NATUR VÅRDS VERKET

SWEDISH
ENVIRONMENTAL
PROTECTION
AGENCY

2024-06-20

Bilagor till analys av vargens referensvärde i fråga om populationsstorlek för gynnsam bevarandestatus

Innehåll

SWEDISH
ENVIRONMENTAL
PROTECTION
AGENCY

Analyses regarding the reference value of wolves in Sweden

In May 2022, the Swedish Government commissioned the Swedish Environmental Protection Agency (SEPA) to investigate, based on the best available knowledge and scientific expertise, if and under what circumstances, the reference value for wolf regarding population size, as defined for favourable conservation status according to the Habitats Directive, could be in the interval between 170 - 270 individuals, as stated in the parliament proposition from 2012 (prop. 2012/13:191).

The most recent evaluation of reference value for wolf in Sweden was conducted in 2015 (Bruford 2015; Liberg et al. 2015; Mills & Feltner 2015). In the commission from the Government, it is clearly stated that there is no reason to question the results from these analyses but that new knowledge regarding the Scandinavian wolf population has been developed since the last evaluation. There are thus good reasons to investigate this again.

The results from the analyses outlined in this document will provide the scientific basis for this evaluation. In order to predict the effects of different population sizes and levels of migration, forward modelling approaches should be used. It is important to consider both demographic and genetic viability. Specific data (input parameters) for the Scandinavian wolf population (both genetic/genomic and demographic) will be provided by Swedish and Norwegian researchers (the Skandulv research consortium: SKANDULV - the Scandinavian Wolf Research Project). The most recent scientific data and results (for example on genome wide estimates of inbreeding, levels of inbreeding depression, generation time and relatedness of founder individuals) should be used for analyses. While the reference value is only applicable to the Swedish part of the Scandinavian wolf population, analyses should treat the Norwegian and Swedish parts, biologically, as one homogenous sub-population.

The modelling results should be visualised as clearly as possible to facilitate management decisions, and to evaluate the likely outcomes of choosing different reference values for population size. Below are possible examples of figures visualising results from genetic viability modelling:
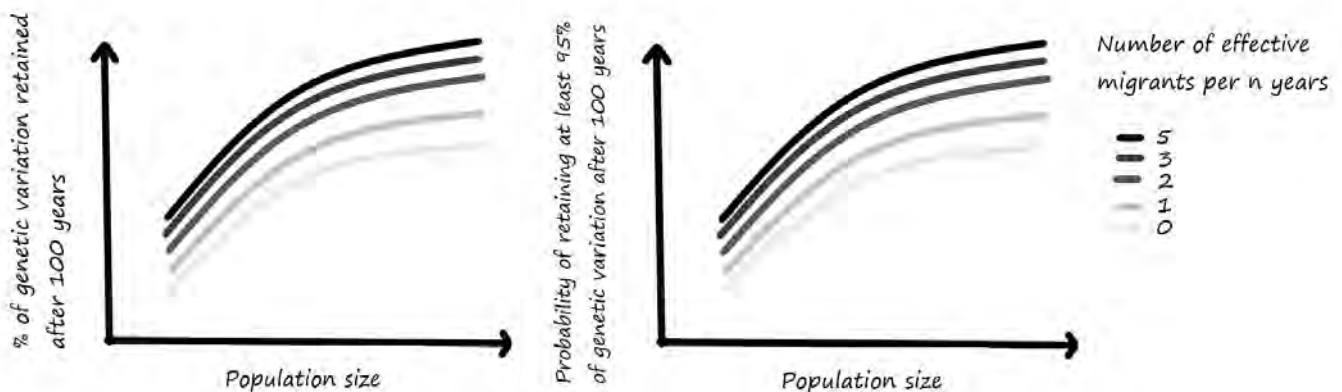
Similar figures should also be produced visualising results for risk of demographic extinction (for example <10% risk of extinction after 100 years).

The range of values for the x-axes (population size) should include at least the range of 170-270 individuals, but could extend further in both directions.

Tabulated values for predicted outcomes should include (but are not restricted to) population sizes of:
170 (lower end of the interval from the parliament proposition)
210 (lower end of interval + 40 individuals in Norway)
270 (higher end of the interval from the parliament proposition)
300 (currently used reference value)
310 (higher end of interval + 40 individuals in Norway)
340 (currently used reference value + 40 individuals in Norway)

Other important considerations

It should be noted that the population estimates which will form the basis for analyses refer to the population size during the beginning of the census period (autumn). All results are also expected to be applicable to this time of the year. In modelling of demography, timing of events should thus be entered in the following order: input population size -> mortality -> reproduction -> output population size. In the reporting guidelines for the article-17 reporting to EU it is expressed that population size estimates should only include adults ("mature individuals". In you analyses it is therefore important that you explicitly state if results apply to the whole population (including offspring produced in the spring before the start of the census season), or only to "mature individuals".

Modelling will be carried out independently by two different international research groups. The reports outlining the results should be written in English. After delivering preliminary results from analyses the researchers will be given the opportunity to access and comment on the draft report from the other research group. Draft reports will also be available for comment by the data providers and SEPA. After this the research groups will have time to revise and update analyses before delivering a final report. The two research groups will also be expected to provide a "joint statement", outlining conclusions that can be agreed upon, given the results from both reports. If results from the two research groups differ substantially the possible reasons for the discrepancies should be discussed and clarified. The joint statement will aid the Swedish authorities in making policy and management decisions based on the conclusions from the analyses.

The full draft reports will be subjected to international peer review.

The contracted researchers are expected to be attending at least one digital start-up workshop in late 2022/early 2023 and one physical seminar during 2023 or 2024, funding for travels and accommodation will be provided by SEPA.

Joint Statement on the Results and Implications of Analyses Informing the Designation of Favorable Reference Value for the Wolf (*Canis lupus*) Population in Sweden

Philip S. Miller, Ph.D.
IUCN SSC Conservation Planning Specialist Group
and
Nic Dussex, Ph.D.
Norwegian University of Science and Technology

Project commissioned by:
Swedish Environmental Protection Agency

1 April, 2024

<u>Introduction – Setting the Context for the Analysis</u>

In May 2022, the Swedish government commissioned the Swedish Environmental Protection Agency (SEPA) to investigate, based on the best available knowledge and scientific expertise, if and under what circumstances the reference value for the wolf (*Canis lupus*) regarding population size, as defined for favourable conservation status according to the European Union's Habitats Directive, could be in the interval between 170 and 270 individuals as stated in the parliament proposition from 2012 (prop. 2012/13:191).

We conducted independent analyses using different simulation tools (Miller: Vortex (Lacy and Pollak, 2022); Dussex: SLiM (Haller and Messer 2019)) to explore the demographic and genetic characteristic defining wolf population viability in Scandinavia. Comparing the results from our independent simulations facilitates an assessment of the congruence and robustness of our results which will then be used by SEPA to develop recommendations for wolf population management in Sweden.

Before discussing specific aspects of these analyses, we believe it is important to highlight key underlying concepts and their definitions as presented in the European Union's guidance document on this broad topic (DG Environment 2023) in the context of the commission to SEPA and our associated work as described in this joint statement and in our separate reports. The guidance document defines "Favourable Reference Population" as "*Population in a given biogeographical region considered the minimum necessary to ensure the long-term viability of the species...*" (DG Environment 2023: 21). This definition invokes the concept of estimating a minimum viable population (MVP) size using methods of population viability analysis (PVA) that are customized for the species under consideration and the specific management situation for a given geographic region. The MVP typically represents the abundance required to eliminate or largely minimize the risk of extinction of the population or species over a defined time frame, usually 100 years from the date of analysis (e.g., Gilpin and Soulé 1986). In some instances, this metric also includes consideration of maintaining a minimum level of genetic variation (heterozygosity) over time to avoid detrimental levels of inbreeding and associated fitness impacts (inbreeding depression).

However, the guidance document goes further and recognizes the value of adopting a more expansive definition of viability – one that explicitly incorporates ecological functionality of a species within its native landscape in order to promote more robust ecosystems through time (e.g., Sanderson 2006; Redford et al. 2011). The guidance document (DG Environment 2023: 25) addresses the clear relationship between these two concepts:

> *"It is therefore important for favourable reference populations to reflect the 'long-term viable component of the natural habitat' at the level of the species across its natural range and distribution, rather than solely a minimum viable population."*

We therefore treat our contribution to this SEPA commission as addressing the identification of a minimum viable population size for wolves in Scandinavia. Translation of this MVP value to a population abundance incorporating larger-scale ecosystem functionality and representation – the favourable reference population (FRP) value – requires a process of "upscaling" as described in the guidance document (DG Environment 2023). Furthermore, we understand that this "upscaling" to a larger population abundance value representing the FRP value is outside the scope of the work described by our PVA efforts and is instead to be conducted by SEPA once our work is complete and fully documented.

Another important issue influencing the interpretation of our analyses concerns the ambiguity in the Habitats Directive and supporting documents (e.g., DG Environment 2023) around the explicit definition of population viability. To be fully operational, a definition of viability for a specific population should be quantitative and reflect an acceptable level of risk tolerance over a defined time frame. Because this quantitative definition was provided by neither the EU nor SEPA, we saw it as our task to agree on an operational definition of viability that could ultimately be adopted by SEPA and other management authorities as appropriate to guide wolf management in Sweden. Specifically, we include both demographic and genetic components in our general definition by defining viability as (A) the probability of population extinction of less than 10% over 100 years, and (B) the retention of at least 95% of current estimated genetic diversity over that same time period. Adopting a different set of criteria may lead to some modifications to our general conclusions, but we use here a consistent definition of viability to frame the results and their implications.

Key Features of the Analytical Methods

The simulation models we used to perform this analysis share important similarities that facilitate meaningful comparison of their results. For example, both Vortex and SLiM are individual-based models which track the fates of individual animals and their genetic variants over time. In addition, both models use age- and sex-specific demographic rates derived from detailed analysis of field data including annual rates of reproductive success and, depending on the specific model, survival or mortality. Immigration and/or emigration at a user-specified rate can be included in both models, and the two models allow for population abundance regulation through multiple mechanisms. Genetic processes including loss of genetic variation through inbreeding and genetic drift are also featured in both models, with simulation of inbreeding depression accomplished in SLiM through the accumulation of deleterious mutations and in Vortex through the specification of the number of lethal equivalents in the wolf population genome. In all, the two models used here are effective and flexible tools for evaluating both general demographic and genetic components of population viability in the context of our specific assignment.

Accompanying these similarities are also some features that differ between the two models. SLiM features more complex mechanics compared to Vortex for simulating population genetic parameters. These mechanics include the specification of both wolf-specific deleterious and neutral mutation rates across chromosomes, identification of selection and dominance coefficients for mutations, detailed specification of both masked and realized genetic load (the assemblage of genes responsible for inbreeding depression) and recombination of chromosomes across generations. While Vortex does not feature this level of detail when simulating population genetic processes, it uses the full pedigree for the Scandinavian wolf population since its founding in 1983 (Åkesson et al. 2023) to initialize the genetic structure (relatedness, inbreeding, etc.) of the population with a high degree of realism and accuracy. In terms of population demographic processes, SLiM uses a relatively more simplified treatment of parameters such as breeding system and catastrophic variation in annual mortality rate while Vortex allows the user to explicitly define these demographic characteristics in as

much detail as the data allow. In basic terms, we can conclude that the SLiM model is focused most heavily on detailed population genetic processes, with Vortex focused more heavily on detailed demographic processes with a higher-level treatment of genetic components of viability.

While acknowledging these similarities and differences in basic model structure, it is worth noting here that the two modeling approaches differ markedly in the initial population conditions and the means by which the wolf population is regulated moving forward in time. The SLiM model begins retrospectively with the initiation of the wolf population in Sweden through immigration from Finland/Karelia in the early 1980s and accounts for specific known immigration events between 1990 and 2021. Moving forward in time beginning in 2022, the population abundance is regulated by the use of a traditional carrying capacity (K) value that restricts reproduction and survival to prevent the simulated population from exceeding the specified value value and thus does not explicitly model yearly culling. The values of K used in the SLiM model included the range of abundance values (170 – 270) specified in the commission to SEPA. In contrast, the Vortex model is initiated with the known abundance of wolves in Scandinavia as of 1 October 2022 (N = 463: Milleret et al. (2023)) and the full pedigree of those individuals, establishing the genetic structure of the population based on past immigration events and breeding histories. Because the Vortex model was initialized with an abundance that already exceeded the range of favorable reference population values outlined in the SEPA commission (N = 170 – 270), a process of gradually reducing this current population to those FRP values was employed to simulate controlled hunting. Once the simulated population reached the desired test abundance, ongoing regulation of population abundance is achieved through continued culling. In this way, population management effectively becomes a carrying capacity of sorts that controls future population growth. While the precise methods of population regulation may differ between the two modeling approaches, neither of them explicitly simulate intrinsic ecological limits to population growth but are instead effectively simulating abundance ceilings imposed by population management practices.

Finally, we note that specific demographic inputs to the two models are largely concordant. Inspection of Table 1 in the SLiM simulation report of Dussex and of Table 1 in Miller's Vortex modeling report shows very similar values for a range of parameters governing mean annual rates of reproduction and survival. One demographic rate that differs slightly between the two models is pup mortality, with Dussex using 30.0% and Miller using 32.3% (citing the annual report on wolf population abundance and dynamics published by Milleret et al. 2023). This difference may be attributed to a slightly different definition of the time period over which mortality is imposed within the annual cycle, although this possible explanation is not confirmed.

Results of the Simulation Models: Consensus Findings and Model-Specific Insights

Despite the differences in the two modeling approaches noted above, the general similarities across the two models in both structure and biological input led to consistent results from the analyses. Specifically, the results showed that population viability – as we have provisionally defined it for this analysis – can be achieved with a total wolf population in Scandinavia (as estimated through the census date of 1 October) within the range of 170 to 270 wolves if the following conditions are achieved:

- Demographic performance of the population leads to a long-term expectation of positive population growth through time. Vability cannot be achieved if the population declines in abundance over time through low reproductive success and/or unsustainable mortality; and
- Immigration occurs according to the following specifications:
  - SLiM model:
    The average effective immigration rate (wolves that disperse and reproduce) into Scandinavia from the original source population in Finland/Karelia(Russia) is no less than one to three individuals per decade, or approximately one individual per 1.5 generations;

- Vortex model:
    The average demographic immigration rate (acknowledging that some immigrants may die before reproducing) is no less than three individuals per decade, or approximately one individual per generation, based on the assumed generation length of three to four years (Wikenros et al. 2021).

If these conditions are met, our models show a low probability of population extinction – less than 0.02 over the next 100 years – and a retention of at least 95% of the current genetic diversity present in the Scandinavian population as well as less than 5% increase in inbreeding over that same time horizon. Because a portion of the Scandinavian wolf population resides in Norway, the total population abundance would need to be downscaled appropriately to derive a suitable viable population abundance estimate for the Sweden component of the population. The detailed analysis of Milleret et al. (2023) indicates that the wolf population in Norway constitutes about 15% of the total population in Scandinavia.

As the preferred population abundance threshold (minimum viable population size) increases or decreases across the range specified in this analysis, the demographic conditions needed to achieve viability likewise change. Specifically, maintaining the population at a smaller abundance such as 170 or 200 individuals requires higher levels of immigration from northern sources in order to offset the more rapid rate of loss of gene diversity through more pronounced inbreeding and genetic drift. Our model results explicitly show this phenomenon, which is a fundamental observation in the management of small and isolated wildlife populations subject to the genetics impacts of isolation and inbreeding. In light of this situation, it would generally be advisable (and supported by the quantitative analyses summarized here) that the preferred viable population abundance be set near the high end of the proposed range. This larger abundance means comparatively higher levels of genetic viability can be achieved with relatively lower rates of immigration from an external source.

The difference observed in the threshold immigration rate between the two models reported above most likely results from the more fine-scale treatment of population genetic mechanics in the SLiM model compared to the simpler Vortex-based analysis. Higher rates of immigration as simulated in SLiM also led to the potential introduction of a larger number of deleterious genetic variants (i.e., genetic load) into the Scandinavian wolf population, which could lead to more inbreeding depression as the introduced load is expressed through time via inbreeding. A reduced threshold for immigration of one immigrant per decade as a mechanism for maintaining population viability follows from this analytical result.

Another characteristic that is different in the two models is the estimation of relatedness between the Scandinavia and Finland/Karelia populations. The SLiM model aims to recreate the historical process of founding the Scandinavia population through immigration of individuals from the Finland/Karelia population, thereby creating a kinship between the new population and its source. In contrast, the Vortex model assumes that the Scandinavia and Finland/Karelia populations are unrelated, or at least as unrelated to each other as the individual founders of the Scandinavia population are to one another on a relative scale. This is a pronounced simplification in the Vortex-based approach, as there is clearly a defined ancestral relationship between these two populations. Despite this simplification, both models lead to similar conclusions regarding the value of sustained connectivity between the populations as a mechanism for reducing inbreeding and introducing new genetic variation to sustain prolonged population viability of the Scandinavia wolf population. Results such as these highlight the value of this simulation approach for quantifying the _relative_ change in key population genetic parameters over time, even while the capacity for accurate prediction of absolute population metrics is impractical owing to persistent gaps in our full understanding of genetic and demographic processes in these wolf populations (and wildlife populations in general). This is a key requisite for understanding the true value of population viability analysis as a decision-support tool (e.g., Morris and Doak 2002; McCarthy et al. 2003; Lotts et al. 2004).

For populations at the lower end of the 170-270 threshold, our simulations indicate that migration may represent a risk of introducing new deleterious variation, which could lead to a reduction in fitness if inbreeding remains high. While temporary, this reduction in fitness will induce a purging effect which is relevant to the long-term health of the population. Nevertheless, it is possible that the negative effects of this genetic load may be counter-balanced by a genetic rescue effect where beneficial genetic variation increases fitness. This underlines the need to monitor both changes in genetic load as well as overall fitness following reproduction by immigrants.

Conclusions

That these two independent simulation modeling approaches generate quite similar results as described in the previou section, even in the presence of identified differences in model structure and/or function, should increase confidence among management authorities in the utility of this exercise. Each model has strengths and weaknesses that become apparent when judging their realism against our understanding of the biology of wolves in Scandinavia. Taken together, they provide useful information for broad policy and management decisions regarding wolf conservation in Sweden. At the same time, each individual model can be studied in more detail to provide additional insight into specific populaion dynamics questions for which they are particularly well-suited. Those insights can be found in each of the two full modeling reports accompanying this joint statement.

We end this statement with a final reminder of our understanding of the scope of this analysis. As argued in the Introduction, our intent with these two PVA efforts is to explore the demographic conditions that are necessary to promote viability of the wolf population in Sweden as embodied in the general concept of minimum viable population (MVP) size and as described in the Habitats Directive guidance on using model-based approaches to determine MVP (DG Environment 2023). The time horizon for this evaluation is 100 years from today, which is considerd by many to constitute a "long-term" projection of population performance (e.g., IUCN 2012). However, in keeping with the recommended use of ecological functionality in the estimation of favourable reference population values, the time horizon on which to determine a favorable reference population (FRP) value could extend beyond 100 years. The actual population size required to achieve that favorable status, moreover, will be larger than the estimated minimum viable population size. The Habitats Directive guidance document (DG Environment 2023) includes suggested protocols on upscaling MVP value or range to a corresponding FRP.

Additional analyses beyond the scope of the present demographic/genetic modeling efforts are required to implement this upscaling. These added analyses will likely include habitat suitability modeling, and considerations of landscape-level genetic, geographical and climatological factors that would influence the capacity of wolves to serve their proper ecological role in Sweden over the next century and beyond. To fulfill this role, the population of wolves in Sweden will need to be part of a functioning metapopulation with genetic connectivity to the Finland/Karelia population and perhaps elsewhere. The value of this connectivity for maintaining wolf population viability should not be discounted. Whenever possible, functional connectivity of Scandinavian habitats and those farther to the northeast should be encouraged and maintained to the fullest extent possible. The sociopolitical complexities of such a recommendation are not to be taken lightly, but the biological needs of wolf populations in the region as described in these analyses provide a scientific basis for productive species conservation planning. Considerations on metapopulation conservation of wolves in Fennoscandia by Bruford (2015) and Laikre et al. (2016) are particularly relevant in this regard.

References

Åkesson, M., A. Danielsson, and C.C. Palacios. 2023. Teknisk rapport över genetiska analyser på varg i Sverige år 2022. Grimsö forskningsstation.

Bruford MW. 2015. Additional population viability analysis of the Scandinavian wolf population. Swedish Environmental Protection Agency Report 6639, July 2015.

DG Environment. 2023. Reporting under Article 17 of the Habitats Directive: Guidelines on concepts and definitions – Article 17 of Directive 92/43/EEC, Reporting period 2019-2024. Brussels. 104pp.

Gilpin, M.E., and M.E. Soulé. 1986. Minimum viable populations: Processes of species extinction. Pages 19-34 in: Soulé, M.E. (Ed.). Conservation Biology: The Science of Scarcity and Diversity. Sinauer Associates, Sunderland, MA.

Haller, B.C., and P.W. Messer. 2019. SLiM 3: Forward Genetic Simulations Beyond the Wright-Fisher Model. Mol. Biol. Evol. *36*, 632–637

IUCN. 2012. IUCN Red List Categories and Criteria: Version 3.1. Second edition. Gland, Switzerland and Cambridge, UK: IUCN. iv + 32pp.

Lacy, R.C., and J.P. Pollak. 2023. Vortex: A stochastic simulation of the extinction process. Version 10.6.0.0. Chicago Zoological Society, Brookfield, IL, USA.

Laikre L, Olsson F, Jansson E, Hössjer O, Ryman N. 2016. Metapopulation effective size and conservation goals for the Fennoscandian wolf (*Canis lupus*) population. Heredity 117:279-289.

Lotts, K.C., T.A. Waite, and J.A. Vucetich. 2004. Reliability of absolute and relative predictions of population persistence based on time series. Conservation Biology 18:1224-1232.

McCarthy, M.A., S.J. Andelman, and H.P. Possingham. 2003. Reliability of relative predictions in population viability analysis. Conservation Biology 17:982-989.

Milleret, C., Dupont, P., Brøseth, H., Flagstad, Ø., Kindberg, J., Svensson, L., and Bischof, R., 2023. Estimates of wolf density, abundance, and population dynamics in Scandinavia, 2013–2023. MINA fagrapport 85. 34pp.

Morris, W.F., and D.F. Doak. 2002. Quantitative Conservation Biology: Theory and Practice of Population Viability Analysis. Sinauer Associates, Sunderland, MA.

Redford, K.H., G. Amato, J. Baillie, P. Beldomenico, E.L. Bennett, N. Clum, et al. 2011. What does it mean to succesfully save a (vertebrate) species? Bioscience 61:39-48.

Sanderson, E.W. 2006. How many animals do we want to save? The many ways of setting population target levels for conservation. Bioscience 56:911-922.

Wikenros C, M. Gicquel, B. Zimmermann, Ø. Flagstad, and M. Åkesson. 2021. Age at first reproduction in wolves: different patterns of density dependence for females and males. Proc. R. Soc. B 288: 20210207. https://doi.org/10.1098/rspb.2021.0207

A Demographic and Genetic Analysis of Minimum Viable Population Size to Inform the Population Reference Value for Wolves in Sweden

FINAL REPORT

Analyses conducted by:
Philip S. Miller, Ph.D.
Director of Science, Single-Species Planning
IUCN SSC Conservation Planning Specialist Group

In fulfillment of:
Swedish EPA Contract Number 365 – 23 – 002

1 April, 2024

This page intentionally left blank

TABLE OF CONTENTS

This page intentionally left blank

Summary

In May 2022, the Swedish Government commissioned the Swedish Environmental Protection Agency (SEPA) to investigate, based on the best available knowledge and scientific expertise if, and under what circumstances, the population reference value for the wolf (*Canis lupus*) as defined for favorable conservation status according to the European Union Habitats Directive, could be between 170 and 270 individuals in Sweden as stated in the parliament proposition from 2012 (prop. 2012/13:191). This report details an independent analysis requested by SEPA to inform future decision-making for wolf conservation in Sweden. Based on the distinction between minimum viable population (MVP) and favorable reference population (FRP) value as described in the Habitats Directive guidance documentation, the analyses described in this report specifically address the identification of a minimum viable population size for wolves in Sweden. Translation of this MVP value to a population abundance incorporating larger-scale ecosystem functionality, representation evolutionary genetic considerations – the FRP value – requires a process of "upscaling" to a larger population abundance. The translational process is outside the scope of this analysis and is instead to be conducted by SEPA after receipt of this PVA.

Another important issue governing the interpretation of this PVA concerns the ambiguity around the explicit definition of population viability in the Habitats Directive and supporting documents. To be fully operational, a definition of viability for a specific population should be quantitative and reflect an acceptable level of risk tolerance over a defined time frame. Because this quantitative definition was provided by neither the EU nor SEPA, it is not possible to provide a definitive interpretation of the PVA results in terms of what combinations of characteristics constitute a viable wolf population in Sweden. The process of setting quantitative thresholds for acceptable risk is a complex normative process that must be conducted by policy makers and not within the species research community. In the absence of such a defininition, simulation model results can be viewed on the basis of alternative definitions of viability in order to provide guidance to policy makers in their exploration of attitudes on acceptable risk.

This analysis was conducted using the simulation software Vortex, an individual-based demographic modeling package used around the world for exploring threats to endangered species and evaluating alternative management strategies. The wolf population in Scandinavia, distributed across south-central Sweden and southeast Norway, was considered to be a single population for purposes of simulating population dynamics. In addition, the population of wolves in Finland/Russia was included as a separate demographic unit to simulate occasional immigration of wolves into the Scandinavian population from this source. The core model structure featured two timesteps per year (each six months in duration) in order to more precisely account for reproduction in the spring and the population census to take place in the winter. The dataset of known living wolves in Scandinavia as of 1 October 2022 (N = 463) was used to initialize the predictive models, with the full pedigree of these individuals and their ancestry used to establish the starting population genetic structure. This valuable information influences the rate of retention of genetic variability (gene diversity) into the future as a function of relatedness among individuals and the inbreeding that can occur as adults form pairs in order to reproduce. Average rates of reproduction and survival, including both natural sources of mortality and anthropogenic mortality in the form of legal and illegal culling, were assembled from the literature and used to generate a population dynamics model with an expected realized annual population growth rate of approximately 2% which has been observed in the wild over the past decade of detailed census counts. The model explicitly counts population abundance at a point in the simulated annual cycle that generally corresponds to the actual wild population census taken as of 1 October.

Because the current population of wolves in Scandinavia is larger than the range of population reference values (170 to 340) tested in the analysis, the simulations feature gradual removal (culling) of wolves over the first five to seven years in order to reduce the population to an abundance consistent with

a given minimum population abundance threshold value. After that point in time, the population is maintained at or above the abundance threshold through the use of legal harvest when necessary (i.e., culling is not performed if the population is assessed to be less than the stated threshold). Wolves identified as valuable to the genetic viability of the population, especially immigrants from the Finland/Russia population, are exempt from removal. This selection process works to minimize the genetic costs of the removal program. Occasional immigration of wolves from the Finland/Russia population is simulated using random dispersal mechanics, with average immigration rates ranging from no immigration (an isolated Scandinavian population receiving no more wolves) to, on average, one wolf immigrating into the Scandinavian population every three years (a time interval that is roughly similar to the average generation length for this population). Immigration rates considered in this analysis are the actual rates, with each new migrant being at risk of dying before they successfully reproduce and, therefore, incorporate their genetic variation into the local population. The impact of their immigration on local population genetic viability, however, is observed through their reproductive success (determined by defined probabilities in the stochastic modeling environment) before mortality removes them from the population.

A total of 30 model scenarios, defined by unique combinations of population reference value and mean expected immigration rate, formed the core of the analysis. Demographically, the simulation models performed as expected, with long-term wolf abundance in the Scandinavian population governed by the expected mean rate of population growth and reaching a type of equilibrium after approximately ten years near the appropriate population reference value. This stable abundance was about 20% - 25% larger than the scenario-specific population reference value, owing to the production of new pups in early spring preceding the 1 October census. The simulated populations would decline to a number much closer to that value after the October – April timestep when winter mortality and, if necessary, removal of wolves occur. Because of the relatively larger starting abundance combined with the mean positive long-term population growth rate, extinction risk across the range of scenarios tested here was quite low, exceeding 0.01 over 100 years in just three of the 30 scenarios making up the analysis and never exceeding 0.02.

As expected from theoretical principles of conservation genetics, simulated populations maintained at smaller population abundance threshold values would show a more rapid rate of loss of genetic variation (gene diversity) over time, particularly if future immigration did not occur. Across the range of values tested here, immigration of wolves from the Finland/Russia population improved gene diversity retention over time. More frequent immigration (one wolf every three to six years) resulted in the Scandinavian population retaining at least 95% of the gene diversity present at the start of the simulation over the full duration of the simulation (100 years) across nearly all tested population reference values. When immigration averaged one wolf every three years, this retention increased to 99% to 100.5% of the original value, owing to the infusion of new genetic variation into the Scandinavian population from the Finland/Russia source. The process of retaining high levels of gene diversity in a population is influenced by stochastic (random) variability, however, resulting in a risk that these particular genetic goals may not be achieved even under favorable conditions. Therefore, choosing a genetic criterion for population viability should not only specify the desired level of gene diversity retention, but also the degree of confidence with which that desired level of retention is likely to be achieved.

Given the nature of the current models discussed in this report, and acknowledging the assumptions built into these simulations as described above, the analysis suggests that the wolf population in Scandinavia (south-central Sweden and southeast Norway) can potentially be considered viable within the interval of 170 to 270 individuals in accordance with the broad definitions presented in the European Union's Habitats Directive. However, this condition requires the following processes to be maintained through time:

- The Scandinavian wolf population must have the demographic characteristics to, at a minimum, sustain a positive population growth rate, ideally similar to or greater than what has been observed over the past decade of detailed observations of reproduction and survival (annual growth lambda $\lambda \geq 1.02$, with the possibility of considerably higher growth rates in the absence of legal harvesting of wolves); and
- Immigration of wolves from Finland/Russia into Scandinavia should be, on average, no less than one individual every three years.

The above discussion defines conditions for maintaining a viable population of wolves in Scandinavia. A similarly viable wolf population in Sweden would also require the same general demographic conditions: reproductive and survival parameters that result in a capacity for sustained population growth, and consistent immigration of wolves from the recognized source population in Finland/Russia. However, because the Swedish population represents only a portion of the total wolf population in Scandinavia, any specification of a minimum viable population for the purposes of setting a favorable reference population in Sweden would require proper scaling of the larger regional population. In addition, it is critically important to recognize that the precise demographic characteristics of a viable population in Sweden or elsewhere cannot be specified until a clear demographic and genetic definition of wolf population viability is presented by the appropriate national or regional authorities.

This page intentionally left blank

Introduction

In May 2022, the Swedish Government commissioned the Swedish Environmental Protection Agency (SEPA) to investigate, based on the best available knowledge and scientific expertise if, and under what circumstances, the population reference value for the wolf (*Canis lupus*) as defined for favorable conservation status according to the European Union Habitats Directive, could be between 170 and 270 individuals in Sweden as stated in the parliament proposition from 2012 (prop. 2012/13:191). In response to this commission, SEPA sought out two conservation biologists to conduct independent analyses in order to inform future decision-making for wolf conservation in Sweden. This report details one of those independent analyses.

Key concepts and issues underlying the scope of the SEPA commission

To place the work described in this document in proper context, it is important to highlight key underlying concepts and their definitions as presented in the European Union's (EU) guidance document on implementing their Habitats Directive (DG Environment 2023). The guidance document defines "Favourable Reference Population" as "*Population in a given biogeographical region considered the minimum necessary to ensure the long-term viability of the species...*" (DG Environment 2023: 21). This definition invokes the concept of estimating a minimum viable population (MVP) size using methods of population viability analysis (PVA) that are customized for the species under consideration and the specific management situation for a given geographic region. The MVP typically represents the abundance required to eliminate or largely minimize the risk of extinction of the population or species over a defined time frame, usually 100 years from the date of analysis (e.g., Gilpin and Soulé 1986). In some instances, this metric also includes consideration of maintaining a minimum level of genetic variation (heterozygosity) over time that helps to avoid detrimental levels of inbreeding and associated fitness impacts (inbreeding depression).

However, the guidance document goes further and recognizes the value of adopting a more expansive definition of viability – one that explicitly incorporates ecological functionality of a species within its native landscape in order to promote more robust ecosystems through time (e.g., Sanderson 2006; Redford et al. 2011). The guidance document (DG Environment 2023: 25) addresses the clear relationship between these two concepts:

> "*It is therefore important for favourable reference populations to reflect the 'long-term viable component of the natural habitat' at the level of the species across its natural range and distribution, rather than solely a minimum viable population.*"

In light of the EU's position, the analyses described in this report specifically address the identification of a minimum viable population size for wolves in Sweden. Translation of this MVP value to a population abundance incorporating larger-scale ecosystem functionality and representation – the favorable reference population (FRP) value – requires a process of "upscaling" as described in the guidance document (DG Environment 2023). It is understood that this "upscaling" to a larger population abundance value representing the FRP value is outside the scope of this PVA and is instead to be conducted by SEPA after the current work is completed.

