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

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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 \(\geq 100\).

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 \(\geq 8\) 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 \(\approx 400\) 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 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 ≤ s < -0.01), h = 0.1 for moderately deleterious mutations (-0.01 ≤ 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\(^{-8}\) 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 (E. 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 each of these two models, default values were used (e.g., Field-based empirical estimates labelled as $^{(f)}$) for all other parameters. The Genome focused model also used field-based empirical estimates as default values.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Demography focused</th>
<th>Genome focused</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Carrying capacity ($N_0$)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ancient Finnish/Parvus ($K_{anc,par}$)</td>
<td>8000 ($N_0, N_1 = 0.05; N_2 = 2,000$)</td>
<td>Brodfuehrer (2015); Gopalakrishnan et al. (2017)</td>
<td></td>
</tr>
<tr>
<td>Historical/Modern Finland/Parvus ($K_{h,mod}$)</td>
<td>800$^{(f)}$</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sweden/Norway ($K_3$)</td>
<td>50, 100, 150, 200, 210, 250, 300, 310, 350, 400, 450, 500</td>
<td>NA</td>
<td></td>
</tr>
<tr>
<td>Founding Swedish population and effective migrants</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Founders (Swedish population) - 1952-90</td>
<td>2</td>
<td>2</td>
<td>Smeds et al. (2022)</td>
</tr>
<tr>
<td>Migrants 1990-1991</td>
<td>1</td>
<td>1</td>
<td>Smeds et al. (2022)</td>
</tr>
<tr>
<td>Migrants 2008</td>
<td>2</td>
<td>2</td>
<td>Smeds et al. (2022)</td>
</tr>
<tr>
<td>Migrants 2017</td>
<td>7</td>
<td>7</td>
<td>SKANDULV, unpub. data.</td>
</tr>
<tr>
<td>Migrants 2021</td>
<td>1</td>
<td>1</td>
<td>SKANDULV, unpub. data.</td>
</tr>
<tr>
<td>Reproduction</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Breeding system</td>
<td>Monogamy</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Age of first pups - female (years)</td>
<td>2</td>
<td>2</td>
<td>Ebenhard 2002; Bruford (2015)</td>
</tr>
<tr>
<td>Age of first pups - male (years)</td>
<td>3</td>
<td>3</td>
<td>Ebenhard 2002; Bruford (2015)</td>
</tr>
<tr>
<td>Maximum age of female reproduction</td>
<td>12</td>
<td>12</td>
<td>SKANDULV, unpub. data.</td>
</tr>
<tr>
<td>Maximum male reproduction</td>
<td>12</td>
<td>12</td>
<td>SKANDULV, unpub. data.</td>
</tr>
<tr>
<td>Maximum lifespan</td>
<td>13</td>
<td>13</td>
<td>SKANDULV, unpub. data.</td>
</tr>
<tr>
<td>Sex ratio of pups at birth</td>
<td>0.5</td>
<td>0.5</td>
<td>SKANDULV, unpub. data.</td>
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<tr>
<td>Proportion of adult females breeding each year</td>
<td>30%*$^{(f)}$/63%$^{(f)}$</td>
<td>63%$^{(f)}$</td>
<td>SKANDULV, unpub. data.</td>
</tr>
<tr>
<td>Mean litter size (SD)</td>
<td>3 ± 0.5*$/3.5 ± 1.4^{(f)}$</td>
<td>3.5 ± 1.4$^{(f)}$</td>
<td>SKANDULV, unpub. data.</td>
</tr>
<tr>
<td>Proportion of adult males in breeding pool</td>
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<td></td>
<td></td>
</tr>
<tr>
<td>Density dependence in pairing/breeding</td>
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<td></td>
<td></td>
</tr>
<tr>
<td>Survival (mean, SD)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Females (%)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Juvenile (Age-0 to Age-1)</td>
<td>30.0$/50.0^{(f)}$/30.0</td>
<td>30.0</td>
<td>Chapron, unpub. Data / Bruford (2015)</td>
</tr>
<tr>
<td>Subadult (Age-1 to Age-2)</td>
<td>40.0$/50.0^{(f)}$/40.0</td>
<td>40.0</td>
<td>Chapron, unpub. Data / Bruford (2015)</td>
</tr>
<tr>
<td>Young Adult (Age-2 to Age-7)</td>
<td>25.0$/30.0^{(f)}$/25.0</td>
<td>25.0</td>
<td>Chapron, unpub. Data / Bruford (2015)</td>
</tr>
<tr>
<td>Older Adult (Age-7 to 12)</td>
<td>25.0$/30.0^{(f)}$/25.0</td>
<td>25.0</td>
<td>Chapron, unpub. Data / Bruford (2015)</td>
</tr>
<tr>
<td>Males (%)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Juvenile (Age-0 to Age-1)</td>
<td>30.0$/50.0^{(f)}$/5.0$^{(f)}$</td>
<td>5.0$^{(f)}$</td>
<td>Chapron, unpub. Data / Bruford (2015)</td>
</tr>
<tr>
<td>Subadult (Age-1 to Age-2)</td>
<td>40.0$/50.0^{(f)}$/40.0</td>
<td>40.0</td>
<td>Chapron, unpub. Data / Bruford (2015)</td>
</tr>
<tr>
<td>Young Adult (Age-2 to Age-7)</td>
<td>25.0$/30.0^{(f)}$/25.0</td>
<td>25.0</td>
<td>Chapron, unpub. Data / Bruford (2015)</td>
</tr>
<tr>
<td>Older Adult (Age-7 to 12)</td>
<td>25.0$/30.0^{(f)}$/25.0</td>
<td>25.0</td>
<td>Chapron, unpub. Data / Bruford (2015)</td>
</tr>
<tr>
<td>Longevity (Females and Males)</td>
<td>13</td>
<td></td>
<td>Chapron, unpub. Data / Bruford (2015)</td>
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<tr>
<td>Catastrophe</td>
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<tr>
<td>Disease</td>
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</tr>
<tr>
<td>Frequency</td>
<td>0.01</td>
<td></td>
<td>Nilsson 2013; Murray et al. 1999</td>
</tr>
<tr>
<td>Severity (survival)</td>
<td>0.9</td>
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</tr>
<tr>
<td>Genetic parameters</td>
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<tr>
<td>Mutation rate (site/year)</td>
<td>NA</td>
<td>1.5 x 10$^{-2}$</td>
<td>Koch et al. (2019)</td>
</tr>
<tr>
<td>Mutation properties (dominance h; selection s; proportion)</td>
<td></td>
<td></td>
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</tr>
<tr>
<td>Lethal</td>
<td>NA</td>
<td>0 - 1.0</td>
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</tr>
<tr>
<td>Very strongly deleterious</td>
<td>NA</td>
<td>0 - 0.1</td>
<td>2.6</td>
</tr>
<tr>
<td>Strongly deleterious</td>
<td>NA</td>
<td>0.01 - 0.1 s = -0.01 - 23.8</td>
<td></td>
</tr>
<tr>
<td>Mildly deleterious</td>
<td>NA</td>
<td>0.1 - 0.01 s = -0.01 - 24.2</td>
<td></td>
</tr>
<tr>
<td>Weakly deleterious</td>
<td>NA</td>
<td>0.4 - 0.01 s = -0.01 - 49.1</td>
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<tr>
<td>Neutral</td>
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<td>0.0 - 0.5</td>
<td>43.3</td>
</tr>
<tr>
<td>Migration (dispersal) parameters</td>
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<td>Minimum age at migration</td>
<td>2</td>
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<td></td>
</tr>
<tr>
<td>Migration rate (Nimigrants/year)</td>
<td>0.01, 0.05, 0.08, 0.1, 0.3, 0.5, 0.8, 1</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
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 ($\pi$), mean inbreeding ($F_{ROH}$; sum of runs of homozygosity $\geq 100$kb 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%.
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 $M \leq 0.1$. For $K_S>100$, the probability of extinction dropped to ~1% and only for $M \leq 0.05$ (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 $M \leq 0.1$. 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 $K_S \geq 100$ (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 an 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 ($\pi$), 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 ($\pi$) 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.

