Preventing the extinction of the Dinaric-SE Alpine lynx population through reinforcement and long-term conservation

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Guidelines for Ensuring the Long-term Viability of Lynx in the Dinaric Mountains and South Eastern Alps

(An update for the Common guidelines for Dinaric - SE Alpine population level lynx management, objective "Maintaining of genetic diversity and avoiding inbreeding depression")

Action D.3

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LIFE Lynx

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Introduction

The Eurasian lynx (*Lynx lynx*) is known for its broad ecological adaptability, historically spanning diverse habitats from Scandinavian forests to the Mediterranean and the Black Sea regions (Kratochvil, 1968; Matjushkin, 1978). Until the early 19th century, the lynx persisted in many parts of Western and Central Europe (Kratochvil, 1968), but by the early 20th century human activities and habitat loss led to the lynx extinction from much of this range, including Dinaric mountains. A successful reintroduction was performed in 1973, with six lynxes from Slovakia being released into the Dinaric Mountains. This reintroduction initially flourished, with the lynx population expanding its range and numbers.

However, the reintroduced population remained isolated, which, together with a small number of founders, led to a significant decline in genetic diversity already by the 1990s. The first genetic survey of the Dinaric lynx population (Sindičić et al. 2013) included samples originating from early years after the reintroduction until 2010 and showed that the population had the lowest genetic diversity of all lynx populations studied so far. Already by the early 2000s, the average inbreeding coefficient exceeded 0.25, which is expected in a brother-sister mating. A subsequent pre-reinforcement baseline study within the LIFE Lynx project (Skrbinšek et al., 2019) which used 217 samples from Slovenia, Croatia, and from source populations in Slovakia and Romania, confirmed that inbreeding continued to increase. Very high inbreeding was confirmed in this population also using genomic data (Mueller et al., 2022). Despite an initially successful period following the 1973 reintroduction, evidenced by relatively high effective population size estimates indicative of expansion, the population suffered a significant loss in genetic diversity during the reintroduction bottleneck. High genetic drift caused by the small effective population size and limited number of unrelated mates immediately after the reintroduction caused rapid increase of inbreeding and the related drop in heterozygosity. Although the population seemed stable in the 1980s with an estimated inbreeding coefficient of Fe = 0.176, by the 1990s this parameter reached Fe = 0.192. In the last three years before the 2019 population reinforcement, inbreeding reached Fe = 0.316, with corresponding expected drop in fitness of 85% (Skrbinšek et al., 2019). All in all, the population has not been doing well from the genetic perspective, and the field data indicate that it was going into the “extinction vortex” (Frankham et al. 2002). It’s difficult to predict exactly when the population would go extinct without intervention, but there is little doubt that extinction would be a matter of “when” rather than “if”.

The population reinforcement and recreation of the connectivity between populations can help to mitigate inbreeding depression and, at the end of the day, rescue the population from extinction. From 2019 to 2023 we translocated 18 lynx from the Slovakian and Romanian Carpathians to the Dinarics (12) and Julian Alps (6) within the LIFE Lynx project. Additionally 4 lynx were translocated to the Julian Alps within the ULyCA2 project. Among these 22 translocated lynx, 13 had successfully reproduced by 2024 (Fležar et al. 2024). Translocations to the Alps are considered “stepping stone” reintroduction as there were no remnant lynx in the area, and the Alpine part is partially isolated from the population in the Dinaric Mts. by a linear barrier formed by Ljubljana - Trieste highway. Obtained genetic, telemetry tracking and camera trapping data indicate the highway as a major barrier for animal dispersal and thus potential gene flow (Kuralt et al. 2023, Fležar et al. 2024). During the project, we obtained genotypes of the remnant and translocated lynx as well as genotypes of their offspring to study how the inbreeding would change if the translocated animals managed to successfully reproduce and include their genes into the population. Based on the empirical data, if the introduced animals and their offspring would form 15% of the total population, the inbreeding would drop to 0.18 (Fležar et al. 2023). While this is still high, it is closer to the range we observed in the 1980s when population still seemed viable (Skrbinšek et al. 2019). If the translocated animals and their offspring formed a large part of the population (40% in the sample), inbreeding would drop to 0.15 (Fležar et al. 2023).

These guidelines are an update to the Common guidelines for Dinaric - SE Alpine population-level lynx management (2022) and cover the “Maintaining the genetic diversity and avoiding inbreeding depression” objective. They include the data collected in the final part of the LIFE LYNX project, and take into account the stepping stone population established in the Alps. As such, they should be used in conjunction with the Common guidelines for Dinaric - SE Alpine population level lynx management (2022).