Another important issue governing the interpretation of this PVA concerns the ambiguity around the explicit definition of population viability in the Habitats Directive and supporting documents (e.g., DG Environment 2023). To be fully operational, a definition of viability for a specific population should be quantitative and reflect an acceptable level of risk tolerance over a defined time frame. Because this quantitative definition was provided by neither the EU nor SEPA, it is not possible to provide a definitive interpretation of the PVA results in terms of what combinations of characteristics constitute a viable wolf population in Sweden. The process of setting quantitative thresholds for acceptable risk is a complex normative process that must be conducted by policy makers and not within the species research

community (e.g., Vucetich and Nelson 2018). In the absence of such a defininition, simulation model results can be viewed on the basis of alternative definitions of viability in order to provide guidance to policy makers in their exploration of attitudes on acceptable risk.

Using population viability analysis for conservation decision-making

The work described here uses tools and processes based in population viability analysis (PVA) to critically evaluate the demographic and genetic properties of a simulated wolf population under a range of alternative scenarios. In this case, these scenarios are defined by paired combinations of (1) an assumed population reference value for wolves in Scandinavia, and (2) an assumed rate of immigration of wolves into the Scandinavia population from an external source – here, Finland/Russia. [Note: From this point onwards, the wolf population in Sweden and Norway will be referred to as the Scandinavian population, while the source population of immigrants to the north will be referred to as the Finland/Russia population.] The overall goal of the analysis is to generate information on the demographic and genetic characteristics of a Scandinavian wolf population that would conform with the EU Habitat Directive's general definition of favorable conservation status.

PVA is a valuable tool used by wildlife conservation researchers, non-government organizations, and national and regional government agencies to assess threats to endangered wildlife populations and to evaluate management options designed to improve population or species status in their wild habitats (Beissinger and McCullough 2002; Moris and Doak 2002). These analyses are typically done using computer simulation modeling tools to project wildlife population abundance into the future. In particular, the process of conducting a PVA helps to stimulate information sharing and discussion among species experts, and provides a framework in which assumptions about our collective understanding of the system of interest are expressed and clarified.

That said, it is important to recognize that PVA methodologies are not intended to give absolute and accurate "answers" for what the future will bring for a given wildlife species or population. Many practitioners caution against the exclusive use of absolute results from a PVA in order to promote specific management actions for threatened populations (e.g., Ludwig 1999; Reed et al. 2002; Ellner et al. 2002; Lotts et al. 2004; Lacy 2019). Instead, we can best use PVA results to make comparisons of the relative performance of a simulated population under alternative management activities or different assumptions of environmental conditions. Even in this comparative framework, however, results from PVA efforts can provide a critical base of evidence when deriving meaningful and justifiable quantitative targets for endangered species recovery (Himes Boor 2014; Doak et al. 2015).

General Approach to the Analysis

Initial discussions around project scope, wolf population data availability and PVA model structure began in early 2022 with an online meeting between the author of this report and a group of wolf biologists that are part of the Scandinavian Wolf Research Project (SKANDULV). Participants in this meeting are listed in Appendix I. A very preliminary report on model development was submitted by the author to SEPA in May 2023; following this early progress report, more intensive work on model structure, input data analysis and scenario construction began in June 2023. A draft report was submitted to SEPA in October 2023 and, following revision of the report based on peer review comments, a final report was submitted on 1 April 2024.

Choice of software for PVA

As noted previously, population viability analysis is typically conducted through the use of computer simulation modeling tools. The tool chosen for this analysis is Vortex version 10.6 (Lacy and Pollak 2022). Vortex models population dynamics as discrete, sequential events that occur according to probabilities that are random variables following user-specified distributions. Vortex simulates a population by stepping through a series of events that describe an annual cycle of a typical sexually reproducing, diploid organism: mate selection, reproduction, mortality, increment of age by one year, dispersal among populations, removals, supplementation, and then truncation (if necessary) to the carrying capacity. The software has a number of benefits inherent to its design, including:

- Individual-based model structure allows for explicit simulation of random (stochastic) variation in annual birth and death rates among age-sex classes, and the impact of this variation on the growth dynamics of small populations.
- Ability to define individual, population, or global state variables that can be used to derive complex expressions for age/sex-specific demographic rates, which can change over time in response to evolving environmental conditions, management regimes, etc.
- Capacity for incorporating metapopulation structure, with multiple populations that are linked by occasional dispersal and distributed across a landscape.
- Incorporation of detailed genetic processes, such as mating between known relatives (as tracked through kinship calculations between selected parents) leading to inbreeding depression, or erosion of genetic diversity over time through random genetic drift.

For more information on the features of Vortex and its application to wildlife population management, see Lacy (2000; 2019) and Lacy et al. (2021).

Vortex has been used by the author in recent detailed analyses of wolf population dynamics and management in North America to successfully inform decision-making by government agencies for management of the Mexican wolf (*Canis lupus baileyi*: Miller 2017; USFWS 2022) and the red wolf (*Canis rufus*: Miller 2023; USFWS 2023). More importantly, the current use of Vortex provides a form of continuity with the most recent analysis of Scandinavian wolf population viability (Bruford 2015) that used an earlier version of the software. Substantial and significant changes have been made to the software since Bruford's analysis; while some of the limitations noted in that earlier analysis may be largely addressed in the present analysis, there are likely other aspects of Scandinavian wolf biology and/or management that we cannot fully accurately capture in the present analysis. The potential impacts of these limitations will be noted throughout this report.

Key features of the Scandinavian wolf PVA model

Important characteristics of the population dynamics model are listed below. More detailed information on the specifics of these characteristics is given in the following section.

- The commission from the Swedish Government for this analysis clearly states that the most recent analysis by Bruford (2015) is not being questioned for its validity, but that new information on wolf demographics, etc. favor an update to that analysis. In the spirit of that assessment, some of the input data used in the 2015 analysis are used without modification as appropriate in the present analysis.
- The focus of this analysis is the full Scandinavian wolf population distributed across south-central Sweden and southeast Norway, which can be considered demographically a single population (Figure 1). Therefore, the actual numbers of wolves in each country are not considered here; users of this analysis will need to employ additional information to determine, at any specified time in the future, the estimated proportion of the total Scandinavian population that resides within Sweden.
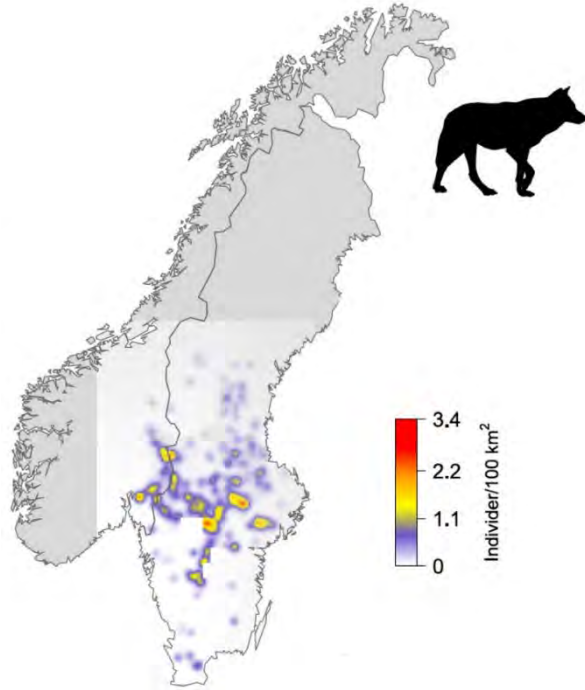
Figure 1. Spatial extent (light gray rectangle) of the Scandinavian wolf population used in this analysis. The figure includes data on wolf density and range for winter 2022-2023, but these data are not used specifically in this analysis. Figure adapted from Milleret et al. (2023).

- A separate population of wolves distributed across northern Finland and Russia is also included as a source of immigrants that occasionally disperse through northern Sweden and Norway to become incorporated into the resident Scandinavian population.
- The census of the wild Scandinavian population is reported as of 1 October, which is some months after the production of wolf pups from that year's breeding season. Therefore, in order to more realistically simulate the timing of annual population monitoring with the underlying population demographics, the model features two distinct timesteps per annual cycle, with each timestep assumed to be six months in duration (Figure 2). This is a structural modification of the default use of Vortex that is typically based on an annual pre-breeding census structure. With this modification, breeding takes place only in the "even-numbered" timesteps corresponding to the time period 1 April – 1 October. In contrast, mortality is imposed in both "even-numbered" and "odd-numbered" timesteps, with estimates of annual survival from the literature (e.g., Milleret et al. 2023) decomposed into numerically equivalent estimates of survival over the shorter six-month time periods.
- Each scenario (defined by a unique combination of input parameters, described in more detail in the following section) making up this analysis begins as of 1 October 2022, which is the date of the most recent available published Scandinavian population size estimate (N_{Total} = 463: Milleret et al. 2023). For the Scandinavian population, this initial population is fully described by a pedigree that lists all the ancestors of each member of the population alive as of the start date. By using this pedigree information, we can accurately describe the current genetic structure of this population (extent of relatedness among living individuals, kinship among paired individuals, mean level of inbreeding in the population, overall retention of gene diversity) and how that structure is expected to change in the future under different minimum population abundance thresholds and the extent of immigration from the Finland/Russia population.
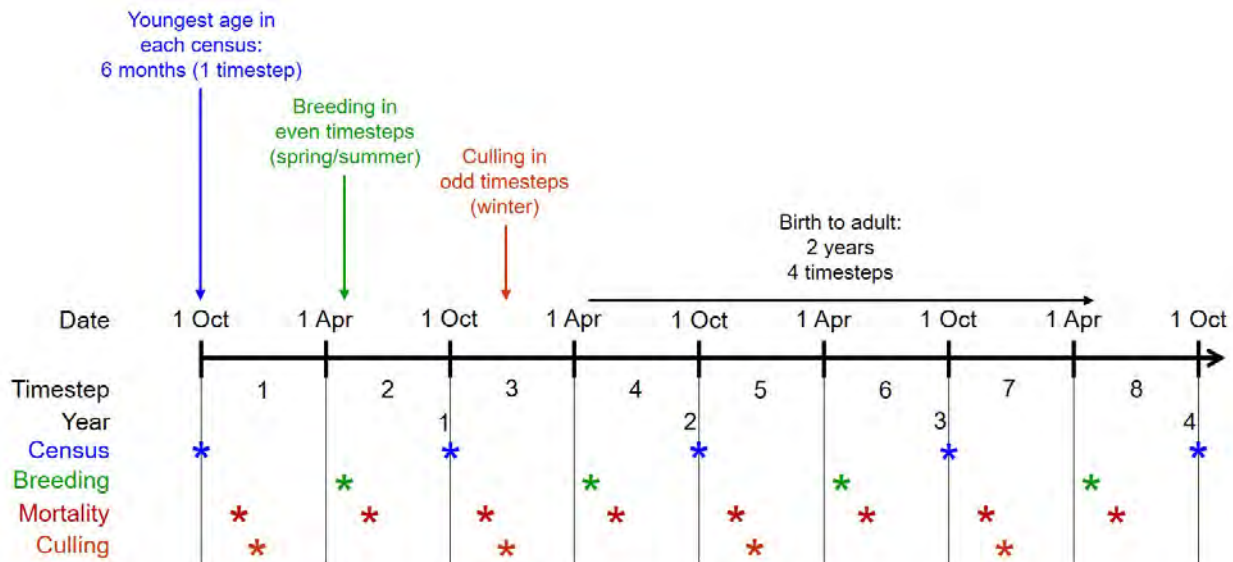
Figure 2. Diagram of Scandinavian wolf PVA simulation timeline, showing the decomposition of the typical annual demographic cycle into two timesteps of equal duration. The timing of each specific event across the annual cycle (designated by asterisks) is relative to other events in that cycle and is not meant to be exact.

- Because each scenario in the analysis is initialized with a wolf population size that exceeds the range of population abundance threshold values examined here, the decision was made to include in this PVA an expanded rate of wolf removal that in the shorter term is designed to reduce the existing wolf population size to the threshold value defining that scenario. A removal rate of reduced intensity can then be implemented in the longer term to maintain the population at the abundance threshold value to be evaluated. Threshold abundances tested in this analysis are:

    o 170 (lower end of the interval proposed by the Swedish government)
    o 210 (lower end of the interval with the addition of 40 individuals in Norway)
    o 270 (higher end of the interval proposed by the Swedish government)
    o 300 (current population reference value for wolves in Sweden)
    o 310 (higher end of the interval with the addition of 40 individuals in Norway)
    o 340 (current population reference value with the addition of 40 individuals in Norway)

  Note that this strategy is a significant departure from the previous analysis of Bruford (2015), which included scenarios that evaluated demographic and genetic characteristics of wolf populations across a wide range of potential population sizes as large as 2000 individuals. In keeping with the author's interpretation of the commission put forward by the Swedish Government, the structure of the present analysis is meant to realistically examine the validity of the proposed population threshold values in the context of the current abundance of wolves across southern Scandinavia.
- The minimum population abundance threshold values used in this analysis are defined on the basis of total population size, in other words, all individuals in the population aged six months and older (in keeping with the timing of reproduction and population monitoring discussed above). All plots of population size in this report will therefore conform to this definition. However, recognizing that the EU Habitats Directive reporting guidelines specify that population size estimates should also be restricted to adults, additional tabulation of adult population size (two years of age and older) is included along with tallies of subadults (between one and two years of age) and juveniles (from birth to one year of age).

- A given minimum population abundance threshold value is interpreted in this analysis to represent the minimum target population size, and not its long-term mean where actual population size could occasionally be less than the target. This interpretation underlies the population harvest mechanics as well as the interpretation of population size output from each scenario.
- In order to generate reasonable and internally consistent estimates of reproduction and age-specific mortality for the Scandinavian wolf population, this analysis uses published population size estimates dating back to the 1 October 2013 census (as reported in Milleret et al. 2023) to calculate a mean annual realized population growth rate (λ, lambda) across the time period 2013 – 2022. The calculated mean growth rate $\lambda = 1.018$ provides a reference point on which to calibrate population demographics with the goal of reproducing that mean growth rate into the future. By using this method, we assume that future population growth will be generally similar to that observed over the past decade. Note that this is a realized growth rate and not a true (much higher) potential growth rate that could be achieved in the absence of various sources of anthropogenic mortality that limit population growth.
- Key model output metrics that can inform the question of minimum population abundance threshold value include:

     o Probability of population extinction, expressed as the proportion of replicate simulations for any given model scenario that decline to zero individuals (or animals of only one sex) within the simulation timeframe.
     o Proportional retention of population genetic diversity (defined here as gene diversity or expected heterozygosity), expressed as both (1) the more theoretical proportion of diversity retained relative to the presumed source of the Scandinavian population, and (2) the proportion of diversity retained in the Scandinavian population relative to the value present in that population at the beginning of the simulation, in other words, 1 October 2022.

Detailed Summary of PVA Model Input

This section gives additional detail on the core numerical input used in the PVA models used for this analysis. A summary of the input parameters can be found in Table 1, with a more detailed presentation of input data for a specific scenario given in Appendix II. Unless specified otherwise, demographic data for the Finland/Russia population are equivalent to those for the Scandinavia population.

Initial population size, structure

Scandinavia: Milleret et al. (2023) used open population spatial capture-recapture (OPSCR) models on an extensive database of non-invasive genetic samples, carcass recoveries, etc. to estimate the number of wolves in Scandinavia as of 1 October 2022 to be 463 individuals (95% credible interval: 441 – 488). In addition, the kinship (relatedness) structure of this population is represented by a pedigree of all animals alive as of 1 October 2022, with their ancestry dating back to the founding immigration event in the 1980s (summarized most recently in Åkesson et al. 2023). A small number of young individuals known or presumed to be alive as of the model start date were not recorded in the pedigree; to account for these wolves, juveniles and subadults were added to the pedigree and assigned sex and parentage at random to bring the final population up to the desired abundance. Finally, adults that were known to be paired as of the model start date were assigned the ID of their mate so that the simulations could begin with an accurate expected breeding structure across the population. With these data included in the PVA model, the starting values of population mean inbreeding coefficient and gene diversity are known quantities. Initial population gene diversity is estimated by "dropping" alleles (two unique alleles per founder) through the known pedigree and calculating expected heterozygosity from the resultant distribution of final allele frequencies (Frankham et al. 2010). A portion of the full pedigree file, listing the original founders and all individuals presumed to be alive as of 1 October 2022, is given in Appendix III.

While a total of ten individuals are known to have immigrated from Finland/Russia into what is now the Scandinavian wolf population, only seven wolves are represented genetically in the current population and are therefore considered as founders. These animals are identified as follows:

- G1-83 (male) and D-85-01 (female) of the Nyskoga pair, immigrated in 1983
- G1-91 (male) of the Gillhov pair, immigrated in 1991
- M-09-03 (male) of the Galven pair, immigrated in 2008
- M-10-10 (male) of the Kynna pair, immigrated in 2008
- G31-13 (female) of the Tivenden pair, immigrated in 2013
- G187-19 (male) of the Setten pair, immigrated in 2021 (alive as of 1 October 2022).

Three additional individuals immigrated into the existing population, but are no longer genetically represented in the current population:

- G23-13 (male) of the Tivenden pair, immigrated in 2013
- G15-16 (male) of the Tunturi pair, immigrated in 2016
- G325-17 (female) of the Svartedalen pair, immigrated in 2017

As is common practice in pedigree analysis methodologies, we assume that all founders are unrelated to one another in the absence of detailed genetic data that might establish more definitive estimates of founder relatedness. This assumption is likely to be inaccurate for certain founder animals, as specific animals may share common recent ancestry.

Finland/Russia: The model includes a generalized estimate of 500 wolves in the Finland/Russia source population. The geographic boundaries of this population, considered in the context of this analysis, are undefined. As a result, the goal here is to create a wolf population of a sufficient size that can serve as a source of immigrants to the Scandinavian population. Because of uncertainty in this parameter, a set of additional scenarios were constructed that assessed the impact of starting the models with a smaller abundance of wolves in this population (see the section titled "Testing model sensitivity to select demographic input parameters" below). Additionally, this population is not initialized with a detailed pedigree; consequently, the model lacks a detailed description of genetic structure in this population. However, in recognition of the fact that some moderate level of inbreeding is likely to be taking place among localized subsets of the population, the mean inbreeding coefficient across all individuals in the population at the start of the simulation is assumed to be 0.1.

To summarize, we have two important assumptions about population genetic structure built into this model: (1) the genetic relatedness among immigrants incorporated into the Scandinavia population in the past – those that are currently identified in the pedigree as founders of that population – is unknown; and (2) the genetic relatedness among future immigrants added to the Scandinavian population from the Finland/Russia source population, and their relatedness to past founders of the Scandinavian population, is also unknown. When considering future loss of gene diversity in the Scandinavian population, the above assumptions imply that the degree of relatedness among new wolves immigrating into Scandinavia from Finland/Russia is no different than the relatedness of founders of the Scandinavian population to each other. This is likely to be a plausible assumption, and should facilitate informative interpretation of the genetic results of the PVA scenarios described here.

Population carrying capacity

In the typical Vortex modeling framework, a population is allowed to increase in abundance under favorable demographic conditions (and without explicit specification of density dependence) until the carrying capacity K is reached. When this occurs, individuals are randomly removed (simulating additional mortality under these limiting conditions) according to the age and sex structure of the

population in order to bring the population back down to the value of K. In this manner, we therefore simulate a ceiling-type density dependence.

Both the Scandinavia and Finland/Russia populations have an assumed carrying capacity of $K = 800$ individuals. These values are somewhat arbitrary, but the overall structure of this PVA means that carrying capacity is not expected to be a factor informing the viability of the Scandinavian wolf population. There is no evidence that the current Scandinavian population is being regulated by natural ecological processes, and the range of identified population abundance threshold values tested in this analysis are lower than the initial abundance used here ($N_{2022} = 463$). Note that the value of K used for the Scandinavian population in the recent analysis by Bruford (2015) was as high as 2000 individuals – likely to be quite unrealistic and uninformative in the analysis described here. Nevertheless, because of some uncertainty in this parameter concerning the Finland/Russia population, a set of additional scenarios were constructed that assessed the impact of starting the models with a smaller value of K in this population (see the section titled "Testing model sensitivity to select demographic input parameters" below).

Reproduction

For the purposes of this PVA, wolves are considered to have a long-term monogamous breeding system, where adult pairs will remain together until one of its members dies. Additionally, female breeding is defined as the birth of a litter of pups. The data presented by Bruford (2015) are used for this analysis, where it is assumed that a mean of $60\% \pm 10\%$ of all adult females will pair with an adult male and produce a litter. The standard deviation given above reflects annual variation around the mean arising from stochastic variability in the environment (for example, annual weather effects, prey availability, individual animal health, etc.). Females that already paired at the beginning of the breeding timestep will retain their mate, while unpaired females have the specified chance of pairing with a similarly unpaired adult male. If one of the pair members dies, the living individual is returned to the pool of available breeders to pair with a suitable mate. Effectively all paired females in a season are assumed to produce a litter. Unpublished data from SKANDULV (time period 2011 – 2022) suggest that the proportion of adult females breeding is slightly higher than 60%. A set of additional scenarios was constructed that assessed the impact of an alternative value for this parameter (see the section titled "Testing model sensitivity to select demographic input parameters" below).

Given our definition of reproduction above, mean litter size reported in the literature – which records the number of pups surviving to 1 October – must be adjusted to reflect the number of pups alive at the beginning of the April – October timestep. In this case, data on the mean number of pups counted in October (taken from Liberg et al. (2005)) and on the mean inbreeding coefficient measured in the population in recent years (taken from Åkesson and Svensson (2022); see below) suggest a mean of 4.5 ± 1.5 pups born in the beginning of the April – October timestep. This mean value was adjusted slightly to 5.3 ± 1.5 pups per successful female in order to generate a simulated population growth near the rate observed over the past decade as reported in Milleret et al. (2023).

Inbreeding depression affecting pup survival

Liberg et al. (2005) examines the effects of inbreeding on the number of pups per litter that survive and are counted in winter (1 October census). The authors of that study transformed their data to generate a standardized estimate of the intensity of inbreeding depression, expressed as the number of lethal equivalents per diploid genome (Morton et al. 1956). The Scandinavian wolf population was assessed to have 6.04 lethal equivalents affecting survival of pups to winter, with 50% of that load comprised of fully lethal alleles (and, therefore, more easily purged from a population through the process of purging). This framework for describing the demographic effects of inbreeding depression is explicitly included in the Vortex model structure. Note that a more recent examination of inbreeding in Scandinavian wolves (Smeds and Ellegren 2022) emphasizes the significance of genetic load in this population, although

techniques for estimating fitness effects like those reported by Liberg et al. (2005) are not currently available.

Mortality

The non-invasive genetic sample data used to estimate Scandinavian wolf population size was also used by Milleret et al. (2023) to summarize key population demographic processes (see Table A.7 in their report). Specifically, they estimated annual age-specific survival rates for "scent-marking" individuals and for other individuals across the time period 2013 – 2022. Additionally, they separated legal culling as a separate source of mortality from the more general category of "other" mortality (both natural and anthropogenic causes other than culling). This "other" mortality was used to specify base age-specific mortality in the model, with culling mortality treated separately (see "mechanics of wolf removal" below). Data for scent-marking individuals were used to classify mortality for paired adults, and data for other individuals used to classify mortality for unpaired adults and younger individuals. As observed in the Milleret et al. (2023) dataset, and in keeping with the previous analysis of Bruford (2015), there is no difference in mortality rates between males and females. Note that the present estimate of annual pup mortality (0.323) is significant greater from the equivalent estimate in Bruford (2015) of 0.05. Details of how that original rate was estimated are unavailable but is likely to follow from a slightly different functional definition of reproduction that already accounts for early mortality in the annual cycle.

Timestep-specific survival rates were obtained by calculating the square root of the annual rates, therefore assuming a constant mortality risk across the full annual cycle. Finally, survival estimates were transformed to their corresponding mortality rates for use in the model. This assumption applies only to the specification of "other" mortality per Milleret et al. (2023), and not to the bulk of culling mortality which is treated separately in the model as discussed above. Small adjustments were made to age-specific mortality rates where appropriate in an attempt to generate the desired population trajectory (realized annual growth rate $\lambda = 1.018$) as described earlier.

Catastrophic variation in demographic rates

An outbreak of a generic infectious disease was included here as a "catastrophe", defined in the context of PVA models as a rare event but with the potential for significant negative impacts to rates of reproduction and/or survival. Every year of the Vortex simulation, a random number draw is used to determine if a catastrophe occurs in that year and, if so, a specified multiplicative modifier is applied in that year to normal rates of reproduction and/or survival.

Without detailed data to the contrary, the catastrophe used in the Bruford (2015) PVA was carried forward to this analysis. When an outbreak occurs, the impacts include a complete elimination of successful reproduction in that year (reproduction multiplicative modifier = 0) as well as a 50% reduction in survival across all age and sex classes (survival multiplicative modifier = 0.5). Despite the significant fitness impacts in the year of the catastrophe, the event's very low frequency of occurrence results in a minimal impact of this catastrophe on the long-term trajectory of population abundance.

Metapopulation connectivity through inter-population dispersal

The Scandinavian and Finland/Russia wolf populations are assumed to make up a metapopulation, with occasional exchange of individuals through dispersal. For the purposes of this model, it is assumed that dispersal occurs only from the Finland/Russia population to the Scandinavian population. This may be an unrealistic assumption, as there are reports of wolf immigration from Scandinavia to Finland (Mäntyniemi et al. 2022). However, these data were not available for review for this analysis, and it is unclear if the recorded immigration events involved wolves that were residing in the Scandinavian population analyzed

here, or if they were transients that were returning to the Finland/Russia population before integrating into the Scandinavian population.

The model simulates only natural dispersal and not human-managed translocation. This natural dispersal is implemented as a stochastic process in the Vortex PVA environment by specifying a probability that a given individual in the Finland/Russia population of either sex will disperse to the Scandinavian population within the six months defining the model timestep. The dispersal event is assumed to take place only in the October-April (odd-numbered) timestep, with all male and female subadults and adults capable of dispersing. Individuals that have dispersed to the Scandinavia population are assumed to be immediately subject to the same rates of reproduction and survival as native wolves born into that population.

This PVA includes a range of expected mean immigration (dispersal) rates that are in keeping with previous analysis such as Bruford (2015) and are broadly consistent with observed rates of individual wolves dispersing into the Scandinavian population from Finland and neighboring areas. Specifically, immigration rates tested here include:

- No dispersal (isolated populations)
- One wolf every 12 years (annual probability of dispersal = 0.083)
- One wolf every 9 years (annual probability of dispersal = 0.111)
- One wolf every 6 years (annual probability of dispersal = 0.167)
- One wolf every 3 years (annual probability of dispersal = 0.333)

Note that this mechanism of metapopulation connectivity explicitly simulates demographic immigration; it is possible that some wolves will immigrate to the Scandinavian population and die before reproducing. While some may see this as a simplification of the model, the consequences of this true dispersal with subsequent reproduction when possible are readily apparent in the genetic components of the model results (see next section). Also note that this dispersal mechanic does not account for individuals that leave the Russia/Finland population but die before reaching the Scandinavian population. In other words, all individuals in the simulation that leave the Russia/Finland source are successful in immigrating into the Scandinavian population. Therefore, there may be a slight underestimate of the genetic cost to losing individuals from the northern source that are not integrated into the Scandinavian wolf population.

Mechanics of wolf removal

For the purposes of this PVA, "removal" refers to legal harvest (culling) of wolves in Scandinavia. This process occurs only in odd-numbered timesteps (October-April) as this is the time of the year when the bulk of the legal culling process takes place (R. Ekblom, pers. comm.). The initial base scenario used to calibrate demographic rates and, by extension, recent population growth rate, includes a separate culling (harvest) process in which a proportion of each age-sex class is removed from the population. This proportion is based on the estimated culling mortality rates presented in Table A.7 of Milleret et al. (2023). Therefore, this mortality is treated distinctly from the "other" mortality from Milleret et al. (2023) that is used to specify base annual mortality rates.

The removal process used here features a number of specific constraints within any given model scenario in an attempt to improve the realism of the simulation (note that these constraints apply only to the Scandinavian population):

- Culling occurs only when the number of wolves across all age classes exceeds a numerical threshold value that is equivalent to that scenario's designated minimum abundance threshold

value. If the total population abundance is less than this threshold, legal culling of any kind does not occur.

- Individuals that immigrate from the Finland/Russia population – new founder animals – are not removed in order to retain their genetic value while they are alive. This is a simplified rule relative to what is practiced in reality, where offspring of founders are also exempt from culling. The precise parameters defining this rule, e.g., if this applied only to first-generation offspring or more distant descendants, is not known and were therefore not employed here.

- There is an additional genetic constraint on removals, in which wolves are targeted for removal only if their mean kinship value (the average degree of relatedness of that individual to all other living individuals in the population) is greater than 50% of the average mean kinship within that population. This constraint – intended to simulate actual decisions on culling individuals from the Scandinavian population – is designed to remove individuals that are relatively less genetically valuable to the population as they have a relatively high degree of relatedness to many individuals in the population. Because of this constraint, the retention of population-wide gene diversity is improved over a removal strategy that does not take individual mean kinship into account. As this may not be consistently applied in practice, a set of additional scenarios was constructed that assessed the impact of relaxing this rule and allowing culling of any individual except for living immigrants from the Finland/Russia population (see the section titled "Testing model sensitivity to select demographic input parameters" below).

- In order to reduce the initial population size to a given minimum abundance threshold value, an attempt is made to somewhat gradually remove individuals over the first few years of the simulation. Specifically, removal is relatively more gradual during the first six years, after which time the removal rate is increased to accomplish the necessary reduction and then maintain the population at or near the desired target reference value for the remainder of the simulation.

- If removal is triggered during a specific timestep (total population size is greater than the minimum population abundance threshold value), both female and male wolves are removed in approximate proportion to the expected age-class distribution of a population growing at the current rate calculated from Milleret et al. (2023): pups, 36%; subadults, 31%; adults, 33%. Since removal is implemented probabilistically, the actual number of wolves removed may be greater or less than the target number.

Additional model characteristics

The specific software package used in this analysis is Vortex version 10.6.0.0 (10 May 2023). Each model scenario was run with 1000 replicate iterations to account for stochastic variability in demographic rates, population genetic processes, etc. Output discussed in the next section shows the mean outcome of those iterations with, where appropriate, estimates of variation around the mean (standard deviation). All scenarios were run for 100 years (200 timesteps), with results presented graphically from even-numbered timesteps that corresponds to the October population census (in model terms, at the end of those timesteps).

A Note on PVA Scenario Structure

As noted previously, the present PVA is based on the previous analysis of Bruford (2015) in terms of software choice as well as general structure and function of the simulations. Insights from the earlier analyses exploring the sensitivity of model output to uncertainty in such input parameters as the number of lethal equivalents making up the population genetic load, the proportion of that load composed of lethal alleles, characteristics of catastrophes, etc. are expected to remain valid in the present analysis. Consequently, this analysis does not repeat those explorations of model sensitivity.

Instead, this PVA attempts to focus on the central question commissioned by the Swedish Government: the necessary conditions for a wolf population in Sweden to be maintained at an abundance of between 170 and 270 and to be considered to satisfy the broad definition of viability per the EU Habitat Directive guidelines. To accomplish this task, this PVA includes a set of 30 distinct scenarios that feature different combinations of six different minimum population abundance threshold values (see page 5) paired with five different assumed immigration rates (see page 10). Where appropriate, additional scenarios have been developed to explore identified areas of uncertainty and their impact on insights into the central question posed above.

Table 1. Summary of core input parameter values used in simulation models of wolf population dynamics.

Model Parameter	Parameter Value		Source (Scandinavia)
	Scandinavia	Finland/Russia	
Initial population size	463	500	Milleret et al. (2023)
Carrying capacity	800	800	This study
Reproduction[1]			
Age of first reproduction	2 years	2 years	Wikenros et al. 2021
Maximum age of reproduction	13 years	13 years	2022 pedigree
Mean adult females breeding (%)	60 ± 10	60 ± 10	Bruford (2015)
Mean litter size[2]	5.3 ± 1.5	5.3 ± 1.5	Liberg et al. 2005
Age-specific mortality (annual)[3]			
Pups (Age-0 to Age-1)	0.323 ± 0.05	0.323 ± 0.05	Milleret et al. (2023)
Subadults (Age-1 to Age-2)	0.300 ± 0.05	0.300 ± 0.05	Milleret et al. (2023)
Adults (Age-2+)	Paired: 0.25 ± 0.06 Unpaired: 0.36 ± 0.06	Paired: 0.25 ± 0.06 Unpaired: 0.36 ± 0.06	Milleret et al. (2023)
Inbreeding depression	6.04 LEs; 50% lethal	6.04 LEs; 50% lethal	Liberg et al. (2005)
Catastrophe			
Annual frequency	0.01	0.01	Bruford (2015)
Severity	Breeding: 0.0 Survival: 0.5	Breeding: 0.0 Survival: 0.5	Bruford (2015)

1. Reproduction defined as birth of pups, not the time of onset of sexual maturity or the time of mating.

2. Pups assumed to be present at birth, not at subsequent 1 October count.

3. Related to "other" mortality reported in Milleret et al. (2023). Legal culling mortality treated separately.

Results of PVA Simulations

Demographic performance of the base scenario

The base scenario in this analysis features the demographic input described in the previous section and projects the pedigreed Scandinavian population forward in time. All rates of reproduction and survival – reflecting both culling mortality and other sources of mortality – are assumed to continue into the future. In addition, the model includes occasional immigration of Finland/Russia wolves (approximately one wolf every six years). The simulation runs for ten years (20 timesteps) to match the number of years comprising the Milleret et al. (2023) dataset used to generate recent population size estimates.

