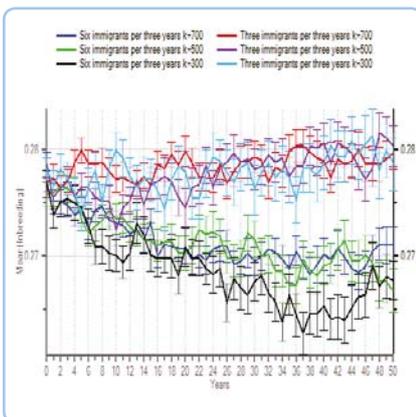


Additional Population Viability Analysis of the Scandinavian Wolf Population

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REPORT 6639 • JULY 2015



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Internet: www.naturvardsverket.se

ISBN 978-91-620-6639-0

ISSN 0282-7298

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Print: Arkitektkopia AB, Bromma 2015

Cover photos: Wolf (*Canis lupus*) photo: Astrid Bergman Sucksdorff (myra.eu). A Swedish wolf cub (*Canis lupus*) in a wolf researcher's knee, photo: Magnus Kristoffersson, Naturvårdsverket.



Förord

Art- och habitatdirektivet (92/43/EEG), där varg ingår i bilagorna II och IV, ställer bland annat krav på att medlemsstaterna ska se till att de arter och livsmiljöer som omfattas av direktivets bilagor uppnår och bibehåller en gynnsam bevarandestatus. I direktivets artikel 1 anges att en arts bevarandestatus är summan av de faktorer som påverkar arten och som på lång sikt kan påverka den naturliga utbredningen och storleken av artens populationer. Bevarandestatusen ska anses gynnsam när dels den berörda artens populationsutveckling visar att arten på lång sikt kommer att förbli en livskraftig del av sin livsmiljö, dels artens naturliga utbredningsområde varken minskar eller sannolikt kommer att minska inom en överskådlig framtid, dels att det finns – och sannolikt kommer att fortsätta att finnas – en tillräckligt stor livsmiljö för att artens populationer ska bibehållas på lång sikt.

EU-kommissionens riktlinjer för bedömning och rapportering enligt artikel 17 i art- och habitatdirektivet använder referensvärden som nyckelbegrepp vid utvärderingen av huruvida arten har gynnsam bevarandestatus. Referensvärdena grundas på vetenskapliga fakta och kan förändras mellan rapporteringstillfällena om kunskapsunderlaget förbättrats. Referensvärdet för populationsstorlek (Favourable Reference Population, FRP) är storleken på artens population som bedöms vara det minimum som är nödvändigt för att på lång sikt säkerställa artens livskraft i medlemsstaten. FRP-värdet bör baseras på artens ekologi och genetik. Om en sårbarhetsanalys för att beräkna minsta livskraftiga populationsstorlek (Minimum Viable Population, MVP) har genomförts, kan den användas som stöd för att bestämma referensvärdet FRP. Enligt riktlinjerna är MVP för en population per definition alltid mindre än FRP.

Bedömningarna av MVP och referensvärdet FRP för varg i Sverige har inneburit och medfört mycket diskussion, likaså frågan om hur stor genetisk inverkan som invandringen av vargar från Finland och Karelen har. Bland annat har en mer skraddarsydd modellering av de skandinaviska vargarnas genetik och inavelsgrad efterlysts, och att de invandrande vargarna då har genetiska egenskaper likt de finsk-karelska. Mot den bakgrunden finansierade Naturvårdsverket analyser gjorda av professor Michael W. Bruford vid Cardiff University vilka presenteras i denna rapport. Analysprojektet hade ett Mid-Term Review-möte med inbjudna skandinaviska forskare i april 2014, och rapportutkast har sedan granskats vetenskapligt av dessa forskare och utomstående oberoende populationsgenetisk expertis (se Appendix 3). Per Sjögren-Gulve var verkets projektledare och managing editor. Naturvårdsverket framför sitt varma tack till alla medverkande för deras arbete och bidrag i processen som lett fram till denna rapport.

Stockholm, juni 2015

Maria Hörnell-Willebrand
Enhetschef, Analys- & forskningsavdelningen

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Sammanfattning

Naturvårdsverkets bedömning av ”minsta livskraftiga populationsstorlek” (MVP; Minimum Viable Population) och ”referensvärdet för populationsstorlek för gynnsam bevarandestatus” (FRP; Favourable Reference Population¹) för varg i Sverige har diskuterats. Naturvårdsverket finansierade därför ytterligare sårbarhetsanalyser av den skandinaviska vargstammen för att bedöma hur populationsstorleken respektive olika frekvenser av reproducerande immigranter från östligare vargbestånd påverkar stammens inavelsgrad och genetiska variation. Man ville få svar på fem frågor (här översatta och något förkortade):

- (1) Hur stark är den genetiska effekten av invandrade vargar som reproducerar sig i den skandinaviska populationen när den har storleken 200-300 vargar? Förhindrar 1 ny reproducerande invandrad varg per generation betydande inavel och förlust av genetisk variation, eller behövs 1-2 eller 3-4 vargar per generation?
- (2) Naturvårdsverket föreslog 2012 att FRP åtminstone behövde uppfylla en genetisk MVP², som då uppskattades motsvara minst 417 vargar kombinerat med minst 3,5 nya reproducerande immigranter per varggeneration. Bekräftar eller förkastar modellering av en vargpopulation, som genetiskt och demografiskt är som den skandinaviska, med helt obesläktade eller finsk-karelska invandrade reproducerande vargar dessa siffror?
- (3) Tillämpliga genetiska kriterier för referensvärdet FRP i det här sammanhanget behöver utgå ifrån
 - avsnitten III.a.ii och IV.b.vii i EU-Kommissionens artikel-17-riktlinjer¹
 - deras referens till Laikre m.fl. (2009)
 - det svenska miljömålet *Ett rikt växt- och djurliv* som anger att ”Arter ska kunna fortleva i långsiktigt livskraftiga bestånd med tillräcklig genetisk variation”
 - att den skandinaviska vargstammen återetablerats genom 3+2 invandrade reproducerande vargar t.o.m. år 2009.Givet dessa förutsättningar – vad är tillämpliga kriterier för FRP?
- (4) Under en workshop 26 april 2013 diskuterades även betydelsen av sällsynta gener för populationers livskraft. Vilken populationsstorlek och immigrationsfrekvens krävs för att bevara tillräckligt med genetisk variation i detta avseende?
- (5) Genom propositionen 2012/13:191 beslutade Sveriges riksdag att FRP-värdet för varg ska ligga i intervallet 170-270 i Sverige. Hur kan en populationsstorlek i detta intervall, kombinerat med viss invandring, tillgodose FRP-kriterierna från artikel-17-riktlinjerna¹?

¹ Evans & Arvela (2011) <https://circabc.europa.eu/sd/a/2c12cea2-f827-4bdb-bb56-3731c9fd8b40/Art17%20-%20Guidelines-final.pdf>

² genetiskt kriterium på Minimum Viable Population, förslaget av Allendorf och Ryman (2002)

Den modell som slutligen användes (AFD-modellen) gav dessa svar på frågorna:

(1) Med en skandinavisk vargstam på 300 individer (varav 270 i Sverige) och 1 ny reproducerande immigrant varje 6-årsintervall under 100 år, reduceras förlusten av gendiversitet med 8,2 % jämfört med om stammen är helt utan immigration.

Simuleringarna indikerade att då förloras totalt sett mindre än 5 % av gendiversiteten på 100 år [jämför med Allendorf och Rymans (2002) MVP-kriterium²]. Den genetiska effekten av 2 ytterligare invandrade vargar per 6-årsperiod som reproducerar sig var mycket stor; det reducerade gendiversitetsförlusten på 100 år mer än om ytterligare 400 vargar fanns i den skandinaviska populationen (jämför Tabell 9b med 9a).

(2) Med minst 417 vargar och minst 3,5 nya reproducerande immigranter varje varggeneration (vilket motsvarar 2 nya reproducerande immigranter per 3-årsperiod) under 100 år så förlorades ca 1,7 % av gendiversiteten, och vargstammens inavelskoefficient minskade med 2,6 % till 0,265 (Tabell 9b). Detta skulle med marginal tillgodose det genetiska MVP-kriteriet att mindre än 5 % av variationen förloras på 100 år². I dessa simuleringar förlorade populationen mindre gendiversitet än en isolerad population med den genetiskt effektiva storleken (N_e) 500, som förväntas förlora 1,9%.

(3) Frågan är väldigt svår att svara på. Det beror på hur stor del av den nordeuropeiska metapopulationen av varg (i Sverige, Norge, Finland och NV Ryssland) som den skandinaviska populationen ska utgöra. Den skandinaviska populationens genetiskt effektiva storlek motsvarade nyligen ca. 100, och 500 är den effektiva storlek som metapopulationen minst behöver ha för att anses bibehålla sin förmåga till evolution och evolutionär anpassning.

(4) Sällsynta genvarianter (sällsynta alleler) förloras snabbare över generationer än gendiversiteten (heterozygotigraden) i små populationer. I Tabell 9a framgår att 1 ny reproducerande immigrant varje 3-årsintervall gör att 96,5% av gendiversiteten återstår efter 100 år om den skandinaviska populationen består av ca 300 vargar. Om den istället består av 700 vargar återstår 97,1 % (0,6 % mer) av gendiversiteten men antalet återstående alleler efter 100 år ökade med 28% jämfört med i den mindre populationen (från 10,8 i genomsnitt till 13,9) – dvs. en betydligt starkare effekt av populationsstorleken på de sällsynta generna. Även mängden immigration påverkar, se Tabell 9b.

Fråga (5) liknar fråga (3). Eftersom FRP = 270 i Sverige beslutades av Naturvårdsverket (ca. 300 vargar i Skandinavien), visar Tabell 9b den effekt som olika invandringstakter hade på den skandinaviska vargstammens gendiversitet, inavelsgrad och antal alleler efter 100 år vid den populationsstorleken. För den långsiktiga överlevnaden av den skandinaviska stammen – och för att kunna anses uppfylla referensnivån FRP – behöver den nordeuropeiska metapopulationen av vargar som de skandinaviska vargarna tillhör ha en genetiskt effektiv populationsstorlek på minst 500. Den skandinaviska populationens genetiskt effektiva storlek enskilt beräknades för ett par år sedan vara någonstans mellan 80 och 130. Det är därför viktigt att bedöma hur stor andel av

den nordeuropeiska metapopulationens effektiva storlek (inklusive Finland och Karelen) som den skandinaviska vargstammen utgör. Först då kan man fastställa preciserade målsättningar för populationsstorlekar och genflödet mellan ländernas delpopulationer.

Så här genomfördes undersökningen

Undersökningen lades upp som ett projekt med en halvtidsgranskning. Då hade rapportförfattaren gjort inledande analyser och simuleringar för att testa bl.a. hur bra den konstruerade VORTEX-modellen förmådde återge den populationstillväxt och inavelsökning som dokumenterats hos den skandinaviska vargpopulationen i verkligheten från dess återetablering 1983 till år 2008. Detta återges i kapitlen ”*Fitting a model to past observations*” och ”*Model refinement*”.

I kapitlet ”*Forward modeling without gene frequencies*” redovisas modellering av den skandinaviska vargpopulationens genetiska variation och inavelsgrad framåt i tiden från år 2012 vid olika populationsstorlekar och med simulerade katastrofhändelser (avsnittet ”*Catastrophes*”). Modelleringen utfördes med VORTEX standardinställningar och utan specifika inputdata om populationens genfrekvenser (allelfrekvenser) och inavelsgrad. Resultaten liknar de som förväntas i teoretiska modeller med liknande antaganden. Invandringen av vargar modellerades då med VORTEX supplementerings-tillval där de tillförda immigranterna är helt obesläktade och har gener som är unika och inte finns i mottagarpopulationen. Mottagarpopulationen har vid simuleringarnas början 100 % genetisk variation och vid simuleringarnas slut ser man hur mycket variation som återstår. Men eftersom den skandinaviska vargpopulationen återetablerades av vargar från Finland/Karelen – från samma östliga population som immigranterna senare också kommer ifrån – och den skandinaviska populationen inte har så hög genetisk variationsgrad, bedömdes det möjligt att resultaten skulle kunna vara missvisande.

Därför utfördes simuleringar med två ytterligare VORTEX-modeller – *pedigree + supplementation (PS)* och *allele frequencies + dispersal (AFD)*. I dessa slutliga modeller simulerades, på grund av begränsningar i VORTEX-programmet, invandringen av vargar genom supplementering i PS-modellen och genom immigration av vargar med finska genfrekvenser i AFD-modellen. I PS-modellen var individerna i mottagarpopulationen besläktade precis som 320 stambokförda vargar i den skandinaviska populationen 2012; i AFD-modellen hade mottagarpopulationen de genfrekvenser som beräknats för den skandinaviska vargpopulationen år 2012 och dess inavelsgrad beräknades ur genfrekvenserna. Resultaten från simuleringarna jämfördes eftersom de två modellerna inte gick att kombinera. PS-modellen visade som förväntat betydligt starkare effekter av immigrationen än vad AFD-modellen gjorde (jämför Supplementary Table 3 med Supplementary Table 4). PS-modellen visade även realistiskt låg populationstillväxt (”stochastic r”). Detta, plus att immigranterna från Finland/Karelen inte kommer att ha enbart unika gener och vara helt obesläktade, gjorde att PS-modellens resultat inte framstod som trovärdiga. Summerat bedömdes AFD-modellens resultat (Tabell 9a och 9b) vara mest trovärdiga.

Summary

Population modeling was carried out to estimate the effects of immigration into the Scandinavian wolf population using realistic genetic assumptions and to examine the trajectory of genetic diversity under a variety of scenarios. Initial modeling sought to establish the most up to date demographic parameters and genetic data, particularly focusing on ways to adequately model inbreeding within the population, and sought to examine the effects of varying these parameters on the outcomes of models by following the population from foundation in 1983 until 2008. The first set of forward modeling parameters were examined for a range of population sizes and immigration rates without using allele frequencies for the population and with immigrants being modeled using supplementation (that assumes immigrants are genetically unique compared to the population they are immigrating into). The results concluded that an acceptable loss of gene diversity and an increase in inbreeding coefficient of within 10% could be achievable in larger population sizes (370 and greater) with as little as one effective migrant per generation. However, since the genetic dividend of immigration was probably too optimistic assuming complete genetic uniqueness of the immigrants, two further models were developed to better utilize the genetic data available.

The first of these two models used supplementation as before, but this time using pedigree data and individual inbreeding coefficients for a sample of the population in 2012. The second model used allele frequencies that were estimated from genetic marker data from the population in 2012, and for the most proximate immigrant (Finnish) population, and used these genetic data to simulate changes in genetic diversity under different dispersal scenarios. It was not possible to combine these two approaches in a single model using Vortex, so their outputs were compared and contrasted but more emphasis has been placed on the allele frequencies based model.

The allele frequencies based model showed that modest levels of immigration (one effective migrant per six years or 0.83 per generation) over both a ten and twenty generation period was sufficient to maintain gene diversity at acceptable levels (0.95 of its current state), regardless of the population size when it was varied between 300 and 700, and was able to constrain mean inbreeding coefficient in the population to values below 0.31 (current estimate 0.27). This result is conservative in the sense that it is predicated on the assumption that immigrants have similar reproductive success to residents (there is circumstantial evidence that immigrant can outperform residents). However, 'effective' immigration implies individuals that arrive in the population survive and breed and this has been a rare occurrence in the Scandinavian wolf population since its re-establishment during the last thirty years.

For the long-term survival of the Scandinavian wolf and to conform to Favourable Reference Population status, an effective population size of 500 should be achieved for the meta-population to which it belongs and the current effective size of the Scandinavian wolf is between 80 and 130. It is therefore important to establish the fraction of the entire metapopulation's effective size (including Finland and Karelia) that is represented in Scandinavia so that appropriate targets can be established.

