Genetic diversity of Barbary lion based on genealogic analysis

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The aim of this study was to evaluate the state of genetic diversity in population of Barbary lion based on the genealogical analysis. Currently, this lion subspecies does not occur in the wild, and its population is considered to be critically endangered. The pedigree file consisted of 545 animals, while the reference population included 445 individuals. Alongside pedigree completeness, the parameters derived from common ancestor were used to analyse the state of genetic diversity in target population. The completeness of pedigree data had significantly decreasing tendency with increasing generations. The pedigree completeness index was the highest in the first generation (68%). The average value of the inbreeding coefficient was very similar in the reference population and the pedigree file (F = 0.05). Across generations, the trend of inbreeding increase was positive mainly due to the long-term use of specific lines and families for mating. The relative high average relatedness among individuals (AR = 0.06) only reflected the individual increase in inbreeding (3.18%). As expected the higher level of individual increase in inbreeding was found in the pedigree file (3.41%). The effective population size at level 26.66 confirmed that the Barbary lion is critically endangered by the loss of diversity. Because of this, the future continuous monitoring of genetic diversity of this subspecies is necessary, especially for long-term conservation purposes.

Keywords: Barbary lion, diversity, endangered species, pedigree analysis

1   Introduction

Conservation of biodiversity has become one of the most important objectives, since diminishing diversity is considered to be a global problem addressed by many international programs, organizations, and strategies (Kadlečík et al., 2016).

The lion (*Panthera leo*) belongs to mammals, from the taxonomic point of view to species from the genus Panthera and family *Felidae* (Linnaeus, 1758). It is quite different from other species belonging to this family. The lion is characterized by short, close-fitting leather and at the end of the tail with the long hairs. The Lions have a characteristic sexual dimorphism; the male is distinguished by a massive body structure, and the mane that extends from head to chest which sometimes covers a part of the chest (Alden et al., 1998; IUCN, 2005).

Nowadays, lions are found in fragmented populations inhabiting the geographical area of Sub-Saharan Africa and Western India (Riggio et al., 2013). From a historical point of view, the population of the Barbary lion represented a relatively large population. But, since 1990,

its population size declined by 43% over the past 21 years (1993–2014). Subpopulations lions approximately totalled an estimated 7,500 Lions in 2014 and comprise a substantial portion of the total species population in applying observed trends to the species as a whole as well as on a regional basis (IUCN, 2010). The North African-Asian population of lions is only represented by today's Asiatic lion (350 wild individuals and 100 zoo captives), so the potential significance of captive Moroccan Royal lions (90 individuals) is not trivial (Black et al., 2013). Although the cause of such rapid decline is not fully understood, the loss of habitats and conflicts with humans are the biggest causes (Riggio et al., 2013). As shown in many studies of wild as well as domestic species, the decrease of population size is mostly accompanied by the significant loss of genetic diversity. That is important for ensuring the adaptive potential of species and preventing the occurrence of inbreeding depression over a long time period (Kadlečík et al., 2016).

This study is focused on the populations of Barbary lion (*Panthera leo leo*) living in captivity. Barbary lions have been considered extinct in the wild since the mid-1960's

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(Black et al., 2013). One of the most important features of the Barbary lion is a very dark and long-haired mane that passes over a shoulder to belly (Hemmer, 1974). The last known wild Barbary lion was shot in the Moroccan part of the Atlas Mountains in 1942 (Black et al., 2013). Barbary lions were offered to royal families and were known as "royal" lions. There are several lions in European zoos that are considered to be partial descendants of Barbary lions, but not purebred Barbary lions because of crossbreeding with other species of lions. Over the last three decades, many observations have been made about of origin purebred Barbary lions, but none of them has been genetically proven (Black, 2009).

The term biodiversity or biological diversity refers to the diversity of all life forms, evolutionary outcomes occurring in nature (Wilson, 1992). Genetic diversity can be defined as the variety of genotypes and alleles present in populations of individuals, which may subsequently manifest themselves in morphological, physiological and behavioural differences among individuals within a given population of the species (Frankham et al., 2002). Individuals of a particular species differ in their genotypic and phenotypic properties. As a result of genetic diversity, the gene pool of a given species is improved, species are being developed and individuals are able to adapt to changing environment conditions. Today's biodiversity status of a given species is a result of its long-term evolution especially adaption to specific environments (Kadlečík et al., 2016).