The results of this scenario are shown in Figure 3. Note the slight reduction in mean population size in the first two years of the prospective trajectory; this is likely caused by the initial age structure of the population, particularly regarding the addition to the starting pedigree of a group of younger individuals that have yet to reach the adult stage. Once the age structure begins to readjust after a few years, the population grows in a generally consistent manner compared to the actual ten years of data compiled in Milleret et al. (2023) – mean growth rate $\lambda = 1.022$. In addition, specific iterations of the prospective trajectory occasionally show significant changes in population size, which is likely to result from a combination of environmental variability in mean demographic rates, a rare infectious disease outbreak, and proportional culling rates that may differ markedly from annual expectations. Overall, however, the results of this scenario provide a satisfactory level of confidence that the demographic rates used as model input result in a simulated Scandinavian wolf population that grows at a similar rate to that observed in the recent past. Consequently, this model can be used as the foundation for additional scenarios that explore the impacts of a range of population reference values and immigration rates on demographic and genetic measures of population viability.

Figure 3. Trajectory of the Scandinavian wolf population size (census as of 1 October) as estimated using analysis of non-invasive genetic sampling over the time period 2013 – 2022 (line and scatter plot: data from Milleret et al. 2023) and as simulated using the demographic and genetic input data comprising this PVA over the time period 2022 – 2032 (heavy solid line). Vertical bars in the historic dataset are 95% credible intervals around the population size estimate. Red dashed line shows the expected mean population trajectory assuming a constant realized growth rate (lambda, λ) of 1.018 as calculated from the historic data. Light gray lines are ten representative iterations of the PVA model base scenario. See text for more information on model structure and input data.
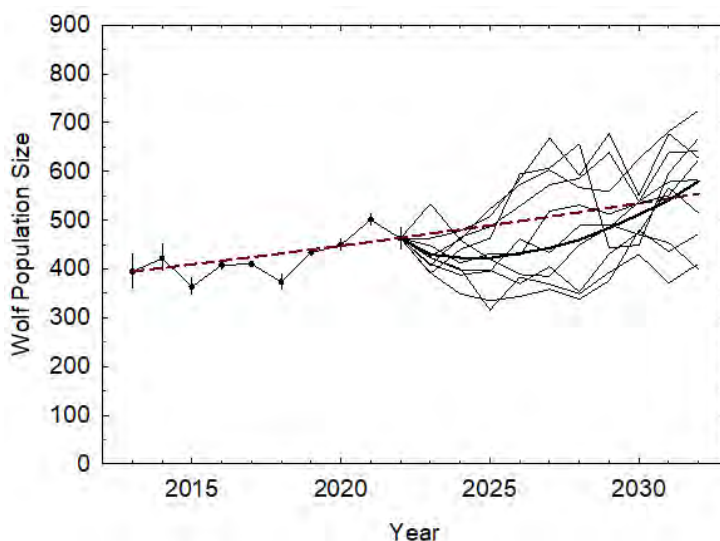
The demographic data used for this base model were also used in a companion scenario with a simplified structure that allowed the calculation of mean generation length. This companion scenario gave a mean generation length of $T = 3.8$ years (detailed results not shown here), which is consistent with recent observations of decreasing generation length in Scandinavian wolves (Wikenros et al. 2022).

Demographic performance of the Scandinavian population without immigration

An initial set of scenarios was created that features management of Scandinavian wolves across the range of minimum population abundance threshold values, but without immigration of individuals from the Finland/Russia source. This serves as a type of "control" to better compare the relative response of the simulated population to increasing levels of occasional immigration from the source population.

Following a period of population reduction through wolf removal over approximately seven years (in keeping with the removal mechanic described previously), each of the simulated populations reaches a stable mean abundance that, on average, is maintained across the duration of the simulation timeframe (Figure 4). This stability results from the inherent capacity for population growth given the underlying demographic rates used in the base scenario (see Figure 3). Measures of statistical variability around the mean abundance values have been omitted from Figure 4 for clarity; detailed inspection of the results show that the standard deviation of mean population size after 100 years is typically around 20% to 25% of the mean value (see Table 2). The number of adults (i.e., individuals at least two years of age) in the population consistently make up approximately 38% - 40% of the total abundance as calculated at the end of the simulation (Table 2).
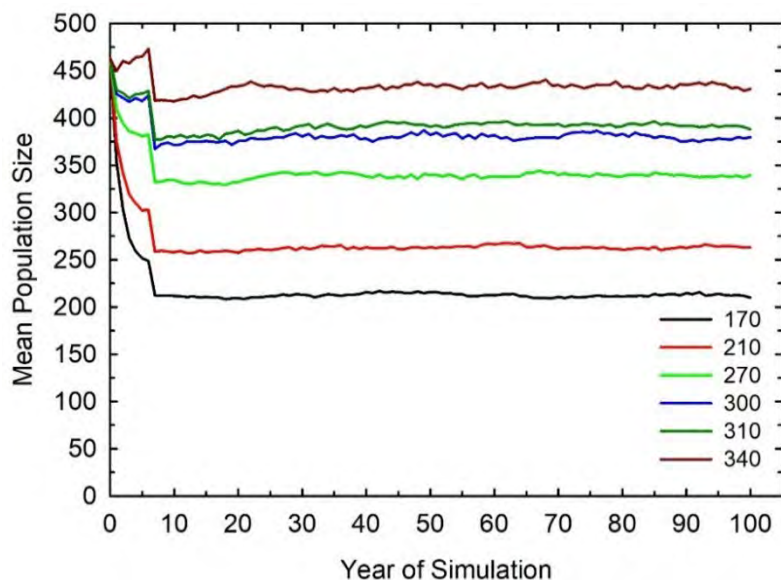
Figure 4. Projections of mean extant population size (census as of 1 October) for the Scandinavian wolf population across the range of minimum population abundance threshold values and assuming no immigration from Finland/Russia. Extant population size for a given scenario is calculated using only those iterations that did not become extinct. See accompanying text for more information on model structure and function.

While the long-term wolf population size shows significant stability over time in these simulations, the mean abundance is consistently larger than the minimum population abundance threshold value used to guide maintenance of the population at that desired abundance. For example, when the threshold is set at 170 individuals, the long-term average population size seen in Figure 4 is approximately 210 animals – about 23% greater than the population reference value. At larger reference values, the long-term mean abundance is approximately 25% greater than the corresponding reference value. This occurs because that the model's annual census event takes place in October which is after the production of pups in the spring, which is itself after the legal culling of wolves the preceding winter (see Figure 2 for the graphical depiction of the model's annual cycle). A pre-breeding census taken just before reproduction in the spring would show population abundance closer to the desired threshold value.

This dynamic can be examined in more detail by showing the total population abundance at each model timestep in order to see the impacts of different events that occur across the annual cycle (Figure 5). The figure shows a segment of just five years (ten timesteps), beginning just after the population census in October at the onset of model year 40. The first timestep features a general mortality event

(including both natural mortality and some illegal hunting mortality), which is then followed by a culling event that is conditional on the population abundance relative to the specified minimum threshold. If the abundance exceeds the threshold, culling occurs as is evident in the first timestep shown in Figure 5. The population is now at or very near the threshold as of 1 April. The second timestep (April – October) now features a breeding event followed by mortality. These events will increase population size which will be recorded in the subsequent October census at the beginning of model year 41. In this next year, pup production declines stochastically, meaning that the mortality event takes the population below the minimum threshold by the 1 April model census. Therefore, culling was not necessary in this second year. Another round of reproduction increases the population to an abundance of about 200 individuals at the 1 October census that initiates model year 42. Because of the timing of the 1 October census in the model, as in reality, and if only the 1 October census data are displayed graphically as model output, the active maintenance of the population at or near the minimum threshold as reflected in the model's 1 April census is not readily apparent. It is recognized that the exact mechanism of setting annual culling targets in the model does not precisely replicate those mechanisms in reality, but the overall intention and outcome of the simulated culling process is generally realistic.

Figure 5. Five-year segment of a single iteration from the scenario featuring a minimum abundance threshold of 170 wolves (horizontal dashed line). Plots show the total wolf population abundance (black) and the number of wolves culled (dark gray) in the two timesteps making a single calendar year. The symbols represent specific events within each timestep, with mortality (red) and culling (orange) occurring in the October-April step and breeding (green) and mortality occurring in the April-October timestep. The population census event (blue) takes place in October. See accompanying text for more information on model structure and function.
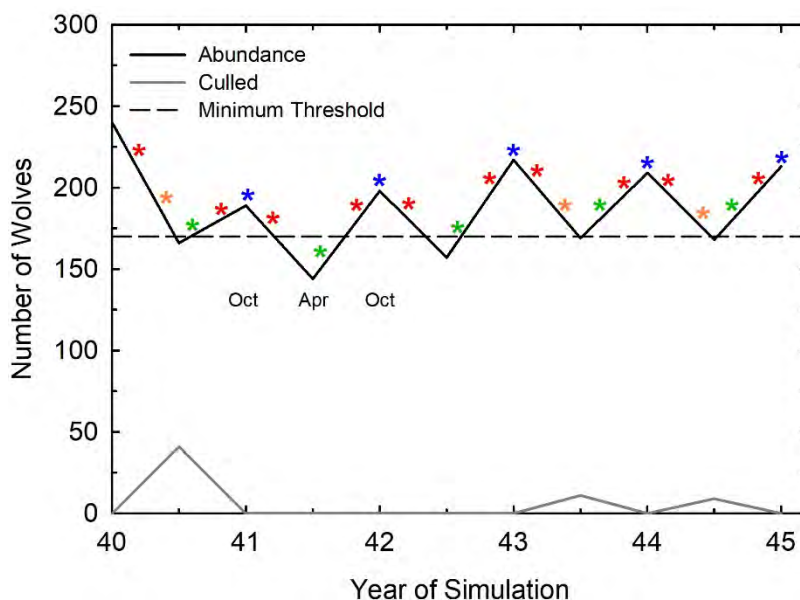
Genetic output from the simulated populations in the absence of immigration from Finland/Russia shows short-term improvement in both the population mean inbreeding coefficient (Figure 6) and the retention of gene diversity (Figure 7). The initial gene diversity is estimated to be 0.802, with some very slight variation across scenarios resulting from the stochastic process of randomly "dropping" unique founder alleles through the known pedigree to initialize population genetic structure. After this improvement over six to seven years, models show consistent increases in mean inbreeding levels and a gradual erosion of gene diversity over the timeframe of the simulations. The short-term improvement is likely a result of the genetic contributions of the newly-arrived founders to the Scandinavian population and, to a lesser extent, the removal of genetically less valuable wolves through selective culling. The populations that are maintained at the smaller abundances accumulate inbreeding and lose gene diversity through random genetic drift more rapidly than those populations maintained at larger target abundances. It is important to observe here that even those simulated populations maintained at the largest abundance values (310 to 340 individuals) show higher levels of inbreeding and a gradual loss of gene diversity. While these populations are relatively large on the scale examined here, the consequences of isolation will inevitably result in reduced genetic viability over time.
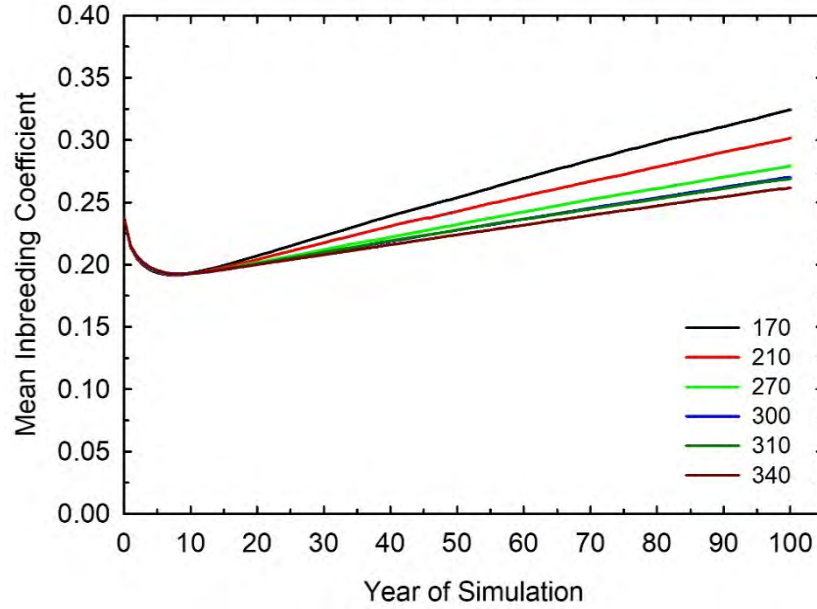
Figure 6. Projections of mean inbreeding coefficient in the Scandinavian wolf population across the range of minimum population abundance threshold values and assuming no immigration from Finland/Russia. Initial values of mean inbreeding in the population are based on pedigree information used as input for all simulations. See accompanying text for more information on model structure and function.
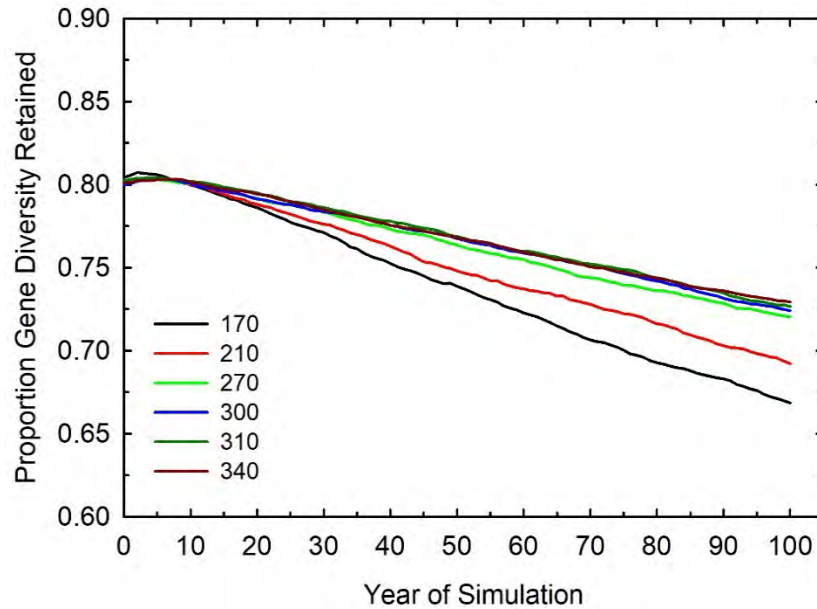
Figure 7. Projections of proportional gene diversity (GD) retained in the Scandinavian wolf population across the range of minimum population abundance threshold values and assuming no immigration from Finland/Russia. Initial GD values in the population are based on pedigree information used as input for all simulations. Retention values reported in this figure give the estimated theoretical proportion of diversity that remains in the population relative to the ancestral population source (Finland/Russia). See accompanying text for more information on model structure and function.

Analysis of genetic benefits of immigration into the Scandinavian wolf population

The simulations discussed in this report do not feature changes to the underlying rates of reproduction or age-specific mortality among Scandinavian wolves. As described in the section "Demographic performance of the base scenario" (page 12), the simulated Scandinavian wolf population grows at a mean realized annual rate of approximately 2% (in the presence of natural and anthropogenic sources of mortality including licensed hunting), which is consistent with the past ten years of data analyzed by Milleret et al. (2023). Consequently, it is assumed that the observed rate of population growth in simulations either excluding or including occasional immigration of wolves from the Finland/Russia population into the Scandinavian population will be effectively identical. This assumption has been verified in observations of mean population growth rate and mean long-term population size in simulations featuring the range of immigration rates tested as part of this analysis (detailed results can be found in Table 2). Therefore, analysis of scenarios featuring immigration into the Scandinavian population will focus on the potential genetic benefits of that immigration process.

Across the full range of minimum population abundance threshold values and immigration rates tested in this analysis, occasional addition of wolves from the source Finland/Russia population leads to a reduced rate of gene diversity loss over time in the Scandinavian population, compared to a "control" scenario excluding immigration (Figure 8, Table 2). This benefit is more pronounced at the lower end of the range of population abundance threshold values (170 to 270 individuals), where smaller isolated populations lose gene diversity more rapidly through increased rates of inbreeding and accelerated genetic drift. As expected, increased frequency of immigration into the Scandinavian population leads to an enhanced genetic effect – lower rates of inbreeding and increased retention of population gene diversity.

The absolute values of proportional gene diversity retained that are shown in Figure 8 are calibrated to the starting value of gene diversity expected to be captured from the historical set of founders that immigrated into the Scandinavian population from Finland and Russia ($GD_0 = 0.802$). More precisely, this starting value is the expected heterozygosity in the population at the start of the simulation, determined by the historical data on founder immigration events and the mating structure among individuals as given by the full population pedigree dating back to the first immigration event in the early 1980s. As explained previously, a statistical technique is used to "drop" unique founder alleles through the pedigree to estimate current allele frequencies, and, by extension, expected heterozygosity. In addition to this presentation of absolute gene diversity retention, another expression of gene diversity retention can be calculated as the relative proportion of the starting value that is retained in the Scandinavian population at the end of the 100-year simulation. This relative retention metric can readily identify situations where introgression of new genes from recent immigrants can effectively offset the loss of existing diversity from past founders.

Figure 9 summarizes gene diversity outcomes for the full range of scenarios making up this analysis. Proportional gene diversity retained over 100 years relative to the simulation starting conditions ranges from a minimum of 0.834 (minimum population abundance threshold value of 170 in the absence of immigration) to 1.003 – 1.005 (minimum population abundance threshold values of at least 270 and with an average of one immigrant entering the Scandinavian population every three years).

Proportional gene diversity retention values of 0.95 or 0.90 over 100 years can be used as candidate measures of genetic viability for the purposes of interpreting these results. Using the 0.95 retention metric, the results in Figure 9 indicate that if the mean immigration rate equates to one wolf added to the Scandinavian population every three years, then all minimum population abundance threshold values within the tested range exceed the genetic viability criterion. For a mean immigration rate of one wolf every six years, minimum population abundance threshold values of 270 and above satisfy the 0.95 genetic viability criterion. A population reference value of 340 wolves can satisfy the 0.95

criterion when the immigration rate is reduced to an average of one wolf added to the Scandinavian population every nine years on average. If the 0.9 retention metric is used, only isolated populations below the threshold of 300 and a population threshold of 170 with the lowest immigration rate fail to meet the genetic viability criterion (see Table 2 for the full results).
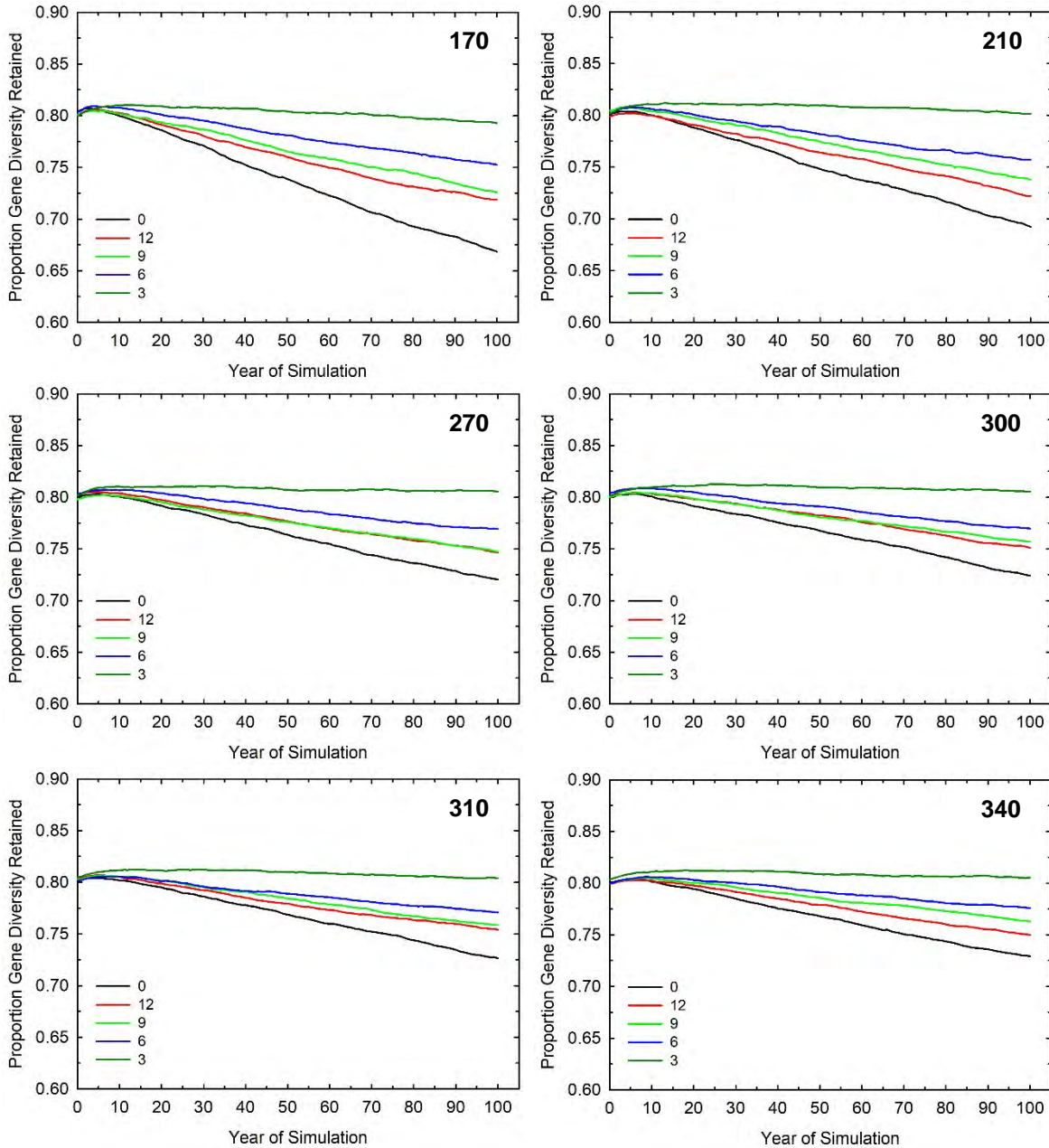
Figure 8. Projections of proportional gene diversity (GD) retained in the Scandinavian wolf population across the range of minimum population abundance threshold values and expected rates of immigration of wolves from Finland/Russia. The value in the top-right corner of each panel is the minimum abundance threshold value for that set of scenarios. Each plot legend defines the expected immigration rate, with numerical values equal to the average number of years between immigration of one individual (male or female) into the Scandinavia population. Initial population GD values are based on pedigree information used as input for all simulations. Retention values reported in this figure give the estimated theoretical proportion of diversity that remains in the population relative to the ancestral population source (Finland/Russia). See accompanying text for more information on model structure and function.
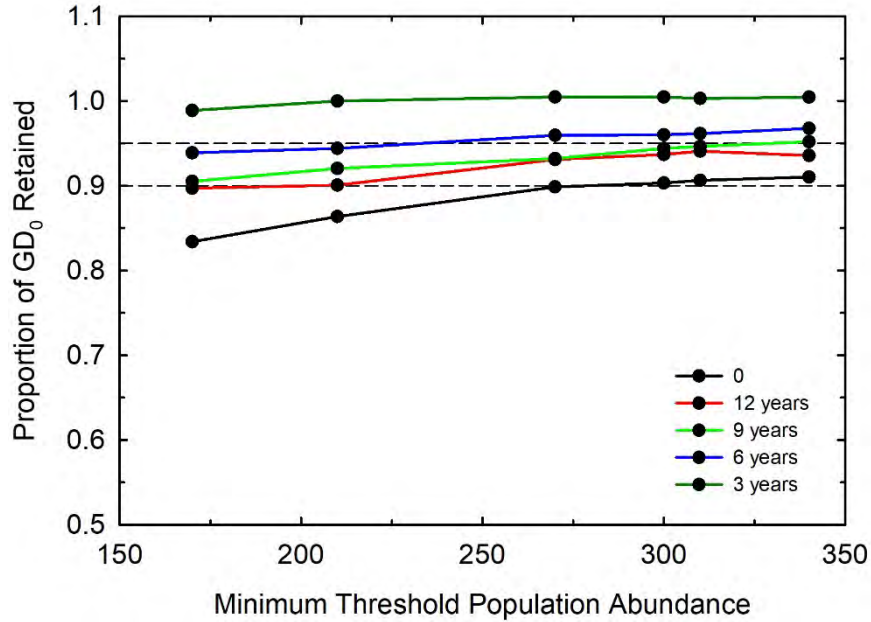
Figure 9. Scandinavian wolf population gene diversity (GD) retained after 100 years, expressed as the proportion of the estimated initial value in the population at the beginning of the simulations. Horizontal dashed lines identify 90% and 95% GD retention levels often used for identifying genetic viability metrics. See accompanying text for more information on model structure and function.

The gene diversity retention results displayed in Figure 9 are mean values calculated over 1000 model iterations for each scenario. The specific probability that the final gene diversity would actually meet or exceed a candidate genetic viability criterion for any given scenario is given in Figure 10. This probability is calculated by storing the final GD value at model year 100 for each of the 1000 iterations run for a given scenario, and calculating the proportion of those values that meet or exceed a candidate criterion. When considering the smallest population abundance threshold of 170 individuals, the probability of meeting or exceeding the 0.95 retention criterion is 0.711 under the highest rate of immigration (one individual every three years on average) and declines to just 0.172 when immigration is absent (Figure 10A). The probability of achieving or exceeding the 0.90 retention criterion increases to 0.837 under the highest immigration rate and to 0.368 assuming no immigration from Finland/Russia (Figure 10B). If the population abundance threshold is increased to 340, the probability of achieving or exceeding the 0.95 retention criterion ranges from 0.781 (one immigrant every three years) to 0.450 (no immigration). The 0.9 retention criterion can be achieved or exceeded with a probability ranging from 0.891 (one immigrant every three years) to 0.645 (no immigration).

If immigration is absent from the analysis, the proportional retention of gene diversity is a comparatively strong function of the minimum population abundance threshold value. As the immigration rate increases, this relationship is less pronounced; interestingly, under the highest rate of immigration tested here (mean one individual every three years) there is rather little influence of population abundance threshold on the final extent of gene diversity retained. In other words, the models appear to be more sensitive to the rate of introgression of wolves from the Finland/Russia source population than they are to the minimum population abundance threshold imposed across the timeframe of the simulation. It is also important to recognize that the achievement of genetic viability as defined here is observed to be a probabilistic phenomenon and, therefore, is not guaranteed given a specified set of biological and

management conditions. A precise definition of population viability can incorporate metrics on both overall gene diversity retention over a specified period of time and the expressed confidence in achieving that outcome.
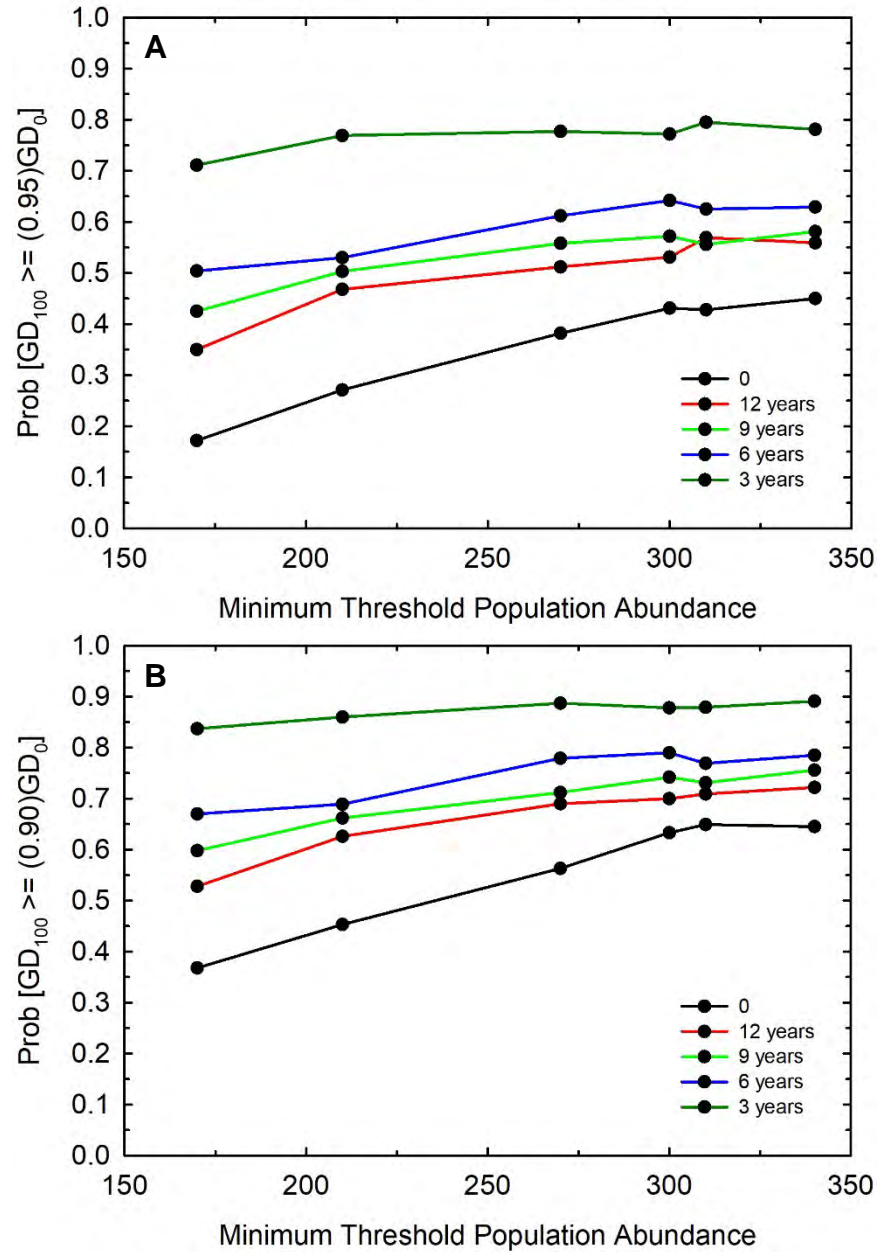
Figure 10. Probability that the gene diversity retained in the Scandinavian wolf population is at least 95% (A, top panel) or 90% (B, bottom panel) of the initial value at the beginning of the simulations. Curves represent different rates of immigration of wolves from the Finland/Russia population, expressed here as the mean time interval between immigration events (single wolf entering the Scandinavian population). See accompanying text for more information on model structure and function.

Table 2. Key output metrics for Scandinavian wolf population dynamics simulations. Shaded cells indicate those scenarios in which the Scandinavian population retains at least 90% (light green) or 95% (darker green) of initial population gene diversity after 100 years.

Column headings: Min N, minimum population abundance threshold; D, mean number of successive years between individual wolves immigrating from the Finland/Russia population into the Scandinavian population; P(E), probability of population extinction within 100 years; $N(Ext)_{100}$ (SD), mean total population size (as of 1 October) across extant populations at 100 years (standard deviation); $N(Ad/Sub/Pup)_{100}$ (SD), mean number of adults/subadults/pups (as of 1 October) across extant populations at 100 years (standard deviation); GD_{100} (SD), mean population gene diversity after 100 years (standard deviation); % GD_0, proportion of population gene diversity retained after 100 years relative to the initial value estimated at the beginning of the simulation; Prob ≥ (0.95/0.90GD_0), probability that the final population gene diversity value is at least 95% / 90% of the initial value at year 0; F, mean population inbreeding coefficient.