Introduction

In accordance with the Decision Letter of the Swedish Environment Protection Agency (SEPA), dated 13/11/2013 I tender my revised final report on the above project. The aim of the PVA modeling was to answer the following questions for SEPA (abbreviated):

1. How strong is the effect of modest numbers of effective immigrants at population sizes of 200–300? How many wolves per generation will prevent significant inbreeding or loss of variation?
2. In 2012 the SEPA proposed a genetic MVP as a minimum value for FRP for the Scandinavian wolves at ≥ 417 combined with effective immigration of ≥ 3.5 reproducing wolves per generation. Does modeling with the Scandinavian population with immigrants as genetically dissimilar as the Karelian and Finnish wolves confirm the result of this assessment?
3. Given the Article-17 guidelines, their reference to Laikre et al. (2009), the Swedish environmental objective “A rich diversity of plant and animal life” which states that “Species must be able to survive in long-term viable populations with sufficient genetic variation”, plus the fact that the present-day Scandinavian wolf population was re-established with founder contributions from 3+2 immigrants until 2009, what are appropriate genetic criteria for FRP from a scientific point of view?
4. The workshop 26 April put emphasis also on maintenance of rare alleles as important genetic aspects of “viability”. Under what combinations of immigration and population size(s) are sufficient amounts of genetic variation also in terms of rare alleles maintained in the population?
5. In a recent bill to the Swedish Parliament, the Swedish government proposed an interval ($170 \leq \text{FRP} \leq 270$), within which the Swedish EPA will be commissioned to decide about the exact value for FRP. How can a number in that interval, in combination with a certain rate of immigration, fulfill FRP criteria that can be derived from the Article-17 guidelines?

This revised Final report follows a Supplementary report that was tendered on 15th January 2015 that received comments from SEPA and reviewers. In accordance with the recommendations, this format of this report now reflects the development of the modeling following discussion with SEPA and revision in accordance with the reviews. The report therefore comprises relevant sections of all reports tendered to date, including the mid-term review (6th April 2014), Final Report (14th June 2014) and the January 2015 Supplementary Report.

For the **Mid-Term Review**, a basic biological model was constructed and evaluated to establish the intrinsic biological parameters that would underpin future modeling. A pack-based model was evaluated (not shown) but it was decided not to carry this forward at the mid-term review. Finally, a past popu-

lation model was simulated to establish whether the parameters chosen could adequately represent the known demographic history of the Scandinavian wolf population since its foundation in 1982. Comments provided by the mid-term review panel are summarized in **Appendix 1**.

For the **Final Report**, the model was refined in line with the recommendations of the mid-term review panel. Inbreeding parameters in particular were refined, based on suggestions from the mid-term review, first by examining the effect of varying the proportion of genetic load accounted for by recessive lethal alleles and second by varying the number of lethal equivalents. A combination of 8 lethal equivalent genes with 30% recessive lethals was identified as that best fitting the known past population trajectory in population size and inbreeding coefficient and was therefore carried forward into all forward (predictive) modeling. The effect of immigration of 1–5 wolf migrants per generation (i.e. 5 years) on genetic diversity at different census sizes (170–570) was then tested (simulated using supplementation in Vortex 9.99).

However, as pointed out in the report and identified by the reviewers (**Appendix 2**), supplementation as a proxy for immigration may overestimate the genetic dividend of immigration because the model assumes that immigrants are genetically unique and does not account for genetic similarity among source and recipient populations. Accordingly for the **Supplementary Report**, two customised modeling approaches were taken that utilized the available genetic data, a partial pedigree with associated individual inbreeding coefficients and microsatellite DNA marker allele frequencies for the Scandinavian, Finnish and Karelian populations. Two modeling approaches using these data were possible, first a model utilizing the allele frequencies and simulating dispersal, and second utilizing the pedigree data and simulating supplementation with totally unrelated wolves. These results and the recommendations are presented.

Finally, in response to reviews received for the **Supplementary Report** some additions and amendments have been made to this section (see comments in **Appendix 3**) for this revised report.

Note: This report is structured in a sequential manner, to enable the reader to understand the development of the modeling approach during the work that was carried out. However, the key results, which are summarized above, are therefore found in the “**Two Further Customised models**” section. Readers who are only concerned with the modeling that has led to the recommendations identified above should therefore focus on the results found in this section, Table 1 and Table 3.

Fitting a model to past observations

The Basic Biological Model

Although grey wolves have been modeled extensively in the past (e.g. Carroll et al. 2014; Lovari et al. 2007), how to use the best input values (parameters) is a constant source of discussion and debate because inaccurate values can lead to misleading model outputs and poor predictions, as has been argued in the past for grey wolves (Patterson and Murray 2008). The first step therefore, was to establish a core set of biological values that were thought to be as realistic as possible for the modeling. In Table 1 the first attempt at biological values for this modeling exercise are laid out and were evaluated. These were based on values produced by SEPA, including values in Nilsson (2004) with comments from Dr Guillaume Chapron (Skandulv data) on certain parameters.

Table 1 – Basic Parameters of the model

Parameter	Value	Comments	Source
Number of populations	1 or 25 (25 packs in one large meta-population)	A single population OR 15 filled packs + 10 vacant territories	
Inbreeding depression	Yes or no		
Lethal equivalent genes	6.04 or 3.02	For testing	Liberg et al. 2005
Percentage of inbreeding depression on survival due to lethal recessive genes	50% or 25%	For testing	
Environmental correlation in reproduction and survival	Yes / No	For testing	
Catastrophes	None		
Mating system	Long-term monogamy		
Age of first offspring males and females	2		
Maximum age of reproduction	15	Skandulv data	
% females breeding in any given year	29%, 58%, 100%	Skandulv data	Nilsson 2004
Density dependent reproduction?	No evidence	Skandulv data	
Mean litter size	4 or 5	Comment from SEPA	Nilsson 2004
First year mortality males and females as a percentage, mean and standard deviation	24, 8		Nilsson 2004
Second year mortality males and females as a percentage, mean and standard deviation	17.5, 5.83		Nilsson 2004

Parameter	Value	Comments	Source
Mortality for all adult males and females (regardless of age) as a percentage, mean and standard deviation	11, 3.67		Nilsson 2004
What proportion of males can breed?	100% of adult males can potentially breed		
Initial population size	200 for the one population model 6 individuals per pack for the pack metapopulation model	The 200 value was arbitrary and used to evaluate model performance	
Age distribution within the population	A stable age distribution was used for the one population model For the pack model, a pack of 6 individuals = 1 adult pair, 2 males, ages 0-1 and 1-2 and 2 females, ages 0-1 and 1-2)	Could have used pedigree data for the one population model (SEPA)	
Carrying capacity (total number of individuals that the environment can allow)	10,000 for one population model, 10 for pack model	Set to be high since no evidence of density dependent growth has been observed and to allow the population to grow without constraint	

The above parameters were used in combination to test the model (see results). The observed growth rate of the population ($\lambda = 1.18$; G. Chapron, Skandulv data) was used to evaluate the different models, along with trends in population size and inbreeding (using, for example, the data from the Stockholm meeting in April 2013).

Once the models had been evaluated pending review at the mid-term meeting, an attempt was made to reconstruct the history of the modern Swedish wolf population with a model starting in 1983 and proceeding until 2008, just prior to the immigration of two new individuals, to further test the validity of the model prior to assessment of the effects of immigration.

Results

The single population model was first evaluated, to test the effects of using a mean of 5 cubs per litter versus 4 and on the inter-birth interval for females, using an annual proportion of 29% (following Nilsson 2004), an intermediate value of 58% and 100% breeding (suggested by G. Chapron, Skandulv data). Density dependent reproduction was not modeled because G. Chapron stated is that there is no evidence for density dependent reproduction in Swedish wolves in the modern era. The results of these simulations are presented in Table 2. Inbreeding depression also tested, to assess its influence in model outputs, since the impact of inbreeding depression in the VORTEX model should be evaluated, given its interactions with specific model outcomes in the software (Lacy 2000).

Table 2 – Deterministic growth results of single population simulations

Model	r	λ	R ₀	Generation time (years)
29% female productivity, mean of 4 cubs	0.174	1.190	2.659	5.61
29% female productivity, mean of 5 cubs	0.221	1.247	3.284	5.39
58% female productivity, mean of 4 cubs	0.341	1.406	5.318	4.90
100% female productivity, mean of 4 cubs	0.501	1.650	9.170	4.43

The model comprising 29% annual female reproduction with a mean of 4 cubs per annum produced a lambda value most in line with expectations (of $\lambda = 1.18$). The stochastic growth rate values were equivalent among models, although simulations involving inbreeding in VORTEX penalize inbred individuals at carrying capacity (which was reached after approximately 20 years in most simulations). Inbreeding levels attained under the model are summarized in **Figure 1**, below. High levels of annual female productivity can be seen to constrain the accumulation of inbreeding over time, although the change was low once the populations reached (unrealistic) carrying capacity levels. As a result of these outputs, the remaining models included 29% female productivity and a mean of 4 cubs per litter.

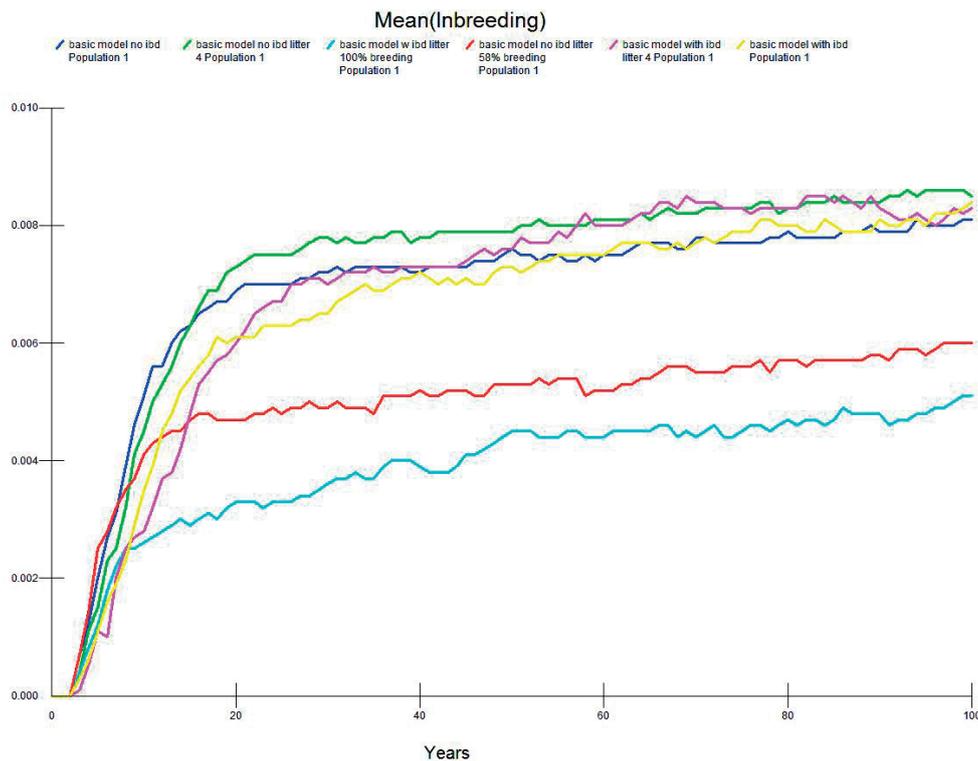


Figure 1 – Change in inbreeding coefficient over time in the basic model

Past Population model

In order to further examine the validity of the model and as a second part of the workflow, I constructed a model designed to mimic the establishment of the Swedish wolf population starting in 1982 with the immigration and breeding of two individuals and the additional arrival of an adult male individual in 1990, modeled forwards until 2008. I did not include the further arrival of two individuals in 2008 to keep the model parameters as simple and as easy to evaluate as possible. The model therefore started at time $T = 0$ (1982) with two founders, assumed to be unrelated and including the addition of an unrelated male at $T = 0+8$ years, assuming a single population model and the biological parameters described above.

Perhaps the most interesting output of the model was the probability of survival of the population up until 2008. In no set of simulations did the survival probability exceed 50% (although the most optimistic were close). The implication of this result, given the parameters used here, is that the persistence of the Swedish wolf population was a statistically unlikely event. Since the Swedish wolf population had grown to approximately 178 individuals at the time of the second immigration in 2008 (Scandinavian population was at 233), it was interesting to test whether, in the absence of carrying capacity constraints and density-dependent reproduction, the simulation could achieve that number in simulation.

I also tested the influence of different inbreeding values on the model outcomes. While the number of Lethal Equivalents has been estimated at 6.04 (Liberg et al. 2005), the percentage of those due to lethal recessives is not known and Bensch et al. (2006) showed that in any case successful pairs have higher heterozygosity than would be predicted by chance for each level of inbreeding tested. I therefore examined the effects of changing inbreeding parameters, testing zero inbreeding, 3 Lethal Equivalent genes (the number of recessive lethal alleles in the genome; LEs), 6 Lethal Equivalents and the percentage of inbreeding depression affecting survival due to lethal recessives (LRs; 50%, the standard model in VORTEX and 25%). **Figure 2** shows the projected population sizes of the population assuming four configurations of inbreeding. The most optimistic model predicted a mean population size of 82 individuals at 2008 – this assumed 3 LEs and 50% LRs.

Figure 3 shows the change in inbreeding coefficient in the same simulations, and indicates that, as expected, inbreeding accumulates more rapidly where the negative effects of inbreeding depression is least severe. However, interestingly the values were of the same order as those indicated by Åkesson et al. in the workshop in April 2013 and show a response to the first immigration event. However, even under the least severe inbreeding model, the projected population size at 2008 was only 57% of the actual value, suggesting inbreeding parameters should be manipulated further.

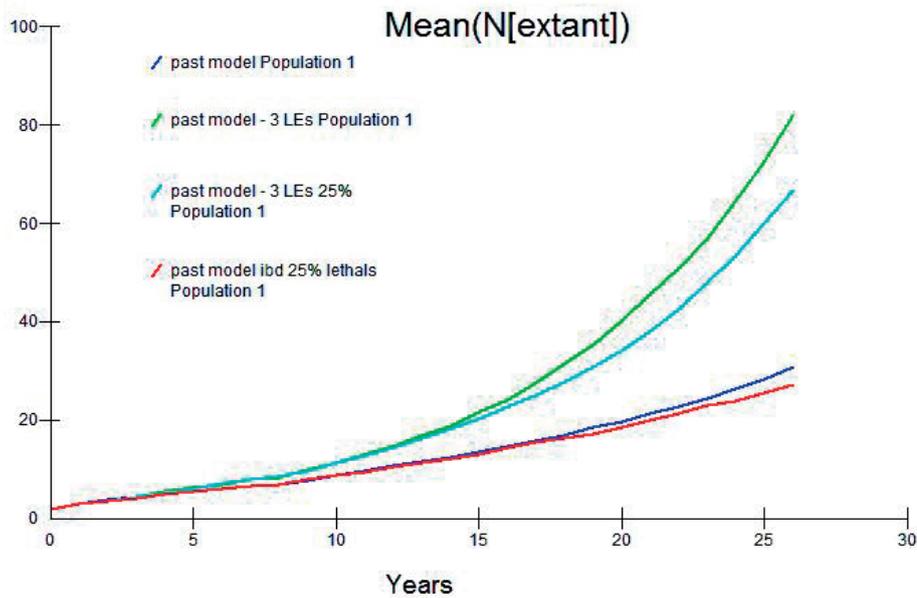


Figure 2 – The influence of inbreeding on the simulated Swedish wolf population size from foundation until 2008 (25 years).

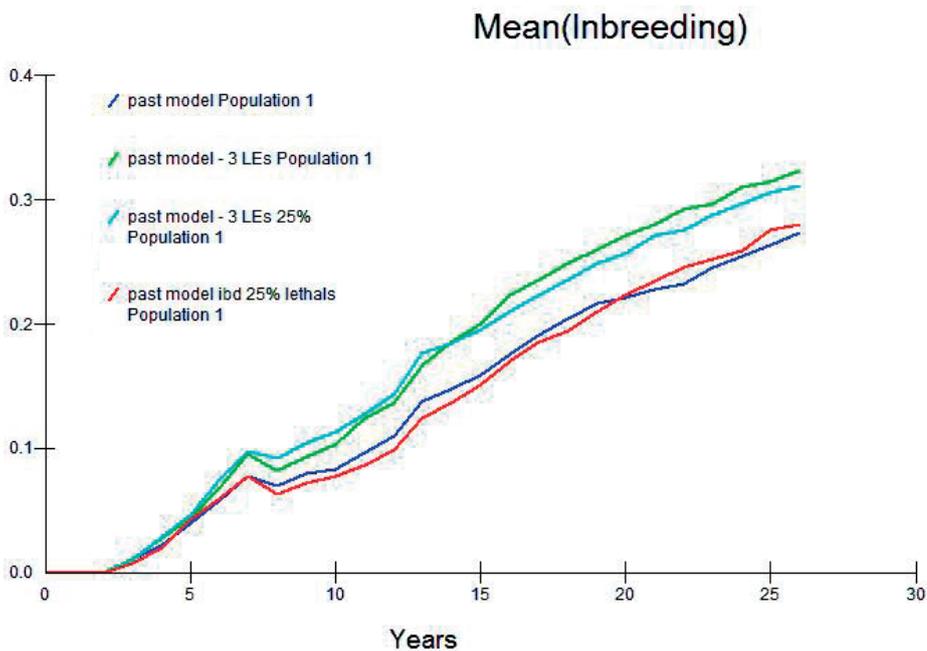


Figure 3 – The influence of inbreeding on the simulated Swedish wolf population inbreeding coefficient from foundation until 2008.