Updated individual-based genetic model

We updated the individual-based genetic model, described in Pazhenkova, Skrbinšek (2021) to include the latest LIFE Lynx genetic data and the newly established Alpine stepping stone. The updated model was used to explore different long-term genetic management scenarios for the Dinaric - SE Alpine lynx population, and provide recommendations.

Modelling process

We performed forward-time individual-based simulations using python library SimuPOP v. 1.1.8svn (Peng and Kimmel, 2005). The modelling basis is described in detail in a previous publication (Pazhenkova and Skrbinšek, 2021). We simulated three populations: Dinaric, SE-Alpine (further: Alpine) and Carpathian. Genotypes for each individual were estimated from the empirical allelic frequencies, updated after the monitoring seasons

2021-2022 and 2022-2023. Genotypes for the Alpine population were sampled from allelic frequencies of the Carpathian population (Romania + Slovakia). While we first considered sampling the "real" allelic frequencies of translocated individuals, the sample size was too low to accurately represent the genome-level genetic diversity of the source populations, causing a considerable sampling bias. We measured inbreeding using the total inbreeding coefficient F_{IT} (Wright 1931).

The simulation start was the year 2021, when the core of the Alpine stepping stone population was already established. We modelled the Alpine population as six founder animals. We simulated reinforcement through translocation of 12 animals (equivalent to the number of lynx released in the Dinaric mountains) from the source (Carpathian) to the Dinaric population over a two-year period, despite the fact that in reality the reinforcement was performed between 2019 and 2023. We made this simplification to explicitly simulate the integration of the translocated animals into the Dinaric population. The effect of the reinforcement on the genetic diversity of the population was similar as we observed in the previous modelling results (Pazhenkova and SkrbinŠek, 2021).

We also modelled a gene flow between the Alpine and the Dinaric population. The gene flow was controlled by two parameters: start of migrations and migration rate, measured as a proportion of migrated individuals from the total population size per year. We tested four scenarios of bidirectional gene flow: no isolation (panmixia), moderate gene flow (10%), low gene flow (1%), and complete isolation. For each of these scenarios we estimated the minimal number of individuals for different time intervals to be translocated from the Carpahtian population to keep the inbreeding coefficient at the optimal level. According to the recommendations for the conservation of the Eurasian lynx, accepted by the Bonn Lynx Expert Group, isolated populations should be managed to keep the F_{IT} below 0.15, and when the inbreeding coefficient increases above 0.25 immediate action is needed (Bonn Lynx Expert Group, 2021). If a simulated translocation scenario succeeded in decreasing the inbreeding coefficient below the 0.15 threshold in the entire period between translocation actions, the scenario was considered successful. We gradually increased the time intervals between translocations (translocations performed each 3, 5, 10, 15 and 20 years), and for each time interval we estimated the minimum effective number of translocated lynx that would provide a successful outcome. Another possible conservation solution is an assisted migration: exchanging animals between Alpine and Dinaric populations. We estimated a necessary minimum number of translocated animals for cases of medium and low gene flow as well as for complete isolation.

Results

Effect of the stepping stone

We estimated the effect of gene flow between Dinaric and SE Alpine subpopulations, assuming no further population reinforcements would be done after the LIFE Lynx project. Even in case of complete isolation of the stepping stone population in the SE Alps (Figure 1, upper left), translocations done within LIFE Lynx significantly delayed the inbreeding increase to the levels that could threaten the population - for 22 years after the project the inbreeding level stayed below the 0.15 threshold. However, in absence of further population reinforcement, after 48 years the inbreeding level exceeded 0.25 threshold, which is equal to the full-sib mating and considered a critical threshold for immediate action (Bonn Lynx Expert Group, 2021). The SE Alpine stepping stone population kept the inbreeding below 0.15 threshold for all the 50 years of the simulations even in case of isolation (Figure 2).