Min N	D	P(E)	$N(Ext)_{100}$ (SD)	$N(Ad)_{100}$ (SD)	$N(Sub)_{100}$ (SD)	$N(Pup)_{100}$ (SD)	GD_{100} (SD)	% GD_0	Prob ≥ (0.95GD_0)	Prob ≥ (0.90GD_0)	F
170	0	0.020	209.9 (109.1)	87.1 (45.7)	48.8 (29.9)	76.3 (50.3)	0.669 (0.121)	83.4	0.172	0.368	0.324
	12	0.010	204.6 (84.3)	83.0 (34.7)	47.9 (25.5)	75.6 (42.0)	0.719 (0.107)	89.7	0.350	0.528	0.296
	9	0.007	211.5 (91.0)	85.6 (39.1)	48.9 (24.5)	79.5 (47.5)	0.726 (0.105)	90.5	0.425	0.598	0.288
	6	0.003	208.8 (72.7)	82.9 (29.8)	49.0 (21.1)	77.9 35.0)	0.753 (0.095)	93.9	0.504	0.670	0.276
	3	0.006	211.3 (63.6)	81.8 (25.2)	49.7 (19.3)	80.4 (31.6)	0.793 (0.076)	98.9	0.711	0.837	0.246
210	0	0.017	262.9 (93.5)	106.0 (39.2)	60.8 (26.9)	98.0 (44.8)	0.692 (0.107)	86.4	0.271	0.453	0.302
	12	0.006	261.5 (92.6)	104.1 (38.6)	61.0 (26.7)	98.1 (43.5)	0.722 (0.101)	90.1	0.468	0.626	0.282
	9	0.009	258.3 (85.9)	102.1 (35.1)	60.3 (24.9)	97.1 (40.9)	0.738 (0.100)	92.0	0.503	0.662	0.274
	6	0.006	262.7 (81.1)	103.1 (32.7)	61.7 (24.1)	99.2 (40.0)	0.757 (0.088)	94.4	0.530	0.689	0.263
	3	0.002	262.6 (63.3)	101.2 (24.4)	61.4 (19.4)	100.7 (34.7)	0.802 (0.073)	100.0	0.769	0.860	0.237
270	0	0.012	339.7 (84.8)	133.6 (34.8)	78.8 (24.5)	129.2 (46.9)	0.720 (0.095)	89.9	0.382	0.563	0.279
	12	0.004	338.1 (85.4)	131.8 (34.0)	80.1 (25.5)	127.9 (46.3)	0.746 (0.090)	93.1	0.512	0.690	0.263
	9	0.001	343.7 (88.4)	132.9 (35.5)	80.6 (25.6)	131.7 (46.6)	0.747 (0.092)	93.2	0.558	0.712	0.258
	6	0.006	337.3 (88.3)	129.7 (34.5)	79.5 (25.9)	129.7 (46.8)	0.769 (0.081)	96.0	0.612	0.779	0.250
	3	0.000	347.2 (65.9)	131.4 (25.9)	82.5 (20.8)	134.0 (38.0)	0.805 (0.068)	100.5	0.777	0.887	0.229
300	0	0.002	379.7 (92.7)	148.3 (38.3)	88.9 (27.6)	144.6 (48.9)	0.724 (0.094)	90.3	0.431	0.633	0.270
	12	0.003	381.2 (91.8)	147.4 ()36.4	89.6 (27.3)	146.1 (50.3)	0.751 (0.087)	93.7	0.531	0.700	0.256
	9	0.001	380.4 (83.9)	146.5 (33.5)	88.6 (26.4)	146.6 (45.3)	0.757 (0.085)	94.4	0.572	0.742	0.253
	6	0.002	380.5 (78.2)	144.8 (30.1)	90.8 (24.5)	145.8 (43.5)	0.770 (0.079)	96.0	0.642	0.790	0.244
	3	0.001	385.9 (76.4)	144.9 (27.3)	91.0 (23.9)	150.5 (43.2)	0.805 (0.067)	100.5	0.772	0.878	0.226

Table 2. (Continued)

Min N	D	P(E)	N(Ext)$_{100}$ (SD)	N(Ad)$_{100}$ (SD)	N(Sub)$_{100}$ (SD)	N(Pup)$_{100}$ (SD)	GD$_{100}$ (SD)	% GD$_0$	Prob ≥ (0.95GD$_0$)	Prob ≥ (0.90GD$_0$)	F
310	0	0.007	388.1 (98.9)	151.6 (39.6)	91.1 (28.7)	147.5 (52.3)	0.727 (0.091)	90.6	0.428	0.649	0.269
	12	0.001	393.1 (88.9)	150.7 (36.2)	93.4 (25.6)	150.2 (46.7)	0.754 (0.084)	94.1	0.569	0.709	0.255
	9	0.002	387.8 (97.8)	149.0 (37.6)	92.0 (27.8)	148.2 (51.7)	0.759 (0.083)	94.6	0.556	0.731	0.251
	6	0.001	399.0 (85.1)	151.9 (32.5)	93.4 (24.9)	154.9 (48.1)	0.771 (0.078)	96.2	0.625	0.769	0.244
	3	0.000	401.3 (81.2)	150.3 (30.3)	95.9 (24.3)	155.9 (46.7)	0.804 (0.073)	100.3	0.795	0.879	0.225
340	0	0.003	430.8 (106.1)	166.8 (41.4)	100.7 (31.9)	165.3 (53.9)	0.729 (0.097)	91.0	0.450	0.645	0.262
	12	0.001	431.7 (103.1)	165.4 (41.2)	101.8 (31.2)	167.7 (57.4)	0.750 (0.087)	93.6	0.559	0.722	0.250
	9	0.000	427.9 (95.8)	163.3 (36.7)	101.8 (29.6)	164.0 (50.3)	0.763 (0.079)	95.2	0.581	0.756	0.246
	6	0.001	436.2 (90.8)	165.8 (33.9)	101.2 (27.0)	170.4 (51.4)	0.776 (0.078)	96.8	0.629	0.785	0.239
	3	0.002	439.0 (87.9)	164.7 (33.1)	102.4 (26.3)	173.0 (50.2)	0.805 (0.069)	100.5	0.781	0.891	0.223

Testing model sensitivity to select demographic input parameters

Reviewers of the initial draft version of this report identified a subset of input parameters with relatively higher levels of uncertainty that, as a result, could be the subject of additional sensitivity testing in order to determine the impact of this uncertainty on model outcome. Three parameters were chosen for this additional analysis:

- Proportion of adult females breeding: All models in this PVA assume that, on average, 60% of adult females produce a litter of pups each year. This estimate was carried over from the most recent PVA model developed by Bruford (2015). Unpublished population data compiled by SKANDULV over the time period 2011 to 2022 indicates that this estimate of reproductive success is in fact 64%. A subset of new models was run created this as the new mean value for the percentage of adult females breeding (producing pups) each year.

- Genetic rule for population culling: Based on conversations with members of the PVA model development team (Appendix I), the assumption was made that culling of individuals each year was conditional on their genetic makeup – measured by their mean kinship to other members of the population. In particular, individuals that are deemed to be relatively more genetically valuable, as measured by a low mean kinship value, would not be subject to legal removal. Subsequent discussions during the review process suggests that this practice is not presently practiced, although regulations do in fact specify that immigrants and their offspring are exempted from legal harvest. A subset of new models was created that removed the genetic condition on harvesting individuals, while retaining the restriction on harvesting immigrants. [Note that model restrictions do not allow for identification of the full range of immigrant offspring for similar harvest restrictions.]

- Size of the source population in Finland/Russia: The core set of models set the initial abundance of the Finland/Russia population at 500 individuals, and with a habitat carrying capacity (K) of 800. These figures were chosen somewhat arbitrarily, primarily with the goal of maximizing the ability of that population to successfully serve as a source of immigrants to the Scandinavia population. Subsequent discussion argued for a smaller population in Finland/Russia, as well as a reduced value for the habitat carrying capacity. In light of these discussions, and informed by a recent report on analysis of wolf population viability in Finland (Mäntymiemi et al. 2022), a subset of new models was created in which the initial abundance in the Finland/Russia population is assumed to be 300, and with a habitat carrying capacity of 600.

The set of five scenarios featuring a minimum population abundance threshold value of 270 individuals was used as the basis for this analysis, as this represents a value approximately mid-way across the range of tested abundances. Each of the three parameters identified above were revised to their alternate values independently in order to evaluate their specific impact on population performance. Proportional retention of gene diversity relative to the starting value was chosen as the output metric for analysis.

Overall, the identified modifications to each of the three input parameters resulted in only very small changes in the chosen output metric, with the changes generally observed to shift gene diversity in the predicted direction (Figure 11). Relaxing the genetic rules governing removal of individuals each year – effectively defined as random removal of living non-immigrants – would be expected to result in removing some individuals of relatively higher genetic value and, consequently, a higher rate of gene diversity loss in the population. The model results confirm this expectation, although the absolute magnitude of reduced gene diversity retention is quite small: final proportional gene diversity retained differed from the baseline scenario results by 0.2% to 1.7%. Similarly, a smaller source population in Finland/Russia would, over time, be expected to result in immigrants entering the Scandinavian population with a higher mean relatedness and, by extension, a comparatively smaller positive contribution to gene diversity in the Scandinavian population. Again, the models generally confirm this expectation, although not with the same level of consistency and magnitude as was seen in the scenarios

featuring a modification to the population culling rules (gene diversity retention change of 0.4% to 0.9% depending on immigration rate). Increasing the proportion of females producing pups each year was expected to produce minimal changes to the population genetic output metric. This change would result in a small increase in population growth rate; however, because of the mechanic built into these models that regulates population abundance at or just above the minimum population abundance threshold value, this apparent benefit would be largely offset by the correspondingly larger number of individuals subject to culling to keep the population at the desired abundance (gene diversity retention change of 0.1% to 0.8% depending on immigration rate).
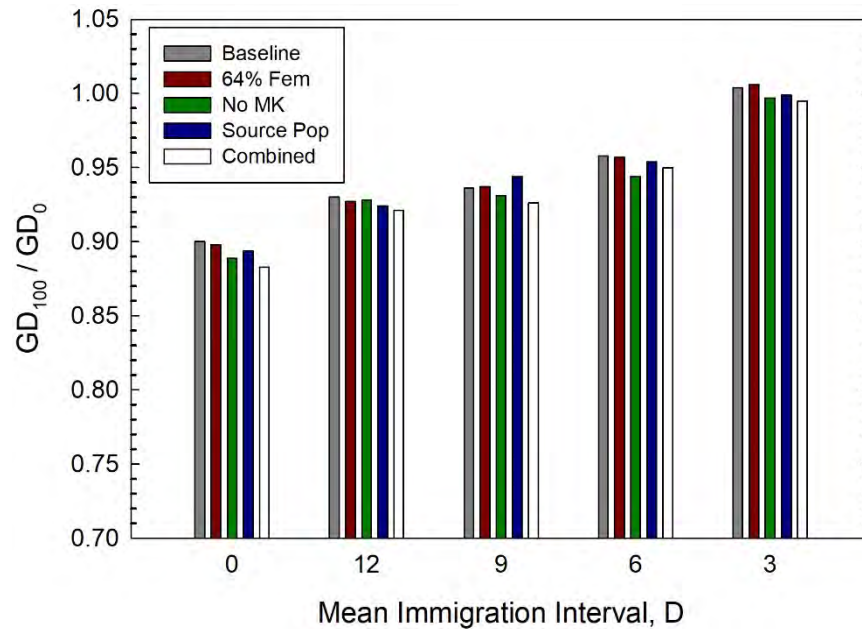
Figure 11. Results of sensitivity analysis scenarios, expressed as the proportional retention of population gene diversity at 100 years (GD_{100}) relative to the gene diversity present in the Scandinavian population at the beginning of the simulation (GD_0). See accompanying text for more information on sensitivity scenario parameter values and overall model structure.

Combining each factor into a single set of scenarios – featuring higher female breeding success, relaxing the genetic-based culling rule, and reducing the size of the Finland/Russia source population – yields results that generally conform to expectations. Gene diversity retention is reduced for each level of immigration relative to the baseline scenarios, but as before the absolute difference remains small at 0.8% to 1.9% compared to the baseline retention value, depending on the immigration rate.

Discussion and Conclusions

Interpreting the language of the EU Habitats Directive for this analysis

The key question posed by the Swedish Government in the context of guiding the construction and implementation of this population viability analysis (PVA) focuses on the conditions that are required for the reference value for the Scandinavian wolf population size, as defined for favorable conservation status according to the European Union Habitats Directive, to lie within the interval of 170 – 270 individuals. In order to properly address this question, it is important to understand the intended meaning of certain words and phrases in the above sentence.

First, the Habitats Directive guidance document (DG Environment 2023) considers the conservation status of a species to be "favorable" when "...the species is maintaining itself on a long-term basis as a viable component of its natural habitats". The author of this report assumes that the concept of favorable conservation status as applied to species in the above statement can also be applied to individual populations as is being done here for the Scandinavian wolf population. Furthermore, the phrase "...the species is maintaining itself on a long-term basis..." implies that the species or population is not declining in abundance and, at minimum, is demonstrating a mean trend in abundance over some specified time period that is at least stable (in other words, neither growing nor declining) and preferably increasing. Secondly, the Directive defines the concept of a favorable reference population (FRP) as the "...population in a given biogeographical region considered the minimum necessary to ensure the long-term viability of the species...".

Note that both of these definitions invoke the concept of viability. The Directive does not provide definitive guidance on how to precisely define the concept of viability and its dependence on a timeframe within which it is measured. In the absence of this definitive guidance, it may be necessary to refer to existing frameworks within which demographic and genetic definitions of viability have been proposed. For example, the Red List Categories and Criteria as defined by the International Union for Conservation of Nature (IUCN) define the risk-based boundary between threatened and non-threatened taxa at a 10% risk of species or population extinction within 100 years (IUCN 2012). The relatively newer addition of explicit genetic concerns in relation to wildlife population viability takes into account the retention of a quantitative threshold of genetic diversity in a population over a specified period of time. These thresholds – typically in the range of retaining 90% to 95% of the original founder gene diversity over a period of 100 years – originate from concepts guiding management of captive populations of endangered species where intensive management of breeding dynamics can be used to promote viability (for example, Lees and Wilcken 2009, 2011). As a working framework for interpreting the results of the present analysis, it may be instructive to adopt similar definitions of both demographic and genetic viability as diagnostic of favorable conservation status as presented in the European Union's Habitats Directive. The official definition of viability generated by government authorities may differ from this example.

Key attributes of PVA model structure and interpretation of results

It is important to clarify the mechanisms by which the range of minimum population abundance threshold values used in the simulations described here – serving as precursors to the associated range of favorable population reference values – are implemented. Before that can be done, however, it must be remembered that all scenarios in this PVA begin in October 2022 with a population of wolves in Scandinavia (Sweden and Norway: N = 463) that is <u>larger</u> than the upper bound of population reference values tested here. Because of this reality, and in light of the question posed by the Swedish Government that frames this analysis, the simulations include a mechanism by which wolves are removed on an annual basis in order to bring the population down to an abundance that is more compatible with the identified reference value. This removal of wolves is therefore considered a necessary activity within the modeling framework to examine a range of future population abundance thresholds while simultaneously acknowledging the need

to initialize the simulations under existing conditions. Establishing hunting regulations in Sweden is certainly not without controversy (e.g., Laikre et al. 2022), and the best scientific information must be used to inform responsible decision-making for management of this endangered population. In implementing this component of the model, the author neither supports nor condones any level of wolf harvest or similar form of management.

The removal process implemented in these simulations is similar in theory to the "selective harvest" of Liberg and Sand (2012), in which genetically valuable animals are exempt from harvest in order to retain their genetic representation in the living population. In the Vortex simulations used here, all new immigrants from the Finland/Russia population (genetic founders to the Scandinavian population) are exempt from this harvest, as are individuals that have low levels of relatedness to other wolves as measured by individual mean kinship (calculated from the initial and derived pedigree over time). The current analysis does not explicitly restrict F1 offspring of new founders from harvest, but these individuals are almost certainly implicitly exempt due to their low levels of kinship to the rest of the population. All in all, the harvest mechanic used here made every attempt to be as realistic as possible and to reduce its impact on the genetic characteristics of the Scandinavian population.

Given this harvest element of the model, it should be recognized that the minimum population abundance threshold value used in any given scenario should not be interpreted as a form of population carrying capacity. The carrying capacity of a population (or, more precisely, the habitat within which the population resides) acts ecologically as a ceiling, restricting the population from further growth under favorable conditions. Instead, in this PVA the population abundance threshold value is meant to act as a floor – the lower bound of abundance, below which the population of interest should not decline. Harvest (set to occur in the winter timestep) only occurs if the population is larger than the reference value, thereby encouraging the population to grow to that value. In the case of this wolf population, the underlying realized annual growth rate of 1.5% to 2.0% should help the population recover from declines due to random drops in survival and/or reproduction or the occasional severe event like a disease outbreak. This growth rate can be significantly larger when the population is below the desired abundance threshold, as legal culling is no longer employed (both in the model and in reality) in favor of facilitating growth to the desired minimum abundance. When population abundance exceeds the threshold to a significant extent, culling is designed to reduce the population closer to that minimum abundance value.

Because this harvest is probabilistic, the simulation of this process will sometimes result in a smaller number of wolves removed than expected. This will result in a population count in the following October that is not reduced to the exact population reference value. Additionally, the observed abundance as of 1 October is the outcome not only of wolf removal the preceding winter, but also of the addition of new surviving pups in the spring, just before the October census is taken. As a result of the combined effects, the long-term abundance therefore equilibrates at a value that is greater than the desired abundance threshold. But this is consistent with the spirit of the Habitats Directive's broad definition of "viable population" as the minimum abundance needed to reduce of an adverse demographic or genetic outcome for the population in the timeframe of the analysis (100 years). To satisfy this viability criterion, the population abundance should ideally remain greater than the stipulated minimum, thereby reducing the likelihood of sustained reductions below that value and risking the destabilizing effects of the "extinction vortex" (Gilpin and Soulé 1986). In this way, the abundance threshold value is truly acting not as a cap on population abundance, but as a floor – below which the population should not decline.

The detailed description of model structure in the report by Bruford (2015) is often unclear, so it is difficult to reconstruct the process by which any type of similar harvest mechanic was implemented to examine different population reference value targets. Some of those models also did not use a pedigree file specifying the ancestral relationships among animals that were alive as of the start date of that analysis. Moreover, that earlier report included many simulations that featured carrying capacity values as

high as 2000 – values significantly greater than the upper bound of the range of population reference values tested in this analysis. It is therefore not practical to make direct comparisons between the results of the present analysis and the earlier Bruford analysis.

While acknowledging the difficulties in comparing the present model structure to the most recent analysis of Bruford (2015), it is instructive to consider some potential weak points of this Vortex model and how those weaknesses could impact the results. At a fundamental level, there may be concerns that the relatively more "generic" structure of the software may not capture the potentially complex nuances of species like wolves that feature socially-stratified breeding dynamics. The basic Vortex framework does not include facilities for including this social stratification, but it can be done if the specific analytical questions call for it and if the relevant data are available. The ability in the current wolf model to specify long-term monogamous breeding, to accurately portray the starting population genetic structure by incorporating the historic pedigree data, and the ability to develop complex rules around population regulation are likely sufficient to create projections of wolf population dynamics that are useful for the larger decision-making process.

When investigating model structure and function at a finer scale, a few input parameters can be highlighted in a discussion of model accuracy and realism:

- Extent of genetic load: The specification of genetic load – the demographic cost of inbreeding, quantified by the number of lethal equivalents – is based on relatively old data from Liberg et al. (2005) that could potentially be updated if new information exists. The study by Bensch et al. was considered for this analysis, but the uncertainty around the precise demographic mechanism of an apparent inbreeding effect was a key factor in ultimately deciding to not include this potential effect in the present PVA. The authors of that study acknowledged that they were unable to determine of the higher success of heterozygous wolves to recruit to the breeding population was caused by selection on survival to breeding age or instead on factors determining pair formation and successful mating. Because of this ambiguity, there appeared to be a significant risk of overestimating the inbreeding effect, primarily by adding the signal detected by Bensch to the existing pup survival effect reported by Liberg et al. (2005). This risk of "double dipping" is real and, given some ambiguity about the mechanism of incorporating this study into our demographic description of wolf population dynamics, the choice was made to exclude it from the analysis.
- Catastrophes: The frequency of a generic catastrophic event as simulated in this analysis – occurring just once on average in a 100-year period – that reduces both survival and reproduction in Scandinavian wolves may be underestimated. Reed et al. (2003) suggested a much higher frequency of a significant event impacting vertebrate populations, and Miller (2017) specified a similarly higher frequency of an infectious disease outbreak in a PVA for Mexican wolves. If a catastrophic event with a higher frequency were included in this analysis, the impact would likely be more deleterious that considered here. The downstream consequences for identification of the minimum viable population abundance are less clear.
- Mean litter size: The mean litter size used for this analysis – an average of 5.3 pups born in April to successful females – may be an overestimate of the true estimate of fecundity in this population. Sand et al. (2014) estimates a mean of 3.5 pups per litter. An assumption for the present PVA is that this value represents an estimate of pups alive as of the 1 October census, which is a different parameter than what is used in this analysis (number of pups alive at birth in April). Assuming a survival rate of approximately 0.82 in the April – October time period (in line with the analysis of Milleret et al. (2023)), the estimated number of live pups at birth would be 4.3. This is in fact quite similar to the original estimated number of live pups at birth for this analysis (4.5) based on the analyses of Liberg et al. (2005). This number was adjust upwards to 5.3 to facilitate a simulated population growth rate matching that reported by Milleret et al. (2023). The fact that we see a wolf population in Scandinavia that is consistently growing in abundance, and given the rather robust

survival estimates presented in Milleret et al. (2023), the uncertainty in mean litter size in this population is likely to be of little consequence in the larger analysis.

Another key element of the present analysis is the metapopulation structure defined by the presence of a second population representing wolves in Finland/Russia. In the Vortex model used here, the demographic and genetic characteristics of this population are not specified with the same level of detail as the Scandinavian population; this second population is designed primarily to serve as a source of animals that would occasionally disperse to the west and become immigrants into the Scandinavian population. This mechanism of probabilistic dispersal is believed to be more realistic than the analysis of Bruford (2015) in which immigration was typically simulated as a deterministic "supplementation" of a specified number of individuals over a specific time interval. Although it is unlikely within the model that more than one individual would immigrate into the Scandinavian population in any given year, the timing of those immigration events is random across years and across iterations so that greater or fewer numbers of wolves could immigrate into the population over the duration of the simulation.

Perhaps the most significant assumption influencing the results of this PVA concerns the relatedness among the founders of the Finland/Russia population and the kinship of that population to the Scandinavian wolves. Since we do not have a detailed pedigree for the Finland/Russia population, we are unable to specify the genetic relationships among those individuals. [This is also, of course, the assumption concerning the seven founders of the current Scandinavian population.] The model does specify that all individuals in the Finland/Russia population start with inbreeding coefficients and kinships amongst each other equal to 0.1, but this only acts to recalibrate the mean level of inbreeding in the starting population away from 0.0 in the default scenario where all starting individuals are unrelated. In turn, this will lead to immediate impacts of inbreeding in that population. This detail, however, does not set levels of relatedness among Scandinavian and Finland/Russia wolves.

There is strong reasoning to assume that wolves immigrating from Finland/Russia share some level of explicit genetic ancestry with the Scandinavian wolves, as the current population is derived from that geographic source (see Smeds and Ellegren 2022 for a review and new data concerning this topic). It is important to remember, though, that the necessary assumption regarding pedigree data is that the founders that started the Scandinavian population, as well as those continuing to immigrate to the west, are also unrelated to each other and to existing Scandinavian wolves. If new data become available that demonstrate a specific common ancestry among the identified Scandinavian population founders, then it would be possible to revise the existing 1 October 2022 pedigree with this new information and update the analyses. Without those data, we are forced to make the assumption that the degree of relatedness among new wolves immigrating into Scandinavia from Finland/Russia is no greater than or less than the relatedness of founders of the Scandinavian population to each other. In this comparative context, it is difficult to estimate the degree of error in the results of the current analysis, especially when assessing the suitability of candidate reference population values.

Even with this recognized uncertainty, there is an argument to be made that the key metric of interest in evaluating a genetic component of population viability is the <u>relative rate of loss</u> of population-level gene diversity, and not the <u>absolute value</u> of that diversity that is retained. Numerous examples exist in the wildlife conservation literature where current levels of gene diversity are already below thresholds commonly used to define viability (e.g., retention of 90% wild population gene diversity). For example, captive populations of both the Mexican wolf (*Canis lupus baileyi*) and the red wolf (*Canis rufus*) have gene diversity values that are below the standard gene diversity retention targets, owing to small founder numbers, earlier extinction of the taxa in the wild, and challenges in proper population management through the early phases of the breeding programs (USFWS 2022; 2023. Despite this undesirable starting point, the goal of population management is to minimize the loss of existing gene diversity in order to

maximize opportunities for continued population growth and to improve parameters for long-term viability.

Finally, it may be instructive to note that the mean inbreeding coefficient in the Scandinavian population, as reported in Åkesson and Svensson (2022), has decreased from a maximum value of approximately 0.31 in 2006 to 0.24 in 2021 and has remained rather constant at the latter value since 2014. [Importantly, this analysis also appears to assume no genetic relatedness among founders since 1983.] This observation could imply that the assumptions built into the current Vortex model regarding relatedness among wolves within and between these populations may be reasonable.

Implications of PVA results for identification of MVP value for wolves in Sweden

The analysis described here is a dedicated attempt to create a sufficiently robust and realistic simulation of population dynamics and management that can ultimately inform the question of a favorable reference population (FRP) value for Scandinavian wolves. However, as described elsewhere in this report, the estimation of the FRP value must first be preceded by an analysis of the ecological and management conditions that can support maintenance of a minimum population abundance that can be considered viable according to specific criteria. A detailed demographic dataset was used to construct realistic rates of annual reproduction and survival for the population, and the genetic structure of the population was characterized by a complete pedigree of all living individuals and their ancestors dating back to the population's beginning about 40 years ago. Despite the depth of the dataset, the analysis required specification of some key assumptions about kinship among animals in the Scandinavian and Finland/Russia populations, and how this genetic ancestry may impact the benefits of immigration into Scandinavia from the source population to the north.

Given the nature of the current models discussed in this report, and acknowledging the assumptions built into these simulations as described above, the analysis suggests that the wolf population in Scandinavia (south-central Sweden and southeast Norway) can potentially be considered viable within the interval of 170 to 270 individuals in accordance with the broad definitions presented in the European Union's Habitats Directive. However, this condition requires the following processes to be maintained through time:

- The Scandinavian wolf population must have the demographic characteristics to, at a minimum, sustain a positive population growth rate, ideally similar to or greater than what has been observed over the past decade of detailed observations of reproduction and survival (annual growth lambda $\lambda \geq 1.02$, with the possibility of considerably higher growth rates in the absence of legal harvesting of wolves); and
- Immigration of wolves from Finland/Russia into Scandinavia should be, on average, no less than one individual every three years.

The above discussion defines conditions for maintaining a viable population of wolves in Scandinavia. A similarly viable wolf population in Sweden would also require the same general demographic conditions: reproductive and survival parameters that result in a capacity for sustained population growth, and consistent immigration of wolves from the recognized source population in Finland/Russia. However, because the Swedish population represents only a portion of the total wolf population in Scandinavia, any specification of a minimum viable population for the purposes of setting a favorable reference population in Sweden would require proper scaling of the larger regional population. For example, if a minimum viable population for Scandinavia were to be set at 250 wolves, and if we take the latest published data from Milleret et al. (2023) to assign approximately 85% of those wolves to Sweden, we would then identify a minimum viable population of wolves in Sweden equal to 210 to 215 individuals.

It is critically important to recognize, however, that the precise demographic characteristics of a viable population in Sweden or elsewhere cannot be specified until a clear demographic and genetic definition of wolf population viability is presented by the appropriate national or regional authorities. This is clearly demonstrated through detailed inspection of the results for gene diversity retention across the range of scenarios analyzed here. A demographic component of viability – often defined as risk of population extinction below a particular threshold value – appears to be met across the full range of scenarios evaluated here. Extinction risks do not exceed 0.02 over a timeframe of 100 years, which is far below the large majority of typical definitions of demographic viability found in the literature (e.g., Doak et al. 2015). In contrast, choice of a genetic viability criterion markedly changes the parameters for viability. If the criterion is 95% retention of today's gene diversity over 100 years, a rather restricted set of satisfactory candidate population abundance thresholds and immigration rates can be identified (see Figure 9). If 90% gene diversity retention is adopted as a viability criterion, a much more expansive set of satisfactory candidate population abundance thresholds and immigration rates can be identified. Moreover, the choice of which populations can be considered viable may also be constrained by the confidence one sees in the ability to achieve the specified genetic criterion. For example, authorities may wish to be at least 80% confident that their gene diversity retention can be achieved. As laid out in Figure 10, this decision on certainty significantly restricts the conditions within which population viability can be achieved.

The introgression of new genetic variation by immigration from the Finland/Russia population can be effective in offsetting the inevitable loss of variation in the Scandinavian population through inbreeding and random genetic drift. If immigrating wolves have a high degree of relatedness to the specific founders of the Scandinavian population, the benefits of this introgression will be reduced. It may be worth noting here that the immigration process being discussed is restricted to natural dispersal of individuals between populations. This process, therefore, does not technically fall under the definition of genetic rescue (Hedrick and Fredrickson 2010), in which unrelated individuals from another population are selected for managed introduction into the target population to reduce genetic load. The process of deciding if and when to adopt this more intensive approach to metapopulation management is beyond the scope of this analysis.

The simulations described here project future population characteristics over a time span of 100 years. The likelihood that current conditions for wolves in Scandinavia will persist in their current form for 100 years is, of course, extremely low. Researchers working in the field of PVA model development and improvement recognize the values – and the difficulties – of forecasting the ecological landscape for endangered wildlife as a function of the even more complex description of future economic, sociocultural, and political variables that determine the human-mediated natural environment (Miller and Lacy 2003; Lacy et al. 2013). Nevertheless, typical forecasts of population viability of both wild and captive populations are conducted for 100 years under the recognition that observing the predicted outcomes of alternative scenarios over many decades can help to strengthen the scope and intensity of recommended management activities designed to improve population viability in the shorter term (Frankham et al. 2010, 2014).

Acknowledgements

References

Åkesson, M., A. Danielsson, and C.C. Palacios. 2023. Teknisk rapport över genetiska analyser på varg i Sverige år 2022. Grimsö forskningsstation.

Åkesson, M., and L. Svensson. 2022. Sammanställning av släktträdet över den skandinaviska vargpopulationen fram till 2021. Rapport från SLU Viltskadecenter 2022–3.

Beissinger, S. and D. McCullough (Eds.). 2002. Population Viability Analysis. Chicago, IL, USA: University of Chicago Press.

Bijlsma, R.J., E. Agrillo, F. Attorre, L. Boitani, A. Brunner, P. Evans, R. Foppen, S. Gubbay, J.A.M. Janssen, A. van Kleunen, W. Langhout, R. Noordhuis, M. Pacifici, I. Ramirez, C. Rondinini, M. van Roomen, H. Siepel, and H.V. Winter. 2019. Defining and applying the concept of Favourable Reference Values for species and habitats under the EU Birds and Habitats Directives: Technical Report. Wageningen, Wageningen Environmental Research, Report 2928. 94pp.

Bruford, M.W. 2015. Additional population viability analysis of the Scandinavian wolf population. Final report to the Swedish Environmental Protection Agency.

DG Environment. 2023. Reporting under Article 17 of the Habitats Directive: Guidelines on concepts and definitions – Article 17 of Directive 92/43/EEC, Reporting period 2019-2024. Brussels. 104pp.

Doak, D.F., G.K. Himes Boor, V.J. Bakker, W.F. Morris, A. Louthan, S.A. Morrison, A. Stanley, and L.B. Crowder. 2015. Recommendations for improving recovery criteria under the US Endangered Species Act. Bioscience 65:189-199.

Ellner, S. P., J. Fieberg, D. Ludwig, and C. Wilcox. 2002. Precision of population viability analysis. Conservation Biology 16:258–261.

Frankham, R., J.D. Ballou, and D.A. Briscoe. 2010. Introduction to Conservation Genetics, second edition. Cambridge, UK: Cambridge University Press.

Frankham, R., C.J.A. Bradshaw, and B.W. Brook. 2014. Genetics in conservation management: Revised recommendations for the 50/500 rules, Red List criteria and population viability analyses. Biological Conservation 170:56-63.

Gilpin, M.E., Soulé, M.E., 1986. Minimum viable populations: processes of species extinction. Pages 19-34 in: Soulé, M.E., and B.A. Wilcox (Eds.), Conservation Biology: An Evolutionary-Ecological Perspective. Sinauer, Sunderland, MA.

Hedrick, P.W., and R. Fredrickson. 2010. Genetic rescue guidelines with examples from Mexican wolves and Florida panthers. Conservation Genetics 11:615-626.

Himes Boor, G.K. 2014. A framework for developing objective and measurable recovery criteria for threatened and endangered species. Conservation Biology 28:33-43.

IUCN. (2012). IUCN Red List Categories and Criteria: Version 3.1. Second edition. Gland, Switzerland and Cambridge, UK: IUCN. iv + 32pp.

Lacy, R.C. 2000. Structure of the Vortex simulation model for population viability analysis. Ecological Bulletins 48:191-203.

Lacy, R.C. 2019. Lessons from 30 years of population viability analysis of wildlife populations. Zoo Biology 38:67-77.

Lacy, R.C., and J.P. Pollak. 2023. Vortex: A stochastic simulation of the extinction process. Version 10.6.0.0. Chicago Zoological Society, Brookfield, IL, USA.

Lacy, R.C., P. S. Miller, P.J. Nyhus, J.P. Pollak, B.E. Raboy, and S.L. Zeigler. 2013. Metamodels for transdisciplinary analysis of wildlife population dynamics. PLoS ONE 8(12):e84211. doi:10.1371/journal.pone.0084211.

Lacy, R.C., P.S. Miller, and K. Traylor-Holzer. 2021. Vortex 10 User's Manual. 30 March 2021 update. IUCN SSC Conservation Planning Specialist Group, and Chicago Zoological Society, Apple Valley, Minnesota, USA.