Evaluation of the first model: analysis of the past population shows that the Swedish wolf is a population that, given the parameters used here, might have been expected to go extinct. The fact that it did not could either be a result of chance or to a factor in its population dynamics that was not captured in the model. Of particular interest is the balance between inbreeding cost, fecundity and inter-birth interval. At the mid-term review, G. Chapron has

surmised that there is no reason why Swedish wolves should not be breeding each year. The implied deterministic lambda values, however, imply that it is unlikely that this is the case, and more likely favour the 29% female breeding value proposed in Nilsson (2004). I used a stable age distribution for the modeling, and an arbitrary population size, to enable the general attributes of the model to be discerned. There seemed to be general agreement around the 4–5 cubs per litter value, and the model was not very sensitive to this parameter, although a realistic standard deviation value needs to be applied. The inbreeding values are perhaps of more interest, and have a profound impact on the model, which is not surprising given its demographic history.

Model Refinement

Revised basic model

Following discussions at the mid-term review meeting (**Appendix 1**), a number of adjustments were made to the basic life history parameters used in the model, to reflect the best estimates available for the Swedish wolf population (**Table 3**).

Table 3 – Modified model parameters . OL = Olof Liberg, pers. comm., GC = Guillaume Chapron, pers. comm.

Parameter	Value	Source
Number of populations	1 for Scandinavia 1 for Finland and/or Karelia	
Lethal equivalent genes	6.04, (8, 10)	
Percentage of inbreeding depression on survival due to lethal recessive genes	0, 0.25, 0.5, 0.75, 1	
Catastrophes	Disease: 1% probability per year, 50% survival, 0% in-year reproduction OR 1% probability per yr, 75% survival, 0% in-year reproduction	Nilsson 2013 Murray et al. 1999
Maximum age of reproduction	12	OL
% females breeding in any given year	60%	OL
Mean litter size	6.5	Appendix 2
First year mortality males and females as a percentage, mean and standard deviation	5, 3	GC
Second year mortality males and females as a percentage, mean and standard deviation	40, 8	GC
Adult mortality males and females as a percentage, mean and standard deviation	25, 3	GC
Carrying capacity	2,000 for Scandinavia, 8,000 for Finland / Karelia	

Structured sensitivity testing was carried out to re-evaluate the population size, extinction probability and genetic diversity trajectories and their standard deviations for the Scandinavian population alone (analysed for consistency between 1983 and 2008), without the inclusion of catastrophes in the first instance. Two inbreeding parameters were first analysed independently, since this is known to be a major issue for this population (Ellegren 1999) and inbreeding is poorly incorporated into many population viability assessments (Frankham et al. 2013). First the percentage of genetic load attributable to recessive lethal genes was examined. This was because some discussion occurred at the mid-term review on the likelihood that genetic load could have been purged (removed by selection) during the history of the Scandinavian wolf population. As expected, when the percentage load due to lethal equivalents was high, the population recovered more rapidly due to the effects of purging (removal of genetic load due to the death of homozygous individuals).

Recent research (e.g. Kennedy et al. 2014) has, however, questioned the role that purging plays in natural populations, especially when inbreeding levels have accumulated rapidly, as in the Scandinavian wolf. This is thought to be because inbreeding depression for many traits could involve many genes of weak deleterious effect. For the simulations carried out here, increasing the proportion of lethal recessive genes involved in genetic load had a predictable and positive consequence for mean population growth (Figure 4). Using a percentage of recessive lethal values of 75 and 100, produced unrealistic population growth rates (standard deviations did not overlap with observed value of approximately 140 individuals at 2008), I confined further analysis to Recessive Lethal percentages of 50 and lower.'

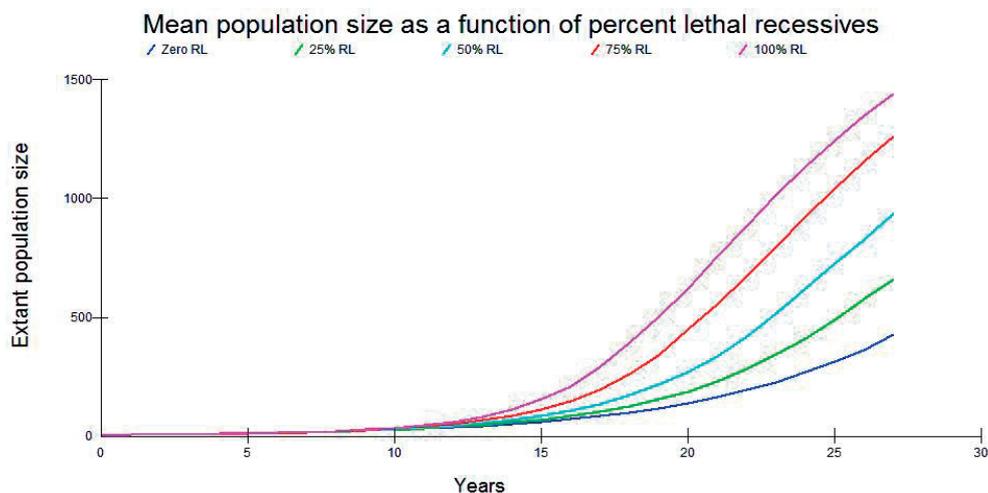


Figure 4. The effects of purging of genetic load on the Swedish wolf population simulated from 1983 to 2008 (25 years) on extant population size (means of 1,000 simulations are shown). RL = recessive lethals.

Next, the number of Lethal Equivalent genes (estimate 6.04; Liberg et al. 2005) was tested to explore higher values to account 1) for additional mortality during period between December and the following May in the first year of life when additional mortality may occur but which cannot be censused in the field, and 2) inbreeding depression due to the lower probability of pairing that has been seen for inbred adults (Åkesson, pers. comm.). The number of lethal equivalent genes was set at 6.04, 7, 8, 10 and 12 to cover realistic values seen from other vertebrate species (O'Grady 2006). These were covaried with 0, 10%, 25% and 50% of genetic load attributed to lethal recessive alleles. Figure 5 shows the results for 10%, 25% and 50% Lethal Recessives.

Although the standard deviations among some of the simulations overlapped (not shown), the parameters best fitting the observed population data at 2008 were 8 Lethal Equivalents for up to 50% Lethal Recessives; this number of equivalents is similar to the more recent estimate by Sand et al. (2014; their Fig. 15). I therefore chose to model these parameter combinations in further analysis.

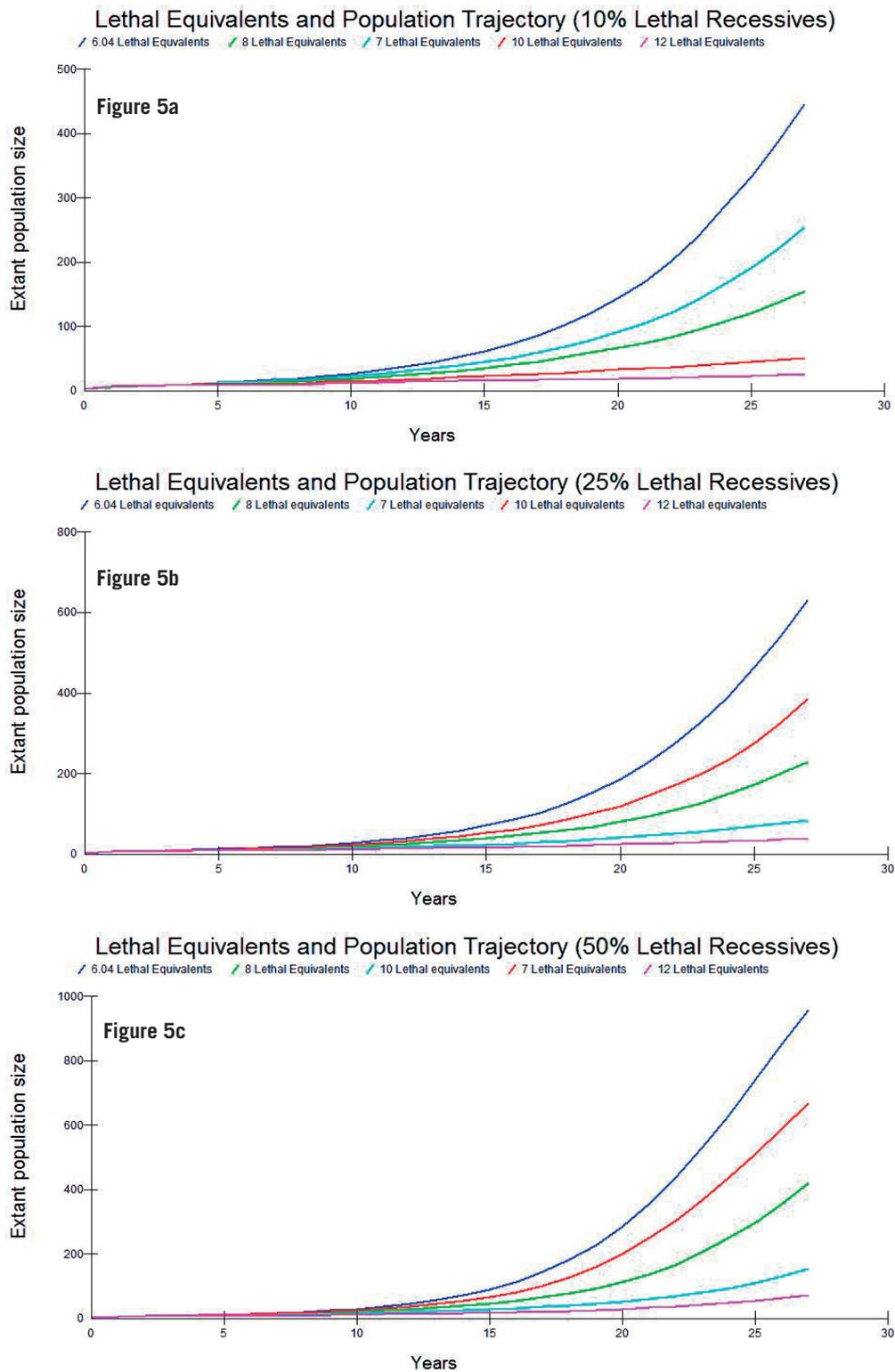


Figure 5. Lethal equivalents and their effect on population trajectory for a) 10%, b) 25% and c) 50% Lethal Recessives (means of 1,000 simulations are shown).

The mean stochastic intrinsic growth rate estimates for these models varied between 0.107 and 0.153, all of which are below the recently estimated value of 0.181 (Chapron et al. 2012, estimated without inbreeding depression modeling, although some of my standard deviations overlapped with that value), however the model estimates comprised the entire 25 year period between 1983 and 2008. A value of 10% recessive lethal genes has been previously observed by Liebl et al. (2006), albeit for synaptogenesis mutants in *Drosophila melanogaster*, however such estimates are in general very difficult to find in the literature. O’Grady et al. (2013) found an average overall effect of 8.4 diploid lethal equivalent genes for survival to sexual maturity in a meta-analysis of vertebrate data.

To further evaluate the percentage of lethal recessives, the model was extended to 2014, thereby including a further immigration event in 2008, when two males immigrated into the population (Laikre et al. 2013). For this model, the percent lethal recessives was further refined to 10, 25, 30, 40 and 50. Table 4 summarises the most relevant results from these models. It should be noted that a limitation of the Vortex software is that deterministic immigration events are modeled by Supplementation. This option does not permit variation in the number of individuals entering the population between supplementation events. Therefore in this instance only the addition of a single adult male in 2008 (in addition to the individual in 1991) could be modelled. An alternative analysis involving the supplementation of two adult male individuals into the population was included, but this produced very similar results (not shown) to those presented below.

Table 4. Model parameters from Lethal Recessive analysis using eight lethal equivalents and five scenarios with different percentages of lethal recessives. Stoc-r : observed intrinsic growth rate, SD(r): standard deviation among simulation replicates, PE: probability of extinction for each model, N-extant: mean size of the surviving populations at the end of the simulation time-point, SD(Next): standard deviation, GeneDiv: recorded mean gene diversity of the population by the end of simulations, AllelN: number of extant alleles in the loci modeled, Mean F: average value of the population inbreeding coefficient (F) by the end of simulations.

Scenario	stoc-r	SD(r)	PE	N-extant	SD(Next)	GeneDiv	AllelN	Mean F
LR 10	0.114	0.422	0.618	233.62	357.59	0.6905	5.62	0.265
LR 25	0.13	0.414	0.59	358.92	496.38	0.692	5.79	0.284
LR 30	0.136	0.412	0.603	425.97	533.03	0.6745	5.82	0.295
LR 40	0.146	0.406	0.582	574.68	687.84	0.6844	5.84	0.273
LR 50	0.152	0.415	0.606	688.12	730.9	0.6684	5.8	0.309

Figures 6a and b show the trajectories in population size and inbreeding coefficient to 2014 under the same assumptions. The closest model to current observed estimates for population size assumes eight lethal equivalents and 30% lethal recessives. All models produced realistic results given the values that have been estimated from pedigrees. Mean inbreeding for litters of breeding wolf pairs in 2013 was approximately 0.25 (Åkesson, pers. comm.) whereas for the model, values for ranged between 0.265 and 0.309 – however

this value is a mean across all surviving individuals and average inbreeding of litters are lower than expected from the potential parents since immigrant offspring have shown a higher pairing success (Åkesson, pers. comm.). Therefore the model chosen for further analysis was the one that most closely follows the current population trajectory, with a number of lethal equivalents of 8 and percentage lethal recessives of 30.

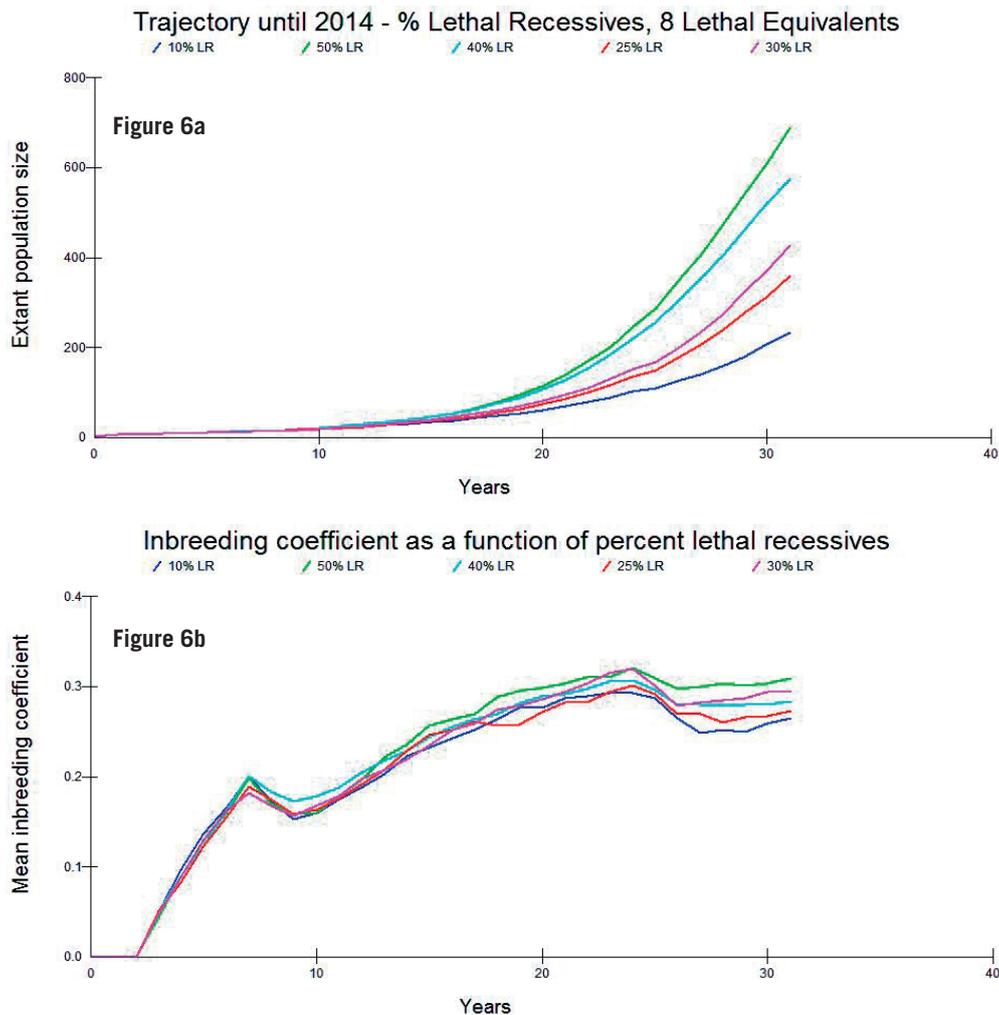


Figure 6. Population size (a), and inbreeding trajectory (b) as a function of lethal recessive percentages (means of 1,000 simulations are shown).

