Chapter 3.3. Ex situ genetic management

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3.3.1 Goals of ex situ genetic lynx testing

In several previous reintroduction attempts of lynx, lack of molecular genetic marker systems and/or unawareness of its importance has led to the release of founder animals of unclear genetic status. Today, genetic testing is considered of pivotal importance in reintroduction programmes. There are three equally important goals of genetic testing of captive lynx in the frame of reintroduction programmes:

1. *Identification of the suitable genetic lineage/subspecies*: Lynx reintroductions in Central and Western Europe should be done either with purebred Carpathian lynx (e.g., within the Alpine and low-mountain regions) or Baltic lynx (in the northern and northeastern flatland region) (see chapter 1 *Introduction*; Bonn Lynx Expert Group 2021). Genetic tests using appropriate marker sets will ensure the selection of suitable animals for establishing pure breeding lines and test animals selected for later reintroduction. No untested animals should be selected for reintroduction, unless studbook data allows for unequivocal reconstruction of ancestry and genetic status (see section *3.3.3 EEP Guidelines*).

2. *Relatedness, genetic diversity and inbreeding*: As stated in chapter *2.2 Genetic monitoring*, the release of related animals should be avoided within the same reintroduction project to avoid inbreeding and maximize the level of genetic diversity in the founder population. Given the limited number of founder animals in lynx reintroduction programmes, genetic testing will ensure the selection of animals leading to the highest possible N_e (effective population size) in the founder population, reducing negative effects of the initial founder effect on short-term (inbreeding) and long-term (genetic diversity in the population) viability.

3. *Linking ex situ and in situ conservation*: Comprehensive genetic testing of the ex situ lynx population and in particular all animals considered for reintroduction will facilitate genetic monitoring of the emerging population in the field and help to provide a baseline for genetic lynx monitoring in the field.

3.3.2. Recommendations for genetic ex situ testing procedure

Effective genetic testing is not a trivial task and needs careful consideration of the chosen laboratory and genetic marker system, as well as professional collection of DNA samples. The following recommendations should provide guidance for genetic ex situ testing:

1. *Choice of laboratory* - It is important to note that not every genetic laboratory will be capable of providing reliable genetic testing of the ex situ lynx population. Suitable labs need to be capable of obtaining reliable genotypes from noninvasively collected wildlife samples with low-DNA content, and have experience in lynx genetics, including the availability of reference samples and/or genotypes from different lynx lineages/subspecies. To enable linking genotypes obtained from ex situ testing with later genetic monitoring of the reintroduced population, genetic testing should be performed by a laboratory within the CElynx consortium (Linking Lynx Genetics Working Group). CElynx uses harmonized marker systems for a common genetic monitoring of central European lynx populations. Within the Linking Lynx network, genetic testing was performed by the Wildlife Genetics

Centre, Senckenberg Research Institute in Gelnhausen, Germany. Prior to testing, the Senckenberg Wildlife Genetics Centre (wildtiergenetik@senckenberg.de) or another CElynx laboratory should be contacted to check for availability, costs, and details regarding the testing procedures.

2. *Marker systems* – Various genetic marker types are used in lynx genetics, ranging from short mitochondrial sequence markers to genome wide assessments (e.g., Mueller et al. 2022). We recommend the use of a combination of sequencing a fragment of the mitochondrial control region to obtain the haplotype (following the nomenclature of Hellborg et al. (2002)), and either microsatellite or SNP analysis (or a combination of both) of the nuclear genome. While the mitochondrial haplotype can be easily linked to the subspecies/ region of origin of the animal, only the application of assignment tests based on multilocus genotype data obtained from microsatellites and/or SNP allows for safe assignment to the correct subspecies and reveal potential admixture. Markers for microsatellite analyses may vary, but should contain the core marker set used by the CElynx consortium. An alternative marker system based on 93 SNPs selected from a genome wide SNP data set (Mueller et al. 2022) has proven useful for testing ex situ lynx during the genetic testing procedure conducted by the Linking Lynx network.

3. *Choice of animals and genetic certificate*. All animals considered for reintroduction or breeding with the goal of reintroducing progeny should be genetically tested beforehand to ensure that they fulfil criteria of correct genetic lineage and unrelatedness. Although testing may not seem necessary in case of available studbook data and/or genetic testing of direct ancestors within the breeding lineage, it is still strongly recommended. Consequently, only animals with a genetic certificate (see Appendix II) should be considered for release. The genetic certificate contains the respective studbook number and information, if available, as well as genotypes and the results of subspecies/lineage testing.

