Transnational cooperation for the genetic monitoring of the transboundary Eurasian lynx population in France, Germany and Switzerland:

Review of knowledge and prospects for the future

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Context

Large carnivores in Europe

Large carnivores are widespread but scarce and occur at low densities. They have large home ranges due to their position at the top of the food webs. As flagship species of pristine ecosystems, large carnivores are usually iconic animals. They are admired but also, paradoxically, the group of species for which long-term conservation is the most challenging (Ripple *et al.* 2014). Their populations and associated geographic ranges have massively declined over the last two centuries. In Europe for instance, Brown bear (*Ursos arctos*), Grey wolf (*Canis lupus*) and Eurasian lynx (*Lynx lynx*) (hereafter, lynx) were widespread across the continent until the 18th century, when these species faced near extinction. In Western, Central and Northern Europe particularly, these species displayed smallest population sizes and ranges due to direct persecution, prey extermination and habitat fragmentation in the 19th and early to mid-20th century (Boitani & Linnell 2015). Since then, the implementation of National and European legislations has changed public opinion towards wildlife conservation; hunting management plans have allowed wild herbivores to recover and forest management policies have helped to increase forest cover again. These positive actions have shaped favorable ecological and legislative environments and have thus led to the natural recovery of large carnivores in their habitats in many areas across Europe, through the recolonization of remaining individuals (Boitani & Linnell 2015). Additionally, increasing concerns about the conservation status of these species have led to several reintroduction programs. For example, under the aegis of an informal Lynx International Group, such operations were conducted in the 1970s, during which a few lynx from the Carpathian Mountains in Slovakia were released into the Alps and in the adjacent mountain ranges of Switzerland, Slovenia, Italy, Austria and France (Breitenmoser-Würsten & Obexer-Ruff 2003, Herrenschmidt 1990).

Conservation status of the Eurasian lynx, threats, and needs for a transnational cooperation

Thanks to ongoing conservation efforts, the felid is now widely distributed across the continent. However, despite reintroductions and natural expansion, the conservation status of lynx remains unfavorable and conservationists have to face new conservation challenges (von Arx *et al.* 2021).

Threats to lynx populations in Europe are similar to those for Brown bear and Grey wolf: habitat loss and fragmentation due to infrastructure development, low social acceptance due to conflicts with hunters and/or farmers that trigger economic issues and psychological distress, illegal killing and accidental mortality. However, compared to bears and wolves, lynx have a low ecological valence, and are more specialized in their habitat requirements and prey selection (Boitani *et al.* 2015). The species is therefore much more sensitive to habitat-related threats. Additionally, intrinsic ecological factors further threaten lynx such as its low dispersal capacity (Schnidrig *et al.* 2016) or the loss of genetic diversity observed in the reintroduced populations (Breitenmoser-Würsten & Obexer-Ruff 2003, Mueller *et al*. 2022).

As the majority of lynx populations in Europe is transboundary (von Arx *et al.* 2021) a common strategy is required for the long-term conservation of the species. In this frame, Bonn Lynx Expert Group (2021) have recently made recommendations for the long-term conservation strategy of Eurasian lynx and the required transnational cooperation.

Apart from better informing lynx conservation strategy and management decisions, transnational cooperation is important for the improvement of human-large carnivore co-existence, which is the core mission of the EU LIFE EuroLargeCarnivores project. Specifically, action B3 focuses on enhancing experience-sharing and learning from each other, and encourages the development of common monitoring practices between countries at the population level. Additionally, the project values the use of participatory sciences and involves various stakeholder groups, which contributes to social acceptance of large carnivores.

The case of Western Europe

France, Germany and Switzerland share three reintroduced lynx populations affiliated to the proposed "Carpathian Evolutionary Significant Unit": the Vosges-Palatinian and the Jura populations belonging to the "upper Rhine metapopulation", and the Alpine population (Bonn Lynx Expert Group 2021, von Arx *et al.* 2021). The genetic status of these populations calls for further investigations through transnational monitoring and first cooperative steps have been initiated in that sense (Drouet-Hoguet *et al.* 2021). However, even though those are positive and encouraging actions towards a transnational cooperation within a broader collaborative genetic monitoring of lynx populations in Western and Central Europe, some gaps remain. Data are missing about the species itself at the European scale, but there is also a poor understanding of the projects running in neighboring countries and the methods they are applying. In addition, transboundary cooperation also relies on a well-organized monitoring in each country. National management/action plans are supposed to be tools for the implementation of conservation and management actions in each country. Yet, management institutions tend to have a low capacity in terms of monitoring (Boitani *et al.* 2015) and a diversity of methods are applied, often with a lack of transparency and communication.

Within this context, the LIFE EuroLargeCarnivores supported the writing of this report to 1) recall the genetic challenges in lynx, 2) present an overall view of the current lynx genetics projects in France, Germany and Switzerland, their respective goals and methods, 3) share information between partners and identify possible ways to harmonize methods, 4) offer bases and perspectives for a transnational lynx monitoring project based on genetics, demography and health surveillance.

Eurasian lynx populations in Western Europe

Lynx is the largest wild cat in Europe. The species has a very broad distribution spanning 11 populations in 23 countries (Figure 1). The last assessment estimated 9 000-10 000 lynx in the continent, excluding Russia and Belarus, and most lynx populations in Europe are generally stable (Breitenmoser *et al.* 2015). However, monitoring effort varies in methods and quality across countries and regions and thus, does not allow reliable estimates and comparisons between populations (Kaczensky *et al.* 2013).