Catastrophes

With the basic parameters established, a model was constructed to examine population growth parameters in the absence of immigration for the coming 100 years (approximately 20 wolf generations) including and excluding catastrophes (here modeled as disease outbreaks), following Nilsson (2013) and Murray et al. (1999). Nilsson (2013) modeled one severe disease epizootic per 100 years with 50% survival and 100% loss of reproduction for the year

of event, while Murray estimated that population size changed by 25.2 ± 7.8 (SE)% for *Canis lupus*. Two models were therefore run, with the first following the Nilsson model and the second modeling 75% survival. A comparison of model outcomes in terms of population trajectory and inbreeding coefficient is shown in **Figure 7a** and **b** below. As can be seen, catastrophes had very little impact on the final size of surviving populations, which reached carrying capacity at Year 75 and where inbreeding coefficient remained at between 0.3 and 0.4 for the forecasting period. I therefore retained the catastrophe model described by Nilsson (2013) as the most conservative option.

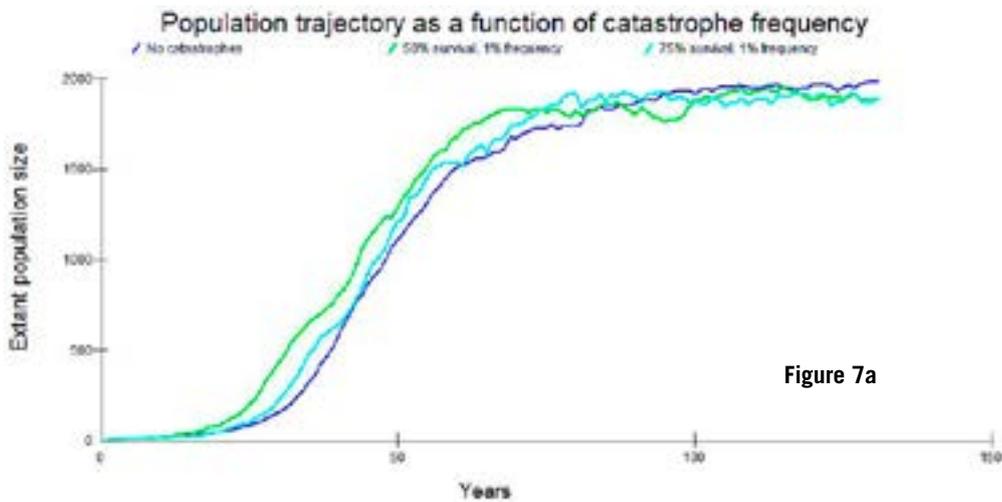


Figure 7a

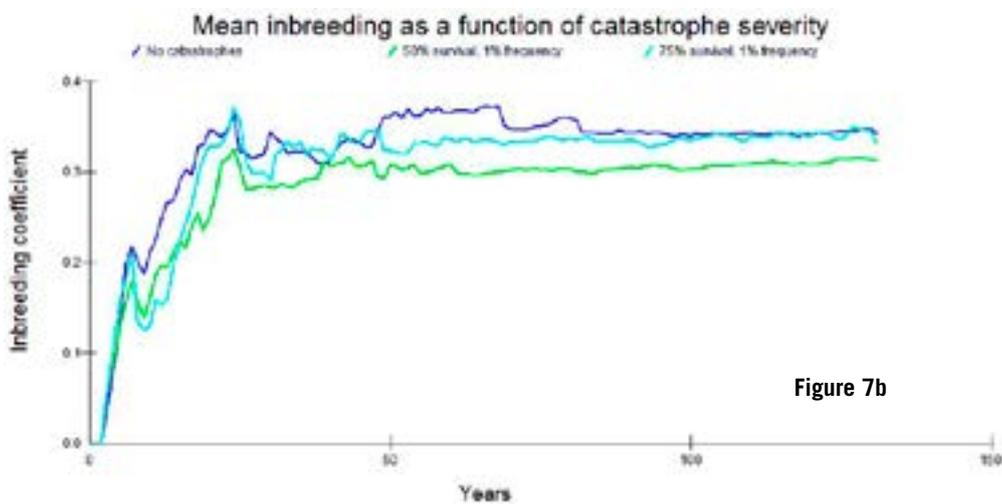


Figure 7b

Figure 7. Forward modeling of different catastrophe scenarios, a) population size and b) inbreeding coefficient (means of 1,000 simulations are shown).

Forward modeling without gene frequencies

Modeling immigration to the Scandinavian population

The current Scandinavian population has a size of approximately 420 individuals with an inbreeding coefficient between 0.25 and 0.30. However, the question posed by SEPA is how strong is the effect of modest numbers of effective immigrants at population sizes of 170 (the lower bound of the Swedish Parliament's Favourable Reference Population or **FRP** definition) through to greater than 417 (SEPA's genetic minimum viable population size or **MVP** as a FRP) and within these value ranges, how many reproducing immigrants per generation will prevent significant inbreeding or loss of variation?

A structured analysis was therefore next carried out constraining the Swedish wolf population to 170, 270 (the Swedish Parliament's upper bound FRP), 370, 420 (SEPA's lower bound MVP is 417), 470 and 570. This was simulated as a managed translocation via regular and directed supplementation of 1, 2, 3, 4 and 5 migrants per generation (defined here as 5 years). Where possible even numbers of both sexes were added. This model did not include gene frequencies, and assumed individuals at the start of the modeling were unrelated. The questions in italics were specifically addressed with this modeling.

How strong is the effect of modest numbers of effective immigrants at population sizes of 200–300? How many wolves per generation will prevent significant inbreeding or loss of variation?

Changes in genetic diversity were estimated using gene diversity, inbreeding coefficient and allelic diversity. **Figure 8** below shows the change in inbreeding coefficient predicted for population sizes 170, 270 (FRP bounds) and 370 under one and two immigrants per generation. The strongest effect could be seen in population size, since the two simulations featuring populations sized 370 showed the only below 10% increase in mean inbreeding coefficient ($\delta F = 0.0894$ for one immigrant per generation and 0.0824 for two immigrants). This suggested that under modest immigration rates, population size (genetic drift) plays an important role in the maintenance of genetic diversity. The final values over 100 years for all three parameters are presented in **Table 5**.

Further, if an aim is to conserve > 90% of gene diversity over 20 generations (or even 40 generations if a 200 year criterion is used; Soulé et al. 1986) with modest immigration rates (sometimes cited as a minimum target for short-term maintenance of genetic diversity in small populations), these results indicated that a population size of 370 individuals would be minimally needed (**Table 5**). While the effects of maintaining allelic diversity in natural popula-

tions have only been demonstrated for a few cases, it is worth noting that while allelic diversity inevitable declines steeply in populations undergoing genetic drift, the highest number of alleles are also maintained in larger population sizes (exceeding 20 only in populations of 370 individuals).

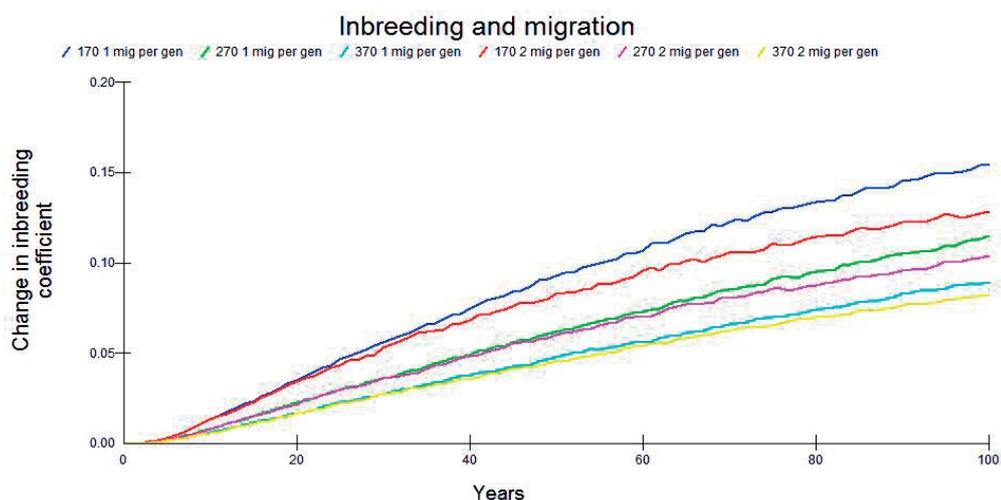


Figure 8. Inbreeding as a function of modest immigration at population sizes of 170, 270 and 370 (means of 1,000 simulations are shown).

Table 5. The effect of modest numbers of immigrants per generation on key genetic parameters after 100 years.

	Proportion of gene diversity retained	Increase in mean inbreeding coefficient	Allelic variation
170 1 imm. per gen	0.8346	0.1541	12.95
270 1 imm. per gen	0.8778	0.115	16.95
370 1 imm. per gen	0.9041	0.0894	20.91
170 2 imm. per gen	0.86	0.1281	16.27
270 2 imm. per gen	0.8888	0.1037	19.84
370 2 imm. per gen	0.911	0.0824	24.02

In 2012, SEPA proposed a genetic MVP as a minimum value for FRP for the Scandinavian wolves at ≥ 417 combined with effective immigration of ≥ 3.5 reproducing wolves per generation. Does modeling confirm the result of this assessment?

To address this question the same parameters were assessed by comparing the effects of higher immigration rates (3 and 4 per generation) across a range of population sizes from 170 to 570 (Table 6). In particular the proportion of genetic diversity retained at a population size of 420 (SEPA's MVP) was compared to realistic and policy relevant values above and below that number.

SEPA's MVP value of 420 individuals was predicted to retain greater than 92% of current gene diversity over 100 years while constraining an increase in inbreeding coefficient to approximately 7% over 20 wolf generations, and retaining approximately 50% more allelic diversity than the 170 lower limit FRP value.

Table 6. The effect of larger numbers of immigrants (3, 4) per generation on key genetic parameters after 100 years as a function of population size. SEPA's MVP value is shown in bold.

	Proportion of gene diversity retained	Increase in mean inbreeding coefficient	Allelic variation
170 3 imm. per gen	0.8753	0.1127	18.78
270 3 imm. per gen	0.898	0.095	23.05
370 3 imm. per gen	0.9153	0.0793	26.95
420 3 imm. per gen	0.9234	0.0705	29.13
470 3 imm. per gen	0.9289	0.0663	30.99
570 3 imm. per gen	0.9382	0.0574	35.03
170 4 imm. per gen	0.8873	0.1005	21.34
270 4 imm. per gen	0.9054	0.0867	25.66
370 4 imm. per gen	0.9203	0.0734	29.66
420 4 imm. per gen	0.9266	0.0671	31.48
470 4 imm. per gen	0.9308	0.0636	33.57
570 4 imm. per gen	0.9407	0.0554	37.73

It is the case that larger population sizes, as expected, retain ever-greater levels of diversity (as much as 94% of gene diversity and constraining inbreeding to a 5.5% increase over 100 years for a population of 570 and four migrants every five years).

Under relatively high rates of immigration (4 per generation), the upper bound FRP size proposed by the Swedish parliament is predicted to retain slightly in excess of 90% initial gene diversity and increase its mean inbreeding coefficient by 8.7%. Retention of 90% of initial heterozygosity over 200 years has been cited as a minimal requirement for maintenance of genetic diversity in captive breeding programs (Soulé et al. 1986) – it is worth noting that a population size of 270 was not predicted to reach this threshold for one, two or three migrants per generation, but was predicted to be achieved with four effective migrants and higher. The likelihood that this level of effective immigration can be achieved on the ground might therefore be the deciding factor on the population size that should be retained.

The definition of “sufficient genetic variation” for short-term population viability is a matter of considerable scientific debate. The Scandinavian wolf population descends from five individuals and Frankham et al. (2013) argue that an effective population size of greater than 100 is required to limit loss in total fitness to 10%. The modeling presented here shows that a balance will need to be struck between population size and immigration to reach this goal. It seems from these modeling results that a loss of gene diversity and an increase in inbreeding coefficient of within 10% to be achievable in larger population sizes (370 and greater) with as little as one effective migrant per generation.

The modeling methods used here were by no means exhaustive and would be improved in a number of ways. Importantly, the second modeling objective identified at the mid-term review, to simultaneously model the Scandinavian and Finnish/ Karelian population has been attempted but did not perform reliably due to difficulties in implementing a complex historical scenario with forward casting in VORTEX (not reported here), uncertainties on the demographic history of the Karelian population and its demographic relationship with the Finnish population. However this work is ongoing and will be added for comparison.

Two further customised models

Reviews of the report describing the **Forward Modeling Without Gene Frequencies** were received from five independent reviewers and one group, plus SEPA. The reviews included suggestions for modifications and clarifications of in final report including reporting of the modeling results and textual changes, but notably included suggestions for additional modeling and requests to more specifically address the legal issues and questions in SEPA's decision letter. A summary description of consensus points from the reviews with specific issues highlighted of importance for the revised modeling is reported in **Appendix 2**.

Basic parameters

Basic values for the models described in this section were established at the mid-term review and are summarized in **Table 1**. There was broad consensus among the reviewers that the biological parameters used adequately represented current knowledge of the Swedish wolf population. However, a number of changes were suggested. The first change concerned altering the mating system specified from 'Monogamy' (monogamy in any given year but a random probability of mating with the same individual in following years) to 'Long-term Monogamy' (the same individuals breed together annually until one dies). This change did not affect the outcome of a subset of Final Report models that were tested (see **Supplementary Table 1**): results obtained were very similar to those obtained using short-term monogamy. Long-term monogamy was retained for further modeling to better reflect the breeding biology of the species. These parameters were also used to examine for any (unexpected) systematic differences in modeling outcomes for Vortex v9.99 *versus* Vortex v10 because further modeling utilized functionality for handling additional genetic data that has been improved in Vortex v10 by RC Lacy. As expected, the results were almost identical, recovering the same or very similar values for all models tested, as could be expected under stochastic simulation (**Supplementary Table 2**).

Model Structure

The most important and fundamental changes in the modeling approach, identified by several of the reviewers (and highlighted in the Final Report) was the need to include more pedigree information, and to more accurately reflect genetic differentiation between the Scandinavian wolf population and the likely source populations for future immigration (Finland / Karelia). However, computational capacity and software functionality constraints in Vortex currently prevent these two issues being modeled simultaneously. Specifically, 1) it is not possible to model multiple populations with different demographic

profiles (described using a pedigree file), even if such data are available (which they are not for Finland and Karelia) and 2) because inbreeding depression, which is computationally intensive to model, must be applied to all populations, it was not possible using the computational capacity available to model three populations simultaneously – only two could be included.

To address the first issue, additional modeling was carried out using pedigree data kindly supplied by Dr Mikael Åkesson (Grimsö Wildlife Field Station, Swedish University of Agricultural Sciences) for a subset (320) of living animals from the Swedish wolf population of 2012. These data, which included individual relationships and inbreeding coefficients, were linked to Vortex to test the impact of different levels of immigration, which could only be modeled using Vortex's Supplementation routine due to functionality constraints when pedigree files are used, and using different carrying capacities for the Scandinavian population (200–700), specified by SEPA following review of the report on the Forward Modeling Without Gene Frequencies. The disadvantage with using the supplementation option in this context (as pointed out in reviews of the final report) is that the alleles carried by supplemented individuals are unique, which in this case is likely to overestimate the genetic benefit of immigration because in reality the allele frequencies of natural immigrants would be expected to be correlated with the Scandinavian population, reflecting the genetic similarity of subpopulations within the northern European metapopulation.

To address the above issue, additional modeling was carried out for the Scandinavian population and a notional second population (with size 1,000 and carrying capacity 2,000 to constrain computational load) that genetically resembled the Finland/Karelian population. Allele frequencies for ten microsatellite genetic profiling markers (kindly supplied by Dr Mikael Åkesson) were utilized for the Scandinavia and Finland/Karelia populations. Here, Scandinavian and Finnish allele frequencies were used with the Vortex genetic management utility, and combined with bidirectional and unidirectional dispersal, and different carrying capacities in Scandinavia for a subset of simulations (see below).