Laikre, L. F.W. Allendorf, J. Aspi, C. Carroll, L. Dalén, R. Fredrickson, C.H. Wheat, P. Hedrick, K. Johannesson, M. Kardos, R.O. Peterson, M. Phillips, N. Ryman, J. Räikkönen, C. Vilá, C.W. Wheat, C. Vernest, and J.A. Vucetich. 2022. Planned cull endangers Swedish wolf population. Science 377:162.

Lees, C., and J. Wilcken. 2009. Sustaining the ark: The challenges faced by zoos in maintaining viable populations. International Zoo Yearbook 43:6–18.

Lees, C., and J. Wilcken. 2011. Global programmes for sustainability. WAZA Magazine 12:2–5.

Liberg, O., H. Andrén, H.-C. Pedersen, H. Sand, D. Sejberg, P. Wabakken, M. Åkesson, and S. Bensch. 2005. Severe inbreeding depression in a wild wolf (*Canis lupus*) population. Biology Letters 1:17-20.

Liberg, O., and H. Sand. 2012. Effects of migration and selective harvest for the genetic status of the Scandinavian wolf population. Report to the Swedish Environmental Protection Agency. 19pp.

Lotts, K.C., T.A. Waite, and J.A. Vucetich. 2004. Reliability of absolute and relative predictions of population persistence based on time series. Conservation Biology 18:1224-1232.

Ludwig, D. 1999. Is it meaningful to estimate a probability of extinction? Ecology 80:298–310.

Mäntyniemi, S., M. Valtonen, I. Helle, H. Johansson, S. Ponnikas, V. Nivala, J. Harmoinen, A. Herrero, S. Heikkinen, L. Kvist, J. Aspi, I. Kojola, and K. Holmala. 2022. Determining reference values for the favorable conservation level of the Finnish wolf population: Final report 2022. Natural resources and bioeconomy research 80/2022. Natural Resources Center. Helsinki. 147 pp.

Miller, P.S. 2017. Population viability analysis for the Mexican wolf (*Canis lupus baileyi*): Integrating wild and captive populations in a metapopulation risk assessment model for recovery planning. Report submitted to the U.S. Fish and Wildlife Service. 76pp.

Miller, P.S. 2023. Population viability analysis of the red wolf (*Canis rufus*): Integrated management of in situ and ex situ populations in support of species recovery in a mixed canid landscape. Report submitted to the U.S. Fish and Wildlife Service. 107pp.

Miller, P.S., and R.C. Lacy. 2003. Integrating the human dimension into endangered species risk assessment. Pages 41-63 in: F.W. Westley and P.S. Miller (eds.), Experiments in Consilience: Integrating Social and Scientific Responses to Save Endangered Species. Island Press, Washington DC.

Milleret, C., Dupont, P., Brøseth, H., Flagstad, Ø., Kindberg, J., Svensson, L., and Bischof, R., 2023. Estimates of wolf density, abundance, and population dynamics in Scandinavia, 2013–2023. MINA fagrapport 85. 34pp.

Morris, W.F., and D.F. Doak. 2002. Quantitative Conservation Biology: Theory and Practice of Population Viability Analysis. Sunderland, MA: Sinauer.

Redford, K.H., G. Amato, J. Baillie, P. Beldomenico, E.L. Bennett, N. Clum, et al. 2011. What does it mean to succesfully conserve a (vertebrate) species? Bioscience 61:39-48.

Reed, D.H., J.J. O'Grady, J.D. Ballou, and R. Frankham. 2003. The frequency and severity of catastrophic die-offs in vertebrates. Animal Conservation 6:109-114.

Reed, J.M., L.S. Mills, J.B. Dunning Jr., E.S. Menges, K.S. McKelvey, R. Frye, S.R. Beissinger, M.-C. Anstett, and P.S. Miller. 2002. Emerging issues in population viability analysis. Conservation Biology 16:7-19.

Sand, H., O. Liberg, Ø. Flagstad, P. Wabakken, M. Åkesson, J. Karlsson, and P. Ahlqvist. 2014. Den skandinaviska vargen - en sammanställning av kunskapsläget från det skandinaviska vargforskningsprojektet SKANDULV 1998 – 2014. Rapport till Miljødirektoratet, Trondheim. 118pp.

Sanderson, E.W. 2006. How many animals do we want to save? The many ways of setting population target levels for conservation. Bioscience 56:911-922.

Smeds, L., and H. Ellegren. 2022. From high masked to high realized genetic load in inbred Scandinavian wolves. Molecular Ecology 00:1-14. https://doi.org/10.1111/mec.16802.

Swedish Environmental Protection Agency (SEPA). 2015. Bilagor till Delredovisning av regeringsuppdraget att utreda gynnsam bevarandestatus för varg (M2015/1573/Nm). 148pp.

U.S. Fish and Wildlife Service. 2022. Mexican Wolf Recovery Plan, Second Revision. Region 2, Albuquerque, New Mexico, USA.

U.S. Fish and Wildlife Service. 2023. Revised Recovery Plan for the Red Wolf (*Canis rufus*). U.S. Fish and Wildlife Service, Atlanta, Georgia. 34 pp.

Vucetich, J.A., and M.P. Nelson. 2018. Acceptable risk of extinction in the context of endangered species policy. Pages 81–103 in: Cohen, A.I. (Ed.), Philosophy and Public Policy. Rowen and Littlefield International, New York NY.

Wikenros C, M. Gicquel, B. Zimmermann, Ø. Flagstad, and M. Åkesson. 2021. Age at first reproduction in wolves: different patterns of density dependence for females and males. Proc. R. Soc. B 288: 20210207. https://doi.org/10.1098/rspb.2021.0207

This page intentionally left blank

Appendices

Appendix I
January 2023 Online Meeting Participants

Name	Organization
Robert Ekblom	Swedish Environmental Protection Agency
Øystein Flagstad	Norwegian Institute for Nature Research
Inari Helle	Natural Resources Institute Finland
Helena Johansson	Natural Resources Institute Finland
Baharan Kazemi	Swedish Environmental Protection Agency
Samu Mäntyniemi	Natural Resources Institute Finland
Phil Miller	IUCN SSC Conservation Planning Specialist Group
Håkan Sand	Swedish University of Agricultural Sciences
Linnéa Smeds	Uppsala University, Sweden
Hanna Dittrich Söderman	Swedish Environmental Protection Agency
Mia Valtonen	Natural Resources Institute Finland
Petter Wabakken	Inland Norway University of Applied Sciences
Camilla Wikenros	Swedish University of Agricultural Sciences

Appendix II
Example Vortex PVA Scenario Input Summary

The information presented below is taken from a selected scenario-specific input file created by Vortex – in this case, the scenario features a Scandinavian wolf population reference value (PRV) of 170 with a mean immigration rate of one wolf entering the population every six years. Additional interpretation of model input requires some knowledge of Vortex and the syntax for coding specific customized input variables and/or output metrics. More detailed explanations of this example input is available from the author upon request.

```
VORTEX 10.6.0.0 -- simulation of population dynamics

Project: ScandinavianWolves

Scenario: PopRV170_6yrs

25/10/2023

   2 populations simulated for 200 years for 1000 iterations

   Undocumented options: Q I1

Sequence of events in each time cycle:
   EV
   Breed
   Mortality
   ISUpdate
   PSUpdate
   GSUpdate
   Harvest
   Disperse
   PSUpdate
   Age
   Supplement
   rCalc
   Ktruncation
   Census


Extinction defined as no males or no females.

EV correlation between reproduction and annual survival - left at default 0.5

Inbreeding depression with a genetic load consisting of
   6.04 total lethal equivalents per individual, of which
   50% are due to recessive lethals, and the remainder are lethal equivalents not subjected to removal by selection.
```

```
Global state variables
  GS1: ReferenceValue      Initialization: 170      Transition: 170

Populations:

  SwedenNorway
  Population state variables
  PS1: PopInbrCoeff        Initialization: 0.238   Transition: =IMEAN2
  PS2: NumPups             Initialization: 176      Transition: =ITOT3
  PS3: NumSubadults        Initialization: 140      Transition: =ITOT4
  PS4: NumAdults           Initialization: 147      Transition: =ITOT5
  PS5: Culled              Initialization: 0        Transition: =HARVESTS
  PS6: Incoming            Initialization: 0        Transition: =IMMIGRANTS
  PS7: Outgoing            Initialization: 0        Transition: 0

  FinlandRussia
  Population state variables
  PS1: PopInbrCoeff        Initialization: 0.1     Transition: =IMEAN2
  PS2: NumPups             Initialization: 180      Transition: =ITOT3
  PS3: NumSubadults        Initialization: 120      Transition: =ITOT4
  PS4: NumAdults           Initialization: 200      Transition: =ITOT5
  PS5: Culled              Initialization: 0        Transition: =HARVESTS
  PS6: Incoming            Initialization: 0        Transition: 0
  PS7: Outgoing            Initialization: 0        Transition: =EMIGRANTS


  Individual state variables
  IS1: MATE               Initialization: -1               Birth function: -1   Transition: =IS1
  IS2: InbrCoeff          Initialization: =I               Birth function: =I   Transition: =IS2
  IS3: Pup                Initialization: =A<2             Birth function: 1    Transition: =A<2
  IS4: SubAdult           Initialization: =(A=2)OR(A=3)    Birth function: 0    Transition: =(A=2)OR(A=3)
  IS5: Adult              Initialization: =A>3             Birth function: 0    Transition: =A>3
  IS6: Origin             Initialization: =P               Birth function: =P   Transition: =IS6


Correlation of EV among populations = 0.5


Both sexes disperse, from age 3 to age 25
Survival during dispersal: 100

Dispersal rates (as counts), from source (row) to destination (column):
                   SwedenNorway                FinlandRussia
  SwedenNorway                                      0
  FinlandRussia    =((Y%2)=1)*0.167
```

```
Reproductive System:

  Monogamy, with pairs maintained across years
  Females breed from age 4 to age 26
  Males breed from age 4 to age 26
  Maximum age of survival: 26
  Sex ratio (percent males) at birth: 50


Correlation of EV between reproduction and survival = 0.5


EV sampled from binomial distributions.


Population specific rates for SwedenNorway

  Percent of adult females breeding each year: =((Y%2)=0)*60
    with EV(SD): =((Y%2)=0)*10
  Percent of adult males in the pool of breeders: 75
  Normal distribution of brood size with mean: 5.3 with SD: 1.5

  Female annual mortality rates (as percents):
    Age 0 to 1:    18 with EV(SD): 1.5
    Age 1 to 2:    17.5 with EV(SD): 1.5
    Age 2 to 3:    16.3 with EV(SD): 1.5
    Age 3 to 4:    16.3 with EV(SD): 1.5
    After age 4:    =((MATE>-1)*13.4)+((MATE<0)*20) with EV(SD): 2

  Male annual mortality rates (as percents):
    Age 0 to 1:    18 with EV(SD): 1.5
    Age 1 to 2:    17.5 with EV(SD): 1.5
    Age 2 to 3:    16.3 with EV(SD): 1.5
    Age 3 to 4:    16.3 with EV(SD): 3
    After age 4:    =((MATE>-1)*13.4)+((MATE<0)*20) with EV(SD): 2
```

```
Catastrophe 1: Disease outbreak
    Local impact
    Frequency (%): 1
    Reproduction reduced by severity multiplier: 0
    Survival reduced by severity multiplier: 0.5

  Initial population size:
    Age   0  1  2  3  4  5  6  7  8  9  10  11  12  13  14  15  16  17  18  19  20  21  22  23  24  25  26      Females
0 0 0 0 0 0 0 0 0 0 0  0   0   0   0   0   0   0   0   0   0   0   0   0   0   0   0   0
  Males   0  0  0  0  0  0  0  0  0  0  0   0   0   0   0   0   0   0   0   0   0   0   0   0   0   0   0

  Carrying capacity: 800
    with EV(SD):  0

  Harvest from year 1 through year 200 by increments of 2
    when =N>GS1
    of individuals that =(IS6=1)*(MK>(0.5*(1-(G/100))))
        Age          0      0
      Females        1      =((Y<12)*(0.15*(((N-GS1)/2))))+((Y>12)*(0.4*(((N-GS1)/2))))
                     2      0
                     3      =((Y<12)*(0.15*(((N-GS1)/2))))+((Y>12)*(0.3*(((N-GS1)/2))))
                     4      =((Y<12)*(0.15*(((N-GS1)/2))))+((Y>12)*(0.4*(((N-GS1)/2))))

      Males          1      =((Y<12)*(0.15*(((N-GS1)/2))))+((Y>12)*(0.4*(((N-GS1)/2))))
                     2      0
                     3      =((Y<12)*(0.15*(((N-GS1)/2))))+((Y>12)*(0.3*(((N-GS1)/2))))
                     4      =((Y<12)*(0.15*(((N-GS1)/2))))+((Y>12)*(0.4*(((N-GS1)/2))))


Population specific rates for FinlandRussia

  Percent of adult females breeding each year: =((Y%2)=0)*60
    with EV(SD): =((Y%2)=0)*10
  Percent of adult males in the pool of breeders: 75
  Normal distribution of brood size with mean: 5.3 with SD: 1.5
```

```
Female annual mortality rates (as percents):
    Age 0 to 1:    18 with EV(SD): 1.5
    Age 1 to 2:    17.5 with EV(SD): 1.5
    Age 2 to 3:    16.3 with EV(SD): 1.5
    Age 3 to 4:    16.3 with EV(SD): 1.5
    After age 4:    =((MATE>-1)*13.4)+((MATE<0)*20) with EV(SD): 2

  Male annual mortality rates (as percents):
    Age 0 to 1:    18 with EV(SD): 1.5
    Age 1 to 2:    17.5 with EV(SD): 1.5
    Age 2 to 3:    16.3 with EV(SD): 1.5
    Age 3 to 4:    16.3 with EV(SD): 3
    After age 4:    =((MATE>-1)*13.4)+((MATE<0)*20) with EV(SD): 2

  Catastrophe 1: Disease outbreak
    Local impact
    Frequency (%): 1
    Reproduction reduced by severity multiplier: 0
    Survival reduced by severity multiplier: 0.5

  Initial population size:
    Age   0  1  2  3  4  5  6  7  8  9  10  11  12  13  14  15  16  17  18  19  20  21  22  23  24  25  26
Females   0 90  0 60  0 33  0 23  0 16   0  12   0   7   0   3   0   3   0   2   0   0   0   1   0   0   0
 Males    0 90  0 60  0 33  0 23  0 16   0  12   0   7   0   3   0   3   0   2   0   0   0   1   0   0   0

  Carrying capacity: 800
    with EV(SD):  0

  Harvest from year 1 through year 200 by increments of 2
        Age      0              1                  2                  3                      4
    Females      0  =0.13*((PS2)/2)               0      =0.13*((PS3)/2)           =0.1*F
     Males       0  =0.15*((PS2)/2)               0      =0.15*((PS3)/2)           =0.1*M

Genetics options:

  Initial population obtained from: ScandWolf_1Oct2022_Revised.txt
  Studbook population appended to any default initial population

  Genetic management for population: FinlandRussia
  Initial kinships set to: 0.1
```

Appendix III
Pedigree Input File (Founders and Living Animals Only: 1 October 2022)

(See Notes at the end of this listing for details on column headings)

ID*	Sire	Dam	Sex	Alive	Age**	Population	IS1***
D-85-01	WILD	WILD	F	0	10	1	-1
G1-83	WILD	WILD	M	0	10	1	-1
G1-91	WILD	WILD	M	0	10	1	-1
M-09-03	WILD	WILD	M	0	10	1	-1
M-10-10	WILD	WILD	M	0	10	1	-1
G23-13	WILD	WILD	M	0	10	1	-1
G31-13	WILD	WILD	F	0	10	1	-1
G15-16	WILD	WILD	M	0	10	1	-1
G325-17	WILD	WILD	F	0	10	1	-1
G187-19	WILD	WILD	M	1	9	1	G120-21
G10-23	G159-17	G7-20	M	1	1	1	-1
G104-23	G104-11	G3-19	M	1	1	1	-1
G107-23	G11-17	G253-17	F	1	1	1	-1
G110-22	G34-20	G80-20	M	1	1	1	-1
G111-22	G160-16	G64-19	M	1	1	1	-1
G113-22	G64-19	G160-16	M	1	1	1	-1
G114-22	G64-19	G160-16	F	1	1	1	-1
G116-22	G64-19	G160-16	F	1	1	1	-1
G121-22	G11-17	G253-17	F	1	1	1	-1
G12-23	G114-21	G27-21	F	1	1	1	-1
G1-23	G159-17	G7-20	M	1	1	1	-1
G129-22	G86-17	G48-17	M	1	1	1	-1
G131-22	G139-21	G38-20	M	1	1	1	-1
G132-22	G139-21	G38-20	M	1	1	1	-1
G13-23	G239-19	G118-21	F	1	1	1	-1
G133-22	G139-21	G38-20	M	1	1	1	-1
G134-22	G139-21	G38-20	M	1	1	1	-1
G135-22	G139-21	G38-20	F	1	1	1	-1
G136-22	G86-17	G48-17	F	1	1	1	-1
G137-22	G11-17	G253-17	F	1	1	1	-1
G138-22	G226-19	G55-17	M	1	1	1	-1
G139-22	G226-19	G55-17	F	1	1	1	-1
G140-22	G199-19	G151-19	F	1	1	1	-1
G142-22	G85-22	G161-21	M	1	1	1	-1
G143-22	G85-22	G161-21	F	1	1	1	-1
G144-22	G85-22	G161-21	F	1	1	1	-1
G145-22	G24-20	G29-16	M	1	1	1	-1
G146-22	G68-17	G96-19	F	1	1	1	-1
G147-22	G139-21	G164-17	M	1	1	1	-1
G148-22	G139-21	G164-17	M	1	1	1	-1
G149-22	G112-20	G198-21	F	1	1	1	-1
G150-22	G112-20	G198-21	M	1	1	1	-1
G151-22	G22-14	G113-12	F	1	1	1	G96-22
G15-23	G11-17	G253-17	M	1	1	1	-1
G156-22	G71-18	G236-17	M	1	1	1	-1
G157-22	G71-18	G236-17	F	1	1	1	-1
G159-22	G71-18	G236-17	F	1	1	1	-1
G160-22	G71-18	G236-17	F	1	1	1	-1
G162-22	G71-18	G236-17	F	1	1	1	-1
G168-22	G54-21	G35-18	M	1	1	1	-1
G169-22	G54-21	G35-18	F	1	1	1	-1
G172-22	G226-19	G55-17	M	1	1	1	-1
G17-23	G226-19	G55-17	F	1	1	1	-1
G173-22	G78-20	G26-20	M	1	1	1	-1
G175-22	G68-20	G6-21	M	1	1	1	-1

ID	Sire	Dam	Sex	Alive	Age*	Population	IS1**
G176-22	G226-19	G55-17	M	1	1	1	-1
G177-22	G22-14	G113-12	F	1	1	1	-1
G179-22	G83-20	G13-20	F	1	1	1	-1
G180-22	G315-17	G51-16	F	1	1	1	-1
G181-22	G234-17	G15-18	M	1	1	1	-1
G182-22	G22-14	G113-12	F	1	1	1	-1
G18-23	G78-20	G26-20	F	1	1	1	-1
G183-22	G83-20	G13-20	M	1	1	1	-1
G185-22	G112-20	G198-21	M	1	1	1	-1
G187-22	G139-21	G164-17	F	1	1	1	-1
G188-22	G139-21	G164-17	F	1	1	1	-1
G189-22	G108-19	G110-19	F	1	1	1	-1
G191-22	G55-16	G56-16	F	1	1	1	-1
G192-22	G129-21	G58-21	M	1	1	1	-1
G19-23	G78-20	G26-20	F	1	1	1	-1
G193-22	G68-17	G96-19	M	1	1	1	-1
G194-22	G68-17	G96-19	M	1	1	1	-1
G195-22	G226-19	G55-17	F	1	1	1	-1
G196-22	G165-17	G24-19	F	1	1	1	-1
G198-22	G12-19	G25-18	M	1	1	1	-1
G199-22	G92-16	G113-16	F	1	1	1	-1
G200-22	G92-16	G113-16	M	1	1	1	-1
G201-22	G112-20	G198-21	M	1	1	1	-1
G202-22	G234-17	G15-18	M	1	1	1	-1
G20-23	G24-20	G29-16	F	1	1	1	-1
G203-22	G55-16	G56-16	M	1	1	1	-1
G204-22	G79-18	G62-20	M	1	1	1	-1
G205-22	G79-18	G62-20	M	1	1	1	-1
G207-22	G226-19	G55-17	M	1	1	1	-1
G208-22	G132-21	G84-18	F	1	1	1	-1
G211-22	G54-21	G35-18	F	1	1	1	-1
G212-22	G54-21	G35-18	M	1	1	1	-1
G21-23	G24-20	G29-16	M	1	1	1	-1
G213-22	G315-17	G51-16	F	1	1	1	-1
G214-22	G159-17	G7-20	M	1	1	1	-1
G215-22	G124-16	G37-16	F	1	1	1	-1
G217-22	G108-21	G233-19	M	1	1	1	-1
G218-22	G108-19	G110-19	M	1	1	1	-1
G219-22	G315-17	G51-16	F	1	1	1	-1
G220-22	G132-21	G84-18	M	1	1	1	-1
G221-22	G165-17	G24-19	F	1	1	1	-1
G222-22	G69-21	G5-21	F	1	1	1	-1
G2-23	G7-21	G218-19	F	1	1	1	-1
G223-22	G226-19	G55-17	M	1	1	1	-1
G224-22	G159-17	G7-20	M	1	1	1	-1
G225-22	G95-21	G97-21	F	1	1	1	-1
G226-22	G89-16	G64-17	F	1	1	1	-1
G227-22	G12-19	G25-18	M	1	1	1	-1
G231-22	G89-16	G64-17	F	1	1	1	-1
G232-22	G165-17	G24-19	M	1	1	1	-1
G23-23	G74-21	G82-21	F	1	1	1	-1
G233-22	G104-11	G3-19	F	1	1	1	-1
G234-22	G234-17	G15-18	F	1	1	1	-1
G235-22	G79-19	G250-17	M	1	1	1	-1
G236-22	G74-21	G82-21	M	1	1	1	-1
G24-23	G12-19	G25-18	M	1	1	1	-1
G26-23	G34-20	G80-20	M	1	1	1	-1
G27-23	G24-20	G29-16	M	1	1	1	-1
G28-23	G24-20	G29-16	M	1	1	1	-1
G29-23	G69-21	G5-21	F	1	1	1	-1
G30-23	G69-21	G5-21	M	1	1	1	-1
G32-23	G50-20	G194-19	M	1	1	1	-1

ID	Sire	Dam	Sex	Alive	Age*	Population	IS1**
G3-23	G34-20	G80-20	F	1	1	1	-1
G33-23	G50-20	G194-19	M	1	1	1	-1
G35-23	G34-19	G100-20	F	1	1	1	-1
G36-23	G55-16	G56-16	F	1	1	1	-1
G37-23	G10-20	G88-21	F	1	1	1	-1
G38-23	G24-20	G29-16	F	1	1	1	-1
G41-23	G234-17	G15-18	M	1	1	1	-1
G42-23	G55-16	G56-16	M	1	1	1	-1
G4-23	G239-19	G118-21	M	1	1	1	-1
G44-23	G11-16	G251-19	M	1	1	1	-1
G45-23	G34-19	G100-20	M	1	1	1	-1
G46-23	G55-16	G56-16	F	1	1	1	-1
G47-23	G222-17	G4-20	M	1	1	1	-1
G48-23	G222-17	G4-20	M	1	1	1	-1
G50-23	G139-21	G164-17	F	1	1	1	-1
G52-23	G11-17	G253-17	F	1	1	1	-1
G5-23	G91-21	G29-21	M	1	1	1	-1
G56-23	G104-11	G3-19	M	1	1	1	-1
G57-23	G68-20	G6-21	M	1	1	1	-1
G58-23	G139-21	G164-17	F	1	1	1	-1
G59-23	G159-17	G7-20	M	1	1	1	-1
G62-23	G12-19	G25-18	M	1	1	1	-1
G63-23	G108-16	G68-13	F	1	1	1	-1
G64-23	G34-19	G100-20	M	1	1	1	-1
G65-23	G7-21	G218-19	M	1	1	1	-1
G66-23	G10-20	G88-21	M	1	1	1	-1
G67-23	G199-19	G151-19	F	1	1	1	-1
G68-23	G226-19	G55-17	F	1	1	1	-1
G70-23	G132-21	G84-18	M	1	1	1	-1
G71-23	G201-21	G152-15	F	1	1	1	-1
G7-23	G55-16	G56-16	F	1	1	1	-1
G75-23	G187-19	G120-21	F	1	1	1	-1
G76-23	G187-19	G120-21	M	1	1	1	-1
G77-23	G79-18	G62-20	M	1	1	1	-1
G78-23	G201-21	G152-15	F	1	1	1	-1
G79-23	G201-21	G152-15	M	1	1	1	-1
G80-23	G201-21	G152-15	F	1	1	1	-1
G81-23	G201-21	G152-15	F	1	1	1	-1
G82-23	G201-21	G152-15	F	1	1	1	-1
G8-23	G78-20	G26-20	M	1	1	1	-1
G83-23	G201-21	G152-15	M	1	1	1	-1
G84-23	G211-19	G33-21	F	1	1	1	-1
G85-23	G62-20	G79-18	F	1	1	1	-1
G87-23	G211-19	G33-21	F	1	1	1	-1
G88-23	G211-19	G33-21	M	1	1	1	-1
G89-23	G211-19	G33-21	F	1	1	1	-1
G90-23	G201-21	G152-15	F	1	1	1	-1
G9-23	G78-20	G26-20	F	1	1	1	-1
G93-23	G187-19	G120-21	M	1	1	1	-1
G94-23	G187-19	G120-21	M	1	1	1	-1
G95-23	G199-19	G74-19	F	1	1	1	-1
G96-23	G124-16	G37-16	F	1	1	1	-1
G97-23	G25-18	G12-19	F	1	1	1	-1
JuWo01	G34-20	G80-20	F	1	1	1	-1
JuWo02	G79-18	G62-20	M	1	1	1	-1
JuWo03	G91-21	G29-21	M	1	1	1	-1
JuWo04	G86-17	G48-17	F	1	1	1	-1
JuWo05	G83-20	G13-20	M	1	1	1	-1
JuWo06	G89-16	G64-17	M	1	1	1	-1
JuWo07	G95-21	G97-21	M	1	1	1	-1
JuWo08	G159-17	G7-20	M	1	1	1	-1
JuWo09	G74-21	G82-21	F	1	1	1	-1

ID	Sire	Dam	Sex	Alive	Age*	Population	IS1**
JuWo10	G315-17	G51-16	M	1	1	1	-1
JuWo11	G69-21	G5-21	F	1	1	1	-1
JuWo12	G226-19	G55-17	M	1	1	1	-1
JuWo13	G139-21	G164-17	M	1	1	1	-1
JuWo14	G108-19	G110-19	F	1	1	1	-1
JuWo15	G95-21	G97-21	M	1	1	1	-1
JuWo16	G79-18	G62-20	F	1	1	1	-1
G100-22	G68-19	G35-18	M	1	3	1	-1
G101-22	G68-19	G35-18	F	1	3	1	-1
G104-22	G154-15	G37-18	F	1	3	1	G37-22
G105-22	G71-18	G236-17	M	1	3	1	G191-21
G106-22	G234-17	G15-18	F	1	3	1	-1
G108-22	G244-19	G36-20	M	1	3	1	-1
G112-22	G64-19	G160-16	F	1	3	1	-1
G118-22	G86-17	G48-17	F	1	3	1	-1
G119-22	G244-19	G36-20	F	1	3	1	-1
G120-22	G22-14	G113-12	F	1	3	1	G92-23
G1-22	G168-17	G124-13	M	1	3	1	G101-19
G12-22	G159-17	G7-20	F	1	3	1	G22-23
G122-22	G241-19	G40-20	F	1	3	1	G240-19
G123-22	G244-19	G36-20	F	1	3	1	-1
G126-22	G68-17	G96-19	M	1	3	1	-1
G130-22	G315-17	G51-16	M	1	3	1	-1
G138-21	G234-17	G15-18	F	1	3	1	G108-20
G140-21	G86-17	G48-17	F	1	3	1	-1
G141-21	G86-17	G48-17	F	1	3	1	-1
G141-22	G315-17	G51-16	F	1	3	1	-1
G14-23	G165-17	G24-19	F	1	3	1	-1
G148-21	G124-16	G37-16	F	1	3	1	-1
G150-21	G79-18	G62-20	M	1	3	1	-1
G153-21	G58-19	G151-17	F	1	3	1	G46-21
G160-21	G264-17	G259-17	M	1	3	1	G173-21
G161-21	G264-17	G259-17	F	1	3	1	G85-22
G16-22	G28-16	G12-20	F	1	3	1	-1
G162-21	G264-17	G259-17	F	1	3	1	-1
G16-23	G315-17	G51-16	F	1	3	1	-1
G164-22	G55-16	G56-16	F	1	3	1	-1
G166-21	G234-17	G15-18	M	1	3	1	G38-20
G166-22	G205-17	G70-19	M	1	3	1	G8-21
G167-21	G234-17	G15-18	M	1	3	1	-1
G169-21	G104-11	G3-19	M	1	3	1	-1
G170-21	G68-19	G35-18	F	1	3	1	-1
G170-22	G40-19	G133-19	F	1	3	1	-1
G173-21	G175-19	G9-18	F	1	3	1	G160-21
G174-22	G69-21	G5-21	M	1	3	1	G106-21
G175-21	G11-16	G251-19	F	1	3	1	-1
G178-22	G124-16	G37-16	F	1	3	1	-1
G180-21	G108-16	G68-13	M	1	3	1	G39-20
G181-21	G108-16	G68-13	M	1	3	1	-1
G182-21	G50-20	G194-19	F	1	3	1	-1
G183-21	G50-20	G194-19	M	1	3	1	G197-19
G184-21	G108-16	G68-13	F	1	3	1	-1
G184-22	G55-16	G56-16	F	1	3	1	-1
G186-22	G28-16	G12-20	M	1	3	1	-1
G190-22	G108-19	G110-19	F	1	3	1	G25-19
G192-21	G222-17	G4-20	F	1	3	1	-1
G193-21	G222-17	G4-20	F	1	3	1	-1
G197-22	G165-17	G24-19	M	1	3	1	-1
G206-22	G172-19	G48-18	F	1	3	1	-1
G209-22	G68-17	G96-19	F	1	3	1	-1
G210-22	G315-17	G51-16	F	1	3	1	G125-17
G21-22	G79-18	G62-20	F	1	3	1	-1

ID	Sire	Dam	Sex	Alive	Age*	Population	IS1**
G213-21	G187-19	G168-16	M	1	3	1	-1
G214-21	G187-19	G168-16	F	1	3	1	-1
G216-22	G11-17	G253-17	M	1	3	1	G65-22
G220-21	G315-17	G51-16	F	1	3	1	-1
G22-22	G125-17	G140-13	F	1	3	1	G238-21
G222-21	G264-17	G259-17	F	1	3	1	-1
G22-23	G11-17	G253-17	M	1	3	1	G12-22
G224-21	G12-19	G25-18	F	1	3	1	-1
G225-21	G108-19	G110-19	M	1	3	1	-1
G227-21	G168-17	G124-13	F	1	3	1	-1
G228-22	G58-19	G151-17	F	1	3	1	G92-20
G229-21	G159-17	G7-20	M	1	3	1	-1
G229-22	G55-16	G56-16	M	1	3	1	-1
G230-22	G11-16	G251-19	M	1	3	1	-1
G23-22	G12-19	G25-18	M	1	3	1	-1
G233-21	G168-17	G124-13	M	1	3	1	-1
G237-21	G124-16	G37-16	F	1	3	1	-1
G238-21	G226-19	G55-17	M	1	3	1	G22-22
G239-21	G79-19	G250-17	M	1	3	1	-1
G240-21	G68-17	G96-19	F	1	3	1	-1
G242-21	G12-19	G25-18	M	1	3	1	-1
G243-21	G104-11	G3-19	M	1	3	1	-1
G244-21	G104-11	G3-19	M	1	3	1	G243-19
G249-21	G24-20	G29-16	F	1	3	1	-1
G252-21	G24-20	G29-16	M	1	3	1	-1
G25-23	G9-20	G10-15	M	1	3	1	-1
G254-21	G92-16	G113-16	M	1	3	1	G55-23
G255-21	G79-19	G250-17	M	1	3	1	-1
G259-21	G79-18	G62-20	M	1	3	1	G165-22
G31-23	G88-17	G188-17	M	1	3	1	-1
G34-23	G226-19	G55-17	M	1	3	1	-1
G37-22	G12-19	G25-18	M	1	3	1	G104-22
G39-23	G108-16	G68-13	F	1	3	1	-1
G40-23	G104-11	G3-19	M	1	3	1	G6-21
G4-22	G89-16	G64-17	F	1	3	1	-1
G43-23	G172-19	G48-18	F	1	3	1	-1
G44-22	G71-18	G236-17	M	1	3	1	G1-21
G49-23	G104-11	G3-19	M	1	3	1	G115-21
G51-23	G199-19	G74-19	M	1	3	1	-1
G53-22	G124-17	G239-17	M	1	3	1	-1
G53-23	G315-17	G51-16	M	1	3	1	-1
G54-23	G68-17	G96-19	F	1	3	1	-1
G55-22	G64-19	G160-16	M	1	3	1	-1
G55-23	G11-17	G253-17	F	1	3	1	G254-21
G59-22	G71-18	G236-17	M	1	3	1	-1
G60-23	G79-19	G250-17	M	1	3	1	-1
G61-23	G92-16	G113-16	M	1	3	1	-1
G6-23	G226-19	G55-17	M	1	3	1	-1
G65-22	G11-17	G253-17	F	1	3	1	G216-22
G66-22	G165-17	G24-19	M	1	3	1	-1
G69-22	G165-17	G24-19	M	1	3	1	-1
G72-22	G199-19	G74-19	F	1	3	1	-1
G72-23	G89-16	G64-17	M	1	3	1	-1
G75-22	G88-17	G188-17	F	1	3	1	-1
G77-22	G2-20	G35-20	M	1	3	1	-1
G86-22	G55-16	G56-16	M	1	3	1	-1
G89-22	G12-19	G25-18	M	1	3	1	-1
G9-22	G226-19	G55-17	M	1	3	1	-1
G92-22	G125-17	G140-13	M	1	3	1	-1
G92-23	G50-20	G194-19	M	1	3	1	G120-22
G94-22	G68-19	G35-18	M	1	3	1	-1
G95-22	G159-17	G7-20	M	1	3	1	-1