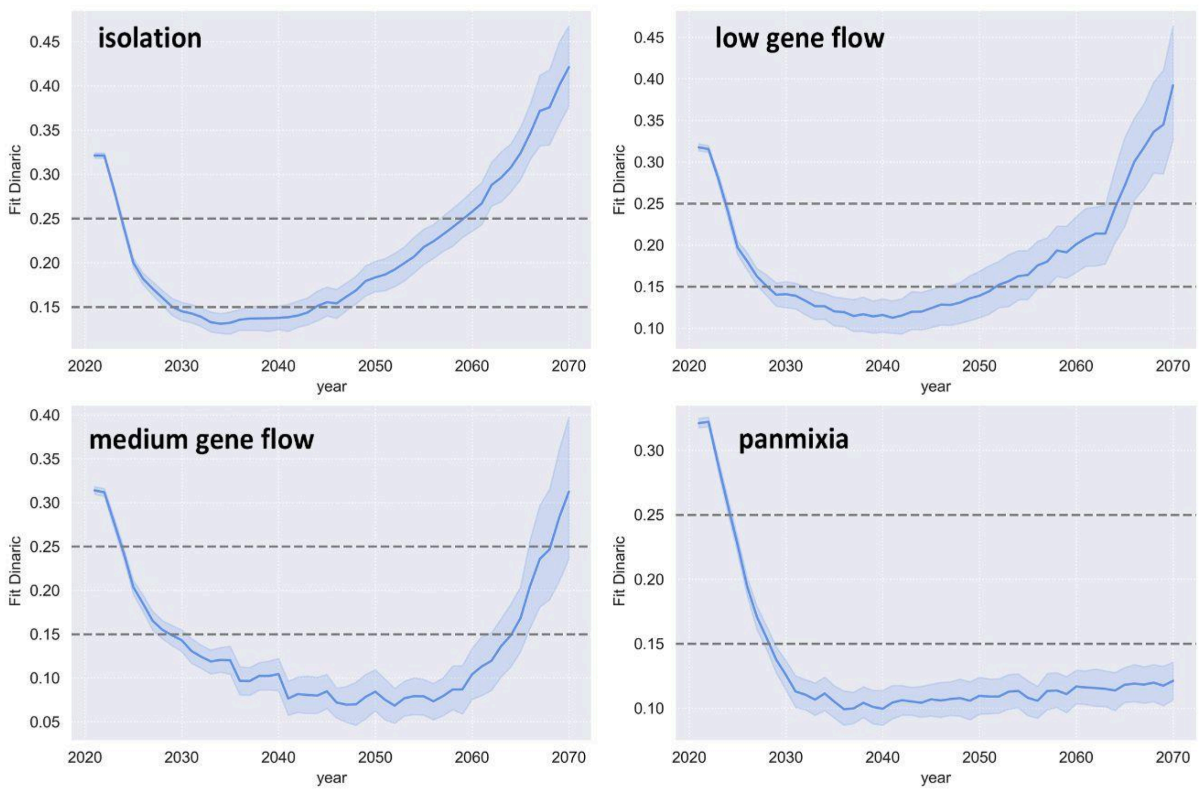

Figure 1. The inbreeding, measured as Fit of the Dinaric lynx population with different modelled levels of gene flow between Dinaric and Alpine populations. Grey dashed lines indicate upper and lower inbreeding thresholds.

If we consider Dinaric and SE Alpine subpopulations having a completely shared gene pool (panmixia), the inbreeding stays low for at least the next 50 years according to the model in the absence of any additional factors, affecting the survival (Figure 1, lower right). However, this scenario is not realistic because of limited landscape permeability (particularly the linear barrier posed by the Ljubljana - Trieste highway), and actual gene flow between both subpopulations should be estimated through further monitoring. We modelled two more realistic scenarios: moderate (exchange of individuals between both subpopulations 10% of the stepping stone population size each year) and low (1%). Even the low gene flow delayed the increase of inbreeding above the 0.15 threshold for approximately 30 years after the reinforcement (Figure 1, upper right). Moderate migration between both subpopulations kept the inbreeding below the threshold for 43 years after LIFE Lynx translocations (Figure 1, lower left).