In the biodiversity assessment, two approaches can be generally observed; the use of diversity indices and a description of population diversity using models. The main difference between models and indices of diversity is based on the fact that the indices try to summarize the diversity of the population in one numerical value, in contrary the models, which try to avoid such information and focus on describing the overall shape of the population curve. It is more logical to use the maximum information contained in the data, however, in some cases, it is more practical and advantageous to use the generally index of diversity combining all the parameters in one value (Jarkovsky et al., 2012).

The ongoing development molecular and statistical methods are providing the scientific community with a wealth of tools for revealing the demographic history of a population. In particular, by bottlenecks, population expansions or declines, as well as effective population (N_e) sizes can now, by a variety of methods, be estimated for natural populations (Spong et al., 2000).

The effective population size represents the number of individuals in populations that vary depending on the inbreeding increase, or the increase in gene frequency

changes in the population (Gutiérrez et al., 2009b; Cervantes et al., 2008). The effective population size is generally accepted as one of the most important parameters reflecting the loss of genetic diversity. According to N_e values the population can be regarded as critically endangered ($N_e \leq 50$), threatened ($50 > N_e < 200$), monitored ($200 > N_e < 1,000$) and non-endangered population ($N_e > 1,000$) (Oravcová et al., 2006).

The aim of this study was to analyse the state of genetic diversity in populations of Barbary lion as one of the critically endangered wild life species by using genealogical analysis. In the evaluated database, we also measured the values of the relative inbreeding intensity (F), the coefficient of average relatedness (AR), the individual increase in inbreeding (ΔF).

2 Material and methods

2.1 Analysed population

The pedigree file consisted of 545 individuals that were registered in studbooks from 2011 to 2017 and monitored by Adrian Harland (Director of the Port Lympne Reserve for Animals). The genealogical information was obtained mainly from the Moroccan studbooks of a Barbary lion and then supplemented by information in cooperation with various European zoos. The reference population covering living animals in captivity consisted of 455 individuals.

2.2 Analysis of genetic diversity

One of the main factors that affect the reliability of genetic diversity indices is the completeness of pedigree. The completeness of the genealogical information was evaluated based on the pedigree completeness index (PEC) according to the following formula (MacCluer et al., 1983):

$$PEC = \frac{2C_p C_m}{C_p + C_m}$$

where:
C_p and C_m – are contributions from the paternal and maternal line but individually:

$$C = \frac{1}{d}\sum_{i=1}^{d} a_i$$

where:
a_i – the share of known ancestors in generations i
d – represent the number of generations which was counted

To assess the state of genetic diversity the parameters derived from a common ancestor (effective population size N_e, inbreeding coefficient of an animal F, individual increase in inbreeding ΔF_i, average relatedness AR)

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were calculated by using ENDOG v4.8 (Gutiérrez and Goyache, 2005). The inbreeding coefficient of an animal (*F*) is defined as the likelihood that an individual has two identical alleles from one common ancestor Provided that the analysed individuals and their common ancestors did not arise from the mating of relatives, *F* is calculated according to the equation of Wright (1922):

$$F_x = \sum 0.5^{n_1 + n_2 + 1}$$

where:

n_1 – the number of generations from the individual *X* to the common ancestor on the father's side

n2 – the number of generations from individual *Y* to the common ancestor on the mother's side

Inbreeding is considered to be the main genetic factor that threatens the life of the population in the short period. Estimation of the inbreeding coefficient depends on the depth and availability of information in the pedigree, whereas the increase in inbreeding depends on the relative increase from one generation to the next (Toro et al., 2011). Gutiérrez et al. (2008) defined the individual increase in inbreeding (Δ*F*) between two discrete generations as:

$$\Delta F = \frac{F_t - F_{t-1}}{1 - F_{t-1}}$$

where:

F_t and F_{t-1} – the averages at *t* and *t* – 1 generations.