ID	Sire	Dam	Sex	Alive	Age*	Population	IS1**
G96-22	G124-16	G37-16	M	1	3	1	G151-22
G97-22	G145-19	G223-17	M	1	3	1	G34-21
G20-22	G199-19	G74-19	M	1	3	1	-1
G194-21	G222-17	G4-20	F	1	3	1	-1
G60-22	G71-18	G236-17	F	1	3	1	-1
G212-21	G187-19	G168-16	M	1	3	1	-1
G54-22	G124-17	G239-17	M	1	3	1	-1
G91-23	G124-17	G239-17	F	1	3	1	-1
G71-22	G86-17	G48-17	M	1	3	1	-1
YeWo01	G11-16	G251-19	M	1	3	1	-1
YeWo02	G11-16	G251-19	M	1	3	1	-1
YeWo03	G79-18	G62-20	M	1	3	1	-1
YeWo04	G205-17	G70-19	M	1	3	1	-1
YeWo05	G68-19	G35-18	M	1	3	1	-1
YeWo06	G68-19	G35-18	F	1	3	1	-1
YeWo07	G124-16	G37-16	M	1	3	1	-1
YeWo08	G159-17	G7-20	F	1	3	1	-1
YeWo09	G199-19	G74-19	M	1	3	1	-1
YeWo10	G124-17	G239-17	M	1	3	1	-1
YeWo11	G108-16	G68-13	F	1	3	1	-1
YeWo12	G241-19	G40-20	F	1	3	1	-1
YeWo13	G68-17	G96-19	F	1	3	1	-1
YeWo14	G124-16	G37-16	M	1	3	1	-1
G101-21	G92-16	G113-16	M	1	5	1	-1
G106-21	G92-16	G113-16	F	1	5	1	G174-22
G108-20	G165-17	G24-19	M	1	5	1	G138-21
G108-21	G11-17	G253-17	M	1	5	1	G233-19
G110-21	G11-17	G253-17	M	1	5	1	-1
G111-21	G11-17	G253-17	F	1	5	1	G13-22
G112-20	G68-19	G35-18	M	1	5	1	G198-21
G113-20	G159-17	G169-16	F	1	5	1	G100-15
G114-20	G159-17	G169-16	F	1	5	1	G146-21
G114-21	G58-17	G31-19	M	1	5	1	G27-21
G115-21	G11-17	G253-17	F	1	5	1	G49-23
G117-22	G124-16	G37-16	F	1	5	1	-1
G118-21	G92-16	G113-16	F	1	5	1	G239-19
G120-21	G2-19	G213-17	F	1	5	1	G187-19
G1-21	G205-17	G77-17	F	1	5	1	G44-22
G13-22	G92-16	G113-16	M	1	5	1	G111-21
G132-21	G40-19	G133-19	M	1	5	1	-1
G144-21	G43-17	G135-16	F	1	5	1	G78-22
G146-21	G11-17	G253-17	M	1	5	1	G114-20
G165-21	G315-17	G51-16	F	1	5	1	G2-22
G165-22	G22-14	G113-12	F	1	5	1	G259-21
G191-21	G22-14	G113-12	F	1	5	1	G105-22
G198-21	G58-19	G151-17	F	1	5	1	G112-20
G201-21	G22-14	G113-12	M	1	5	1	G152-15
G2-22	G168-17	G124-13	M	1	5	1	G165-21
G226-21	G168-17	G124-13	M	1	5	1	-1
G27-21	G124-17	G239-17	F	1	5	1	G114-21
G29-21	G154-15	G37-18	F	1	5	1	G91-21
G33-21	G154-15	G37-18	F	1	5	1	G211-19
G34-21	G154-15	G37-18	F	1	5	1	G97-22
G41-22	G205-17	G70-19	F	1	5	1	G88-17
G44-21	G2-19	G213-17	M	1	5	1	G251-19
G46-21	G27-19	G85-19	M	1	5	1	G153-21
G5-21	G104-11	G3-19	F	1	5	1	-1
G54-21	G22-14	G113-12	M	1	5	1	G35-18
G55-21	G104-11	G3-19	M	1	5	1	-1
G58-21	G89-16	G64-17	F	1	5	1	-1
G6-21	G104-11	G3-19	F	1	5	1	G40-23
G62-21	G88-17	G188-17	F	1	5	1	-1

ID	Sire	Dam	Sex	Alive	Age*	Population	IS1**
G69-21	G28-16	G12-20	M	1	5	1	G216-19
G7-21	G22-14	G113-12	M	1	5	1	G218-19
G74-21	G168-17	G124-13	M	1	5	1	G82-21
G78-20	G156-16	G135-17	M	1	5	1	G26-20
G78-22	G281-17	G9-18	M	1	5	1	G144-21
G80-20	G315-17	G51-16	F	1	5	1	-1
G81-21	G88-17	G188-17	M	1	5	1	-1
G8-21	G22-14	G113-12	F	1	5	1	G166-22
G82-21	G108-16	G68-13	F	1	5	1	G74-21
G83-20	G159-17	G169-16	M	1	5	1	G13-20
G88-21	G123-14	G31-13	F	1	5	1	G10-20
G90-20	G68-17	G96-19	M	1	5	1	-1
G91-21	G177-19	G223-17	M	1	5	1	G29-21
G92-20	G43-17	G135-16	M	1	5	1	G228-22
G93-21	G2-19	G213-17	F	1	5	1	G297-17
G95-21	G165-17	G24-19	M	1	5	1	G97-21
G97-20	G104-15	G88-16	F	1	5	1	-1
G97-21	G165-17	G24-19	F	1	5	1	G95-21
G98-21	G92-16	G113-16	F	1	5	1	-1
G100-20	G156-16	G135-17	F	1	5	1	G34-19
G67-21	G22-14	G113-12	F	1	5	1	-1
G101-19	G159-17	G169-16	F	1	7	1	G1-22
G107-21	G58-17	G31-19	M	1	7	1	G57-21
G108-19	G166-14	G97-15	M	1	7	1	G110-19
G110-19	G166-14	G97-15	F	1	7	1	G108-19
G13-20	G92-16	G113-16	F	1	7	1	G83-20
G139-21	G156-16	G135-17	M	1	7	1	G164-17
G194-19	G168-17	G124-13	F	1	7	1	G50-20
G197-19	G168-17	G124-13	F	1	7	1	G183-21
G211-19	G176-16	G87-17	M	1	7	1	G33-21
G216-19	G23-16	G14-19	F	1	7	1	G69-21
G2-20	G45-19	G147-15	M	1	7	1	G35-20
G226-19	G45-19	G147-15	M	1	7	1	G55-17
G229-19	G315-17	G51-16	F	1	7	1	-1
G233-19	G92-16	G113-16	F	1	7	1	G108-21
G239-19	G92-16	G113-16	M	1	7	1	G118-21
G240-19	G11-17	G253-17	M	1	7	1	G122-22
G243-19	G123-14	G31-13	F	1	7	1	G244-21
G244-19	G89-16	G64-17	M	1	7	1	G36-20
G251-19	G11-16	G132-16	F	1	7	1	G44-21
G258-19	G169-17	G136-17	M	1	7	1	G262-17
G34-20	G123-14	G31-13	M	1	7	1	-1
G35-20	G125-17	G259-17	F	1	7	1	G2-20
G36-20	G159-17	G169-16	F	1	7	1	G244-19
G38-20	G123-14	G31-13	F	1	7	1	G166-21
G39-20	G237-17	G10-15	F	1	7	1	G180-21
G4-20	G222-17	G7-18	F	1	7	1	G222-17
G57-21	G90-17	G272-17	F	1	7	1	G107-21
G62-20	G34-18	G129-17	F	1	7	1	G79-18
G7-20	G159-17	G169-16	F	1	7	1	G159-17
G85-22	G125-17	G140-13	M	1	7	1	G161-21
G218-19	G96-12	G26-15	F	1	7	1	G7-21
G50-20	G35-16	G80-17	M	1	7	1	G194-19
G10-20	G43-17	G135-16	M	1	9	1	G88-21
G12-19	G22-14	G113-12	M	1	9	1	G25-18
G12-20	G83-14	G147-15	F	1	9	1	G28-16
G172-19	G6-12	G77-14	M	1	9	1	G48-18
G25-19	G43-17	G135-16	M	1	9	1	G190-22
G26-20	G156-14	G53-16	F	1	9	1	G78-20
G3-19	G123-14	G31-13	F	1	9	1	-1
G34-19	G97-17	G168-16	M	1	9	1	G100-20
G64-19	G86-16	G166-16	M	1	9	1	G160-16

ID	Sire	Dam	Sex	Alive	Age*	Population	IS1**
G71-18	G132-11	G74-11	M	1	9	1	G236-17
G74-19	G29-15	G173-16	F	1	9	1	-1
G79-18	G124-17	G172-14	M	1	9	1	G62-20
G79-19	G58-17	G175-17	M	1	9	1	G250-17
G84-18	G18-15	G89-15	F	1	9	1	-1
G15-18	G123-14	G31-13	F	1	11	1	G234-17
G164-17	G123-14	G31-13	F	1	11	1	G139-21
G165-17	G123-14	G31-13	M	1	11	1	-1
G168-17	G12-16	G66-12	M	1	11	1	G124-13
G222-17	G6-12	G77-14	M	1	11	1	G4-20
G234-17	G123-14	G31-13	M	1	11	1	G15-18
G236-17	G6-12	G77-14	F	1	11	1	G71-18
G239-17	G13-16	G56-11	F	1	11	1	G124-17
G25-18	G47-10	G18-10	F	1	11	1	G12-19
G253-17	G26-16	G123-16	F	1	11	1	-1
G262-17	G47-16	G48-17	F	1	11	1	G258-19
G266-17	G123-14	G31-13	M	1	11	1	-1
G297-17	G72-16	G152-15	M	1	11	1	G93-21
G315-17	G104-15	G88-16	M	1	11	1	G51-16
G35-18	G122-14	G110-14	F	1	11	1	G54-21
G125-17	G155-14	G139-14	M	1	13	1	G210-22
G159-17	G90-15	G80-13	M	1	13	1	G7-20
G250-17	G68-15	G10-15	F	1	13	1	G79-19
G48-18	G156-14	G53-16	F	1	13	1	G172-19
G55-17	G89-16	G64-17	F	1	13	1	G226-19
G68-17	G96-12	G26-15	M	1	13	1	G96-19
G96-19	G151-15	G99-16	F	1	13	1	G68-17
G108-16	G95-10	G16-12	M	1	15	1	G68-13
G113-16	G108-14	G1-14	F	1	15	1	-1
G124-17	G63-15	G64-15	M	1	15	1	G239-17
G152-15	G86-11	G144-14	F	1	15	1	G201-21
G28-16	G104-11	G13-15	M	1	15	1	G12-20
G51-16	M-09-03	G68-13	F	1	15	1	G315-17
G56-16	G84-11	G12-12	F	1	15	1	G55-16
G86-17	G164-13	G113-12	M	1	15	1	G48-17
G88-17	G61-14	G66-12	M	1	15	1	G41-22
G100-15	G37-10	G85-13	M	1	17	1	G113-20
G160-16	G24-12	G86-15	F	1	17	1	G64-19
G48-17	G11-13	G49-12	F	1	17	1	G86-17
G55-16	G39-11	G12-09	M	1	17	1	G56-16
G64-17	G133-13	M-10-08	F	1	17	1	G89-16
G67-16	G39-11	G12-09	F	1	17	1	-1
G89-16	G24-12	G86-15	M	1	17	1	G64-17
G68-13	G22-12	G12-10	F	1	21	1	G108-16
G124-13	G7-10	D-11-17	F	1	25	1	G168-17

Notes:
*   Individuals beginning with JuWoXX and YeWoXX are juveniles and yearlings,
     respectively, added to the population in order to bring the total abundance up to
     the desired value as determined by recent census methodologies.
**   Age is defined here in the context of two 6-month timesteps per calendar year.
     Because the model begins on 1 October, assumed to be six months after the
     production of pups the previous spring, the youngest individuals in this initial
     population are one timestep in age. It follows that the age of all individuals is
     expressed in odd numbers, corresponding to the odd-numbered timesteps in which
     the population census is taken.
*** IS1 = Individual State Variable 1 = the ID of the mate of the given individual
     assigned at the beginning of the simulation. Mate assignments are symmetrical, so
     that the mate of X is Y and the mate of Y is X.

Minimum Viable Population Analysis to inform the Favourable Reference Value for wolves in Sweden

FINAL REPORT – April 2024

Nicolas Dussex, Ph. D.
Researcher
Norwegian University of Science and Technology
Trondheim, Norway

In fulfilment of:
Swedish EPA Contract Number 365 – 23 – 002

Summary

The aim of this report, commissioned by the Swedish Environment Protection Agency (SEPA), was to evaluate if and under which conditions a Favourable Reference Value (FRV) of 170 to 270 wolves (*Canis lupus*) represents a viable population in Sweden. To address this question, I performed a Population Viability Analysis using forward-in-time genome-informed simulations implemented in SLiM. I modelled a large population in Karelia and a smaller population in Scandinavia (i.e., including wolves from both Sweden and Norway) and examined the demographic and genetic viability of the population. I first modelled the effects of survival, reproductive output, population size and migration rates on the probability of extinction of the population. Secondly, using field-based empirical estimates for demographic and life-history traits, I examined the effect of varying population size and migration rates on genome-wide diversity (e.g., nucleotide diversity, inbreeding and two components of genetic load) as proxy for viability.

Simulations indicate that with reduced survival rate and female reproductive output, the risk of extinction would range between 22 and 32% for a population size of 50 and between 1 and 10% for a population size of 100. However, when using higher survival rates and female reproductive output values based on field-based estimates, the risk of extinction was close to 1% for a population size of 50 and no extinction was reported for a population size ≥ 100.

Furthermore, for a population size of 170 to 270 wolves in Sweden (i.e., 210 and 310 for the whole Scandinavian population), between 1 to 3 effective (i.e., reproducing) immigrants per decade would be needed remain within a 5% window of loss in nucleotide diversity and increase in inbreeding. However, while migration rates above a threshold of 1-3 effective immigrants per decade would increase genetic diversity and would potentially induce a genetic rescue effect, it would also represent a risk of introducing new deleterious variation, especially for lower population sizes. Moreover, the simulations showed that larger population sizes would be more immune to loss of diversity. Yet, there would also be a non-negligible risk of introduction of new deleterious variation with ≥ 8 migrants per decade. Consequently, the trade-off between genetic rescue and introduction of deleterious variation needs to be taken into account when determining a target population size for management.

Introduction

Small populations are exposed to a number of threats. On the one hand, demographic stochasticity can increase the risk of population collapse and extinction [1]. On the other, genetic processes will accelerate population decline and increase the risk of extinction through genomic erosion [2,3], which includes the loss of adaptive variation as well as the increase in deleterious variation (i.e., inbreeding depression). The combination of these demographic and genetic effects can thus trap species into an extinction vortex.

Many small populations are the remnants of larger populations that have been declining through the effects of habitat modification and direct human interference. However, newly established populations can remain relatively small due to a reduced number of founder and low immigration. This is particularly the case for populations that have been locally extirpated and that have re-established naturally through small number of individuals but that remain small due to unsuitable habitat or intense conflicts with humans from various interest groups (e.g., hunters, farmers, etc.). The resulting lack of connectivity with large and genetically-diverse populations can thus hamper population recovery and increase the risk of extinction through demographic and genetic effects [4].

As a case in point, the wolf (*Canis lupus*) population in Scandinavia was probably functionally extinct by 1966 [5]. A new population was founded by two breeding individuals from Karelia in 1982-90. Later migration of one breeding male in 1990-91 [6,7] and of four additional breeding individuals between 2008-2021 [8] contributed to an increase in population reaching ~400 individuals today. The Favourable Reference Value (FRV) for the population has been set at 170 to 270 individuals for the Swedish population while a value of 300 would be preferred to guarantee its long-term viability based on the last report on the Swedish wolf population [9].

The Scandinavian wolf population in Sweden and Norway has overall been increasing since its establishment in the 1980s, thanks to local reproduction and the arrival of breeding individuals over the

past 10 years. However, the population is highly inbred [8,10] and there is evidence for inbreeding depression with a reduced pup survival during the 1983–2002 period [11]. Consistent with evidence for inbreeding depression, recent genomic data indicate that the population had a higher proportion of deleterious variation expressed compared to the larger population in Russia and Finland during the five generations following the establishment of the population by the two founders [8]. While the arrival of new breeding immigrants since 2008 induced a reduction in the expression of deleterious variation and an increase in fitness (i.e., genetic rescue effect, [7]), this effect was only temporary and increased inbreeding in the descendants of those immigrants has again, led to the exposure of deleterious mutations [8]. These results thus highlight the importance of continuous gene flow for the reduction of inbreeding and inbreeding depression and for the viability of the wolf population in Scandinavia

It has been proposed that a minimum effective population size (N_e) should be in the range of 70 to reduce the risks of inbreeding depression [12], whereas the 50/500 rule (i.e., goal of short term N_e >50, long term N_e >500) has been used to assess the minimum viable N_e [13]. While useful, these values are impractical if the ratio of effective to census size (i.e., N_e/N_C) is unknown or if this ratio varies through time. Consequently, targeting appropriate population sizes for management and defining Favourable Reference Values (FRV) can be challenging. In contrast, thanks to their versatility and ability to model complex demographic scenarios incorporating various life-history traits and genetic parameters, simulations can provide important insights into the factors impacting population viability. For instance, simulations can quantify the effect of specific demographic or life-history traits values on the probability of extinction and of retaining a certain amount of genetic variation. Based on these results, one can then infer a target population size that would allow to minimise the risk of demographic collapse and inbreeding depression and to maximise the retention of genetic diversity.

The ultimate conservation goal for the Swedish wolf population is to assess an appropriate FRV that would maximise the long-term viability of the population. However, the models presented here simulate various Minimum Viable Population (MVP; i.e., demographic and genetic concepts) sizes focusing on the 170-270 interval. Importantly, once a MVP is defined, it should then be upscaled to include other ecological parameters not examined here (e.g., habitat size, prey availability, competition with other predators, etc.) to determine a target FRV. Thus, this report does not aim at recommending a FRV. Instead, SEPA will determine an appropriate FRV based on the results presented here.

The goal of this report thus is to determine if and under which conditions a size of 170 to 270 individuals for the Swedish wolf population represents a viable population over a 100 years period. While discussing the definition of viability of a population is beyond the scope of this report, the specific goal of the simulations presented here is to examine the combined effects of some demographic parameters, different population sizes and migration rates on the probability of demographic collapse and on the retention of genetic variation. Specifically, the aim of these simulation was to answer the following questions:

- What is the effect of varying age-specific survival, female reproduction output, population size and migration on the probability of population extinction?

- What are the combined effects of population sizes and migration on indices of genome-wide diversity?

- Under which conditions does a population size of 170-270 represent a viable Swedish population where the probability of demographic collapse and genome-wide diversity loss and the introduction of deleterious variation is minimized?

Methods

The aim of the models was to examine the effects of varying age-specific survival, female reproductive output (i.e., proportion of breeding females and litter size), population sizes and migration rates on MVP sizes for the Swedish wolf population. While the current report focuses on the viability of the Swedish wolf population, these simulations were performed for a Scandinavian population including wolves from the Norwegian population (see point 3).

I designed two types of models. First, I examined the effect of age-specific survival, female reproductive output, migration and population sizes on the probability of population extinction, thereafter referred to as *Demography focused*. Secondly, I used the most realistic and field-based empirical estimates for age-specific survival and female reproductive output (SKANDULV, Chapron, *unpub. data*; [9]; Table 1) and examined the effects of migration and population sizes on the rate of loss in genetic variation in the population, thereafter referred to as *Genome focused.*

1. Model description

1.1 Population history and model building

To examine the effects of population size and migration rates on the viability of the Swedish wolf population, I used forward-in-time simulations in SLiM v4 [14]. In recent years, this this tool has been increasingly used to examine the extinction risk of small populations in a number of threatened species (e.g., Isle Royale wolf [15]; vaquita [16]; kākāpō [17]; Channel Island fox; [18]; Svalbard reindeer [19]) as it allows for fine-tuning of realistic demographic and genetic parameters. While this tool as mostly been for *a posteriori* inference of demographic parameters that could have induced an observed genetic signature (e.g., population size, magnitude and duration of bottlenecks), it can also be used to predict future population and genetic trends under various demographic scenarios [14,20].

I thus created a model recapitulating the population history of wolf as summarised in Smeds & Ellegren [8] since the establishment of the wolf population in Sweden and Norway. For this model, I first simulated a large ancestral Karelian population (i.e., Finland and Russia) and ran the simulations for a 200'000 years burnin period to generate genetic variation and to allow the population to reach mutation-drift-migration equilibrium. Based on the demographic reconstruction from Gopalakrishnan *et al.* [21], the N_e for gray wolves ranged from 1,000 to 4,000 over the past 2,000 years. Assuming a conservative N_e/N_C ratio of 0.25 [9,22] and a N_e value of 2,000, this corresponds to a N_C of ~8,000. For all simulations, the carrying capacity K for the ancestral Karelian population ($K_{Anc-Karelia}$) was thus set to ~8,000 individuals (Table 1). Even though the current Karelian population may number fewer than ~8,000 individuals, this population size was chosen to represent the long-term effective population size (N_e) and to simulate the high genetic diversity characteristic of a large ancestral and panmictic population. I then modelled a population split c. 200 years BP and a bottleneck to ~800 individuals (SKANDULV, *unpub. data*) corresponding to the separation of modern Karelian population from this large ancestral Karelian population ($K_{Karelia}$). The Scandinavian population (K_S) was then founded by two individuals in 1982-90 from this Karelian population. This foundation event was followed by the immigration of one male in 1990-91 [6,7], two additional individuals in 2008, one in 2017 and one in 2021 (R. Ekblom, *unpub. data.*). All migrants were established and produced offspring in the population. From the time of this last migration event, statistics were recorded and calculated for another 100 years until 2121, by varying population sizes and migration rates (see point 3).

1.2 Non-Wright-Fisher models

Individual-based simulations were conducted using the non-Wright-Fisher (nonWF) model in SLiM v4. This model differs from the classical Wright-Fisher model implemented in SLiM v3 and previously used in other population genomics studies in important ways. First, generations in this model are overlapping and each cycle in the model corresponds to a year. Thus, the mutation rate obtained from the scientific literature and estimated per generation needs to be converted into per year rates by dividing it by the generation time for wolf (i.e., 3 years [23–25]). Secondly, the probability of an individual

surviving from one year to the next is given by its absolute fitness, which ranges from 0 to 1 and which is determined by its genetic composition, age, and by density-dependence . Third, contrary to a Wright-Fisher model, one cannot set the population size (N) which is an emergent parameter and is the outcome of a stochastic process of reproduction and viability selection. Instead, carrying capacity (K) needs to be set and N fluctuates around K. For instance, for a K=100, N will range between ~90 and ~110. When N>K, the model rescales the absolute fitness downward by the ratio of K/N. Therefore, these models do not allow for population growth. While not allowing for population growth may not seem biologically realistic, allowing N to fluctuate around the maximum bound of K is valid for the purpose of this report for two reasons. First, N is considered as an explanatory variable in the model (see point 3) as we want to evaluate the impact of the size of the Swedish wolf population on the population extinction risk and on the retention of genetic diversity. Secondly, since the wolf population is intensively regulated through culling, it is likely that in reality the population will not be allowed to grow substantially beyond a certain size and that population growth will be limited. In other words, because N is limited by K in the model, the yearly culling to reach a target population size is implicit to the model for any given K value used.

2. Input parameters

2.1 Life-history traits and demography

Reproduction: As default values for female reproduction, I used the field-based empirical estimates (SKANDULV, *unpub. data*; Table 1). At the start of the reproduction event, every reproductive-age female (i.e., ≥2 years) had a 63% probability of reproducing with a randomly-selected reproductive-age male (≥2 years). Furthermore, each successful mating produced a litter size of 3.5 pups (SD: 1.4) with a sex-ratio of 0.5. I assumed a monogamous mating system where one male reproduces with only one female at every reproductive cycle (i.e., every year). The models assumed random mating and allowed one mating event per year. To take into account reproductive advantage of immigrants [9], I only allowed 80% of resident males to reproduce during the year of the reproduction event. The minimum and maximum ages at reproduction 2 and 12 years, respectively for a maximum lifespan of 13 years. Importantly, all immigrant individuals reproduced the year following the migration event and were thus effective migrants contributing progeny to the next generation.

For the *Demography focused* model, I also built a variation with '*reduced female reproductive output*' with only 30% reproductive probability for females and a litter size of 3 pups (SD: 0.5). For the *Genome focused* model, I only used the default values as described above (Table 1).

Mortality: As default values for age-related mortality, I used the field-based empirical estimates (Chapron, *unpub. data*) except for the value from 0 to 1 years old, which was increased to 30% based on reviewers' comments on the preliminary report (Table 1). Mortality was modelled by rescaling fitness (i.e., survivability) by a value corresponding to each age-class survival probability. For instance, the fitness of a new-born was rescaled by subtracting a value of 0.3 to the survivability value (i.e., 1-0.3 = 0.7), that of a yearling using value of 0.4 (i.e., 1-0.4 = 0.6), whereas that of a 13 years old adult using a value of 1 (i.e., 1-1 = 0), since maximum longevity is of 13 years (SKANDULV, *unpub. data*; Table 1). To incorporate density-dependence fitness, the population fitness was then rescaled by K divided by the number of individuals multiplied by their survivability (i.e., K/N*survivability). If N>K or N<K, individual fitness is rescaled downward or upward, respectively. However, when N<K, the model rescales fitness upward, resulting in absolute fitness being rescaled to 1.0 (i.e., the chance of survival = 100%) for all individuals given the large ratio of K/N, meaning that viability selection would not occur. In order to ensure viability selection when N<K, I prevented upwards rescaling of fitness from occurring and rescaled fitness by choosing the minimal value between K/N and 1 (i.e., min(K/N, 1.0)). This ensured that the impacts of inbreeding depression were expressed in the simulations. These rescaling factors (i.e., age-specific survival and density-dependence) were then multiplied by the absolute fitness of each individual, which also varies as a function of age (i.e., older individuals tend to have higher fitness given that they have survived many years of viability selection).

For the *Demography focused* model I also built variation of the model with '*reduced survival*' with 50% mortality from 0 to 2 years old and 30% mortality from 2 years old until death (Table 1). For the *Genome focused* model, I only used the default values as described above.

Viability selection: After reproduction, viability selection occurs through a fitness rescaling procedure. Each individual survives with a probability determined by its absolute fitness multiplied by any scaling factors for age or density dependence (K), as well as the selection coefficients of deleterious mutations.

Migration: individuals were allowed to migrate/disperse by the age of 2 years old.

Catastrophes: In order to account for diseases outbreaks, I included catastrophe events occurring with a 1% yearly probability and reducing survival by 50% (Table 1; [26,27]).

2.2 Genetic parameters

For the *Genome focused* model, I randomly generated 3000 genes (i.e., ~15% of a complete wolf exome, assuming ~20'000 genes) for each individual wolf. I generated genes of a size of 1750 base pairs (bp) containing randomly generated introns and exons. For recombination rate, I assumed no recombination within genes, a rate of $1e^{-9}$ between genes, and free recombination between chromosomes based on Kyriazis *et al.* [28].

Mutation types: I randomly generated deleterious (non-synonymous) and neutral (synonymous) mutations in exonic regions following based on Kyriazis *et al.* [28]. Deleterious (non-synonymous) mutations occurred in exonic regions at a ratio of 2.31:1 to neutral (synonymous) mutations [29] making for 43.3 proportion of synonymous mutations (Table 1). Furthermore, the proportion of different deleterious mutation types (lethal , very strongly, strongly, moderately and weakly deleterious) was based on distribution of fitness effects (DFE) for humans [29,30] (Table 1). The selection coefficients (*s*) of non-synonymous mutations (i.e., very highly deleterious to weakly deleterious) were drawn from distributions based on estimates in humans [30] using a gamma distribution a mean s $=-0.01314833$ and shape= 0.186 (Table 1). For neutral and lethal mutations *s* was set to 0 and 1, respectively. For dominance coefficients (*h*), we assumed an inverse relationship between *h* and *s* [31,32] with $h = 0.0$ for lethal and very strongly deleterious mutations ($s < -0.1$), $h = 0.01$ for strongly deleterious mutations ($-0.1 \leq s < -0.01$), $h = 0.1$ for moderately deleterious mutations ($-0.01 \leq s < -0.001$), and $h = 0.4$ for weakly deleterious mutations ($s > -0.001$).

Mutation rate and genomic elements: Several mutation rates have been used in wolf genomics studies based on fossil calibrated molecular clocks or *de-novo* mutation rates estimated from pedigreed data and range from 1×10^{-8} to 4×10^{-8} per generation [25,33–35]. Here, I used a mutation rate of 1.5×10^{-9} mutations/year based on the rate of 4.5×10^{-9} mutations per generation estimated from pedigree data [34] and assuming a generation time of 3 years [23–25].

3. Explanatory variables

To assess various MVP sizes including for the interval of 170 to 270 individuals for the Swedish wolf population, explanatory variables included age-specific survival, female reproductive output, population size (i.e., carrying capacity for the Scandinavian population, K_S) and migration rates (M).
   For the *Demography focused* model, I varied age-specific survival (i.e., '*reduced survival*' model; see point 2.1; Table 1) and female reproductive output (i.e. '*reduced female reproductive output*' model; see point 2.1; Table 1), K_S and M. For the *Genomic focused* model, I only varied K_S and M and used the field-based empirical estimates for age-specific survival and female reproductive output as default values (see point 2.1; Table 1).
   K_S values ranged between 50 and 500 and included the 210 and 310 values for the Scandinavian population (Table 1). It is important to note that while this report focuses on the 170 and 270 values for the Swedish population, the Scandinavian wolf population is not constrained by countries boundaries

and should also include wolves from Norway. Thus, assuming that the Norwegian part of the Scandinavian population is of at least 40 individuals (R. Ekblom, *unpub. data.*), the simulated K_S values considered should be of 210 and 310, when examining the 170 to 270 range for the Swedish population. The models were designed to include the migration rates M ranging between 0 to 1 effective immigrants per year (over a 100 years period; Table 1). Because SLiM does not use migration rates for models with overlapping generations, migration was incorporated in the model by modelling actual individuals migrating at different time intervals. Thus, a number of migrants was randomly assigned to a given decade, starting with only 1 migrant over 100 years period (i.e., M=0.01 migrant/year) to 100 migrants over 100 years (i.e., M=1 migrant/year). All immigrant individuals reproduced the year following the migration event and are thus effective immigrants contributing progeny to the next generation (see point 2.1). For each model, I ran 100 replicates for each K_S-M combination and varied the seed value to ensure randomization of starting values.

Table 1. Description of input parameters for the *Demography focused* and *Genome focused* models. For the *Demography focused* model, ◊ refers to values used for a '*reduced survival*' alternative model and * refers to those used for a '*reduced female reproductive output*' alternative model. For these two models, default values were used (e.g., Field-based empirical estimates labelled as [Est]) for all other parameters. The *Genome focused* model also used field-based empirical estimates as default values.