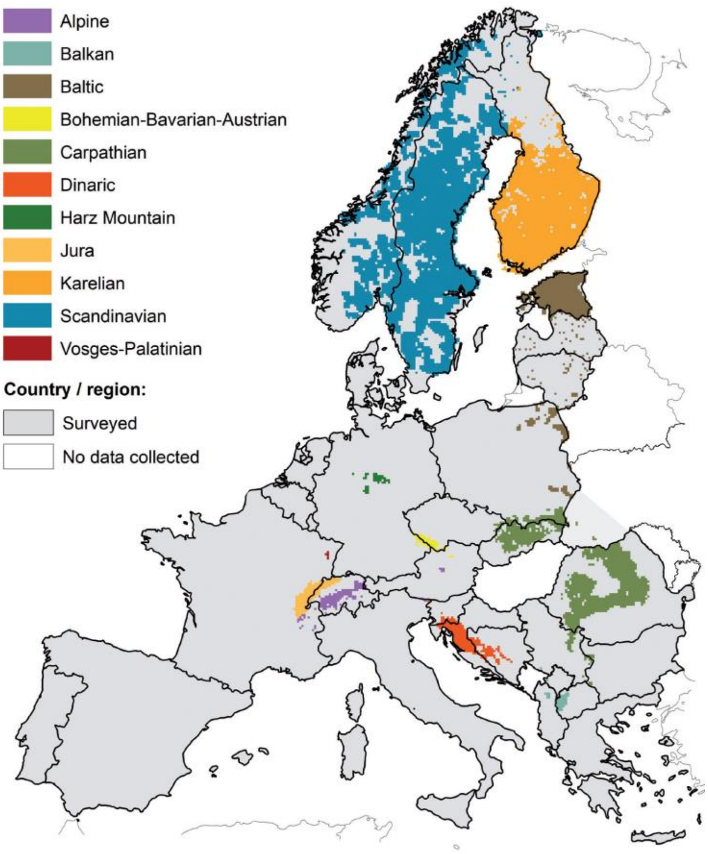


Figure 1*: Distribution of the Eurasian lynx populations in Europe (permanent presence 2012-2016). Source: Kaczensky et al. 2021.*

Although the Eurasian lynx is listed as "Least Concern" in the IUCN Red List of Threatened species at the European scale (Breitenmoser *et al.* 2015), conservation statuses diverge locally and the situation of some populations is preoccupying. In Scandinavia and the Baltics, autochthonous populations have shown declining trends in the past decade, while the Balkan population was assessed as "Critically Endangered". As for reintroduced populations, they remain small and isolated (von Arx 2020) while major threats (habitat fragmentation, illegal killing and accidental mortality) put them at risk of stochastic demographic events, genetic loss and inbreeding (Boitani *et al.* 2015, Premier *et al.* 2021). In Western Europe* particularly, all populations are "endangered" (Alpine, Jura) or "critically endangered" (Vosges-Palatinian, Harz) (von Arx *et al.* 2021) (Table 1). Within this context, implementation of appropriate conservation actions are required and constant monitoring is needed to ensure the recovery of the species (Boitani *et al.* 2015).

** Here, lynx populations in Western Europe refer to the ones only occurring and/or shared by France, Germany and Switzerland.*

***Table 1**: Current status and origins of Eurasian lynx populations in Western Europe. It is important to note that the success of these operations is hard to evaluate and that the exact number of released individuals was not always known, even for the best documented reintroductions. Source: Gatti 2021, von Arx et al. 2021, Charbonnel & Germain 2020, Linnell et al. 2009, Vandel et al. 2006.*

Population	Range countries and approx. % of the shared population	Estimated size (nb. independent individuals) (2012-2016)	Trend (2012-2016)	IUCN Red List Category (2018)	Reintroduction, translocation and reinforcement	References
Alpine	Switzerland (77%) France (10%) Italy (7%) Austria (3%) Slovenia (3%)	163	↗	Endangered	1970-1976: release of **14** free-ranging lynx from the Carpathian Mts. in Slovakia to the Swiss Alps 2001-2021 (stepping-stone projects): • 12 lynx translocated from NW Alps and Jura Mts. to NE Switzerland (LUNO project 2001-2008) • 5 lynx translocated from NW Alps and Jura Mts. to the Kalkalpen National Park in Austria (2011-2013) • 3 lynx translocated from Jura Mts. to SE Alps in Italy (ULyCA project 2014) • 5 lynx translocated to SE Alps in Slovenia (LIFE Lynx 2021)	Breitenmoser *et al.* 1998 Breitenmoser & Breitenmoser-Würsten 1990 Molinari *et al.* 2021
Harz	Germany (100%)	46	↗	Critically endangered	2000-2006 (Harz lynx project): release of **24** captive-bred lynx from German and Swedish zoos and wildlife parks	Anders & Middelhoff 2021 Mueller *et al.* 2020
Jura	France (70%) Switzerland (30%)	140	↗	Endangered	1972-1975: release of **8-10** free-ranging lynx from the Carpathian Mts. in Slovakia	Breitenmoser *et al.* 1998 Breitenmoser & Breitenmoser-Würsten 1990
Vosges-Palatinian	Germany (90%) France (10%)	<30 *	↘ *	Critically endangered	1983-1993: release of **21** free-ranging lynx from Carpathian Mts. in Slovakia and kept in captivity in zoos temporarily, in addition to supposed illegal releases 2015-2021 (LIFE Luchs Pfälzerwald): translocation of **20** free-ranging lynx from Switzerland and Slovakia	Vandel *et al.* 2006 Idelberger *et al.* 2021

* This population size estimate takes into account the reintroductions carried out under the LIFE Lynx Palatinate project (2016-2020), while the last assessment of the population trend is from 2012-2016.