The average relatedness coefficient (*AR*) of each individual is defined as the probability that an allele randomly was chosen from the whole population in the pedigree belongs to a given animal. *AR* is an alternative or complement to *F* and can be used to predict the long-term inbreeding of a population because it takes into account the percentage of the complete pedigree originating from a founder at the population level (Kadlečík a Kasarda, 2007; Gutiérrez et al.; 2009a).

Individual increase in inbreeding (Δ*F*) is often expressed as the effective population size (N_e), which is defined by Hill and Zhang (2004) as the size of an idealised population with the same increment in drift or inbreeding per in observed generations. The rate of loss of genetic diversity within a species or population increases dramatically when N_e <100, while a population with N_e <50 is considered to be at high risk of the detrimental effects of inbreeding (Lewis et al., 2015). Gutiérrez et al. (2008) mention N_e as a key indicator for the conservation of genetic resources as it has a direct relationship to the level of inbreeding, the fitness of animals, and the degree of loss of genetic variability due to random genetic drift.

According to Simon and Buchenauer (1993), the effective population size is calculated as:

$$N_e = \frac{4(N_m \times N_f)}{(N_m + N_f)}$$

where:

f – the number of active pure-bred females enrolled in the studbook

m – the number of active pure-bred males used in pure-bred breeding

3 Results and discussion

Pedigree is a record that contains the most important information about the individual and its ancestors, usually in the 3–5th generations, with the parents of a certain offspring being considered as the first generation. It illustrates cognitive relationships between individuals. Pedigrees provide information on the origin and development of zootechnical taxonomic units that are an important part of breeding works, especially in case of individuals with known ancestors listed in the pedigree have a higher breeding value.

Pedigree analyses have to begin with the founders of the population, so individuals with unknown or estimated ancestors, from which come the known members of the population (Lacy, 1989). This analysis found evidence about maximum 7 generations of ancestors. In our opinion when comparing our result to livestock species, this is a relatively high number of generations traced. When evaluating the results of the analysis of pedigree completeness we also observed the percentage of known ancestors by generation. Since observed values are same we cannot talk about a significant improvement. Figure 1 clearly indicates that with the increasing number of generations the proportion of known ancestors significantly decreases. Within both pedigree file and
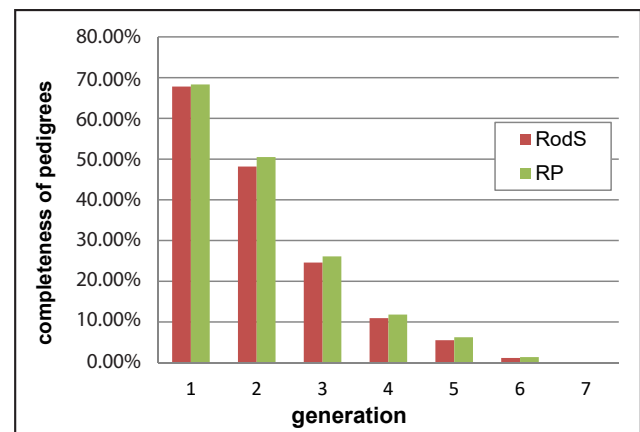
Figure 1 Pedigree completeness index by generations according to MacCluer et al. (1983)
RodS – Pedigree file, RP – Reference population

reference population the highest proportion of known ancestors was observed in the 1st generation (in average 68%), while the lowest values were in the 7th generation (Figure 1).