Parameter	Demography focused	Genome focused	Reference
Carrying capacit (K)			
Ancestral Finland/Karelia ($K_{Anc-Karelia}$)	8000 (N_e/N_C = 0.25; N_e = 2,000)		Bruford (2015); Gopalakrishnan et al. (2017)
Historical-Modern Finland/Karelia ($K_{Karelia}$)	800		
Sweden/Norway (K_S)	50, 100, 150, 200, 210, 250, 300, 310, 350, 400, 450, 500		NA
Founding Swedish population and effective migrants			
Founders (Swedish population) - 1982-90	2	2	Smeds et al. (2022)
Migrants 1990-1991	1	1	Smeds et al. (2022)
Migrants 2008	2	2	Smeds et al. (2022)
Migrants 2017	1	1	SKANDULV, unpub. data.,
Migrants 2021	1	1	SKANDULV, unpub. data.,
Reproduction			
Breeding system	Monogamy		
Age of first pups - female (years)	2		Ebenhard 2000; Bruford (2015)
Age of first pups - male (years)	2		Ebenhard 2000; Bruford (2015)
Maximum age of female reproduction	12		SKANDULV, unpub. data.,
Maximum age of male reproduction	12		SKANDULV, unpub. data.,
Maximum lifespan	13		SKANDULV, unpub. data.,
Sex ratio of pups at birth	0.5		
Proportion of adult females breeding each year	30%* / 63%[Est]	63%[Est]	SKANDULV, unpub. data.,
Mean litter size (SD)	3 ± 0.5* / 3.5 ± 1.4[Est]	3.5 ± 1.4[Est]	SKANDULV, unpub. data.,
Proportion of adult males in breeding pool	100%		SKANDULV, unpub. data.,
Density dependence in pairing/breeding	No		SKANDULV, unpub. data.,
Mortality (mean, SD)			
Females (%)			
Juvenile (Age-0 to Age-1)	30.0 / 50.0◊	30.0	Chapron, unpub. Data / Bruford (2015)
Subadult (Age-1 to Age-2)	40.0[Est] / 50.0◊	40.0[Est]	Chapron, unpub. Data / Bruford (2015)
Young Adult (Age-2 to Age-?)	25.0[Est] / 30.0◊	25.0[Est]	Chapron, unpub. Data / Bruford (2015)
Older Adult (Age-? to 12)	25.0[Est] / 30.0◊	25.0[Est]	Chapron, unpub. Data / Bruford (2015)
Males (%)			
Juvenile (Age-0 to Age-1)	30.0 / 50.0◊	5.0[Est]	Chapron, unpub. Data / Bruford (2015)
Subadult (Age-1 to Age-2)	40.0[Est] / 50.0◊	40.0[Est]	Chapron, unpub. Data / Bruford (2015)
Young Adult (Age-2 to Age-?)	25.0[Est] / 30.0◊	25.0[Est]	Chapron, unpub. Data / Bruford (2015)
Older Adult (Age-? to 12)	25.0[Est] / 30.0◊	25.0[Est] / 30.0◊	Chapron, unpub. Data / Bruford (2015)
Longevity (Females and Males)	13		Chapron, unpub. Data / Bruford (2015)
CATASTROPHE(S)			
Type	Disease		
Frequency	0.01		Nilsson 2013; Murray et al. 1999
Severity (survival)	0.5		
Genetic parameters			
Mutation rate (site/year)	NA	1.5×10^{-9}	Koch et al. (2019)
Mutation properties (dominance *h*; selection *s*; proportion)			
Lethal	NA	0; -1; 0.5	
Very strongly deleterious	NA	0; s< -0.1; 2.6	
Strongly deleterious	NA	0.01; -0.1 ≤ s < -0.01; 23.6	Huber et al. (2017, 2018); Kim et al. (2017); Agrawal et al. (2011); Smeds and Ellegren (2023)
Midly deleterious	NA	0.1; -0.01 ≤ s < -0.001; 24.7	
Weakly deleterious	NA	0.4; s > -0.001; 49.1	
Neutral	NA	0; 0.5; 43.3	
Migrtion (dispersal) parameters			
Minimum age at migration	2		
Migration rate (N/migrants/year)	0.01, 0.03, 0.05, 0.08, 0.1, 0.3, 0.5, 0.8, 1		

4.   Response variables

For the *Demography focused* model, I tested the effect of age-specific survival, female reproductive output, K_S and M on the probability of population extinction, estimated as the proportion of simulations where N reaches 0 within the 100 years period of the simulation. The census size N included individuals of all age classes and was calculated yearly in the autumn (i.e., after reproduction).

For the *Genome focused* model, I tested the effect of K_S and M on the temporal changes in four genetic indices using the field-based empirical estimates of survival and female reproductive output parameters (see point 2.1; Table 1). Statistics were also recorded yearly in the autumn (i.e., after reproduction) from a random sample of 30 individuals including all ages classes and for the period spanning from years 2021 to 2121. Note that year 2021 correspond to the record at time 0 representing the wolf population after the arrival of the last identified effective migrant in 2021. These statistics included, population size (N), mean nucleotide diversity (π), mean inbreeding (F_{ROH}; sum of runs of homozygosity $\geq$100kb divided by genome length), and the number of each mutation type (neutral to lethal). Based on the number of deleterious mutations and their selection and dominance coefficients, I also included two components of genetic load. First, the 'realised load' (i.e., calculated multiplicatively across sites) [36,37], corresponds to the reduction in fitness due to segregating and fixed deleterious mutations in homozygous state [38]. Secondly, the 'masked load' (i.e., calculated additively across sites), also referred to as inbreeding load or potential load since it does not reduce fitness assuming that mutations are recessive or nearly-recessive [36,37], measures the quantity of recessive deleterious variation hidden in heterozygote state and thus can be used to quantify the strength of purging. This component is also important as it represents the load that could be expressed as realised load in the future as they become exposed in homozygous state. It is worth noting that realised and masked load are both components of the genetic load estimated by summing up the selection coefficients of all deleterious mutations [37].

The probability of extinction, temporal changes in N and genetic parameters (i.e., individual replicates and mean over replicates) and the combined effects of K_S and migration rate M on the percentage of change in genetic indices were visualised in R [39]. For the temporal changes in genetic parameters, I calculated the mean value and 95% Confidence Intervals (CI) for each parameter and K_S-M combination. I used a $\pm$5% bracket of change as a goal for maintenance of genetic diversity based on the retention of 95% of heterozygosity considered as a minimal requirement for maintenance of genetic diversity [40]. It is also worth noting that this report does not aim at comparing absolute estimates of genetic parameters with empirical estimates (e.g., [8,10]) but instead focuses on the relative change in these parameters over the 100 years of the simulation

Results

1.   Demography focused model

I first assessed the rate of success for the establishment of the Swedish population by calculating the proportion of simulations failing (i.e., N reaching zero) before the statistics are recorded starting in year 2021 (i.e., year 0 in the simulations). For models of '*reduced survival*' and '*reduced female reproductive output*', 24.9% and 1.4% of simulations failed before 2021, respectively. In contrast, for a model using the field-based empirical estimates for survival rates and female reproductive output based (Table 1), 1.3% of simulations failed before 2021. This suggests that the latter model and default demographic and life-history traits input values are able to recapitulate the population history of the Scandinavian wolf population.

Secondly, I evaluated the impact of age-specific survival, female reproductive output, carrying capacity K_S and migration rate M on the probability of extinction of the Scandinavia population, estimated as the proportion of simulations where N reaches 0 within the 100 years simulation period.. Overall, small populations with K_S<150 were most likely to go extinct but higher migration rates tended to reduce the effect of population extinction at these low K_S values (Fig. 1). For a '*reduced survival*' model but with the field-based empirical estimates of female reproductive output, the probability of extinction was greatest for K_S=50 and low migration rates (i.e., M=0-0.1) and ranged from ~23 to 31%

(Fig. 1a,2; Table S1). In the absence of migration, there was a probability of 31% and 8% of extinction for K_S=50 and 100, respectively (Fig.1a,2; Table S1). However, with migration rates M>0.1, the extinction rate ranged between ~3 and 10%, for K_S=50. For K_S=100, the probability of extinction ranged between 1 and 9% but only for migration rates of M≤0.1. For K_S>100, the probability of extinction dropped to ~1% and only for M≤0.05 (Fig. 1a; Table S1).

For a '*reduced female reproductive output*' model with 29% of adult females reproducing and a litter size of 3 pups (SD=0.5) but with field-based empirical estimates of survival rates, the probability of extinction was greatest for K_S=50 and ranged between 23 and 32% for low migration rates (i.e., M=0-0.1). In the absence of migration, the risk of extinction was of 32% and 10% for K_S=50 and 100, respectively (Fig.1b,2; Table S1). With migration rates of M>0.1, the extinction rate ranged between ~1 and 7% for K_S=50. For K_S=100, the probability of extinction ranged between 5 and 10% but only for migration rates of M≤0.1. For K_S>100, the probability of extinction dropped to ~1%, but only for K_S=150 and K_S=210 for low migration rates (i.e., M=0-0.05; Fig. 1b; Table S1).

Finally, when using the field-based empirical estimates for survival rates female reproductive output, the risk of extinction was of ~1% for a K_S of 50, with no obvious association with migration rate. No extinction was reported for K_S≥100 (Fig. 1c). This indicates that given the empirical field-based estimates of demographic and life-history traits used here and even in cases of population crashes due to catastrophes such as diseases outbreaks, the population seems to be resilient and able to recover quickly.
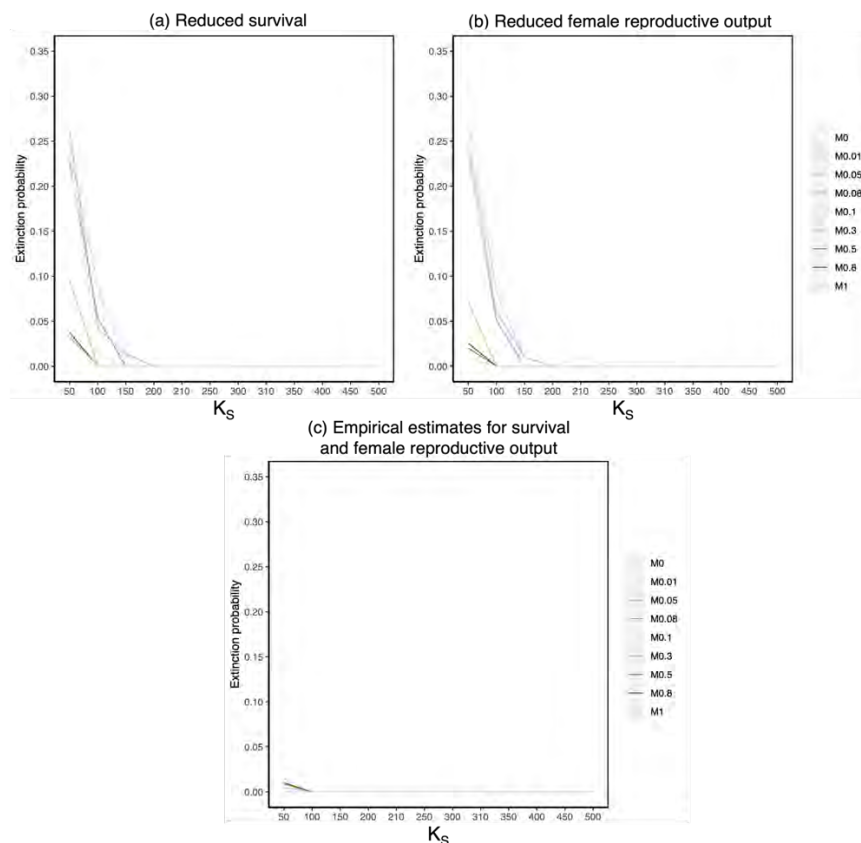
Figure 1. Probability of extinction for K=50-500 and for M=0-1 migrants/year when varying age-specific survival and female reproductive output. (a) *Reduced survival*: 50% mortality from 0-2 years old, 30% from 3 years old, proportion of females mating = 63%,, litter Size = 3.5 (SD: 1.4); (b) *Reduced female reproductive output*: proportion of females mating = 29%, litter Size = 3 (SD: 0.5), 5% mortality from 0-1 years old, 40% mortality from 1-2 years old, 25% from 3 years old; (c) Field-based estimate of age-specific survival and female reproductive output: proportion of females mating = 63%, litter Size = 3.5 (SD: 1.4); 30% mortality from 0-1 years old, 40% mortality from 1-2 years old, 25% from 3 years old. Results are based on 100 replicates per K_S-M combination. Raw data in Table S1.
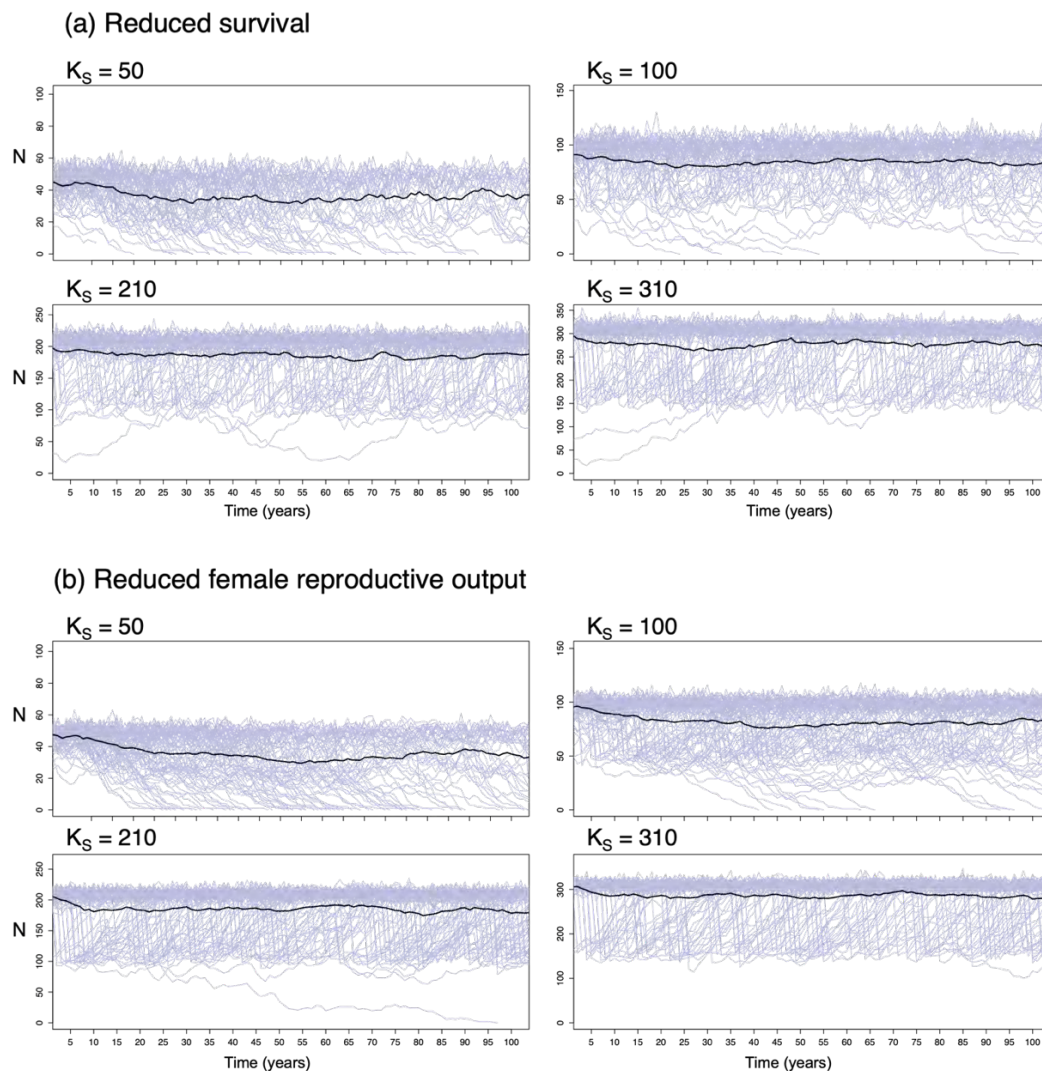
Figure 2. Temporal change in population size N for K$_S$=50, 100, 210 and 310 without migration for (a) *Reduced survival*: 50% mortality from 0-2 years old, 30% from 3 years old, litter Size = 3.5 (SD: 1.4); (b) *Reduced female reproductive output*: proportion of females mating = 29%, litter Size = 3 (SD: 0.5). The black curve represents the mean over the 100 replicates shown in purple. Population crashes are shown with purple curves reaching 0 on the x-axis. Population crashes due to catastrophes are clearly visible with a sharp decline in N.

2. Genome focused model

2.1 A current Swedish population size of 170 or 270 without migration

I first examined the genetic consequences of a worst-case scenario without migration (M=0) for a carrying capacity K$_S$=210 and 310 for the Scandinavian population, corresponding to a Swedish population of 170-270 wolves. These simulations depicting complete isolation of the population over the next 100 years showed a 16% reduction in nucleotide diversity and a 16% increase in inbreeding for K$_S$=210 (Fig. 3; Table S2). For a K$_S$=310, there was a 10% reduction in nucleotide diversity and a 8% increase in inbreeding (Table S3). The realised load remained constant with ~1% increase for K$_S$=210 and K$_S$=310, while the masked load showed a reduction of 25% and 16%, for K$_S$=210 and K$_S$=310 individuals, respectively (Tables S2,S3). This reduction in masked load was most likely facilitated by a relatively higher inbreeding for a K$_S$=210 compared to K$_S$=310.

It is worth noting that the model is bound to show some degree of stochasticity among individual simulations. However, 100 replicates for this model showed that the overall trend among runs is consistent (Fig. 3,4). Furthermore, these simulations clearly showed the effect of catastrophes such as diseases outbreaks with sharp drops in N and realised load (Fig. 3).
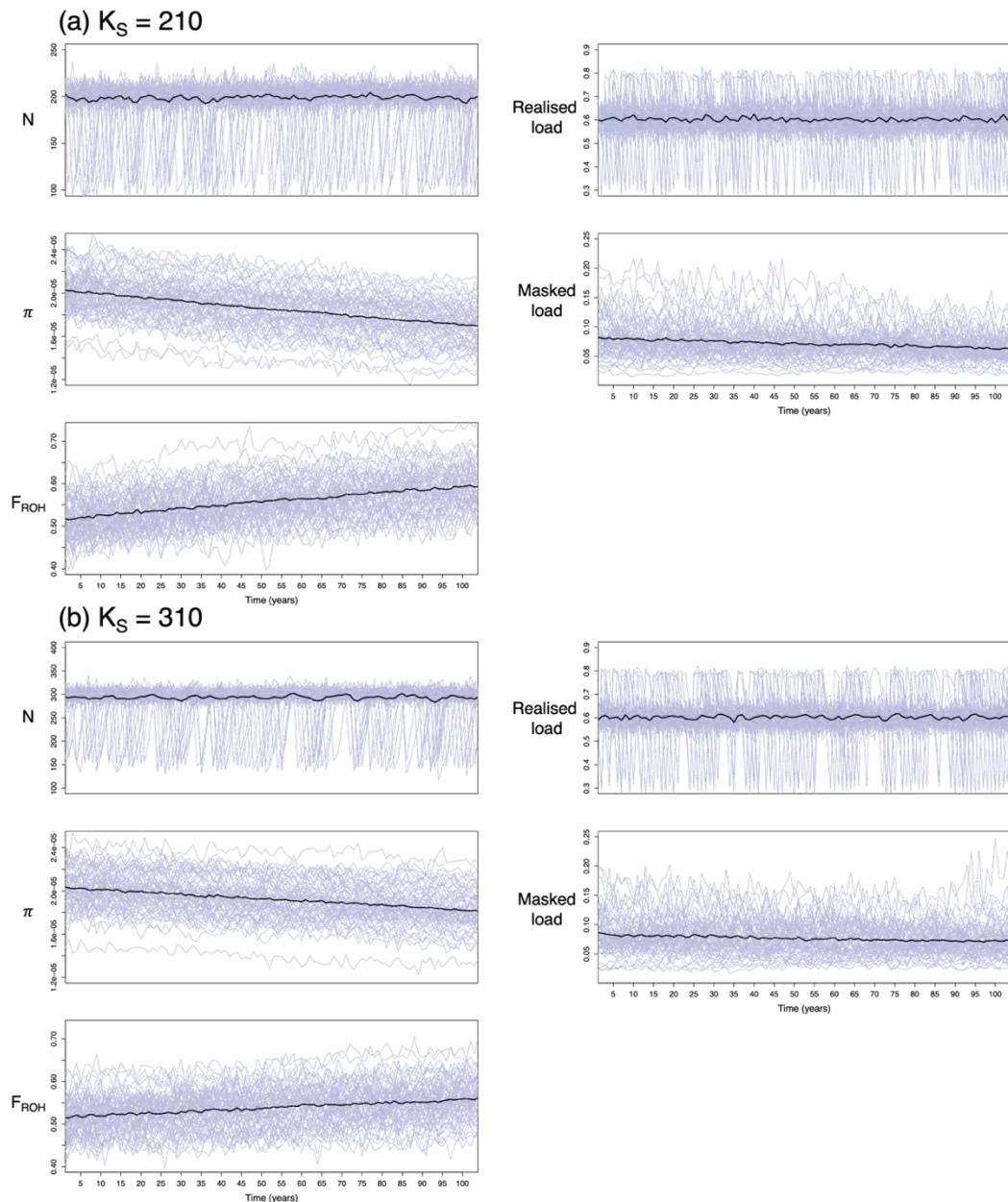
Figure 3. Temporal changes in N, mean nucleotide diversity (π), inbreeding (F_{ROH}), Realised load and Masked load for (a) K_S=210 and (b) K_S=310, corresponding to a Swedish wolf population size of 170 to 270, and assuming no migration over 100 years since the last reported migration event in 2021. The black curve represents the mean over the 100 replicates which are shown in purple. Raw data in Tables S2,S3.

2.2 Impact of migration rates on a population size of 170 or 270

Simulations for K_S=210 and K_S=310 and with migration rates ranging from 0 to 1 migrant(s) per year (M=0-1), showed that migration always increased nucleotide diversity and reduced inbreeding (Fig. 4; Table 2).

Overall, without migration (M=0), a larger K_S was always associated with a lower reduction in nucleotide diversity with -10% and -16% reduction for K_S=310 and K_S=210, respectively (Table 2, S2-3). Similarly, for K_S=210, there was a 16% increase in inbreeding, but for K_S=310, this increase was 8%. In contrast, for a high migration rate (M=1), the increase in nucleotide diversity did not vary substantially. For K_S=210 there was a 26% increase and for K_S=310, there was a 22% increase.
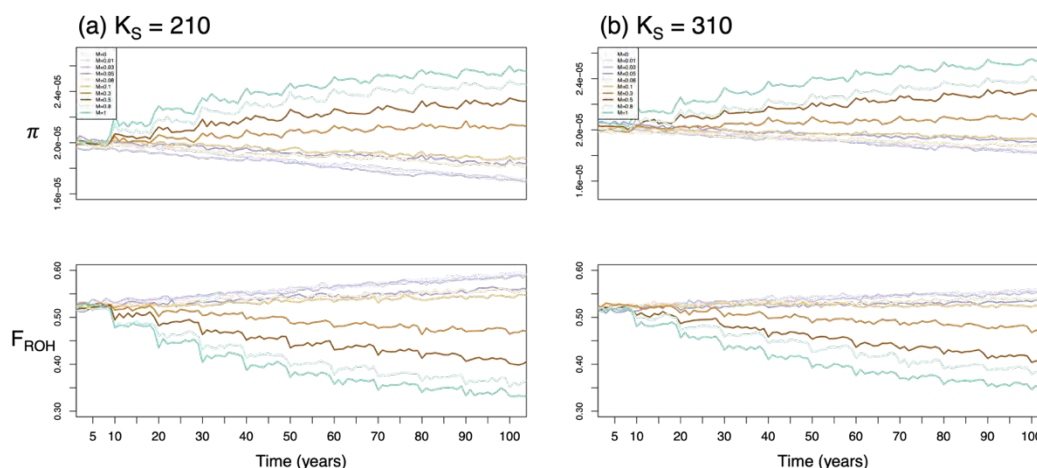
Figure 4. Temporal changes in mean nucleotide diversity (π) and inbreeding (F_{ROH}) for different yearly migration rates (M), for (a) K_S=210 and (b) K_S=310, corresponding to a Swedish wolf population size of 170 to 270. Each curve represents the average value for 100 simulations. Raw data in Tables S2-3.

For both K_S=210 and K_S=310, a minimum of 1 to 3 effective migrants per decade (M=0.1-0.3) would be necessary to avoid a reduction in nucleotide diversity and increase in inbreeding over the next 100 years (Fig. 4; Tables 2, S2-3). Such number of migrants would constrain the change in nucleotide diversity to a -7% to +7% bracket for K_S=210 and to a -6% to +5% bracket for K_S=310. This would also constrain the change in inbreeding to a 4% to -12% bracket for K_S=210 and to a 1% to -10% bracket for K_S=310. However, three effective migrants per decade (M=0.3) would allow for 5 to 7 % increase in nucleotide diversity for K_S=210 and K_S=310, respectively. Similarly, M=0.3 would allow for a 10 to 12% reduction in inbreeding for K_S=310 and K_S=210, respectively (Tables 2, S2-3).

Table 2. Temporal percent change in genetic indices over 100 years for the Scandinavian wolf populations for a K_S=210 and 310. Raw data in Tables S2-3.

Mig. / year	0	0.01	0.03	0.05	0.08	0.1	0.3	0.5	0.8	1
Nucleotide diversity (π)	-16%	-14%	-13%	-11%	-9%	-7%	7%	15%	23%	26%
Inbreeding (F_{ROH})	16%	14%	12%	9%	7%	4%	-12%	-23%	-31%	-37%
Realised load	-1%	-3%	-6%	-5%	-5%	-5%	-7%	2%	-5%	-4%
Masked load	-25%	-14%	-14%	-3%	-17%	-17%	3%	-1%	14%	11%
					$K_S = 310$					
Mig. / year	0	0.01	0.03	0.05	0.08	0.1	0.3	0.5	0.8	1
Nucleotide diversity (π)	-10%	-10%	-9%	-7%	-6%	-6%	5%	14%	19%	22%
Inbreeding (F_{ROH})	8%	7%	6%	5%	4%	1%	-10%	-21%	-25%	-31%
Realised load	0%	-6%	2%	-5%	5%	-1%	-3%	-7%	-7%	-7%
Masked load	-16%	-11%	-12%	-16%	-6%	-8%	-4%	3%	7%	7%

Tracking realised load (i.e., load expressed in individuals and affecting fitness) as a function of migration rate revealed year-to-year fluctuations but little overall temporal changes. Over the whole simulation period the realised load remained on average close to a value of 0.4 for both K_S=210 and K_S=310 (Fig. 5, Tables 2, S2-S3) and the change in realised load ranged from -7% to +5% for either K_S value and for any migration rate. While the realised load did not substantially change over the whole simulation period, the dynamics of this load clearly reflected events of effective immigration. Indeed, there were noticeable peaks in realised load at regular intervals corresponding to each immigration event, which indicates the likely introduction of new deleterious alleles by breeding immigrants (Fig. 5). Each peak was then followed by a drop in this load, showing the effect of selection against newly-introduced and expressed deleterious variation. Consistent with theory, this effect is much stronger in a

small and more inbred population where deleterious variants will be more readily exposed to selection in homozygous state (i.e., K_S=210; Fig. 5). Furthermore, the magnitude of these peaks and drops appeared to be positively correlated with migration rates, with M=1 showing the highest peaks.

In contrast, the dynamics of masked load (i.e., recessive heterozygous deleterious alleles) revealed changes strongly reflecting the effect of migration intensity and K_S values over the 100 years simulation period. Overall, higher migration rates induced a higher increase in masked load and a lower K_S showed greater changes in masked load (Fig. 5). For instance, migration rates below or equal to 1 to 3 effective immigrants per decade (M≤0.1-0.3) were associated with a reduction in masked load which was greater for K_S=210 than for K_S=310 (Fig.5; Table 2, S2-3). Furthermore, for M=0, there was a 25% reduction for K_S=210 but only a 16% reduction for K_S=310. In contrast, for M=0.8-1, the masked load showed an increase of 11-14% for K_S=210 and of 7% for K_S=310 (Fig.5; Table 2, S2-3). This is not surprising since the effect of effective immigration and gene flow will be stronger in a population with lower genetic diversity compared to a population with higher diversity.
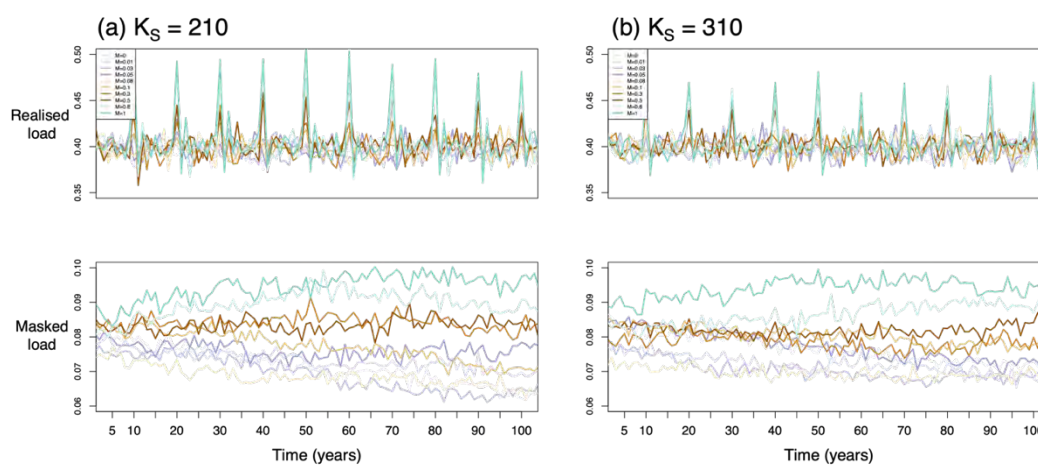
Figure 5. Temporal changes in mean realised and masked load for different yearly migration rates (M), for (a) K_S=210 and (b) K_S=310, corresponding to a Swedish wolf population size of 170 to 270. Each curve represents the average value for 100 simulations. Raw data in Tables S2-3.

These result are consistent with theory that suggests that masked load is more efficiently reduced in small and more inbred populations since recessive mutations are more frequently exposed to purifying selection as homozygotes through purging [37,41]. Conversely, the masked load is likely to be higher in large populations where inbreeding is less frequent and where high heterozygosity masks recessive alleles from selection or in the presence of gene flow increasing the amount of new genetic variation [41]. The results from these simulations are also consistent with empirical genomic data from Smeds & Ellegren [8] that indicate that while immigration induces an increase in masked load through heterozygosis, the number of deleterious mutations in heterozygote state is reduced compared to the Karelian population only a few generations after an immigration event, as inbreeding gradually increases [8], thus confirming a scenario of recurrent purging of load. This point was also raised in the previous report on Swedish wolf viability [9]. Nevertheless, the higher temporal increase in masked load for K_S=210 and for higher migration rates (M≥0.8) compared to simulations with lower migration and for K_S=310 indicates that a smaller population is at greater risk of increasing and expressing new deleterious variation.

Consequently, these simulations suggest that moderate migration rates can have a dual beneficial effect. On the one hand, moderate migration rates allow to reduce the chance of introduction of new deleterious variation in the population. On the other, moderate migration will keep inbreeding high enough to facilitate the removal of deleterious mutations through purifying selection. Nevertheless, it is important to note that there is a trade-off between reduction in deleterious variation via inbreeding and maintenance of adaptive variation via gene flow [42]. While too much gene flow could introduce new deleterious and cause inbreeding depression if future population declines and inbreeding events occur (e.g., [43]), gene flow is also essential for maintenance of adaptive potential and reduction in inbreeding in the population.

Taken together, these results suggest that for a size of 170 to 270 for Swedish wolf population, a migration rate of 1 to 3 effective immigrant per decade (i.e., M=0.1-0.3) would satisfy this trade-off by avoiding a loss in genetic diversity and increase in inbreeding as well as an increase in genetic load (i.e. masked load). However, since it would be preferable to reduce the average inbreeding coefficient (F_P; estimated with pedigree data) from 0.23 to <0.2 (R. Ekblom, *pers. comm.*) corresponding to a ~13% reduction, 3 to 5 effective migrants per decade (i.e., M=0.3-0.5) would be preferable for K_S=210 to 310 (Fig. 5, Tables 2, S2-3).

2.3 Combined effect of population size and migration rates on genetic diversity

Because population size and migration rates will fluctuate naturally and since conservation goals may change over time, it is crucial to assess the combined effects of various population sizes (i.e. below and above the 170-270 range) and migration rates on the medium- to long-term genetic population viability.