In both cases, a key question is what constitutes 'genetically effective' immigration, as opposed to demographic immigration, since equating demographic immigration to gene-flow assumes that all immigrants will remain, survive and breed, which is not the case, since 12 individuals immigrated into Scandinavia between 2002 and 2009 (approx. 1.5 per year; SOU 2012:22) and because between 2008 and 2012, four immigration events occurred and only two of those are known to have been genetically effective (0.4 genetically effective migrants per year, approximately two individuals per generation). Both these '*pedigree + supplementation*' and '*allele frequencies + dispersal*' models used a range of supplementation/ dispersal values to cover most foreseeable scenarios, ranging from zero to two *effective* migrants per year (see below). All modeling reported hereon was carried out using Vortex v10. Using replicated subsets of the simulations, results converged before a total of 1,000 simulation replicates which therefore were used as a standard number in the simulations.

The pedigree + supplementation model

To establish that using the pedigree data yielded more realistic estimates of genetic diversity and inbreeding in the current Scandinavian population, simulations were carried out using these data to examine the magnitude and trajectory of inbreeding and heterozygosity in the absence of immigration with a carrying capacity set at a notional 700 wolves (Figure 9a, b). Simulations were carried out over 50 years (approximately 10 wolf generations). The mean starting inbreeding coefficient was 0.267 ± 0.041 (SD) and as can be seen in Figure 9a, initially the inbreeding coefficient underwent a decline before gradually increasing to a final mean value of 0.348 ± 0.078 (SD). These values and (initial) trajectory are similar to the empirical values that have been reported by Åkesson et al. (pers. comm.). Figure 9b shows the trajectory of gene diversity (heterozygosity) in the absence of immigration and the values estimated from the pedigree genetic data agree closely with current molecular estimates (Åkesson et al.). The final (year 50) value of 0.642 ± 0.075 (SD) implies a mean decline of 0.082 from an initial value of 0.724. However, it should be pointed out that for both parameters, standard deviations overlap substantially for the duration of the model. The mean final population size was 550 ± 237 (carrying capacity 700).

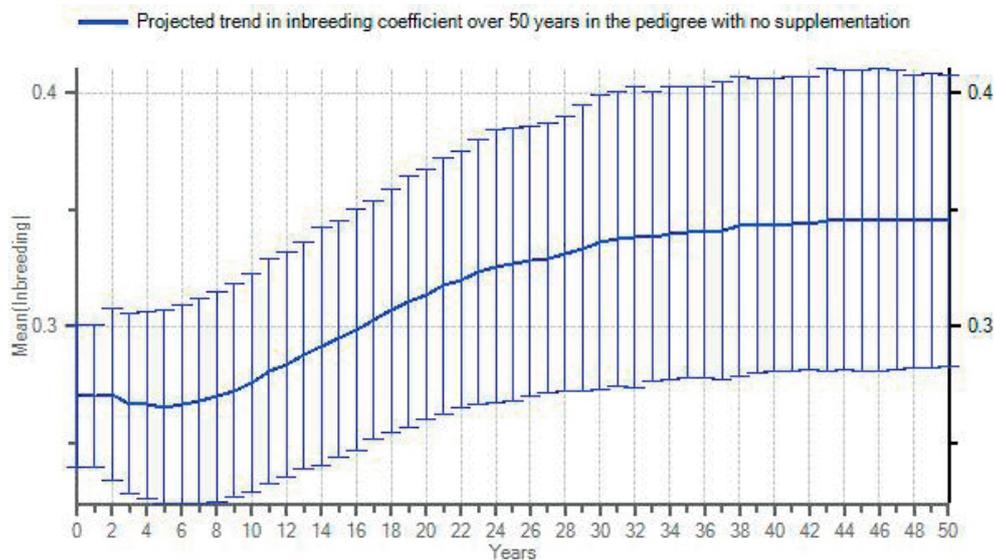


Figure 9a. Inbreeding coefficient in the Scandinavian wolf population starting from 2012 pedigree values in the absence of immigration. Means and standard deviations of 1,000 simulations are presented.

Immigration was then simulated with the pedigreed dataset, using supplementation over a 50-year period, with different frequencies to represent a spread of possible effective immigration rates, with a notional carrying capacity of 700 wolves. Figure 10a shows the effect of different immigration rates, ranging from one female per 12 years (2–3 wolf generations) through to four effective immigrants per three years. In contrast to the results reported using

the idealized population simulated in the Forward Modeling Without Gene Frequencies analysis, as few as one female per three years produced a substantial decrease in the mean inbreeding coefficient compared to its starting value (final value 0.214 ± 0.037 SD) and even immigration of one female per six years (approximately one migrant per generation) resulted in a slight decrease in mean inbreeding coefficient, using a carrying capacity of 700, although again standard deviations overlap considerably (0.250 ± 0.037).

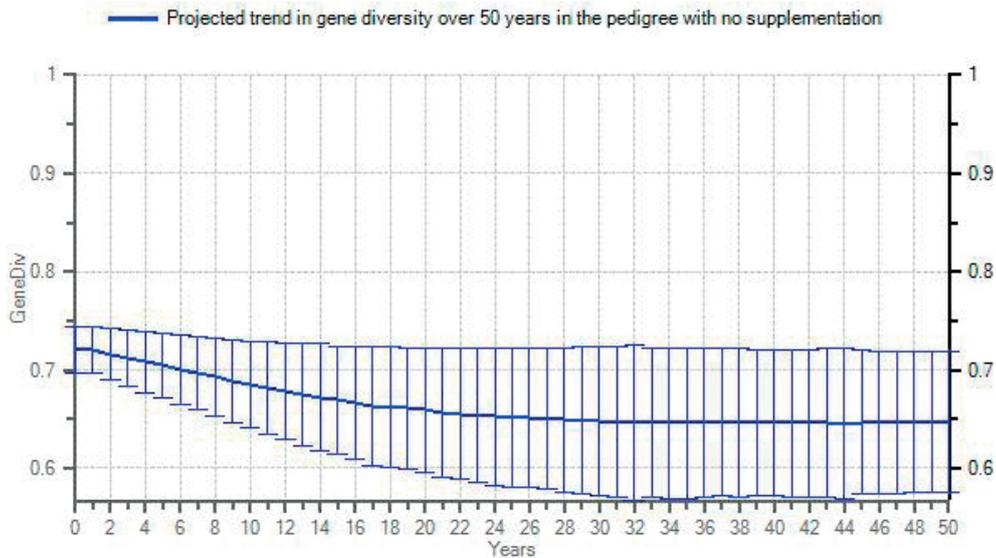


Figure 9b. Gene diversity in the Scandinavian wolf population starting from 2012 in the absence of immigration. Means and SDs of 1,000 simulations are presented.

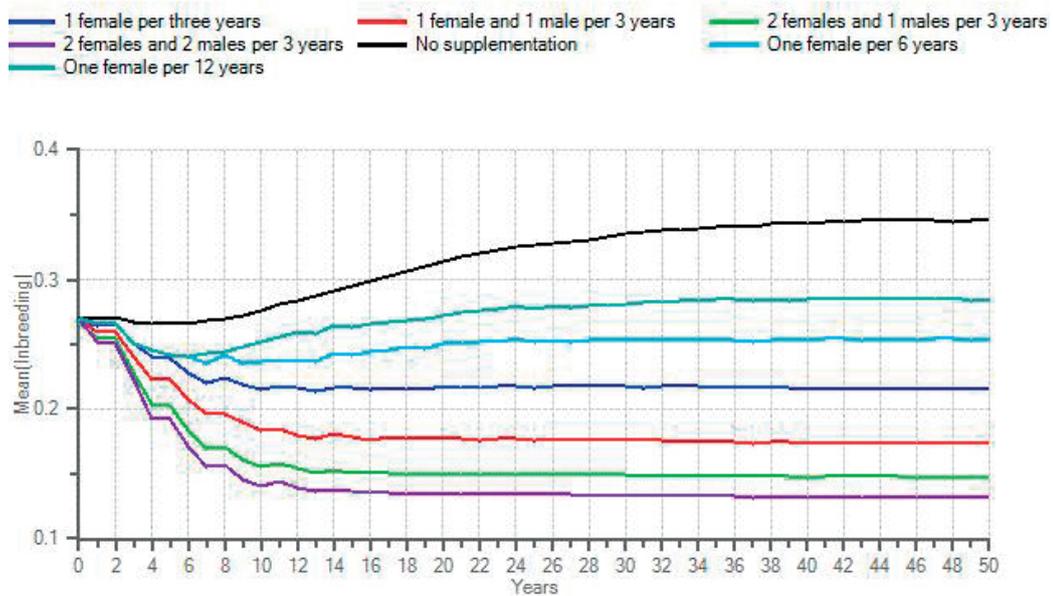


Figure 10a. Inbreeding coefficient in the Scandinavian wolf population starting from 2012 pedigree values using a carrying capacity of 700 with population supplementation. Means of 1,000 simulations are presented.

Figure 10b shows the trajectory in gene diversity for the same set of simulations. The supplementation of one female every 6 years resulted in a slight increase in mean gene diversity to 0.743 ± 0.045 SD. While populations supplemented every 12 and six years did not achieve carrying capacity, although SDs overlapped (628 ± 170 and 662 ± 116 , respectively), all other simulations achieved mean final population sizes very close to 700.

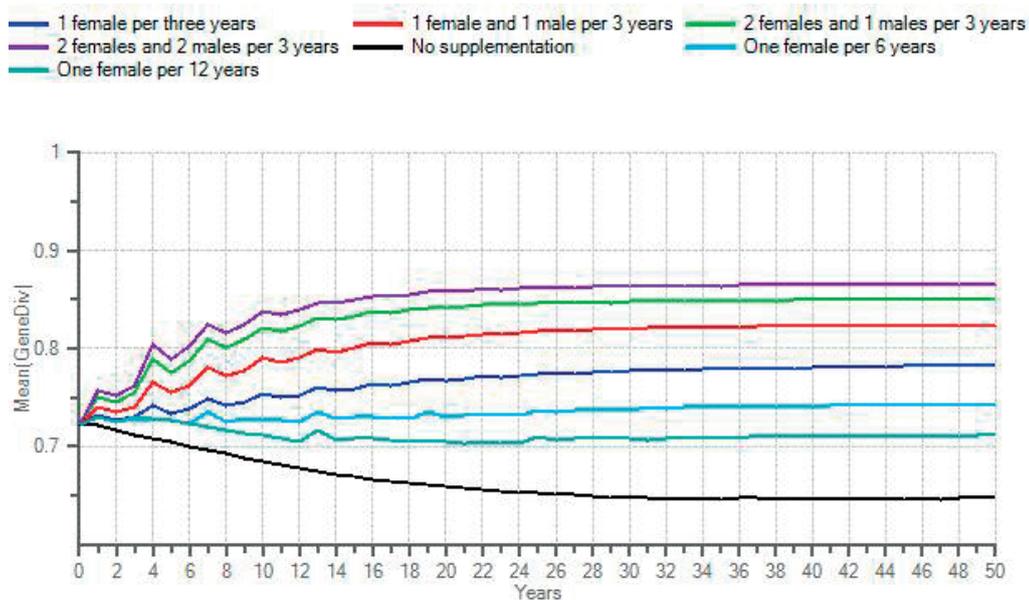


Figure 10b. Gene diversity in the Scandinavian wolf population starting from 2012 pedigree values using a carrying capacity of 700 with population supplementation. Means of 1,000 simulations are presented.

A subset of the supplementation scenarios were then subjected to variation in carrying capacity ($k = 200, 300, 400, 450, 500, 600, 700$) to assess whether a systematic effect of carrying capacity and hence (in most cases) population size could be detected under similar ranges of supplementation, as had been found in the previous modelling. In these simulations, the effect of population size was very moderate compared to the effect of supplementation rate. **Figure 11** shows the change in inbreeding coefficient as a function of supplementation frequency (no supplementation, one female per 12 years, one female per six years and one female per three years), with the effect of carrying capacity explored only in the latter two categories. Surprisingly, no effect on mean inbreeding could be inferred for any given level of supplementation (for a more detailed graph, see **Supplementary Figure 1**) with the smallest carrying capacity ($k = 200$) showing slightly larger increases in mean inbreeding coefficient, although this is not evident in all supplementation combinations (**Supplementary Table 3**), although mean final coefficients declined as a function of supplementation rate (from 0.318 to 0.147; **Supplementary Table 3**).

Table 7 shows summary results for key scenarios in final population parameters for supplementation rates as a function of carrying capacity (full results can be found in Supplementary Table 3). Population sizes reported are means across years as opposed to mean final values. Mean final inbreeding coefficients decreased from present-day values in all cases and mean final Gene Diversity values exceeded present day values in all cases, regardless of carrying capacity. However, while the relative magnitude of these trajectories is informative, especially among supplementation scenario groups, their absolute value is questionable due to the unrealistic assumption of the supplementation regime in Vortex, which is why the ‘*allele frequencies + dispersal*’ model was also explored (below). Stochastic-*r* (the stochastic population growth rate) is calculated using the simulated birth and death rates in the model, whereas the deterministic growth rate simply uses the demographic parameters in the model as fixed. Stochastic-*r* values are almost always smaller than deterministic *r* -values (Lacy et al. 2014), and in Vortex this is because multiplicative effects of population fluctuations in growth and survival, because deterministic growth parameters assume a starting stable age distribution, and most importantly because of density dependent effects and especially inbreeding at small population sizes. In the case of the *pedigree + supplementation* model, it is likely that these inbreeding effects are the predominant cause of the disparity between stochastic and deterministic growth. Identifying the precise demographic factors underpinning lower growth rates in inbred populations is, however, highly challenging (Keller et al. 2012).

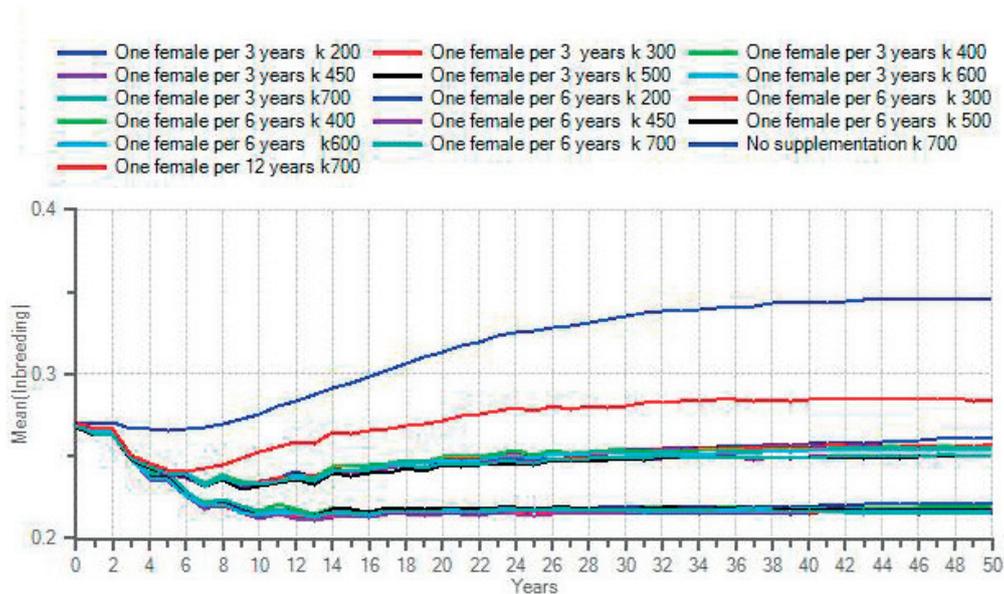


Figure 11. Inbreeding coefficient in the Scandinavian wolf population starting from 2012 pedigree values using carrying capacities of 200–700 with population supplementation ranging from zero to one female per three years. Means of 1,000 simulations are presented.

Table 7. Key model outcomes at 50 years for the Scandinavian wolf population with 2012 pedigree values, carrying capacities of 200–700 with population supplementation. Supplementary Table 3 presents the full range of results.