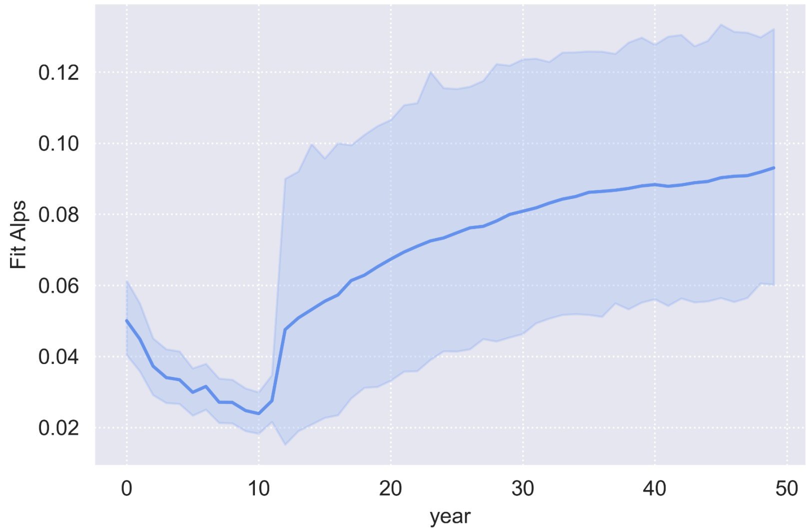

Figure 2. The inbreeding, measured as Fit of the Alpine stepping stone population in case of isolation between Dinaric and Alpine populations.

The establishment of the new population in the Alps seems to make a huge difference for the lynx survival in the entire Dinaric-SE Alpine complex, considerably improving the conservation outcomes. The translocation of the new animals to the Dinaric population, which was after long isolation and low effective population size already extremely inbred, provided only a relatively short-term solution for population's survival. While the first generation descendants of the translocated animals are completely outbred and can be expected to have high fitness, backcrossing through subsequent generations will more quickly increase inbreeding than what we would expect if the population was started with unrelated outbred founders. The gene flow between Dinaric and SE Alpine subpopulations, if established and high enough, can lead to a much more robust conservation outcome, but it is crucial to continue with genetic monitoring of the population to detect actual connectivity between both subpopulations, and adapt management accordingly.

Optimization of the management scenarios

According to our simulations, the reinforcement performed within the LIFE Lynx project had a significant impact on the genetics of the Dinaric lynx population. The simulations suggest that total inbreeding would remain below the optimal threshold of 0.15 for 22 years after the translocations, even without any gene flow between SE Alpine and Dinaric populations or additional reinforcements (Figure 1). This implies that the critical measures to prevent population extinction due to genetic erosion were already successfully done. Nevertheless, to ensure the long-term viability of the population, it is crucial for the conservation measures to continue. Possible strategies include systematic reinforcements with individuals from source populations, as well as facilitating gene flow (assisted or natural migration) between neighbouring populations. Nowadays the most important barriers for lynx movement include densely continuously populated (urban) areas, intensive agricultural lands and transport infrastructure. The possible solution for emphasising population connectivity might be increasing the permeability of Ljubljana - Trieste highway via e.g. building the green bridges. We estimated the minimum number of animals that would need to be integrated into the Dinaric population per action (translocation or assisted migration) to keep inbreeding below the 0.15 threshold, with regard to different levels of connectivity between the Alpine and Dinaric subpopulations. We simulated different time intervals between actions (translocations or exchange of the individuals were performed each 3, 5, 10, 15 and 20 years), and for each time interval we estimated the minimum effective number of translocated lynx that would provide a successful outcome. According to the modelling, a medium gene flow (10%) between Dinaric and SE Alpine subpopulations allows for the minimum reinforcement effort, but this scenario is rather optimistic considering restricted landscape permeability. A more realistic scenario with a 1% migration rate requires at least one effective migrant per five years, either from the stepping stone subpopulation or Carpathian population. If the Dinaric subpopulation remains fully isolated, one migrant per three years is needed to ensure the long-term viability of the population. An acceptable alternative are less frequent actions with more translocated animals (Table 1), but the following criteria should be taken into account to improve the effectiveness of the reinforcement actions: ensure appropriate intervals between actions to avoid changes in management plans on the administrative level, consider the economic feasibility of translocation strategies, and assess the population's robustness in case of a management failure (Pazhenkova & Skrbinsek, 2021).

Table 1. The minimum number of animals integrated into the Dinaric population per action that would allow the inbreeding to remain below the 0.15 threshold under the different gene flow conditions. Symbol ⇄ means the assisted migration between the Alpine stepping stone and Dinaric subpopulations, "translocations" means population reinforcement via translocation from the Carpathian population.

	isolation		***1% migration***		***10% migration***	
Year interval	⇄	translocation	⇄	translocation	⇄	translocation
3	1	1	1	1	1	1
5	2	2	1	1	1	1
10	5	4	3	2	1	1
15	6	5	5	3	2	1
20	8	5	8	5	2	2

Strategy for maintaining of genetic diversity and avoiding inbreeding depression

(updated objective of the "Common guidelines for Dinaric - SE Alpine population level lynx management")