Genetics challenges for the Eurasian lynx

Previous research has shown that in small and isolated populations, the lack of genetic exchange with other populations due to restricted dispersal caused by human activities (*i.e.*, habitat fragmentation, vehicle collisions and illegal killing) leads to a lower genetic diversity. This is due to genetic drift and inbreeding, which can in turn have negative impacts on species fitness through the fixation of deleterious alleles. This affects both individual and population levels and ultimately the viability and survival of the species (Keller & Waller 2002, Kruckenhauser *et al.* 2009*,* Premier *et al.* 2021).

Low genetic diversity has been reported with a tendency for lower levels of heterozygosity and allelic diversity in the reintroduced lynx populations of the Jura Mountains and the Alps, compared to the source population (Breitenmoser-Würsten & Obexer-Ruff 2003, Mueller *et al*. 2022). As a fact, the current reintroduced populations are mainly originating from very few founder individuals from the Carpathian Mountains population of Slovakia (*Lynx lynx carpathicus*) (Table 1), among which some of them were even closely related (Breitenmoser-Würsten & Obexer-Ruff 2003).

European lynx experts have now clearly stated that the reintroduced populations of lynx will not be (genetically) viable in the foreseeable future, and therefore require short- to long-term genetic management (Bonn Lynx Expert Group 2021).

Even though a reduction of adaptive potential and fitness due to low genetic variation and/or inbreeding has not yet been clearly documented for wild lynx, some of their consequences (*i.e.*, genetic disorders, infectious diseases, low fertility) have been recorded in captive individuals (Laikre 1999). In free-ranging lynx in Switzerland, viruses have been observed (isolated cases of FIV, FelV, sarcoptic mange, etc.) but does not seem to be significant, or epidemic so far (reported in SFEPM 2021). However, the possible correlation between the emergence of heart disorders (cardiomyopathy, arteriosclerosis of the coronary arteria and heart murmur) in free-ranging lynx and the loss of genetic diversity is currently being studied (Ryser-Degiorgis *et al*. 2020 and 2021). In the Dinaric population as well, loss of genetic diversity might be responsible for the decline of this reintroduced population, also affected by inbreeding and very low genetic diversity (Sindičić *et al.* 2013). Sindičić *et al.* 2016 speculate that this acted in synergy with high human-induced lynx mortality and has thus led to the critical status of the population. A reduction in birth rate was also observed (R. Černe pers. comm. cited in Schnidrig *et al.* 2016).

In southern Florida in the USA, the Florida panther (*Puma concolor coryi*) is a distressing clear example of what a small population with low levels of genetic diversity and inbreeding depression could actually lead to. At the end of the 20th century, researchers found that individuals showed poor sperm quality, low testosterone levels, poor fecundity and recruitment, high incidence of thoracic cowlicks and numerous atrial septal defects, as well as high parasite load and infectious disease pathogens, among other disorders (Johnson *et al*. 2010). The solution came in 1995 from a genetic rescue of the population led by conservation managers who released eight females from Texas to increase depleted genetic diversity and population size, and reverse indications of inbreeding depression (Johnson *et al.* 2010, van de Kerk *et al.* 2019).

However, past experience has proven that genetic remedy is not always the solution, or a success. For instance, in central Austria, three bears were released by WWF between 1989 and 1993 (Kruckenhauser *et al.* 2009) which has the effect to increase breeding success in the subpopulation. Despite this, the population remained threatened. Additionally to low population size, the problem was that all the individuals detected were descendants from one couple and thus represented only one family, which led to inbreeding and associated complications. This population has been extinct in 2011 as a consequence of the small founder effect, but also due to poaching (European Wildlife 2012).

Actually, when the human dimensions are not considered carefully, reintroductions can prove to be a failure, mainly due to illegal killing, as Drouilly & O'Riain (2021) summarized. On the 15 reintroductions conducted across eight European countries and involving about 170 lynx between 1970 and 2007, only a third is considered successful (Linnell *et al.* 2009). Specifically, "Guidelines for Reintroductions and Other Conservation Translocations" (IUCN/SSC 2013) pointed out that if a species is extinct in an area for a long time, local communities may have no connection anymore to this species unknown to them, and hence may be opposed to any releases. In such cases, special efforts on socio-economic dimension should be made well in advance of such operations. Additionally, if the threat(s) that caused previous extinction have not been identified and removed or sufficiently reduced, translocation is not advised.

Given the relative success of reintroduction or translocation operations, other limitations to genetic rescue have recently been highlighted by two studies. Using a spatially explicit individual-based population model, Premier *et al*. (2020) found that in some cases, lynx movements could lead to a higher loss of genetic diversity and an increase in the genetic structure differentiation between populations. Interestingly, Kyriazis *et al*. (2021) also demonstrated that, rather than concentrating on maintaining a high genetic diversity, management strategies should instead focus on minimizing strongly deleterious mutations. Additionally, they showed that genetic rescue is more effective when translocated individuals come from historically-small source populations where strongly recessive deleterious mutations have been purged.