4. *Collection of DNA samples* - The collection of samples from captive lynx and the professional and timely preservation of the collected material is of decisive importance for a successful genetic analysis. To avoid contamination, the sample collection should always be carried out with the highest care possible.

Several sample types are suitable for genetic testing of captive lynx, such as blood swabs, buccal swabs, hairs and scats (for further instructions see Appendix III). Consider that EDTA blood is not optimal for genetic testing. We recommend to store materials either dried (buccal swabs, blood, hairs) or in 96% ethanol (scats, tissue from dead animals). Sample material should never be sent in an untreated condition. Alternative preservation methods should be discussed beforehand with the respective laboratory. If possible, buccal swabs are the preferred DNA source.

3.3.3. EEP Guidelines for *Lynx lynx carpathicus*:

The European Association of Zoos and Aquariums (EAZA) conducts population management programmes for animal species that are coordinated by EAZA-Members. The aim of these EAZA Ex situ Programmes (EEPs), is to build healthy and self-sustaining zoo populations. To achieve this, controlled breeding is required for wild animals. Experts - so-called EEP coordinators - generate breeding recommendations for endangered species, which zoos must implement within the EAZA framework. To prevent inbreeding, animals must be exchanged and transported between different zoos regularly. Currently, around 400 different animal species are coordinated within their respective EEPs.

The EEP for the Carpathian lynx is part of a coordinated breeding programme for the Eurasian lynx (*Lynx lynx*). The aim of this programme is to maintain a genetically healthy and sustainable population of the Carpathian lynx in captivity. The programme is managed by the European Association of Zoos and Aquaria (EAZA) and is also guided by the principles of the IUCN's Species Survival Commission.

The EEP for the Carpathian lynx has several key objectives. These include:

- Maintaining a physically and behaviourally healthy and genetically diverse population of Carpathian lynx in captivity by developing and implementing a coordinated approach to the management and breeding of two subspecies across different institutions. Captive breeding is a key component of ex situ management for the Carpathian lynx. The Carpathian lynx is bred in captive facilities to maintain and enhance genetic diversity and to provide a source of animals for reintroduction. Careful selection of individuals for breeding is necessary to ensure the retention of as much genetic diversity as possible.
- Providing advice and support to institutions involved in the breeding and management of Carpathian lynx for the specific purposes of either display and education or reintroduction of individuals.
- Conducting research to improve our understanding of the biology and behaviour of the two subspecies, and to inform the public about conservation efforts in the wild.
- Contributing to the educational roles of modern zoos by being an example of a European carnivore that came back from extinction in many locations: The Nordic population started to recover in the 1950s. State bounties removal, better harvest regulations and the expansion of the roe deer population in Scandinavia were responsible for this trend (von Arx et al. 2004); the population of the Carpathian lynx in central and western Europe stem from reintroductions in the 1970s and 1980s (Kaczensky et al. 2013). Ex situ management for the Carpathian lynx also involves the education and engagement of the public. Raising awareness about the reintroduction program and the conservation of the Carpathian lynx can help generate support for the programme and promote the importance of the species and its conservation.
- Supporting reintroduction efforts, that are done in accordance with the IUCN reintroduction guidelines.

The ESB for the Carpathian lynx has been successful in maintaining a genetically healthy population of Carpathian lynx in captivity. The population is managed by the studbook keeper using the Zoo Animal Management Software (ZIMS; Species360 2023) and PMx (Ballou et al. 2022) – a demographic and genetic analysis tool to assist with the management of breeding programmes.

As of February 2023, the Carpathian lynx population managed by the EEP consists of 137 (79.58.0) individuals at 57 Institutions of which 43 are EAZA institutions and 14 are non-EAZA institutions. In the studbook, the ancestry of the Carpathian lynx is 88 percent known and the proportional gene diversity (as a proportion of the source population) is 94.8 percent. This means of all the currently living Carpathian lynx individuals entered in the ZIMS database, 88 percent of their pedigrees can be traced back to known founders.

This captive population has great potential to support the wild population genetically and particularly demographically, and can, thus, contribute to the conservation of the subspecies in the future. By following the guidelines set out by the EEP, institutions can help ensure that the Carpathian lynx population in captivity remains healthy and sustainable. Also, they can make a valuable contribution to the conservation of this subspecies.

To contribute to reintroduction programmes by breeding offspring, the institutions and enclosures need to fulfil further specific requirements (see chapter 3.1 *Breeding Lynx for reintroduction*).

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