The effective population size is required to predict the rate of inbreeding and loss of genetic variation in the specific population. Based on this, analysed population of Barbary lion can be labelled as critically endangered population (Oravcová et al., 2006), since the calculated effective population size is less than 50 (26.66). The low effective population size is related to the high relatedness of animals and consequently to the inbreeding coefficient. A similar conclusion was reached by used to estimate N_e for the 2 remaining populations of the endangered ocelot (*Leopardus pardalis albescens*) occurring in the United States. Janečka et al. (2008) used several methods on calculated N_e (Oravcová et al., 2006), resulting in estimates ranging from N_e = 8.0 to 13.9 for the population located at the Laguna Atascosa Wildlife Refuge in Cameron County, Texas. The ocelot population in Willacy County, Texas, had N_e estimates of 2.9 and 3.1, respectively. When comparing with our result, it must be pointed out that we used the pedigree information for calculation of effective population size. In our study, the effective population size was estimated based on individual increase in inbreeding and through regression on equivalent generations for a given subpopulation according to Gutiérrez et al. (2008). However, the calculation in the ocelot population used genetic data from microsatellite loci. Estimates of N_e provide important information on the status of endangered populations and serve as indicators of genetic viability. Therefore, in line with this idea of endangered populations, it can be concluded that threatened populations are vulnerable to the effects of genetic drift and inbreeding, particularly when gene flow is low and the effective population size is low.

From the point of view of the interpretation of indicators derived from the common ancestor represent a set of indicators enabling the evaluation of genetic diversity. Overview of these indicators can be seen in the Tab.1 The obtained value of the average relatedness among animals (*AR* = 0.58%) is higher than the coefficient of the intensity of the inbreeding (F = 0.5%). We can assume that the genetic diversity will continue to diminish in the future and inbreeding will increase. With regard of the risk of inbreeding increase in future generations, it is necessary to improve attention when mating animals with F value of more than 1.01%. In the results, the average inbreeding intensity coefficient in the reference population was 4.2%; the average *F* = 4.5% in the whole pedigree file. The highest recorded inbreeding coefficient was 9.4% (Figure 2). When we comparing our result with the result in the study Zanin et al. (2016) overall, our method was the one

that obtained the most higher values F. Should be noted Zanit et al. (2016) worked with, the molecular genetic structure of the Puma (Puma concolor) and Jaguar (Panthera onca) in Mexico. Values F (for subpopulations) were at a level, for certain areas for Puma -0.22 to 0.12 and Jaguar -0.13 to -0.6. Values *AR* were at a level, for certain areas for Puma 1.67 to 4, and Jaguar 2.79 to 3.24. Of the results show Pumas exhibited higher variation in genetic diversity than a Jaguar, for both expected and observed heterozygosities. The genetic variation revealed that, in their study area, there is not a uniform and panmictic population for either species. The gradual transition across all sampled locations suggests an isolation-by-distance pattern of genetic variation.

Our results demonstrate two things. First values of average relatedness coefficient of the reference population was 15.6%. Second, a value of the averaged relatedness coefficient in the pedigree file was 15%. It is important to point out that inbreeding is often associated with a decrease in offspring negative fitness (inbreeding depression). These negative effects are most profound in indicators closely linked to reproductive success, including seminal quality and fecundity. In Barbary lion´s population, the effect of inbreeding depression has been manifested in offspring. Total inbreeding had an impact on the survival of the young and their evolution. The records of the studbook have seen trouble cases of milk production in dams or the offspring has problems with the locomotive apparatus.

As shown in Figure 2, we can observe a rising inbreeding trend in populations considered, as the result of fact that each subsequent generation comes from a small number of females. These inbreeding values significantly complicate the possibilities when composing mating plans.

From the results, it can be said that there is a high percentage of inbreeding in the Barbary lion population
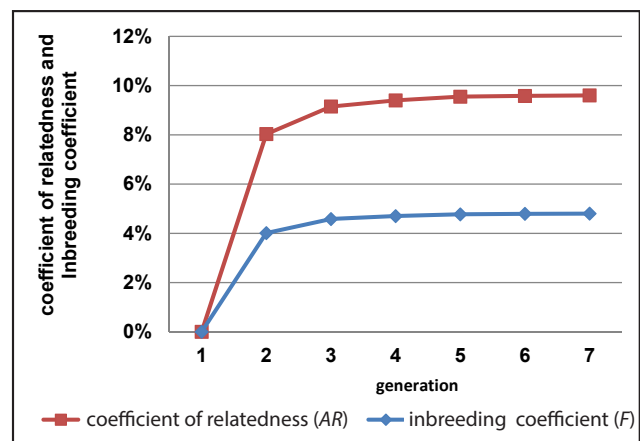