<table>
<thead>
<tr>
<th>Mig. / year</th>
<th>0</th>
<th>0.01</th>
<th>0.03</th>
<th>0.05</th>
<th>0.08</th>
<th>0.1</th>
<th>0.3</th>
<th>0.5</th>
<th>0.8</th>
<th>1</th>
</tr>
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<tbody>
<tr>
<td>Nucleotide diversity ($\pi$)</td>
<td>-16%</td>
<td>-14%</td>
<td>-13%</td>
<td>-11%</td>
<td>-9%</td>
<td>-7%</td>
<td>7%</td>
<td>15%</td>
<td>23%</td>
<td>26%</td>
</tr>
<tr>
<td>Inbreeding ($F_{ROH}$)</td>
<td>16%</td>
<td>14%</td>
<td>12%</td>
<td>9%</td>
<td>7%</td>
<td>4%</td>
<td>-12%</td>
<td>-23%</td>
<td>-31%</td>
<td>-37%</td>
</tr>
<tr>
<td>Realised load</td>
<td>-1%</td>
<td>-3%</td>
<td>-6%</td>
<td>-5%</td>
<td>-5%</td>
<td>-7%</td>
<td>2%</td>
<td>-5%</td>
<td>-4%</td>
<td></td>
</tr>
<tr>
<td>Masked load</td>
<td>-25%</td>
<td>-14%</td>
<td>-14%</td>
<td>-3%</td>
<td>-17%</td>
<td>-17%</td>
<td>3%</td>
<td>-1%</td>
<td>14%</td>
<td>11%</td>
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</table>