Scenario	Stochastic r (growth rate)	SD (r)	Mean Population size	SD Population size	Gene diversity	SD (GD)	Percentage of initial Gene Diversity retained	Inbreeding coefficient
One female/ 3 years k200	0.139	0.23	192.86	23.14	0.769	0.04	106.2	0.223
One female/ 3 years k450	0.141	0.232	435.94	51.66	0.78	0.038	107.7	0.22
One female/ 3 years k700	0.142	0.23	679.06	85.92	0.782	0.037	108.0	0.221
One pair/ 3 years k200	0.171	0.241	196.01	19.31	0.818	0.032	112.9	0.184
One pair/ 3 years k450	0.176	0.235	438.02	49.65	0.823	0.031	113.7	0.186
One pair/ 3 years k700	0.175	0.233	684.94	71.11	0.823	0.033	113.7	0.186
Two females and two males/ 3 years k200	0.214	0.245	195.63	20.54	0.863	0.023	119.2	0.143
Two females and two males/ 3 years k450	0.215	0.238	491.36	46.32	0.867	0.023	119.8	0.145
Two females and two males/ 3 years k700	0.216	0.236	689.84	55.48	0.865	0.024	119.5	0.147

The allele frequencies + dispersal model

To model the potential genetic dividend of immigration into the Scandinavian wolf population using genetic data, microsatellite marker (DNA profile) allele frequencies were used to describe the genetic diversity and differentiation within and among the Scandinavian and Finland/Karelia populations. DNA profile frequencies were provided courtesy of Dr Mikael Åkesson, and these were used with the Vortex genetics module as follows. Ten profiling markers (the maximum number permissible in Vortex) were selected (20, 225, 2001, 2010, 2088, 2168, AHT101, vWF, PEZ03, AHT126) and the allele frequencies for each marker for live individuals from 2012 were used to specify the starting genetic allele frequencies for the model. However, computational capacity prevented all three populations and all ten loci being modeled simultaneously, probably because inbreeding depression can only be applied to all populations simultaneously in the model. I therefore chose to use the Finland population allele frequencies for the second population, as Finland is most proximate and immigrants are most likely to come from this source (see Seddon et al. 2006; Kojola et al. 2009). However, to acknowledge the ongoing gene-flow and connectedness between the populations in Finland and Karelia, I used a notional starting population size of 1,000 individuals with a carrying capacity of 2,000. Allele frequencies for the first five markers were selected although parallel simulations using the second five markers were carried out as a control for a small number of scenarios and the results were similar (data not shown).

First, and to enable a direct comparison of the genetic parameters computed using Vortex for the Scandinavian population in isolation with the ‘pedigree + supplementation’ model, simulations were conducted using starting allele frequencies for the Scandinavian population alone. The initial (2012) population size was specified as 320 individuals (for direct comparison with the pedigree model) with a stable age distribution and simulations were conducted over 50 years (approximately 10 generations) with a notional carrying capacity of 700. **Figure 12a** shows the trajectory in inbreeding coefficient and **Figure 12b** shows the trajectory for gene diversity. The mean starting inbreeding coefficient was 0.277 ± 0.007 and the mean gene diversity was 0.722 ± 0.002 .

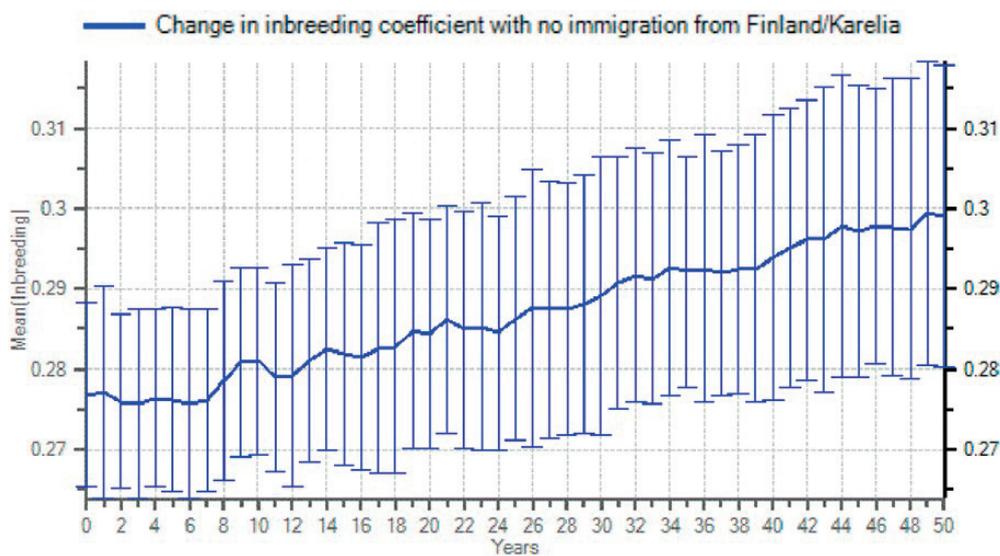


Figure 12a. Inbreeding trajectory in the dispersal model without immigration (carrying capacity 700). Means and standard deviations of 1,000 simulations are presented.

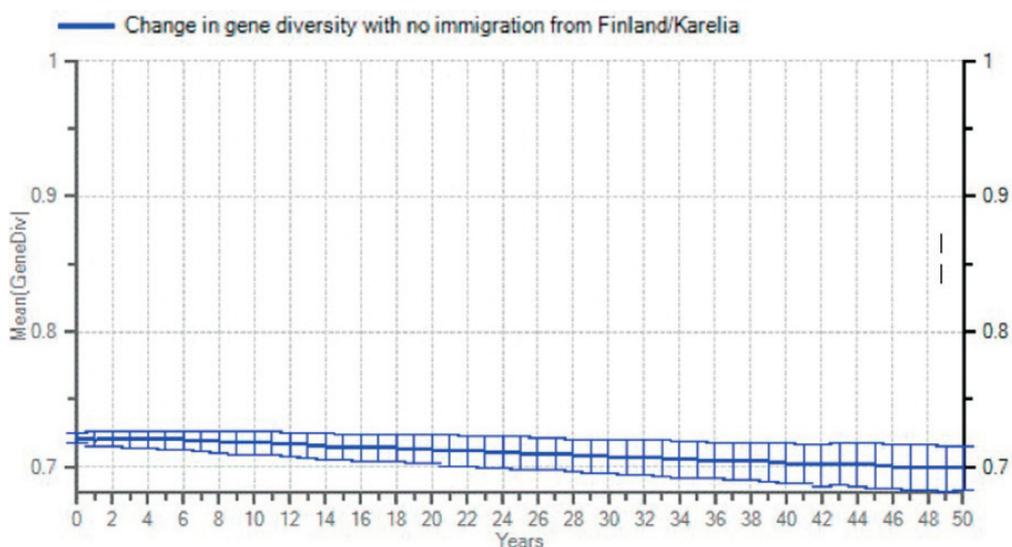


Figure 12b. Gene diversity trajectory in the dispersal model without immigration (carrying capacity 700). Means and standard deviations of 1,000 simulations are presented.

These values, while not identical to those estimated from the pedigree data, are within a single standard deviation and are almost identical for gene diversity. The estimate of mean inbreeding coefficient in this model is calculated using the homozygosity estimated from the user-defined starting allele frequencies (R.C. Lacy, pers. comm.). Note, mean population size across all years of the simulation was 695.41 ± 18.37 . Although the mean final inbreeding coefficient was lower for the dispersal model, the mean final values and their distribution overlapped almost entirely between suggesting that although the source of genetic data in the two models was different, the model assumptions produced convergent estimates of genetic diversity.

Next, immigration was simulated for a Scandinavian population using the same carrying capacity (700 individuals) with immigration rates varying from one immigrant per twelve years to six immigrants per three years (see below). Mean final inbreeding coefficients were surprisingly similar across simulations, ranging from 0.271 ± 0.019 (SD) for six immigrants per three years to 0.291 ± 0.015 (SD) for one immigrant per twelve years and standard deviations overlapped. **Figure 13** shows that for both six and three effective migrants per three years an improvement or parity in mean inbreeding coefficient, respectively, was produced whereas two effective immigrants per three years or less resulted in an increase. The magnitudes of these effects (also evident for gene diversity, not shown) are much smaller for the dispersal model than for the pedigree-based model, albeit at a higher carrying capacity (and population size), reinforcing the importance of the assumptions of genetic similarity of immigrants on genetic diversity indices.

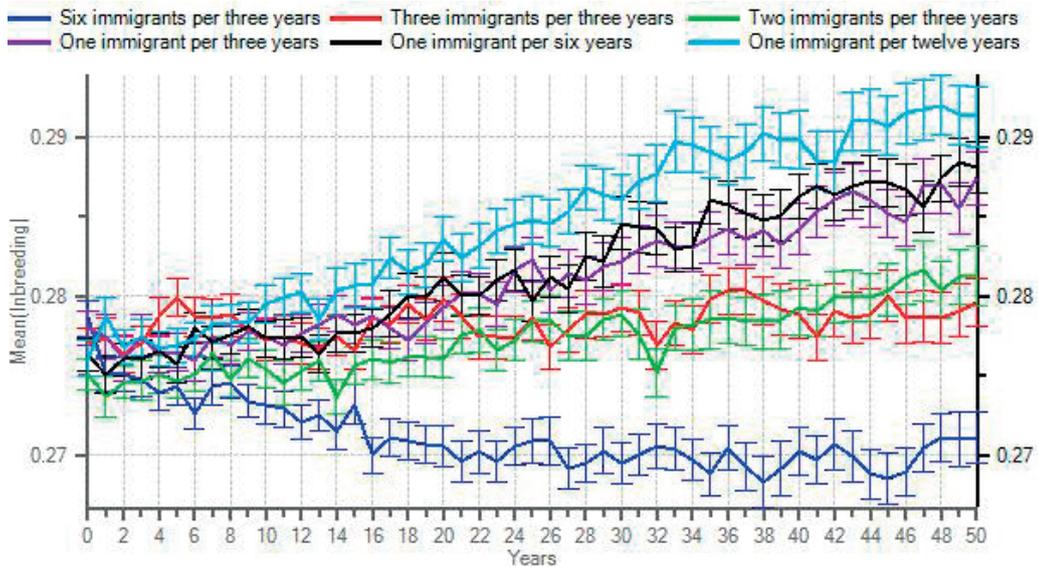


Figure 13. Inbreeding trajectory in the dispersal model with immigration (carrying capacity 700). Means and standard errors of 1,000 simulations are presented.

Two additional carrying capacities were applied, using the immigration rates identified in the $k = 700$ simulation as not resulting in an increase in inbreeding coefficient, in order to establish whether carrying capacity *per se* (hence population size, since all simulations gave mean population sizes within one standard deviation of the carry capacity, not shown) constrained changes in inbreeding and gene diversity and to provide two direct points of comparison ($k = 500$ and $k = 300$) with the *pedigree + supplementation* model. **Figure 14** shows the trajectory of inbreeding coefficient in these simulations and shows that six effective immigrants per three years resulted in a decrease in mean inbreeding coefficient over 50 years with the largest magnitude of change (> 0.01) for carrying capacities of 300 and 500 (0.267 ± 0.010 and 0.268 ± 0.007 , respectively). The mean final gene diversities for these simulations also showed the largest increases (to 0.728 ± 0.015 SD and 0.729 ± 0.16 , respectively). **Table 8** summarises the outcome for these simulations. Key scenarios and full outcomes are reported in **Supplementary Table 4**. In sum, under the assumptions of the *allele frequencies + dispersal* model, the genetic dividend of the highest immigration rates (six effective immigrants per three years) is most strongly felt at lower carrying capacities/population sizes, although these effects remain subtle and standard deviations overlap.

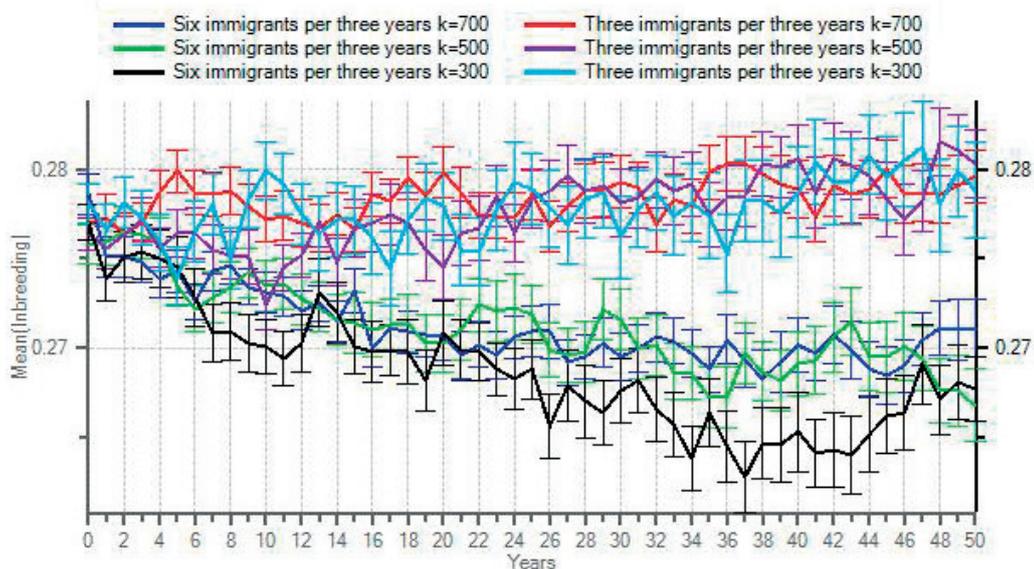


Figure 14. Inbreeding coefficient trajectory for three and six immigrants per three years as a function of carrying capacity (700, 500, 300) for Scandinavia. Means and SE of 1,000 simulations are presented.

Table 8. Key model outcomes at 50 years using the allele frequencies + dispersal and carrying capacities of 700, 500 and 300. Supplementary Table 4 presents the full range of results.

Scenario	Stochastic growth rate r	SD (r)	Mean final Population size	SD Population size	Gene diversity	SD (GD)	Percent GD retained from starting value	Inbreeding coefficient
Six immigrants/ 3 years k700	0.367	0.23	691.4	53.51	0.728	0.016	101.0	0.271
Three immigrants/ 3 years k700	0.363	0.245	683.7	73.92	0.718	0.014	99.4	0.280
Six immigrants/ 3 years k500	0.363	0.239	496.7	19.76	0.729	0.016	101.0	0.267
Three immigrants/ 3 years k500	0.363	0.235	496.8	33.13	0.716	0.015	99.1	0.280
Six immigrants/ 3 years k300	0.360	0.222	299.6	13.04	0.728	0.015	101.0	0.268
Three immigrants/ 3 years k300	0.350	0.24	292.1	38.05	0.715	0.021	99.0	0.279

Discussion

The two further customised modeling approaches that were adopted here are underpinned by different genetic data and have different magnitudes of effect when projecting the maintenance of genetic diversity in the Scandinavian wolf population. Both datasets produced starting population estimates for inbreeding and gene diversity that are close, but not identical to the estimated values from real molecular and pedigree data, and it is worth noting that neither models predicted significant probabilities of extinction in any forward-simulation scenario. The *pedigree + supplementation* model best represented the demographic structure and pre-existing inbreeding in the Scandinavian wolf population, but the supplementation regime used in Vortex assumes unrealistically high levels of genetic differentiation between the recipient population and immigrant individuals, since immigrant genotypes are not sampled from a realistic source population allele distribution. As a result of this, the genetic consequences of even modest numbers of effective migrants were high, with as few as one female per six years (1–2 wolf generations) stabilizing mean inbreeding and gene diversity to 2012 levels over a 50 year simulation (approximately ten wolf generations; **Supplementary Table 3**). Furthermore, and in contrast to the forward modeling without gene frequencies, the rate of supplementation was a far more important explanatory factor in determining the outcome of genetic parameters than population size. These results recall the observation of Vila et al. (2003) who showed the dramatic genetic (and demographic) dividend of a single immigrant in the Swedish wolf population from 1991. However, the wolf population at this time was much smaller than the 2012 population and the demographic and likely genetic impact of rare immigrants (so called ‘genetic rescue’) can be unpredictable, even in other wolf populations (Hedrick et al. 2011). Further, as highlighted, for example, by Whitlock and McAuley (1990) and Whitlock (1992), genetic variation and structure in small populations is strongly influenced by non-equilibrium dynamics and the combination of founder effect, genetic drift and inbreeding often makes trajectories of genetic diversity difficult to predict. Thus a conservative interpretation of the results of the *pedigree + supplementation* models is that they may be more useful for evaluating the relative impact of supplementation genetic rescue than its absolute impact. Of concern is the observation that the predicted inbreeding coefficient using this model did not vary as a function of population size for a given supplementation rate. These two parameters are expected to covary under standard population genetic models, suggesting that this model may not be responding in a predictable manner. Mean final inbreeding coefficient did, however, predictably decrease with increased supplementation rate (**Supplementary Table 3**). In addition, the mean final population size did not achieve carrying capacity in the absence of immigration (mean size of 550 with a carrying capacity of 700), which suggests more profound effects of inbreeding on the model than are being observed in reality (G. Chapron, personal communication). The results of this model should therefore be treated with caution, but are retained here for comparative purposes.