As Breitenmoser *et al*. (2021) highlighted as well, all these research ask various management questions and simply illustrate the overall complexity of the situation, which calls for a transnational consensual strategy for the long-term conservation of Eurasian lynx in Europe.

Call for cross-border monitoring

Wide-ranging species are likely to occur beyond national frontiers and lynx is no exception. Ten out of the eleven lynx populations in Europe are transboundary (von Arx *et al.* 2021). Therefore, when activities such as translocations or reintroductions are undertaken in one population or in one country, it ultimately affects those of neighboring countries. This means that a consensual strategy is needed for the long-term recovery and maintenance of a demographically and genetically viable lynx meta-population. However, this common conservation goal requires defined and accepted standards as well as protocols facilitating the transboundary and interregional cooperation. They also require common guidelines and a coordinated approach at the European landscape-scale, including a careful monitoring of the lynx populations in the range countries (Breitenmoser *et al*. 2021, von Arx *et al.* 2021).

As an example, in the Carpathian population, recent research has shown that the population size was in fact overestimated due to the lack of relevant scientific data. Misleading information about population trend have in turn led to social conflicts and subsequent illegal killing (Kubala *et al.* 2021). While the Carpathian population has been and still is a source for lynx reintroduction and reinforcement, lynx experts and the Carpathian Convention support the development of a Pan-Carpathian conservation strategy between the range countries. The goal is to provide a standardized monitoring system and robust scientific methods applicable in each country to better inform lynx conservation actions at the population scale and not only at the country's level.

Beside standardization of monitoring methods across countries, an absolute requirement is also to allow common interpretation and cross-comparable results among research teams and labs within and across countries. This extends to the analysis of different types of samples when referring to genetics (invasives vs. non-invasives samples) (De Groot *et al.* 2021). Previous projects have actually proven successful in data sharing and standardization of monitoring protocols. In wolverine (*Gulo gulo*) monitoring for instance, transboundary cooperation between Norway and Sweden has led to more accurate estimates in population size, population growth rate, and vital rate (Gervasi *et al.* 2016).

This is why 53 experts gathered in 2019, in Bonn, Germany, formulated recommendations about "monitoring of the conservation status of lynx populations" and "principles for their genetic monitoring and management" (Bonn Lynx Expert Group 2021). Within this context, they stipulated a number of standards and protocols for ongoing and future conservation projects in Western and Central Europe:

- The agreement on "evolutionary significant units" of Eurasian lynx in Continental Europe, their geographic delineation, and the use of appropriate individuals for further translocations
- The management of small and isolated populations, through natural gene flow or assisted dispersal in order to :
    - minimize loss of genetic diversity (heterozygosity, allelic richness)
    - keep the inbreeding coefficient F_{IT} below 0.15
    - keep the effective population size above 100 mature individuals
- The systematic genotyping of individual being selected for release operations
- The implementation of permanent opportunistic sampling across the lynx distribution range to reach a minimum sample-size goal of 30 animals per generation (5 years) per population
- The use of a common panel of 15 microsatellites markers by all laboratories involved in the genetic monitoring of lynx
- The exchange and sharing of calibration samples and calibration table between participating laboratories
- The testing of new marker systems as they become available
- The establishment of a permanent lynx genetics working group including experts from the laboratories involved in lynx genetic monitoring and research

By following these principles, the goal is to "track genetic diversity and inbreeding over time, allowing the assessment of the effective population size and the detection of gene flow between neighboring populations".

Ultimately, the aim is to provide "objective information through monitoring and research to continuously observe the conservation status of each population and to propose the appropriate conservation measures" at the population scale.

Lynx genetics monitoring in France, Germany and Switzerland

Through the LIFE EuroLargeCarnivores, a workshop was organized by the SFEPM (9th December 2021) to discuss the various lynx genetics projects in France, Germany and Switzerland, and to share information between the structures in charge. Table 2 below summarizes the main information gathered during this workshop.

***Table 2**: Review of knowledge on the different lynx genetics projects and research in Western Europe, part 1/2. Source: SFEPM 2021, N. Drouet-Hoguet pers. comm. 08/02/2022.*