<table>
<thead>
<tr>
<th>Mig. / year</th>
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<th>0.01</th>
<th>0.03</th>
<th>0.05</th>
<th>0.08</th>
<th>0.1</th>
<th>0.3</th>
<th>0.5</th>
<th>0.8</th>
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<tr>
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<td>-10%</td>
<td>-9%</td>
<td>-7%</td>
<td>-6%</td>
<td>-6%</td>
<td>5%</td>
<td>14%</td>
<td>19%</td>
<td>22%</td>
</tr>
<tr>
<td>Inbreeding ($F_{ROH}$)</td>
<td>8%</td>
<td>7%</td>
<td>6%</td>
<td>5%</td>
<td>4%</td>
<td>1%</td>
<td>-10%</td>
<td>-21%</td>
<td>-25%</td>
<td>-31%</td>
</tr>
<tr>
<td>Realised load</td>
<td>0%</td>
<td>-6%</td>
<td>2%</td>
<td>-5%</td>
<td>5%</td>
<td>-1%</td>
<td>-3%</td>
<td>-7%</td>
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<tr>
<td>Masked load</td>
<td>-16%</td>
<td>-11%</td>
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<td>-16%</td>
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<td>-8%</td>
<td>-4%</td>
<td>3%</td>
<td>7%</td>
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</tbody>
</table>

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 ($M \leq 0.1-0.3$) 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.

These results 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 ($M \geq 0.8$) 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.

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.
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 $\sim 13\%$ 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, $\geq 1$ effective immigrant per decade (i.e., $M\geq 0.1$) 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 $M\leq 0.05$ 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 $\sim 20$ 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 $M\leq 0.03$, populations with $K_S=50$ to 150 would experience $\sim 55$ to 20\% reduction in nucleotide diversity and $\sim 65$ to 20\% increase in inbreeding (Fig. 6; Tables S4).

![Figure 6](image)

**Figure 6.** Effect of yearly migration rate $M$ (0–1 effective immigrants/year) and $K_S$ values (50–500) on the mean nucleotide diversity ($\pi$) 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 $\pm 5\%$ change and dashed line no change. Raw data in Table S4.

In contrast, for $K_S\geq 150$, 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 $K_S\geq 250$-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 $K_S \geq 150$.

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 masked 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 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 Ks≥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≥0.8-1), even large populations with Ks=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 Ks≥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 Ks 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 Ks=50 and between 2 and 3% for Ks=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 Ks≥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., NAnc-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 NAnc-Karelia values, the proportion of temporal change in these estimates was not affected by the NAnc-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 $K_{Anc-Karelia}$=4,000. 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.
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 ±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., $M_\text{eff} \geq 0.6$) 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 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.
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