644	0.4653	0.4250	
SD				0.2574	0.2790	0.2236	0.2023	
P (0.99)				0.8333		0.9167		

Na, number of alleles; Ho, observed heterozygosity; He, estimated heterozygosity

SD, standard deviation