	FRANCE	FRANCE	GERMANY	SWITZERLAND
Structure in charge of genetic analyses	French Biodiversity Agency (OFB) Analyses are undertaken through a convention with KORA *Public institution*	Chrono-Environment Lab (LCE), University of Franche-Comté *University laboratory*	Center for Wildlife Genetics, Senckenberg Research Institute *National reference research center for large carnivore monitoring*	KORA *Foundation*
Research / Monitoring aims	To feed a global knowledge at the metapopulation scale (cf **SWITZERLAND**) by an integrated approach involving population dynamic, health and genetic monitoring along with a cross-border dimension. The final aim it to provide knowledge's on connectivity at the metapopulation scale	To study the genetic diversity of the French lynx population To inform lynx diet and the importance of secondary preys	To track lynx genetic diversity and inbreeding over time, related to gene flow	To monitor of genetic parameters (genetic diversity, gene flows, degree of kinship and of inbreeding, etc.) in parallel with demography and health surveillance
Time frame	On-going	Since 2019	Since 2010	Since the early 2000s
Partners	KORA Matson's laboratory (USA) for teeth aging only	SFEPM and local partners for the scat collection network	Central European Lynx Consortium (CElynx)	Collaborative effort with KORA, FIWI, OFB and Central European Lynx Consortium (CElynx)
Authority responsible for lynx monitoring	The OFB, under the authority of the Ministries in charge of Ecology, Agriculture and Food	None non-invasive sampling was organized in the country until the LCE started this research project. The use of non-invasive samples does not require an official permit	Non-invasive sampling is organized by the Federal State Environmental Authorities, National Parks conduct different sampling than the State authority	The KORA, on mandate of the Federal Government
Type of samples	**Type:** invasive samples provided to KORA (mostly tissues, also blood depending on collection opportunities) **Source:** mainly from reported dead lynx, occasionally from alive individuals	**Type:** non-invasive samples (scat) **Source:** collected in the wild	**Type:** About 95% are non-invasive samples (hairs, scats, saliva traces from kills, etc.) **Source:** not known	**Type:** invasive samples (tissue and blood) **Source:** from dead lynx and individuals captured for collaring

Table 2: Review of knowledge on the different lynx genetics projects and research in Western Europe, part 2/2. Source: SFEPM 2021, N. Drouet-Hoguet pers. comm. 08/02/2022.

	FRANCE	FRANCE	GERMANY	SWITZERLAND
Sampling strategy	**Type:** opportunistic and on-going sampling at large scale with the contribution of national networks ("Wolf-Lynx" and "SAGIR"). **Sample size*:** n=129	**Type:** opportunistic sampling through the lynx scat collection network coordinated by the SFEPM **Sample size*:** n=32 since the beginning of the project	**Type:** mainly opportunistic and it varies depending on the region. **Sample size:** not known	**Type:** NA **Sample size*:** 30 individuals/year - Alps n=310 - Jura Mts. n=234 - stepping-stone population n=50
Need for additional samples	Yes, because sampling in the Vosges and the Alps mountains is needed	Yes, the network is expanding over the species range in order to collect relevant data	No	No, because very good sampling effort across the country
Markers	(cf **SWITZERLAND**)	1 DNA mitochondrial marker for identifying the species 1 sex marker 18 microsatellites (goal of 25) chosen using the published scientific literature 2 fragments of mTDNA for diet study specifically	4 types of markers (all of them are used for non-invasive samples, except SNPs): 1. Mitochondrial DNA, for sequencing haplotypes 2. Microsatellites, STR: 24 + sex markers divided in 4 multiplex reaction 3. Reduced SNP panels (rSP), for discriminating individuals, sex, populations, lineages, hybridization and subspecies. 4. SNPs (NextRAD) for lineage assignments, genome wide diversity, inbreeding which cannot be reliably assessed by microsatellites	Panel of 27 microsatellites + 2 sex markers, chosen among a list of 150 tested
Challenges	Improving sampling in the Vosges and Alps mountains	Working with scats means low DNA concentration and quality but the lab has a long experience using non-invasive samples for genetics studies	• Need for methods harmonization between three different labs analyzing samples (for instance with the Bavarian forest National Park) • Need for harmonizing data collection between regions	None

* The numbers mentioned are the ones gathered at the time of writing this document.

Towards a greater transnational cooperation in the monitoring of lynx genetics in Western Europe

Long-term cooperation in lynx conservation and monitoring already exists to some extent in Europe (Appendix). More specifically in Western Europe, some collaborations are already set-up about individuals and genetic monitoring between France, Germany and Switzerland, which share lynx populations. The prerequisite for a well-established and open cross-border cooperation about lynx genetics is to find common interests and synergy between respective research questions (Table 3).

***Table 3**: Summary of further cooperation steps about lynx genetics monitoring in Western Europe based on current knowledge of projects. Source: SFEPM 2021.*

	1. Identification of common interest(s)	2. Harmonization of methods
1. Research question(s)	**Studying genetic diversity and inbreeding** seem common to all identified research questions of the different structures (Table 2). Therefore, sharing common molecular markers seem to be an essential first step.	
2. Genetic markers	The number and type of markers used depend on the research question, on the genetic variability of the studied population and on the type of samples used. All partners do not need to share the exact same panel but a **minimum overlap of chosen markers must exist to allow common individual identifications**. Finding this minimum agreement could mean adding a few new markers to the database for new labs joining an established consensus.	**Genetic working group**: Very recently, a cross-border genetic monitoring was initiated through the establishment of the CElynx consortium with four labs (among which three have a long-term experience of non-invasive samples): KORA (Switzerland) with Christine-Breitenmoser, Brno University (Czech Republic) with Jarmila Krorerová-Prokešová, Ljubljana University (Slovenia) with Tomaz Skrbinsek and Senckenberg (Germany) with Carsten Nowak. **Harmonizing markers:** The first goal of CElynx is to formalize an official microsatellite core marker system (minimum of 15 recommended by the Bonn Lynx Expert Group 2021) in 2022. CElynx has a list of markers used by different labs in order to be considered in the markers choice. **Joining CElynx:** Any new lab is welcome to join and to use the common set of markers once it has been defined. **Calibration:** A common ring testing of microsatellites markers will be required through the exchange of calibration samples to test if the results found are the same. It is a crucial step for cross-comparison between samples and between type of samples (invasive / non-invasive). **Continuous regular exchanges** of data and protocols to ensure partners refer to the same individuals, especially for long distance migrants. **Analysis method:** LCE aims to use genotyping by sequencing but the lab is open to discussion regarding the use of SNPs or other appropriate methodology. As the project is relatively recent, they explained that it could still be time to adapt methods if needed.
3. Sampling strategy	Synergy can be found in terms of sampling across a shared population but also within a country. For instance in France, two types of sampling occur (Table 2). It could be **spatially (and maybe temporally) optimized** through a complementary approach of the two methods with a focus in the Vosges and the Alps areas. Another example is that in Germany, lynx monitoring is organized through regional authorities, or National Parks when they are concerned. **Harmonization of the monitoring standards** throughout the country would certainly be beneficial for research and conservation projects.	**Mapping samples locations:** the lynx samples locations have to be mapped to see where there are overlaps or gaps in sampling and therefore develop a complementary strategy and optimize sampling area. This sampling could also be public on an open and updated map such as with the KORA monitoring Center (www.koracenter.ch) **Work meeting:** a meeting between French partners is required to discuss improvement in data collection of invasive and non-invasive samples. This could be done as part of the National Action Plan (PNA).