The *allele frequencies + dispersal* model better represented the genetic differentiation between the Scandinavian wolf population and its neighbours in Finland/Karelia and thus provided a more realistic assessment of the impact of genetic rescue as a function of that genetic differentiation. However this model had a less realistic demographic representation of the Scandinavian population, including its ongoing inbreeding. It was notable that simulations using this model had far higher stochastic growth rates and predicted lower mean inbreeding and higher mean gene diversity in the absence of immigration than the *pedigree + supplementation* model (Table 7, Table 8, Supplementary Table 4). Hence the impact of immigration would be expected to be lower even if the genetic dividend was as high as seen in the *pedigree + supplementation* model. Nonetheless it was surprising that the difference in mean inbreeding coefficient between zero immigration (0.299 ± 0.017) and two immigrants per year (0.271 ± 0.019) was modest at $k = 700$. Constraining carrying capacity to 300 and 500 had a more pronounced effect on genetic diversity and at $k = 300$ mean final inbreeding coefficient was 0.311 ± 0.011 with no immigration and 0.303 ± 0.007 for one immigrant per six years (Supplementary Table 4). The estimated decreases in inbreeding are much lower than those expected values under a Wright's classical Island Model (Skanduly comment), emphasizing that in this model the Scandinavian wolf population is not a drift/mutation equilibrium. A potential explanation for this observation is that it is a consequence of the five genetic marker loci chosen for the allele frequency analysis, and that they produced an underestimate of the genetic benefit of immigration. This could be evaluated by examining alternate combinations of markers sampled from the ten available in the future. If, however, this model shows that the genetic benefit of immigration is conservatively estimated compared to the *pedigree + supplementation* model, it can nevertheless be inferred that two effective immigrants per year maintained 2012 levels of genetic diversity regardless of the three carrying capacity and one effective migrant per three years had only a very slight negative effect on genetic diversity over 50 years at $k = 500$.

Importantly, for both models, it is important to state that differences in genetic outcomes were largely subtle when key parameters were varied, with standard deviations overlapping in most cases. The two modeling approaches suggest different levels of genetic benefit given similar levels of inferred immigration. For both models, the consequence of complete demographic isolation and of one migrant per two wolf generations, especially at small carrying capacities was a substantial loss of genetic variation and increase in mean inbreeding, even if trajectories in the model (and seen empirically) suggest a decline in mean inbreeding during the early years post 2012. However, while the *pedigree + supplementation* model suggests that one effective female immigrant per three years would have a substantial positive impact on genetic diversity and inbreeding in the Scandinavian wolf population, regardless of carrying capacity (tested up to 700), the *allele frequencies + dispersal* model suggests that at a carrying capacity of 700 wolves, a small decrease in genetic diversity and increase in inbreeding coefficient would arise (albeit by less than 5%) over fifty years (Tables 7, 8 and Supplementary Tables 3, 4).

If these models represent extreme ends of a ‘genetic rescue spectrum’, then immigration rates of less than one effective migrant per three years in a population with carrying capacity of less than 500 individuals could result in a decline in gene diversity and concomitant increase in inbreeding coefficient, and this could be more pronounced over 100 years (approximately 20 wolf generations; **Table 9a,b**). Comparing the two model outcomes for one immigrant per six years with a carrying capacity of 300, The *pedigree + supplementation* model predicted an improvement in genetic diversity (decline in mean inbreeding coefficient of 0.017, increase in mean gene diversity of 0.011) while the *allele frequencies + dispersal* model predicted a deterioration (increase in mean inbreeding coefficient of 0.023, decrease in mean gene diversity of 0.034).

A confounding issue for both models is the concept of *effective* immigration as opposed to demographic immigration. According to the data available for 2008–2012, recent effective immigration may at best amount to 50% of demographic immigration, implying, since no immigration related mortality was modeled here, that demographic immigration may need to be double that inferred above (i.e. 2 demographic migrants per three years for carrying capacity 500 and above).

Further potential refinement of the modeling

In ideal circumstances, a combination of pedigree and allele frequency data would be used, but this functionality is not currently available in Vortex, and no pedigree data are available for the Finland/Karelia population. In addition, inbreeding depression can only be universally applied and since modeling this parameter dramatically increases the computational intensity of simulations, this limits capacity in processing complex models and thus the number of parameter combinations that could be computed here with the resources available to the author. Moreover, while inbreeding is known to be a problem in Finnish wolves (Jansson et al. 2012), its magnitude and effects have yet to be reported. An additional observation (Åkesson, pers. comment) is that the offspring of immigrant wolves in Scandinavia have a higher probability of reproduction than the offspring of residents. Future modeling could include this demographic process, if it is accurately quantified – it was not included here. A prediction of the inclusion of this process in the model is that the genetic benefits of immigration would be higher, increasing population viability, perhaps more in line with the predictions of the *pedigree + supplementation* model.

Two potential future modeling approaches could be taken – first a specific wolf model could be elaborated not using Vortex, including all genetic requirements described above and with the potential to incorporate pack structure more explicitly (including spatially) as pointed out by one of the reviewers. Second, Vortex may be modified to circumnavigate some of the limitations described. Discussions with RC Lacy during this process have already resulted in modifications in version 10.0.7.4 that now includes the possibility to incorporate individual molecular genotypes in pedigree files (ultimately not used here due to computational capacity issues).

Effective population size, favourable conservation status and minimum viable population

The genetic effective population size (N_e) can be measured in a number of ways, both from demographic and genetic data. For both demographic and molecular estimates, temporal variation is extremely important because N_e is strongly influenced by a population's lowest numbers and is consequently usually measured as a harmonic mean. In Scandinavian wolves, this is especially relevant because the population was founded by two individuals, has received only three additional genomes subsequently and is currently carrying a mean inbreeding coefficient of approximately 0.25. Thus any N_e estimate based on the number of breeding males and females in the population needs to account for past population demography. Bensch et al. (2006) estimated an effective size for the Swedish wolf population of 45 using molecular data when the census size was approximately 140 individuals and it was subsequently reevaluated at 24% of census size using individual-based modeling (Forslund 2009). Using these two N_e/N_c ratios (N_c = census population size), the effective size of the Scandinavian wolf population in 2012 might have been 100–134 (see also Rääkkönen et al. 2013, who inferred N_e = 80).

Vortex estimates effective population size (N_e) from the loss of gene diversity from year 1 to the last year. Note that this N_e is the size of a randomly breeding population each generation (i.e., tallying only the adults, not the juveniles that constitute the next generation) across the generations. This will be approximately the harmonic mean of N_e at each generation, and will be less than an arithmetic mean N_e across generations if the populations are growing, declining, or fluctuating in size over time. Under conditions of high gene-flow where differences in heterozygosity were very modest, results were inconsistent, especially for the *pedigree + supplementation* scenarios and for all scenarios with high immigration rates. For the *allele frequencies + dispersal* models where immigration was modest (here I report on one female per three and six years), stable estimates were obtainable, but it should be noted that estimating N_e for populations receiving even modest numbers of immigrants can be complex, especially where immigrants originate from a large population (e.g. Mills and Allendorf 1996). For one immigrant per three years, N_e/N_c estimates were 463/697 ($k = 700$; ratio 0.66), 333/495 ($k = 500$; ratio 0.67) and 255/297 ($k = 300$; ratio 0.86). For one migrant per six years, N_e/N_c estimates were 370/694 ($k = 700$; ratio 0.53), 257/489 ($k = 500$; ratio 0.53) and 236/297 ($k = 300$; ratio 0.79). For no immigration, estimates were 219/686 ($k = 700$; ratio 0.32), 180/499 ($k = 500$; ratio 0.36) and 115/297 ($k = 300$; ratio 0.39). These estimates indicate that under modest migration rates over 50 years, N_e could increase from its current top range estimate of 134 to 250–350 assuming a carrying capacity of 500. N_e can also be measured using the formula:

$$\frac{\bar{H}_t}{\bar{H}_0} = \left(1 - \frac{1}{2N_e}\right)^t$$

where \bar{H}_t is the final gene diversity, \bar{H}_0 is its starting value and t is measured in units of 5 years (an estimated wolf generation). Using this approach and the mean population size data in Table 9b, estimates of $N_e:N_c$ are substantially lower, for example for $k = 300$ in the absence of immigration, the ratio is 0.246 and for $k = 700$ is 0.224.

The issue of the establishment of the Scandinavian wolf as a Favourable Reference Population (FRP) is a key issue for SEPA. It is generally agreed that FRPs entail the retention of genetic diversity and population viability over a longer time-scale than is required for a Minimum Viable Population (MVP). Laikre et al. (2012), following from a recommendation of an international expert panel (Hansen et al. 2011) suggested that to attain Favourable Conservation Status the population needed to attain a mean inbreeding coefficient ≤ 0.1 and a N_e value for the region (Sweden, Norway, Finland and Karelia) of 500–1,000. Provided true demographic connectivity is established/maintained between these regions in northern Europe, these targets would appear to be achievable using the modeling results from this exercise and assuming that the population in Finland stabilizes and the population Karelia is maintained. However, the lowest mean inbreeding coefficient range achieved was 0.14–0.15 for Scandinavia, and involved the effective immigration of 2 females and 2 males per three years at a range of carrying capacities with the *pedigree + supplementation* model. Another criterion that has been applied is the retention of 90, 95% or 99% of extant heterozygosity for a defined period of time (periods including 10 generations, 100 years and 200 years have been used). None of the simulations that did not include immigration achieved this threshold. However, none of the simulations involving gene-flow with either model predicted declines of more than 5% of extant heterozygosity over 50 years. The worst-case scenario of one immigrant every 12 years at $k = 300$ for the *allele frequencies + dispersal* model still predicts the retention of 95.57% of starting heterozygosity at the end of 50 years. However, for the limited subset of 100-year simulations carried out, the above simulation resulted in retention of initial gene diversity of only 92.5%.

In 2012, SEPA proposed that a minimum FRP would at least have to fulfil a genetic MVP as equating to 417 individuals (with a likely N_e of 100–134) provided there was an effective immigration rate of ≥ 3.5 individuals per generation (approximately 2 individuals per three years if we assume a 5-year generation time). Analysis using the *pedigree + supplementation* model implies that this would achieve an inbreeding coefficient in the range of 0.18–0.185 after 50 years for a variety of carrying capacities, and analysis of the *allele frequencies + dispersal* model provides a N_e estimate for a carrying capacity of 500 of 411.69.

The MVP concept has attracted, and continues to attract, a great deal of debate and controversy in the conservation genetics literature. It is important to note that MVPs are often primarily defined on the basis of demography, as opposed to genetics, and on the probability of persistence of a population (e.g. 1% extinction risk over 40 generations, Reed et al. 2003), although it is worth noting that it is not clear whether this value is arrived at to mitigate against short-term extinction risk or long-term viability (they suggest a target popu-

lation size of 7,000 adults to ensure long-term viability). However, genetic parameters are also often cited as key indicators within MVP definitions. Traill et al. (2007, 2010) showed that while approximately 60% of MVP estimates include genetic parameters in their estimation, nearly all will underestimate its impact on population persistence, primarily due to limitations on the inbreeding models used. In terms of N_e , MVPs have often been defined by the ‘50/500 rule’, which states that a short-term N_e of > 50 is needed to avoid inbreeding depression and a long-term N_e of > 500 is needed to maintain a population’s evolutionary potential (Franklin 1980). Jamieson and Allendorf (2012) have recently argued that these two figures have very different derivations, with the 50 value coming from the results of animal breeding experiments when mean increasing inbreeding coefficient 2–3% per generation compromised trait gain, whereas the long-term N_e of 500 being a value used as a threshold where additive genetic variation being lost due to genetic drift is replaced by mutation. In terms of population sizes to be maintained, the 500 value would need to be scaled by the N_e/N_e ratio such that a population with a ratio of 0.1 (not uncommonly observed in the literature) would require the maintenance of 5,000 individuals. Jamieson and Allendorf (2012) conclude by stating that MVPs of 500 represent “a long-term aspirational goal for maintaining healthy and genetically robust populations”. This assertion has triggered a flurry of recent publications (e.g. Frankham et al. 2013), including a suggestion to double these values to 100/1,000 (Frankham et al. 2014 and responses), as suggestion that has been both criticized and supported (Jamieson 2014; Rosenfeld 2014), based on its theoretical underpinning and the need to provide pragmatic and realistic targets in a conservation context.

The Scandinavian wolf’s current N_e almost certainly lies between 50 and 500, as a result of recent immigration, and currently mean population inbreeding coefficient is declining (Åkesson, pers. comm.), a trend that was also evident in both kinds of Vortex model reported (Figure 9a, Figure 12), albeit that this trend is only predicted to last 8–12 years (approximately 2 generations) before increasing, in the absence of further immigration. Thus to obviate the effects of inbreeding on population recovery, the maintenance of effective immigration therefore remains crucial.

The scale and rate of immigration depends on when the target of a N_e is required to be met, and how large the Scandinavian wolf population is allowed to become. Using the N_e estimates from Vortex for the *allele frequencies + dispersal* model, a N_e value of 500 for Scandinavia alone may require a carrying capacity of greater than 700 wolves to be achieved within 50 years, assuming modest immigration rates (e.g. one effective migrant per three years). Such a carrying capacity may not be desirable or achievable for the Scandinavian population: at a carrying capacity of 500, achieving a N_e of 500 would assume a 1:1 $N_e:N_e$ ratio, and using the SEPA FRP criteria, the N_e predicted at 50 years was estimated using Vortex as 412. Under certain conditions, $N_e:N_e$ ratios can exceed 1, which means that even populations below 500 could in principle reach a N_e of 500 and approaches that could be used here include ensuring very high rates of effective immigration and/or direct management of breeding within the Scandinavian population using, for

example, selective translocation to maximize genetic diversity and minimize inbreeding. Such scenarios were not explored here and would have required the implementation of a pack-structure model that was decided against at the Mid-term review. It is worth reiterating that most researchers view the N_e of 500–1,000 to refer to metapopulations, of which the Scandinavian wolf is just one component. Future research and FRP status recognition should be predicated on understanding what proportion of the metapopulation effective size for the northern European wolf is represented by wolves in Scandinavia.

For most of the modeling, and due to computational limitations I used 50 years for the simulations, which approximately equates to ten wolf generations. As a response to reviewer requests I was able to model changes in genetic diversity for a subset of the previous *allele frequencies + dispersal* scenarios for 100 years, focusing on the full range of immigration scenarios modeled at a carrying capacity of 300 and one immigrant per three years for carrying capacities at 700, 500, 400 and 300. Tables 9a and b below summarise the outcomes of these simulations. As can be seen from Table 9a, under conditions of one effective migrant per three years, greater than 95% of initial gene diversity (cf. Allendorf and Ryman 2002) is retained for all carrying capacity values. At a carrying capacity of 300, less than one effective immigrant per six years results in less than 95% of initial gene diversity being retained and less than one immigrant per three years resulted in the mean inbreeding coefficient exceeding 0.3.

Table 9a. Key outcomes at 100 years using the allele frequencies + dispersal model and carrying capacities of 700, 500, 400 and 300 for one immigrant per three years.

Scenario	Stochastic r	Mean Pop size	Gene diversity	SD (GD)	Percent GD retained	Inbreeding coeff	Mean final alleles
k = 700	0.354	694.0	0.701	0.021	97.1	0.292	13.89
k = 500	0.347	480.2	0.703	0.020	97.4	0.292	12.43
k = 400	0.343	400.8	0.702	0.027	97.2	0.293	11.37
k = 300	0.335	297.4	0.697	0.027	96.5	0.299	10.76

Table 9b. Key outcomes at 100 years using the allele frequencies + dispersal model with a carrying capacity of 300 for a range of immigration rates and at 417 for two immigrants per three years.

Scenario	Stochastic r	Mean Pop size	Gene diversity	SD (GD)	Percent GD retained	Inbreeding coeff	Mean final alleles
Six immigrants/ 3 years	0.353	297.8	0.730	0.016	101.1	0.266	14.23
Three immigrants/ 3 years	0.343	299.9	0.710	0.026	98.3	0.285	12.32
Two immigrants/ 3 years K=417	0.357	414.0	0.730	0.016	98.3	0.265	15.56
Two immigrants/ 3 years	0.338	294.8	0.709	0.022	98.2	0.286	11.62
One immigrant/ 3 years	0.335	297.4	0.697	0.027	96.5	0.299	10.76
One immigrant/ 6 years	0.329	296.5	0.688	0.027	95.2	0.307	9.56
One immigrant/ 12 years	0.323	291.3	0.668	0.034	92.5	0.327	8.12
No immigration	0.316	293.3	0.628	0.038	87.0	0.369	5.69

SEPA's original questions

1. *How strong is the effect of modest numbers of effective immigrants at population sizes of 200–300? How many wolves per generation will prevent significant inbreeding or loss of variation?*

Both modeling approaches predict that one effective immigrant per three years (1.6 per generation) will retain gene diversity at a level greater than 95%, even over 100 years (Tables 7, 9a,b). The predicted impact on mean inbreeding coefficient at this level of immigration varies from a decline of 20% from the starting value (to 0.22 *pedigree + supplementation*, 50 years, Table 8) to an increase of 10% from the starting value (to 0.299 *allele frequencies + dispersal*, 100 years, Table 9b). In the two further customised models, both *allele frequencies + dispersal* and *pedigree + supplementation* models identified that the immigration parameter explained genetic diversity outcomes more strongly than population size (here approximated by carrying capacity). Hence, effective immigration seems to be the key parameter to maintain genetic diversity in Scandinavian wolves.