Overall, simulations indicate that populations with K_S<150 are most at risk of reduction in nucleotide diversity and increases in inbreeding. For these K_S values, ≥1 effective immigrant per decade (i.e., M≥0.1) would be required for the population to remain within a 5% window of loss of nucleotide diversity and increase in inbreeding (Fig. 6). Furthermore, nucleotide diversity would drop and inbreeding increase sharply for migration rates M≤0.05 for K_S<200 (Fig. 6). These observed reductions are especially sharp for K_S declining from 150 to 50. For instance, for M=0.05, a population with K_S=150 to 50 would experience ~20 to 25% reduction in nucleotide diversity and increase in inbreeding, whereas for K_S=210, these would only be reduced or increased by 10% (Fig. 6; Tables S4). For M≤0.03, populations with K_S=50 to 150 would experience ~55 to 20% reduction in nucleotide diversity and ~65 to 20% increase in inbreeding (Fig. 6; Tables S4).
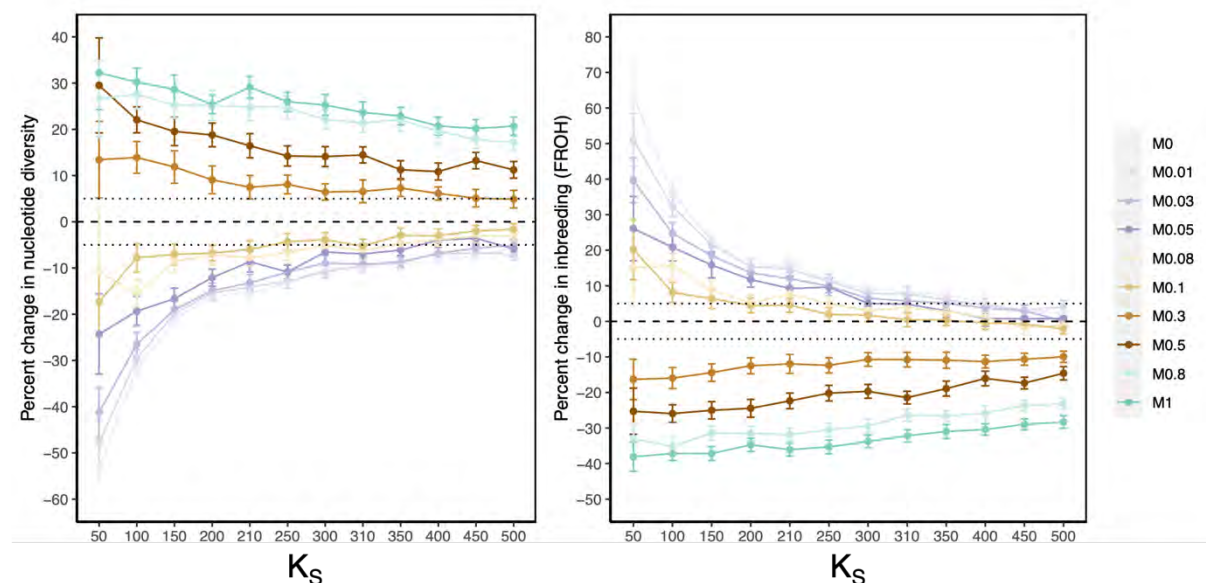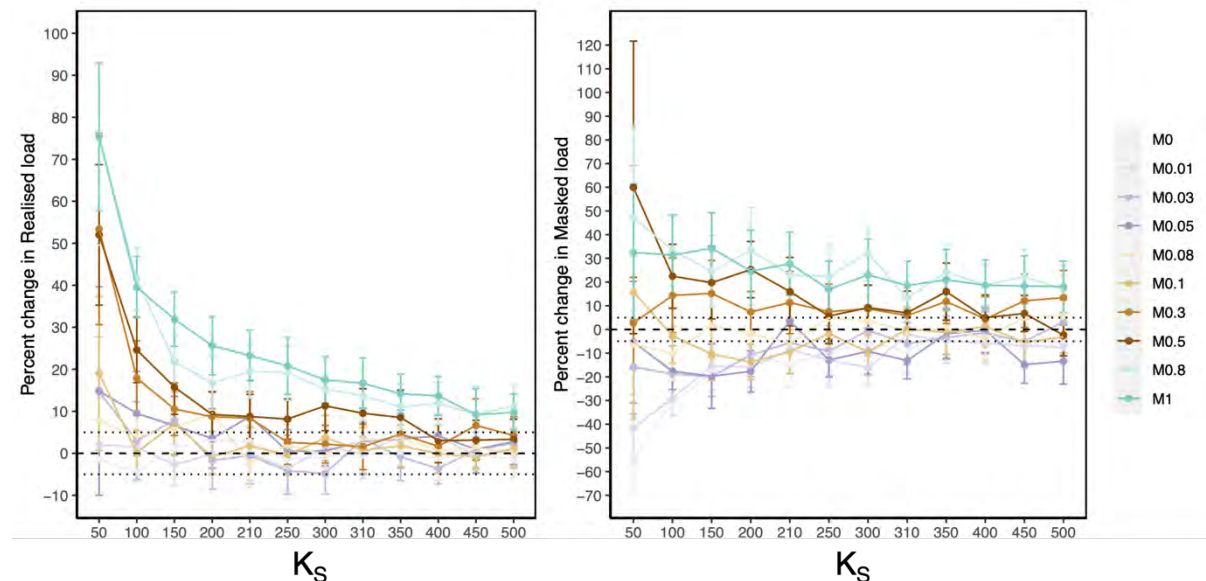

Figure 6. Effect of yearly migration rate M (0–1 effective immigrants/year) and K_S values (50–500) on the mean nucleotide diversity (π) and inbreeding (F_{ROH}), estimated as the percent change over 100 years. Values >0 and <0 indicate increase and reduction, respectively. Points represent mean and whiskers represent the 95% CI. Dotted lines depict a ±5% change and dashed line no change. Raw data in Table S4.

In contrast, for K_S≥150, a minimum of 1 effective migrants per decade (i.e., M=0.1) would be required to for the population to remain within a 5% window of loss in nucleotide diversity or increase in inbreeding (Fig. 6; Tables S4), whereas for K_S≥250-300, even a migration rate of M=0.08 would achieve the same outcome over 100 years.

When evaluating the impact of K_S and migration on genetic load, simulations showed that high migration rates were positively correlated with the two estimates of load through the introduction of a

higher proportion of deleterious variation, even for large K_S values (Fig. 7; Table S4). Overall, high migration rates of M>0.3 showed an increase in realised load (i.e., deleterious variation expressed), which is especially sharp for $K_S \leq 200$. For instance, for K_S=50 and for M=1, realised load increased by 75% (Fig. 7; Table S4). While realised load decreased and stabilised with increasing K_S, there was still a high risk for realised load to increase by ~10-30% for M=0.5-1 and for $K_S \geq 200$.

No population extinction was reported over the 100 years simulation period using the field-based empirical estimates for demographic parameters, except for K_S =50 (i.e., <5%). Yet, the increase in realised load ranging between 10-20% suggests that small populations could be at higher risk of extinction. However, with M=0-0.1, the change in realised load would still remain close or within a 5% change window for $K_S \geq 150$.

Figure 7. Effect of yearly migration rate M (0–1 effective immigrants/year) and K_S values (50–500) on mean realised load and masked load, estimated as the percent change over 100 years. Values >0 and <0 indicate increase and reduction, respectively. Points represent mean and whiskers represent the 95% CI. Dotted lines depict a $\pm$5% change and dashed line no change. Raw data in Table S4.

The migration rate had similar effect on masked load (i.e., recessive deleterious variation not expressed in individuals in a given year but potentially in future generations). This pattern reflected the impact of migration on nucleotide diversity (Fig. 6) but with wider magnitude of change for each K_S-M combination and with more obvious variation among K_S-M combinations (Fig. 7; Tables S4). Similar to the pattern observed for realised load, the change in masked load also stabilised with the increase in K_S values, but this time, for K_S=100 already. Furthermore, there was a risk for an increase in masked load of more than 5% for M$\geq$0.3 even for larger K_S values. There was also a tendency for masked load in populations with $K_S \leq 200$ to be more affected by high or low migration rates. For instance, for K_S=100, masked load increased or decreased by 31% for M=1 and M=0, respectively. In contrast, for K_S=250, masked load increased by 17% and was reduced by 10% for M=1 and M=0, respectively (Fig. 7; Table S4). Furthermore, the reduction in mased load was strongest for K_S=50 and with M=0-0.1, ranging from ~40 to 50%, which indicates that purging is facilitated in small and inbred populations compared to larger populations where deleterious mutations are more likely to be hidden in heterozygous state.

Consistent with the results of Section 2.2, for K_S=210-310, migration rates of between 1 to 3 effective migrant per decade (M=0.1-0.3) would maintain the status quo or restrict fluctuations in nucleotide diversity, inbreeding as well as genetic load to a 5% window. This migration rate would thus favour a trade-off between the a genetic rescue effect and the introduction of new deleterious variation that could reduce fitness in future generations. Nevertheless, a $K_S \geq 310$ would make the population more resilient to a future reduction in nucleotide diversity and increase in inbreeding in case migration

is reduced. For instance, if a migration were to drop to M<0.1, in cases of high conflict with humans along main migration routes for instance, a $K_S \geq 310$ would be preferred to limit the increase in inbreeding while the risk of an increase in masked load would still be limited.

Finally, it is worth noting that if gene flow were to increase to 8 to 10 effective immigrants per decade (M$\geq$0.8-1), even large populations with K_S=200-500 could be exposed to a ~20% increase in masked load. Thus, if the Scandinavian population remains isolated or if gene flow is uneven through time, populations with $K_S \geq 500$ may be needed to mitigate the effects of introduction and expression of deleterious variation, especially if the population fluctuates naturally or through culling. In contrast, for M=0.5-0.3, there would be an increase in load closer to the 5% threshold.

3. Comparison with results from the preliminary report

A preliminary report was assessed by an external panel of reviewers in November 2023. Simulations and analyses were mostly identical to those described above. However, in light of the reviewers' comments, some improvements were made to produce the final report presented here. The main changes included: testing for the effect of a reduced survival probability for the *Demography focused* model, including K_S values of 210 and 310 to account for the Norwegian part of the population (i.e., N=40), the simulation of a population split of the Karelian population c. 200 years BP from a large ancestral population, the addition of random catastrophic events to simulate diseases outbreaks, the removal of two founder individuals that did not reproduce and the use of a 5% threshold when reporting changes in genomic indices instead of a 10% threshold. Furthermore, while the preliminary report presented results based on simulations of single-chromosome genomes, this final report presents simulations of 3000 unlinked genes as it allowed to significantly reduce computational time.

Overall, the preliminary results showed that for a model of *Reduced female reproductive output* (i.e., 30% reproductive probability for females; litter size of 3 pups, SD: 0.5), the risk of extinction ranged between 12 and 37% for K_S=50 and between 2 and 3% for K_S=100 (Fig. 8a). In contrast, when using the field-based empirical estimates for survival and female reproductive output as input values in the simulations (Fig. 8b), the risk of extinction was of 1 and 6% for a population size of 50 and no extinction was reported for $K_S \geq 100$.

From a genomics perspective, a minimum of 1 effective immigrant per decade (i.e., M=0.1) would be required to maintain the current amount of genetic diversity and reduce the risk of increases in inbreeding and genetic load for a target population size of 170-270 individuals (Fig. 9-10). However, it would be preferable to aim for a higher number of effective migrants per generation (i.e., M=0.3-0.5) to favour a long-term genetic rescue effect. Below the migration rate threshold of 1 migrant per decade, the population would likely experience more than 10% reduction in nucleotide diversity and increase in inbreeding. Importantly, these preliminary results were robust to the choice of the size of the ancestral Karelian population (i.e., $K_{Anc\text{-}Karelia}$=4,000 or 8,000; Fig. 9-10). Indeed, while the absolute estimates of genetic diversity (i.e., nucleotide diversity, inbreeding, load) were positively correlated with $K_{Anc\text{-}Karelia}$ values, the proportion of temporal change in these estimates was not affected by the $K_{Anc\text{-}Karelia}$ value chosen.

These preliminary and final results are thus highly consistent for both the *Demography focused* and *Genome focused* models. However, the slightly higher migration rate required in the final results (M=0.1-0.3) indicate that the introduction of catastrophic event, which was not included in the preliminary simulations may impact the viability of the population even if it can recover quickly after a population crash.
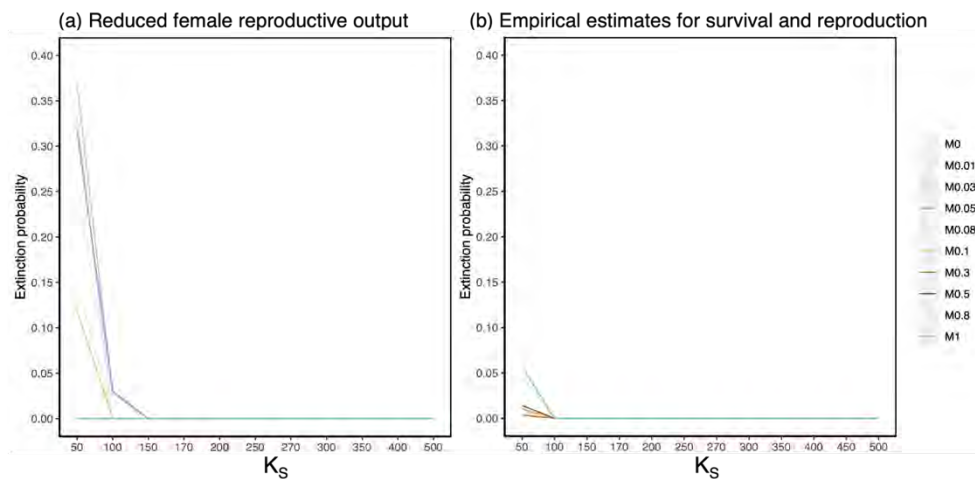
Figure 8. Result summary from the preliminary report (November 2023) (a) *Reduced female reproductive output.* (b) Field-based estimate of age-specific survival and female reproductive output (Table 1).
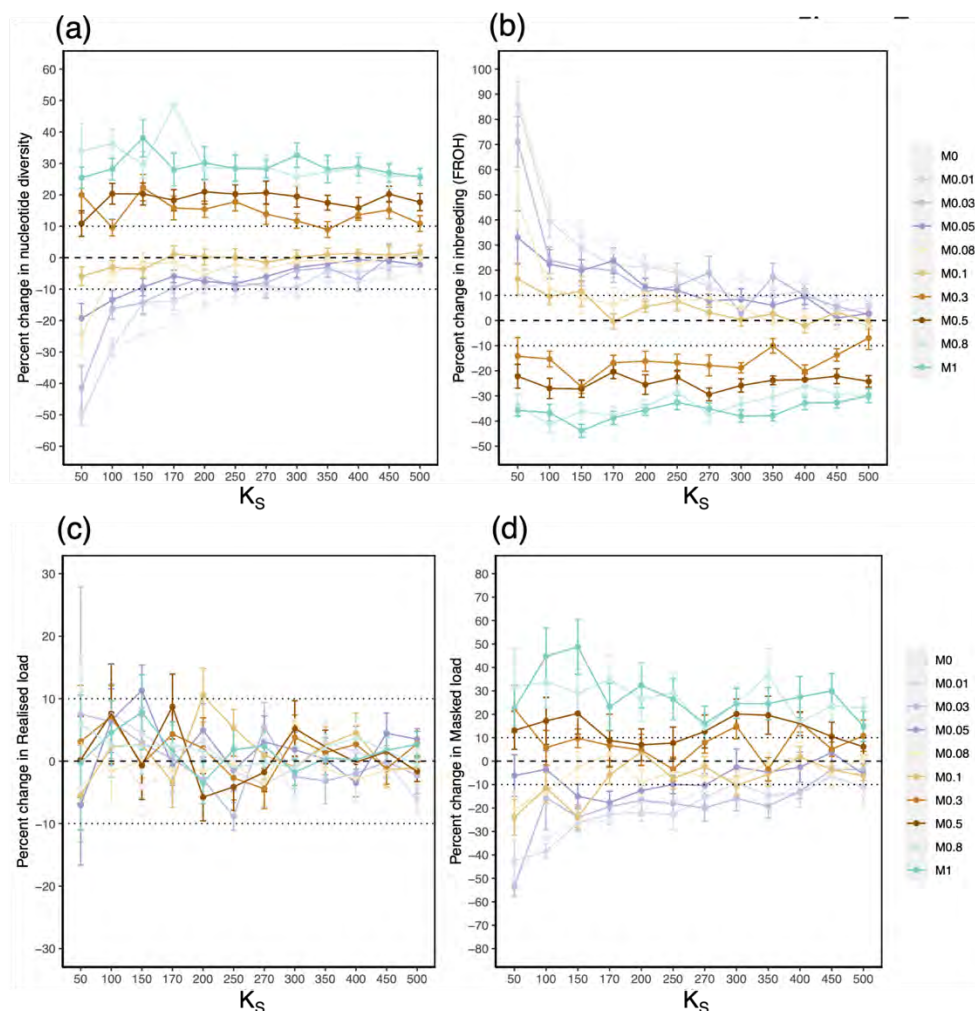
Figure 9. Effect of yearly migration rate M (0 – 1 effective immigrants/year) and K_S values (50 – 500) on (a) mean nucleotide diversity, (b) inbreeding, (c) realised load and, (d) masked load, estimated as the percent change over 100 years for $K_{Anc\text{-}Karelia}$=4,000. Values >0 and <0 indicate increase and reduction, respectively. Points represent mean and whiskers represent standard deviation. Dotted lines depict a ±10% change and dashed line no change.
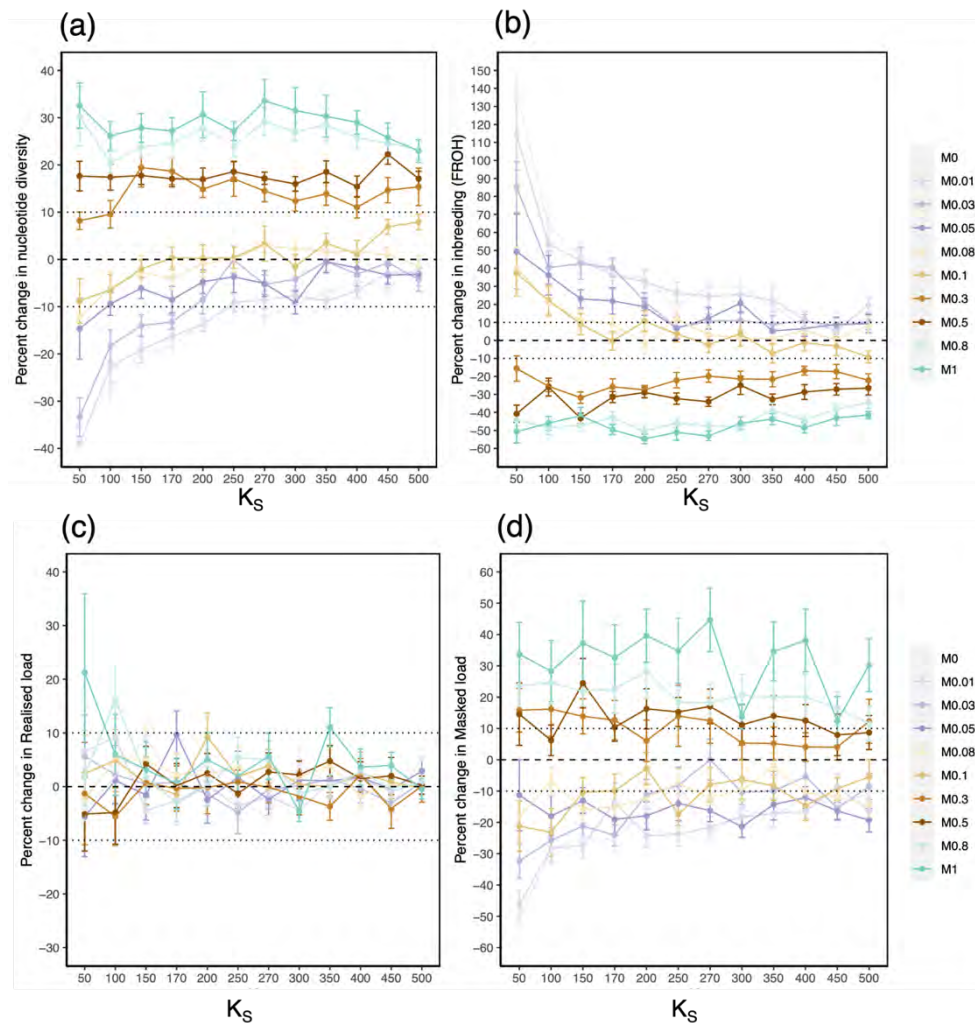
Figure 10. Effect of yearly migration rate M (0 – 1 effective immigrants/year) and K_S values (50 – 500) on (a) mean nucleotide diversity, (b) inbreeding, (c) realised load and, (d) masked load, estimated as the percent change over 100 years for $K_{Anc-Karelia}$=8,000. Values >0 and <0 indicate increase and reduction, respectively. Points represent mean and whiskers represent standard deviation. Dotted lines depict a $\pm$10% change and dashed line no change.

Discussion

Using forward-in-time simulations, I tested the impact of age-specific survival, female reproductive output, population size and migration on probability of demographic collapse and extinction and on several indices of genetic diversity to determine under which conditions a population of 170-270 would represent a viable wolf population in Sweden. Overall, for a model of *reduced survival*, the risk of extinction would mostly affect population sizes with $K_S \leq 100$ and that this risk would range between 5 and 31%. Similarly, a model of *Reduced female reproductive output* showed the greatest probability of extinction for K_S=50 and ranged from 5 to 32%. However, when using the field-based empirical estimates of age-specific survival and female reproductive output, the risk of extinction would remain low, even for K_S=50 and would be close to 1%, thus showing the high degree of resilience of wolf populations to demographic declines.

From a genetic perspective, between 1 and 3 effective immigrants per decade (i.e., M=0.1-0.3) would be required to keep the change in genetic diversity, inbreeding and genetic load within a 5% window for a target population size of 170-270 individuals in Sweden. However, since inbreeding is already high (i.e., mean of 0.27 [10]) and since it would be preferable to reduce it to a mean of <0.2, it would be preferable to aim for a higher number of effective immigrants per generation (i.e., M=0.3-0.5)

to induce a long-term genetic rescue effect [4]. Below the migration rate threshold of 1 immigrant per decade, the population would likely experience more than 5% reduction in nucleotide diversity and increase in inbreeding. These results are consistent with the previous wolf population viability analysis from Bruford [9] who found that one effective migrant per 6 years (i.e., $M \cong 0.6$) would allow to retain 95% of genetic diversity. In contrast, migration rates between the Karelian and Finnish populations range between 1.4 and 3 effective migrants per generation (i.e., 0.5-1 per year assuming a generation time of 3 years; [23–25]), which is considered sufficient to maintain genetic diversity but which is higher than the migration rate needed to avoid >5% loss in diversity and increase in inbreeding based on the present simulations.

 The examination of the dynamics of overall genetic variation (e.g., nucleotide diversity, inbreeding) and deleterious mutations underscores the need to be consider the trade-off between genetic rescue and introduction of new deleterious variation. While the simulations show that immigration will increase genetic diversity and thus potentially inducing a genetic rescue effect and an increase in population fitness, it may also introduce new deleterious variation. This effect is particularly strong in small populations where even a moderate number of breeding immigrants can increase the realised and masked loads substantially. For instance, the migration of a single male wolf into the small and isolated Isle Royale wolf population resulted in a population decline associated with the introduction of detrimental variation [15]. Consequently, situations where K_S is small, inbreeding high and M moderate to high presents a risk of sudden increase in realised load and decrease in fitness. However, the present simulations show that while fitness would initially decline, genetic load would also be purged more efficiently in small populations and with low migration rates compared to situations with high migration. Nevertheless, it is worth stressing that the benefits of genetic rescue are likely to outweigh the negative effects of introduction of deleterious genetic variation [42]. Thus, monitoring individual health based on phenotypic data as well as quantifying changes in load and beneficial variation over the medium- to long-term and following effective migration events, should be considered as important components of the genomic monitoring strategy for wolf.

 From a practical perspective, determining which MVP is most appropriate will depend in great part on real-life migration rates. It is possible that the actual migration rate may be lower, especially if there are conflicts between wolves and other interest groups (e.g., reindeer herders, hunters, etc) and will thus require to update population size targets and MVPs accordingly. Finally, while the present report focuses on MVPs in the 170-270 range, it is important to note that SEPA will have to upscale a target value that takes into account ecological parameters, such as habitat size, prey availability and competition with other predators to define an appropriate FRV for wolf.

Limitations of the simulations

One important limitation of the simulations presented here is that models make a number of assumptions on demographic and genetic parameters used in the model. Nevertheless, we can be confident that the results presented here are realistic for two reasons. First, the Scandinavian wolf population has been closely studied for over 40 years and demographic parameters as well as life-history traits have been validated in a previous report [9] and previous studies [23–25]. Secondly, while there are some uncertainties around the recombination and mutation rates as well as around the proportion of mutations belonging to each selection coefficient category, the present report focuses on the temporal relative change in genetic indices over a 100 years period and not on absolute estimates. Furthermore, the current and preliminary results show that while absolute genetic estimates vary as a function of the ancestral population size, the relative change in these genetic estimates remained consistent among models. Thus, these results are of great value to evaluate the conditions under which the target FRV would represent a viable population. Nevertheless, there is high value in using empirical estimates from genomic data as starting points in future work. This would for instance allow to predict which demographic and life-history traits values could satisfy specific goals to set for the amount of genetic diversity to retain or for the maximum increase in inbreeding or genetic load allowed in the population.

Acknowledgements

References

1.   Melbourne BA, Hastings A. 2008 Extinction risk depends strongly on factors contributing to stochasticity. *Nature* **454**, 100–103.

2.   Lynch M, Conery J, Burger R. 1995 Mutation Accumulation and the Extinction of Small Populations. *The American Naturalist* **146**, 489–518.

3.   van Oosterhout C. 2020 Mutation load is the spectre of species conservation. *Nature Ecology and Evolution* **4**, 1004–1006.

4.   Hohenlohe PA, Funk WC, Rajora OP. 2021 Population genomics for wildlife conservation and management. *Molecular Ecology* **30**, 62–82.

5.   Wikenros C, Gicquel M, Zimmermann B, Flagstad Ø, Åkesson M. 2021 Age at first reproduction in wolves: Different patterns of density dependence for females and males. *Proceedings of the Royal Society B: Biological Sciences* **288**. (doi:10.1098/rspb.2021.0207)

6.   Hagenblad J, Olsson M, Parker HG, Ostrander EA, Ellegren H. 2009 Population genomics of the inbred Scandinavian wolf. *Molecular Ecology* **18**, 1341–1351.

7.   Åkesson M, Liberg O, Sand H, Wabakken P, Bensch S, Flagstad Ø. 2016 Genetic rescue in a severely inbred wolf population. *Molecular Ecology* **25**, 4745–4756.

8.   Smeds L, Ellegren H. 2023 From high masked to high realized genetic load in inbred Scandinavian wolves. *Molecular Ecology* (doi:10.1111/mec.16802)

9.   Bruford MW. 2015 *Additional Population Viability Analysis of the Scandinavian Wolf Population*.

10. Kardos M *et al.* 2018 Genomic consequences of intensive inbreeding in an isolated Wolf population. *Nature Ecology and Evolution* **2**, 124–131.

11. Liberg O, Andrén H, Pedersen HC, Sand H, Sejberg D, Wabakken R, Åkesson M, Bensch S. 2005 Severe inbreeding depression in a wild wolf (Canis lupus) population. *Biology Letters* **1**, 17–20.

12. Caballero A, Bravo I, Wang J. 2017 Inbreeding load and purging: implications for the short-term survival and the conservation management of small populations. *Heredity* **118**, 177–185.

13. Franklin IR, Soulé ME, Wilcox B a. 1980 Evolutionary change in small populations. In *Conservation Biology: An Evolutionary-Ecological Perspective* (eds ME Soule, BA Wilcox), pp. 135–149. Sunderland, Mass.: Sinauer Associates.

14. Haller BC, Messer PW. 2019 SLiM 3: Forward Genetic Simulations Beyond the Wright-Fisher Model. *Molecular Biology and Evolution* **36**, 632–637.

15. Robinson JA, Räikkönen J, Vucetich LM, Vucetich JA, Peterson RO, Lohmueller KE, Wayne RK. 2019 Genomic signatures of extensive inbreeding in Isle Royale wolves, a population on the

threshold of extinction. *Science Advances* **2014**, 440511.

16. Robinson JA. 2022 The critically endangered vaquita is not doomed to extinction by inbreeding depression. , 635–639.

17. Dussex N. 2021 Population genomics of the critically endangered kakapo. *Cell Genomics* **1**, 0–2.

18. Robinson JA, Brown C, Kim BY, Lohmueller KE, Wayne RK. 2018 Purging of Strongly Deleterious Mutations Explains Long-Term Persistence and Absence of Inbreeding Depression in Island Foxes. *Current Biology* **28**, 3487-3494.e4.

19. Dussex N *et al.* 2023 Adaptation to the High-Arctic island environment despite long-term reduced genetic variation in Svalbard reindeer. *iScience* **26**, 107811.

20. Kyriazis1 CC, Robinson JA, Lohmueller KE. 2022 Using computational simulations to quantify genetic load and predict extinction risk. *The American Naturalist* 202.6 (2023): 737-752.

21. Gopalakrishnan S *et al.* 2017 The wolf reference genome sequence (*Canis lupus lupus*) and its implications for Canis spp. population genomics. *BMC Genomics* **18**. (doi:10.1186/s12864-017-3883-3)

22. Forslund P. 2009 Modellering av den skandinaviska vargpopulationens tillväxt och inavelsnivå. Unpublished report to the Swedish Environmental Protection Agency (in Swedish).

23. Aspi J, Roininen E, Kiiskilä J, Ruokonen M, Kojola I, Bljudnik L, Danilov P, Heikkinen S, Pulliainen E. 2009 Genetic structure of the northwestern Russian wolf populations and gene flow between Russia and Finland. *Conservation Genetics* **10**, 815–826.

24. Åkesson M, Flagstad Ø, Aspi J, Kojola I, Liberg O, Wabakken P, Sand H. 2022 Genetic signature of immigrants and their effect on genetic diversity in the recently established Scandinavian wolf population. *Conservation Genetics* , 359–373.

25. Fan Z *et al.* 2016 Worldwide patterns of genomic variation and admixture in gray wolves. *Genome Research* **26**, 163–173.

26. Nilsson T. 2013 Population viability analyses of the Scandinavian populations of bear (Ursus arctos), lynx (Lynx lynx) and wolverine (*Gulo gulo*). SEPA Report 6549.

27. Murray DL, Kapke CA, Evermann JF, Fuller TK. 1999 Infectious disease and the conservation of free-ranging large carnivores. *Animal Conservation* **2**, 241–254.

28. Kyriazis CC, Beichman AC, Brzeski KE, Hoy SR, Peterson RO, Vucetich JA, Vucetich LM, Lohmueller KE, Wayne RK. 2022 Genomic underpinnings of population persistence in Isle Royale moose. *Molecular Biology & Evolution* **40**, 2022.04.15.488504.

29. Huber CD, Kim BY, Marsden CD, Lohmueller KE. 2017 Determining the factors driving selective effects of new nonsynonymous mutations. *Proceedings of the National Academy of Sciences of the United States of America* **114**, 4465–4470.

30. Kim BY, Huber CD, Lohmueller KE. 2017 Inference of the Distribution of Selection Coefficients for New Nonsynonymous Mutations Using Large Samples. *Genetics*. **206**, 345–361. (doi:10.1534/genetics.116.197145)

31. Huber CD, Durvasula A, Hancock AM, Lohmueller KE. 2018 Gene expression drives the evolution of dominance. *Nature Communications* **9**. (doi:10.1038/s41467-018-05281-7)

32. Agrawal AF, Whitlock MC. 2011 Inferences about the distribution of dominance drawn from yeast gene knockout data. *Genetics* **187**, 553–566.

33. Skoglund P, Ersmark E, Palkopoulou E, Dalén L. 2015 Ancient Wolf Genome Reveals an Early Divergence of Domestic Dog Ancestors and Admixture into High-Latitude Breeds. *Current Biology* **25**, 1515–1519.

34. Koch EM, Schweizer RM, Schweizer TM, Stahler DR, Smith DW, Wayne RK, Novembre J. 2019 De Novo Mutation Rate Estimation in Wolves of Known Pedigree. *Molecular Biology and Evolution* **36**, 2536–2547.

35. Song S *et al.* 2017 Ancient European dog genomes reveal continuity since the Early Neolithic. *Nature Communications 8*(1), 16082.

36. Bertorelle G, Raffini F, Bosse M, Bortoluzzi C, Iannucci A, Trucchi E, Morales HE, van Oosterhout C. 2022 Genetic load: genomic estimates and applications in non-model animals. *Nature Reviews Genetics* 23 (8), 492-503.

37. Dussex N, Morales HE, Grossen C, Dalén L, van Oosterhout C. 2023 Purging and accumulation of genetic load in conservation. *Trends Ecol. Evol.*

38. Kirkpatrick M, Jarne P. 2000 The effects of a bottleneck on inbreeding depression and the genetic load. *American Naturalist* **155**, 154–167.

39. 2020 *R: A Language and Environment for Statistical Computing (R Foundation for Statistical Computing)*.

40. Frankham R, Hemmer H, Ryder O a., Cothran EG, Soule ME, Murray ND, Snyder M. 1986 Selection in captive populations. *Zoo Biology* **5**, 129–138.

41. Hedrick PW, Garcia-Dorado A. 2016 Understanding Inbreeding Depression, Purging, and Genetic Rescue. *Trends in Ecology and Evolution* **31**, 940–952.

42. Ralls K, Sunnucks P, Lacy RC, Frankham R. 2020 Genetic rescue: A critique of the evidence supports maximizing genetic diversity rather than minimizing the introduction of putatively harmful genetic variation.

43. von Seth J *et al.* 2021 Genomic insights into the conservation status of the world's last remaining Sumatran rhinoceros populations. *Nature Communications* **12**, 1–32.