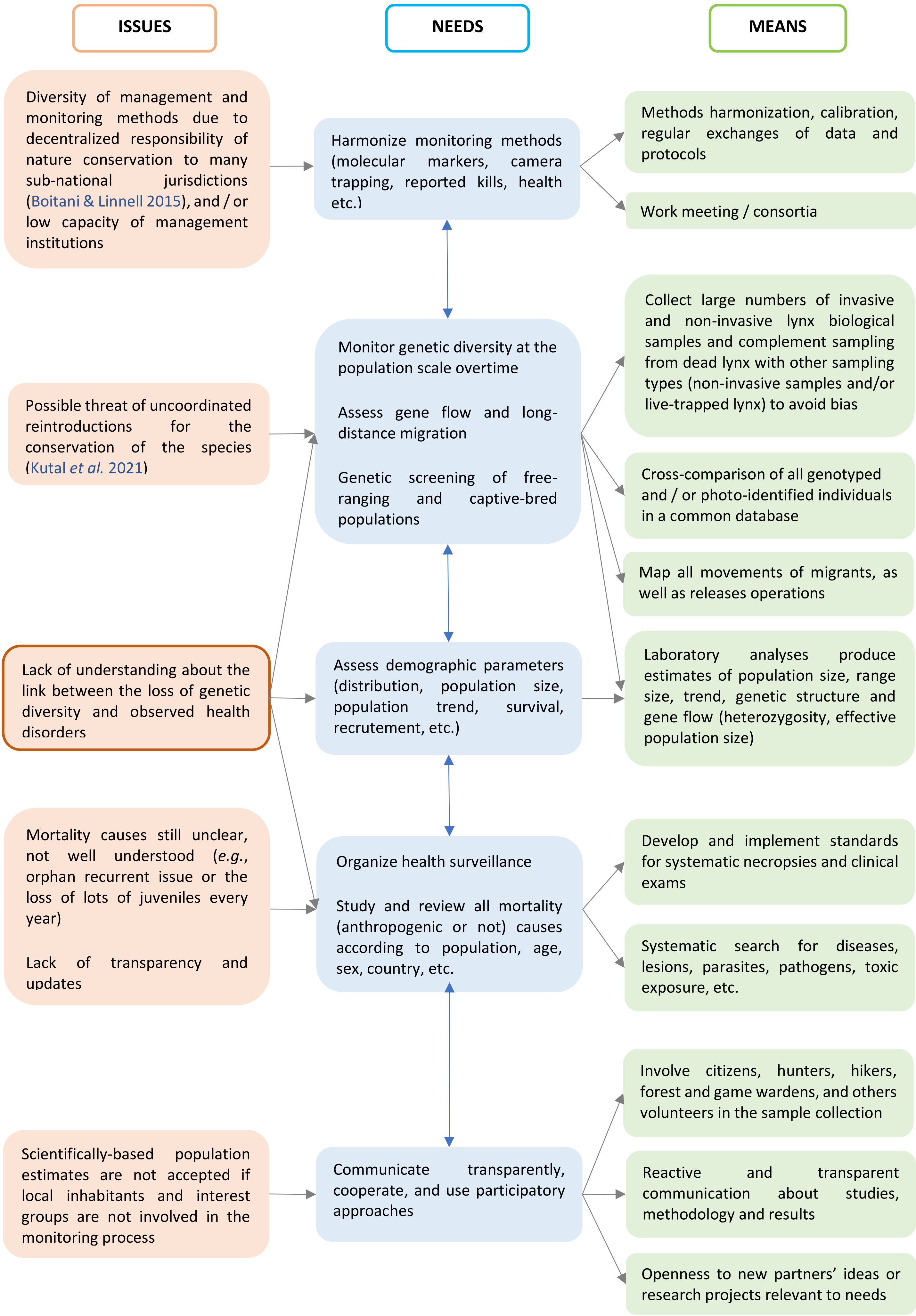
Prospects for a future transnational lynx monitoring project: needs and priorities for action

Compared to the brown bear and the grey wolf, the lynx monitoring has often been neglected in the past. Yet, its populations require precise transboundary monitoring at the relevant scale to adapt conservation actions and ensure the viability of the species. More specifically, lynx systematic genetic monitoring should become a general practice in the next decade (Melovski *et al.* 2021) as it allows very precise estimates relevant for conservation management. For instance, huge amount of information has been gathered about a small brown bear population in Austria over a six years project involving genetic monitoring (number of individuals, sex ratio, genetic variation, relationships, pedigree, ranges, preferred sites, and migration) (Kruckenhauser *et al.* 2009). This has allowed researchers to better understand the status of the population and thus to inform further management decisions and the specific need for international efforts for the long-term survival of the species.