Figure 2 Graphic representation of *F* and *AR* values in the individual recalculated generations

because genetic purity is not entirely certain and the ancestors who consider themselves to be the founders do not have precisely defined relationships between the other ancestors. It can't be denied that the founders already had a certain percentage of inbreeding which they later transferred to their offspring. From these results the average relatedness coefficient of animals for both files, it has a high value, which is higher than the average value of the inbreeding intensity coefficient. In view of the small number of living individuals in the world, an increase in inbreeding intensity may be expected in the future because there will be mating more and more cognate individuals.

In livestock, especially in cattle, such analysis is often given increased attention to the preparation of rescue plans to save the population. Nomura (1999) suggested a suitable alternative so-called "compensatory mating" which is defined as a system when family members/individuals, in which are selected most of the individuals, and those are mating with individuals of families from which at least individuals are selected. The author also introduced the concept of the mating score, defined as the number of selected complete siblings of the individual (including the individual himself). It can be said, therefore, that compensatory mating is formulated as the negative selective mating of individuals, based on a mating score. Due to the fact that the males (sires) are sorted downwards and females (dams) that are ranked ascending according to mating scores are individuals with the same position in a row and mating with each other.

In our work we evaluated the representation of individuals in classes according to the intensity of inbreeding, the groups were divided into three groups, with the highest proportion in both files evaluated individuals reaching $F = 0$–0.39 (Table 1). The most inbreed animals had F values in the range of 12.5–37.5 in the reference population, representing 16.85% of inbreed individuals. In the pedigree file, it was 15.78% inbreeding of individuals (Table 1). In view of the risk of inbreeding growth in future generations, it is necessary to increase attention when mating animals with an F value of more than 0.39%. In the pedigree file, lower values can be seen, since it is made up of ancestors, who have no known parents, and we consider them in our database as basic ancestors. Introduction males (sires) appeared in the reference population, i.e. that they were actively engaged/involved in reproduction. We d not have to forget that individuals who were included in the first class with the lowest inbreeding values, not one hundred per cent pure individuals as their basic ancestors are not known. And we cannot even confirm or refute whether these individuals originated in a related mating and to what extent.

Table 1 Indicators of diversity derived from common ancestors in the pedigree file and in the reference population

Indicators		RP	RodS
Inbreeding coefficient (F)		4.16%	4.80%
Coefficient of average relatedness (AR)		5.73%	5.50%
F by sex			0.072 (0)
		4.613 ♂	0.0402 ♂
		0.042 ♀	0.047 ♀
AR by sex			0.076 (0)
		0.063 ♂	0.052 ♂
		0.057 ♀	0.051 ♀
Representation of individuals in classes F	$F \leq 0$	342	420
	$F = 0.39$–12.5	28	39
	$F \geq 12.5$–37.5	75	86
Individual increase in inbreeding (ΔF)		2.95%	3.41%

4 Conclusions

To evaluate the state of genetic diversity in the population of Barbary lion the indiacators derived from the common ancestor were used. One of the main factors that affect the reliability of genetic diversity indices is the completeness of pedigree. The pedigree completeness index was the highest in the first generation, with the following generations decreasing. The average value of the inbreeding coefficient was the same in the reference population and the pedigree file ($F = 4.79\%$). In individual generations, the development of the coefficient was positive and, in particular, due to the long-term use of mothers and the relatedness of a mating of individuals, because low numbers of people cannot be expected in low numbers. The average relatedness coefficient at 0.06 pointing out to a relatively high degree of affinity among rated individuals. In the population, there is an increase in inbreeding, and the population becomes less demographically stable. Size of the population and inbreeding are related and lead to the loss of genetic diversity. It can be said that the loss of the population is actually the result of the interaction between inbreeding, the impact of human activity, demographic instability and the loss of genetic diversity. It would be appropriate to introduce a system of regular (annual) monitoring of the genetic diversity of lions kept in zoo gardens in the Czech Republic and Slovakia as a joint project in relation to the international database. The results of this work can be taken as an initial assessment.

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