While a one-time reinforcement effort, such as that executed in the LIFE Lynx project, represents a significant step towards genetic rescue within the Dinaric - SE Alpine lynx population, it is important to underline that it alone cannot fully address the complexities of long-term genetic viability. Continuous monitoring of population development and the impact of the translocated animals on genetic integrity is crucial. Special attention must be paid to population genetics metrics, including the inbreeding coefficient (F_{IT}), number of alleles per locus (A), observed and expected heterozygosity (Ho, He), and effective population size (Ne). According to the recommendations for the conservation of the Eurasian lynx by the Bonn Lynx Expert Group, isolated populations should be managed to keep the inbreeding coefficient (F_{IT}) below 0.15, and if the inbreeding coefficient rises above 0.25 immediate action is required (Bonn Lynx Expert Group, 2021).

Regular genetic monitoring plays a critical role in preventing the Dinaric- SE Alpine lynx population from dropping into the “extinction vortex” again. A comprehensive monitoring system will allow us to detect early signs of declining genetic diversity before the population starts spiralling towards extinction. Genetic data can also illuminate other threats, such as habitat fragmentation, which can lead to creation of isolated subpopulations and a further decline in population’s viability. Therefore, it is essential that results provided by regular genetic monitoring form the basis for management decisions.

To prevent genetic erosion and ensure the population's continued viability, a long-term strategy for genetic management of the population must be implemented. Such a strategy should be based on the results provided by genetic monitoring and population development models. It is recommended that such models are revised and the strategy updated when new data on the population development becomes available. Based on the data collected during the LIFE Lynx project, we updated the previously developed model (Pazhenkova & Skrbinsek, 2021). We explored the effects of the stepping stone on the reduction of inbreeding in the Dinaric population, and formulated the scenarios that would ensure long term population viability. In the scenario of 1% migration between the Dinaric and the SE Alpine subpopulation, we would need a translocation of at least one animal from an outbred population (Carpathians) per five years (Figure 1, Table 1). This low-level natural gene flow between Dinarics and the Alps could also be aided by assisted migration through translocation of animals between Dinaric and SE Alps. If the Dinaric part of the population remains fully isolated, one translocated animal from an outbred population per 3 years would be needed to ensure long-term viability (Table 1).

Apart from further reintroductions and assisted migration, the management goals should be directed towards a natural connection of Dinaric - SE Alpine population with other lynx populations in Europe. The population “stepping stone” established in the Julian Alps within the LIFE Lynx project serves this purpose. The Julian Alps are within the average dispersal distance from the current lynx population in the Dinaric Mountains of Slovenia, but improving connectivity between these areas would help maintain adequate natural gene flow between the stepping-stone nucleus and the core population, for which permeability of the Ljubljana-Trieste highway is of particular importance. Lynx expansion in this and other areas should be supported with activities that would increase lynx acceptance among the general public and the key local interest groups. Further stepping-stone nuclei should be created in Slovenia, Italy and Austria with a final goal of connecting the Dinaric-SE Alpine population with other, currently isolated lynx populations in the Alps (Molinari-Jobin et al. 2003). This would create a functional meta-population across the NW Dinaric Mountains and the Alpine arc, and ensure gene flow, reducing the need for further translocations from Carpathians.

Title of the action	Maintaining genetic diversity and avoiding inbreeding depression
Objective(s)	Ensure that inbreeding in the Dinaric and SE Alpine lynx population remains at an acceptable level in the long term. Ensure connectivity of the Dinaric-SE Alpine population, creating a functional meta-population across the Dinarics and the Alpine arc.
Description of the activities	Regular monitoring of the population. Lynx translocations according to the proposed optimal scenarios given the actual Dinaric- SE Alpine population connectivity. Timely updates of the strategy using the monitoring data and new insights from forward-time population development simulations performed with the new data. Creation of new stepping-stone nuclei in Slovenia, Italy and Austria.
Expected results	The population inbreeding level is kept constantly below 0.15. Increased observed heterozygosity as an indicator of genetic variability compared to the baseline value. Improved long-term viability of the population. Connection of the Dinaric – SE Alpine population with other Alpine populations, Balkan and Carpathian populations.
Responsible for implementation	Management authorities of each involved country.
Actions that need to be implemented beforehand	Coordination of translocation planning with monitoring of the genetic status and connectivity of the population. Implementation of the strategy for genetic management of the population.
Means of assessing success	Genetic parameters of the lynx population estimated from empirical data.

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