The present report documents the actual state of knowledge that has been gathered and the first steps for a wider cooperation about lynx genetics monitoring in Western Europe between the identified structures in charge. However, the issues to tackle for lynx conservation do not only concern genetics. Genetic impoverishment starts to be a problem as soon as it leads to health disorders (*e.g.,* increase of susceptibility to infectious diseases, malformations, reproductive problems, histological lesions) and therefore reduces the adaptive potential of the species. Although no systematic malformations and no reproductive problems have been observed as the Jura and Alps lynx populations increase, the relationship between genetics, health and demography is currently being studied (reported in SFEPM 2021). Indeed, on the Swiss side, KORA, joined by Senckenberg (Germany) and more recently by the OFB (France), have identified the following question: “to what degree the loss of genetic diversity and inbreeding impact lynx populations and their long-term conservation in Western Europe?”

Therefore, a priority action would be to establish a transnational project on genetic, health and demography monitoring in Western Europe, while following the recommendations of the Bonn Lynx Expert Group (2021). This project will require to obtain long-term datasets in genetics, and about the life history and fitness of individual lynx in a standardized way across various regions.

The following part does not have the claim to expose all gaps, needs and recommendations prior to set such a project, but lists the ones that have been identified within this preliminary work, in addition to the previous Harmonization of methods (Table 3) between partners.


ISSUES
NEEDS
MEANS
Diversity of management and monitoring methods due to decentralized responsibility of nature conservation to many sub-national jurisdictions (Boitani & Linnell 2015), and / or low capacity of management institutions
Harmonize monitoring methods (molecular markers, camera trapping, reported kills, health etc.)
Methods harmonization, calibration, regular exchanges of data and protocols
Work meeting / consortia
Collect large numbers of invasive and non-invasive lynx biological samples and complement sampling from dead lynx with other sampling types (non-invasive samples and/or live-trapped lynx) to avoid bias
Monitor genetic diversity at the population scale overtime
Assess gene flow and long-distance migration
Genetic screening of free-ranging and captive-bred populations
Possible threat of uncoordinated reintroductions for the conservation of the species (Kutal *et al.* 2021)
Cross-comparison of all genotyped and / or photo-identified individuals in a common database
Map all movements of migrants, as well as releases operations
Lack of understanding about the link between the loss of genetic diversity and observed health disorders
Assess demographic parameters (distribution, population size, population trend, survival, recrutement, etc.)
Laboratory analyses produce estimates of population size, range size, trend, genetic structure and gene flow (heterozygosity, effective population size)
Mortality causes still unclear, not well understood (*e.g.*, orphan recurrent issue or the loss of lots of juveniles every year)
Lack of transparency and updates
Organize health surveillance
Study and review all mortality (anthropogenic or not) causes according to population, age, sex, country, etc.
Develop and implement standards for systematic necropsies and clinical exams
Systematic search for diseases, lesions, parasites, pathogens, toxic exposure, etc.
Involve citizens, hunters, hikers, forest and game wardens, and others volunteers in the sample collection
Scientifically-based population estimates are not accepted if local inhabitants and interest groups are not involved in the monitoring process
Communicate transparently, cooperate, and use participatory approaches
Reactive and transparent communication about studies, methodology and results
Openness to new partners' ideas or research projects relevant to needs

Conclusion

Regardless of conservations policies and protection status, 61% of the 31 largest carnivores occurring around the globe are threatened (vulnerable, endangered, or critically endangered) and at risk of local or total extinction. Some are even disappearing while their role in the ecosystems and their ecological value were still not fully understood (Ripple *et al.* 2014). It is however well known that apex predators, such as lynx, contribute to the ecological functionality of ecosystems and preserve their evolutionary potential. Conserving large carnivores like lynx and their associated ecosystem, is thus all the more important.

After having disappeared from many countries for about 200 years, the lynx is gradually recolonizing Europe and its situation has improved thanks to conservation efforts across Europe. However, numerous conservation plans and projects highlight the fact that most of its populations are still threatened. Key actions spanning in different fields are defined for lynx conservation (Boitani *et al.* 2015) but accurate lynx populations monitoring is an absolute necessity. Yet, monitoring practices are still diverse and vary in applied methods, transparency and quality. This calls, first and foremost, for the need of information sharing, review of knowledge, and more cooperation between countries to get a common understanding of lynx populations and insure the long-term recovery of a large metapopulation. Additionally, although experts are legitimate stakeholders to agree and set up consensual actions in this direction, involvement of relevant authorities, other interest groups and the civil society can be trickier. Accounting the human dimensions is crucial for conservation actions and long-term dialogue is therefore required to meet cooperation efforts and willingness (Drouilly & O'Riain 2021).

France, Germany and Switzerland hold and share four of the lynx populations in Europe which are among the smallest ones on the continent and are thus of genetic concerns. The focus of this document was to report these genetic challenges for the lynx in Western Europe, as well as presenting the projects addressing them in France, Germany and Switzerland. Ultimately, the aim was to assess the perspectives for a transnational cooperation. However, as for the One Health perspective, lynx genetics should not be considered separately from lynx demography and health.

From the state of knowledge gathered in the context of this LIFE EuroLargeCarnivores mission, a wider project involving the cross-border monitoring of lynx in Western Europe should be set up and implemented in the coming years. Some needs and recommendations are exposed in this document as the first basis for such a project, which will require further inputs from experts.

Following the example of similar projects being undertaken on other large carnivores would also be helpful in project planning. For instance, a recent Interreg LoupO project 2020-2022 (www.loupo-eu.com) is currently developing a cross-border monitoring of brown bear and wolf populations in the Pyrenees mountain range, between Spain, France and Andorra. In Central Europe, a Pan-Carpathian conservation strategy is being developed to provide standardized lynx monitoring system across the region (Kubala *et al.* 2021).

This report does not replace expert knowledge and judgement and should only be used to inform potential project decision makers for a transboundary monitoring of the shared lynx populations in Western Europe, in line with the already existing cooperation dynamic. Approval from the lynx genetics workshop participants (SFEPM 2021) is required prior to sharing this report.

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APPENDIX: non-exhaustive list of cooperative projects on Eurasian lynx monitoring (listed from local to continent scale)

NAME	TYPE	COUNTRIES INVOLVED	TIME FRAME	TOPIC
LIFE Lynx Palatinate	LIFE project	France, Germany	2015-2021	Reintroduction of the Carpathian lynx (20 wild born lynx from Switzerland and Slovakia) in the Palatinate forest.
KORA-OFB partnership	Convention in 2020 but historical cooperation for lynx monitoring	France, Switzerland	2020	Improvement of knowledge on genetic diversity and gene flow, as well as on demography and health about lynx Jura populations and neighboring populations.
LIFE Lynx visiting LECA Institute in Grenoble (Source: link and Marjeta Konec pers. comm. 12/01/2022)	Experience-sharing visit	France, Slovenia, Switzerland	2021	Optimizing the wet lab protocols and bioinformatics pipeline in order to end up with a standardized and cost effective method for non-invasive genetic samples. Benefit of the method is that there is no need for calibration between different laboratories and could thus be ultimately applied to large scale (cross-boundary) genetic monitoring for lynx.
Expert Committee Lynx	Expert group established under the Upper Rhine Conference	France, Germany, Switzerland	Since 2016	Demographic and genetic monitoring, interconnection and acceptance for the establishment of an Upper Rhine lynx metapopulation (Jura, Vosges-Palatinate Forest and Black Forest populations).
CElynx (reported in SFEPM 2021)	Consortium / working group with 4 current members : KORA, Brno and Ljubljana Universities, Senckenberg Research Institute	Germany, Czech Republic, Slovenia, Switzerland	Recent (start year is not known)	Cross-border lynx genetic monitoring. It is one implementation of the Bonn Proceedings with the Bonn Lynx Expert Group.
SCALP project (Status and Conservation of the Alpine Lynx Population) (Molinari-Jobin *et al.* 2021)	SCALP expert group founded by KORA	Austria, Bosnia, France, Germany, Italy, Croatia, Liechtenstein, Slovenia, Switzerland	Since the early 1990s	Ongoing program aiming to coordinate the lynx monitoring, conservation and management activities in the Alps, but the monitoring approach has recently been expanded to the neighboring Dinaric, Jura and Vosges Mountains as well as the Palatinate and the Black Forest. Common approach of monitoring data presentation (SCALP criteria) and yearly distribution maps.
EAZA breeding program (Lengger *et al.* 2021)	Genetic screening by Senckenberg Research Institute and Natural History Museum Frankfurt	Not known	Since 2002	Eurasian lynx European StudBook since 2002. Full genetic evaluation (phylogenetic origin, genetic variability) of the current zoo population. Detailed protocols for breeding, husbandry, training and rewilding.
Eurolynx (Heurich *et al.* 2021)	Bottom-up collaborative network of 42 partners	19 countries across Europe	Since 2018	Open collaborative project based on a spatial database to investigate variation in lynx behavioral ecology along environmental gradients or population responses to specific conditions.
Bonn Lynx Expert Group 2021	Group of 53 lynx experts across Europe	19 countries across Europe	Since 2019	Recommendations and standards for the conservation and management of viable populations and metapopulations of Eurasian lynx in Continental Europe. The work of Bonn Lynx Expert Group 2021 was submitted to the Secretariat and the Standing Committee of the Bern Convention, which adopted the Recommendation No. 204 (Standing Committee 2019).
Large Carnivores Initiative for Europe (LCIE)	IUCN SSC (Species Survival Commission) Experts group	Europe	Not known	Conservation of large carnivores in Europe through coordination and networking between projects run by LCIE group members and partners, as well as inspiration and guidance to the wider conservation community. Production of a common report every 6-7 years about the status of the lynx and other large carnivores across Europe.
Cat Specialist Group	IUCN SSC (Species Survival Commission)	57 countries worldwide	Since 1971	Long-term conservation of the 40 wild living cat species and their habitats by means of continuous monitoring and assessment, information-sharing, identification of conservation priorities and facilitation/delivery of these priority actions through collaborative conservation work.

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