2. *In 2012 the SEPA proposed a genetic MVP as a minimum value for FRP for the Scandinavian wolves at ≥ 417 combined with effective immigration of ≥ 3.5 reproducing wolves per generation. Does modeling with the Scandinavian population with immigrants as genetically dissimilar as the Karelian and Finnish wolves confirm the result of this assessment?*

At a carrying capacity of 417 wolves, modelled with 2 immigrants per 3 years over 100 years, the *allele frequency + dispersal* predicted retention of 98.3% gene diversity over 100 years, along with a decline in inbreeding coefficient to 0.265 (by 2.6%), and retention of relatively high allelic diversity (15.56 alleles; Table 9b). Thus if this level of effective immigration could be maintained, the genetic parameters measured using Vortex all indicate that maintenance of genetic diversity, a key MVP parameter, would be achieved.

3. *Given the Article-17 guidelines, their reference to Laikre et al. (2009), the Swedish environmental objective “A rich diversity of plant and animal life” which states that “Species must be able to survive in long-term viable populations with sufficient genetic variation”, plus the fact that the present-day Scandinavian wolf population was re-established with founder contributions from 3+2 immigrants until 2009, what are appropriate genetic criteria for FRP from a scientific point of view?*

This question is extremely difficult to answer and at the least critically depends on what fraction of the total effective population size (N_e) of the Scandinavia/ Finland/ Karelia metapopulation that the Scandinavian population represents, since the metapopulation can be considered to be the unit of assessment for FRP status. The effective size of the Scandinavian population

is approximately 100 currently and 500 is usually considered to be a lower threshold for populations retaining evolutionary potential. Therefore, as a minimum N_e needs to be estimated for the Finnish and Karelian subpopulations before this criterion can be specified.

4. *The workshop 26 April put emphasis also on maintenance of rare alleles as important genetic aspects of “viability”. Under what combinations of immigration and population size(s) are sufficient amounts of genetic variation also in terms of rare alleles maintained in the population?*

Rare alleles are lost more rapidly than gene diversity (or heterozygosity) in small populations. In the simulations, one immigrant per three years maintained 96.5% of initial heterozygosity for a population size of 300 and 97.1% for a population size of 700, a difference approximately 1%. However, the difference in mean number of retained alleles was 28% (10.8 *versus* 13.9; **Table 9a**). Further, for a population size of 300, there was a 2.5-fold difference in the number of retained alleles when comparing zero immigration with six immigrants per three years (**Table 9b**).

The role of rare alleles in population viability is highly debated but may have most relevance in genes with high allelic diversity, for example those important in immunity such as the Major Histocompatibility Complex (MHC; Schwensow et al. 2007). However, in such populations, allelic diversity can be maintained by selection and therefore may not decline the rate predicted under a ‘neutral’ model of genetic drift and inbreeding. Previous analysis suggests that MHC variation in the Scandinavian wolf has followed neutral predictions (Seddon and Ellegren 2004), but more recent data from Finland/Karelia suggests that balancing and allele specific selection is maintaining genetic diversity (Niskanen et al. 2014). However, since it is impossible to anticipate the selective forces that might favour or disfavour certain alleles in the future, it is important to maintain allelic variation wherever possible, despite the fact that this is a very difficult challenge in small populations. Future genomic studies of the Scandinavian wolf should be able to document allelic diversity across the genome and study those allelic diversity for genes underlying fitness in the population.

5. *In a recent bill to the Swedish Parliament, the Swedish government proposed an interval ($170 \leq FRP \leq 270$), within which the Swedish EPA will be commissioned to decide about the exact value for FRP. How can a number in that interval, in combination with a certain rate of immigration, fulfill FRP criteria that can be derived from the Article-17 guidelines?*

This question is very similar to Question 3 in that the fraction of the northern European/Russian wolf metapopulation represented by Scandinavia is a key parameter in this assessment and needs to be measured first.

Acknowledgements

I would like to thank Dr Per Sjögren-Gulve (SEPA) for his assistance, patience and good humour during this process and for organising the production of this report. I would also like to give a special thanks to Mikael Åkesson (Swedish University for Agricultural Sciences) and the Skandulv group for the provision of unpublished genetic and pedigree data for the modelling: without this information I would not have been able to integrate meaningful genetic parameters into the model. Guillaume Chapron provided updated demographic information for the model. Bob Lacy (Chicago Zoological Society) provided guidance on Vortex assumptions and capabilities and even provided an updated version of the software after some of our discussions. The Mid Term Review Group (the scientists: Mikael Åkesson, Torbjörn Nilsson, Guillaume Chapron, Øystein Flagstad, Olof Liberg, Johan Månsson, Nils Ryman, Håkan Sand and Henrik Andrén, the SEPA: Per Sjögren-Gulve, Mark Marissink, Klas Allander, Magnus Kristoffersson, Helene Lindahl and Marie Larsson) provided invaluable guidance at a critical juncture and Torbjörn Nilsson was especially helpful in revision of the final version of the report. Cardiff University and my colleagues in the School of Biosciences allowed me both to take the time and computational capacity needed to complete this project.

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Appendix 1

Summary of comments provided on Mid-Term Report and discussions during the mid-term review

OL: Olof Liberg, **GC:** Guillaume Chapron, **NR:** Nils Ryman, **MB:** Mike Bruford, **TN:** Torbjörn Nilsson, **P S-G:** Per Sjögren-Gulve, Comments also provided for or at the meeting by Håkan Sand, Mikael Åkesson, Øystein Flagstad,

Parameters:

For litter size we should use the intercept value from the OL paper, which is 6.5.

We will revisit the mortality estimates (OL and GC) and provide new values with standard deviations (as opposed to CIs). Guillaume thinks adult mortality should be approx 24%. OL and GC will attempt to provide these values as soon as possible.

For carrying capacity we will assume something in the region of 2,000 for Scandinavia and a much larger value for Finland and Karelia (total of 8,000 but I did not catch how this should be divided between the two populations).

Generation time should be 5.3 years.

Lambda, back corrected for hunted animals, should be 1.21 (since the year 2000, 1.19).

We agreed that LEs of 6.04 was a minimum estimate and that sensitivity testing should be used to explore higher values to account for the period between December and the following May when additional mortality may occur. We also agreed to explore the modelling of additional inbreeding depression due to the lower probability of pairing that has been seen for inbred adults. Mike will explore the options for how to parameterise this in the model.

Mike will sensitivity test a range of lethal recessive % values, ranging from zero to one, since we have no idea what is correct for wolves. Mike and all will have another look in the literature to see what other values have been reported for similar species.

We agreed that on balance although there are some interesting properties of the pack model (NR, MB) that it was an unnecessary complication and that the social structure was largely captured by the use of long-term monogamy as the mating system (TN).

Natal pack dispersal starts from 10 months in the first year of life and is usually complete by the end of the second calendar (not model) year (OL) – dispersal should be modelled as 2/14 for 0–1 and 12/14 for 1–2.

Catastrophes should be modelled using information from Denis Murray's paper in Animal Conservation that reviews carnivore die-offs. Frequencies of catastrophes should be modelled from 1–5% given the 30 years that have elapsed of the population's existence. Candidate diseases are CDV and parvovirus but not mange (OL).

Model Specification:

With the new parameters Mike will:

1. Model the past population again including structured sensitivity testing (PS-G) for the parameters described above in the intrinsic model. Using correct SD values and much longer runs, Mike will re-evaluate the population size, P(E) and genetic diversity trajectories and their standard deviations.
2. Construct a model that simultaneously estimates the population trajectories for the Scandinavian, Finnish and Karelian populations, including known dispersal into Scandinavia, testing F and K as alternative sources, and using allele frequencies for 10 loci that have been randomly sampled from the more extensive data provided by MA (this sampling should probably be done 5 times minimum). Disperser genotypes should be sampled from within the donor population allele frequencies to control for over-estimating genetic differentiation of the dispersers. We know quite a lot about the history of the Finnish population (found in Aspi?) but next to nothing about the Karelian population that is itself in genetic contact with a much larger population to the north.

Appendix 2

Summary of first reviews

It would be informative to have the average population size during the replicates of a simulation reported in the output. It might be better to simulate the various population scenarios choosing a K (ceiling) not equaling the “FRP” (or MVP) – because this might entail an average size being less than K due to the capping combined with stochastic demography – but adjusted so that average population size over time among the replicates, when simulated without catastrophes, is at or above the MVP value in question. ...adjust the K values for the various simulations so that average N of the replicates is as close to the (for example,) 200, 300, 400, 420, 450, 500, 600, 700

Since you have modelled the immigration solely with Vortex’s supplementation subroutine, the immigrants are demographic immigrants and not genetically effective ones.

The parallel modelling of a Karelian and Finnish wolf population(s) that are donor population(s) to the Scandinavian population is necessary.

The initial subpopulations in the modelling will have a subpopulation-F which is around 0.20–0.25 for the Scandinavian population and probably 0 for the interconnected Karelian and Finnish ones.

It would be good to know if the retention of at least (or more than) 90% of the gene diversity (Balmford et al. 1996, Frankham et al. 2013) or of at least (or more than) 95% (Allendorf & Ryman 2002) in a subpopulation is a recommendation or demand from conservation geneticists, or is it $N_e \geq 500$?

All simulations should be (re-)run with “Long-term monogamy”.

The initial population should be chosen at a starting year when the F is around 0.25 or/and with an initial population provided through the Genetic management option to include it from a stud book.

Scandinavia had 12 spontaneously immigrating wolves from Russia and/ or Finland during 2002–2009 (1.5 per year), but before 2008 none of them reproduced because they were killed. Thus, it would be good if your Vortex simulations cover demographic immigration rates up to 7 or 8 per 5-yr-period.

What would be appropriate retention of genetic variability for a (sub)population with the demographic history of recolonization as in the Scandinavian wolves’ case.

It would be good to have some N_e/N estimates.

How many replicates are used here and elsewhere?

The review of O’Grady et al. seems flawed. They appeared to have left out many estimates of low inbreeding depression, even for other traits in the same studies they cite.

The recommendations of Frankham et al. (2013) are unrealistic in many situations, because many endangered species populations have either been started with less than 20 individuals (not N_e) or have gone through bottlenecks of less than 20.

No confidence intervals/ measures of variation are given for any of the simulation data.

Also, it must be more fully and clearly emphasized that the PVA models effective immigrants- it seems that in the real Swedish population few real immigrants actually reproduce and therefore do not contribute genes.

A spatial model or a pack model should be a high priority for future work, and if knowledge is missing on the proper parameters, than research to garner such information is urgent.

The genetic criteria for FRP are discussed only vaguely. A general recommendation for prevention of inbreeding (<10%) and retention of alleles (>90%) is given, although the latter feature is more or less arbitrary.

I think a small section explaining the limitations of the simulations and of the PVA approach in general is important.

Questions 3 and 5, which refer to the Article 17 guidelines, are not handled in the discussion of the results.

Some of the results are not in accordance with basic population genetics expectations and also contradicts what actually has been observed in the population.

The study does not provide an estimate of minimum viable population size.

The author should address this question (#3) and discuss what are the “appropriate genetic criteria for FRP”, given the results of the analyses.

The issue of “sufficient amounts of genetic variation” in the context of maintenance of rare alleles is still left largely unanswered in the text. For example, it is still unclear what importance rare alleles have for populations in the short and the long term perspective. Bruford refers to Frankham et al. (2013), in which it is argued that “an effective population size of greater than 100 is required to limit loss in total fitness to 10%”. This is the revised recommendation of population size required to avoid inbreeding depression in the short term (ca 5 generations). The importance of rare alleles is taken into account first when they argue for a revised recommendation of $N_e > 1,000$ for long term maintenance of evolutionary potential. Moreover, it might be worth mentioning that the recent publication by Jamieson and Allendorf (2012) argue for the applicability of the 50/500 rule.

To answer this question it is vital that the FRP criteria are discussed and defined. The author refers to Balmford et al. (1996), where “...retention of 90% initial heterozygosity has been regarded in the literature as a minimal requirement for maintenance of genetic diversity...”. How does this relate to the Article 17 guidelines.

We are very puzzled by the development of the inbreeding coefficient in the population under a scenario of adding several immigrants per generation (Figure 5, Table 3).

Demographic parameters are well presented in Table 1, but I lack for each figure values like initial population size (and structure), in particular for genetic aspect and also how simulations were performed exactly (e.g. capping populations and at which values).

The finding that under modest migration rates, population size plays a very important role in the maintenance of genetic diversity, is important and deserves being discussed in more detail; I suggest comparing this result with those of Liberg & Sand (2012) and adding a discussion on what may explain the apparently contradicting results.

It should be mentioned that the short-term genetic MVP criterion discussed so far in Sweden is maintaining 95% (not 90%) of gene diversity over 100 years.

I think it would be valuable if the author could elaborate a little on how goals or criteria regarding maintenance of a certain percentage of genetic diversity can be relevant to a population that receives immigrants from a larger and more diverse population.

What are appropriate genetic criteria for FRP? Answering this question should include

- *noting that the Article-17 guidelines quote the EU Commission’s definition of FRP, which says that FRP is the population considered the minimum necessary to ensure long-term viability,*
- *noting that, as pointed out e.g. by Laikre et al. (2009), there is a broad unanimity that at least $N_e > 500$ is required for long-term viability,*
- *providing an estimate of N_e/N for the population.*

Appendix 3

Peer-review statements

SEPA's introductory comment: Below are the final comments of each respective person or group that participated in the peer-review process of this report.

Prof.s Mikael Åkesson, Olof Liberg & Håkan Sand (Swedish Univ. of Agricultural Sciences – Grimsö) and prof. Øystein Flagstad (NINA):

SUMMARY OF COMMENTS

We think the current report (dated 7th April 2015) has been significantly improved as compared to the previous version (dated 29 December 2014) and have provided many interesting results that may be important for the management of the Scandinavian wolf population. Especially the additional modelling effort included and the elaborated discussion has resulted in a much clearer message of the achievements of the work.

However, the report is now quite massive and we think it is quite demanding to read for people that do not have a good background on inbreeding and its effects in small populations. In particular, we still find the disposition of the report confusing to read as the author has included results from several versions. We acknowledge the intension of the author to do this in order to give the readers the opportunity to follow the chronological development of the report. But for the sake of clarity it should at least be clearly marked where the new and final version one starts. We suggest that the final version of the report only contain new and vital information similar to a revised manuscript sent to a peer-referee journal.

From what we can tell from the description of the modelling procedures the model simulations is based on the assumption that pair formation and mating between individual wolves occurs random with respect to genetic constitution and degree of genetic similarity. However, recent research results from the Scandinavian wolf population show that there is a higher probability for offspring resulting from immigrated individuals to pair and successfully reproduce in the population as compared to offspring from inbred individuals. This was also brought up and outlined as an important factor to be considered during the mid-term review but has so far not been included in the modelling procedures presented in the final report. It is good that this is now mentioned in the discussion and suggested to be included in future versions of the Vortex model. The author could also have indicated in what direction this observation likely would impact on the genetic situation of the population.

The results from the *allele frequencies + dispersal* model still show surprisingly small effects of immigration on the equilibrium state of inbreeding whereas the results of the *pedigree + supplementation* model show large effects of even small rates of immigration. These large differences in model results are surprising and puzzling and indeed hard to evaluate. Linked to this is the fact that the *allele frequencies + dispersal* model utilizes genetic data

from the founder population but it is not clear how the model use this information for calculating the level of inbreeding and gene diversity. We think it would have been good for the general transparency of the process if the properties of the model had been better described, including how inbreeding is calculated and what demographic parameters that could be applied for the populations. It is stated that the *allele frequencies + dispersal* model is demographically more unrealistic for Scandinavian wolf population. We find it difficult to evaluate the possible consequences of this, since the model is not described in much detail.

In our comments to the December 2014-version of the report we pointed out that the F-values given in Figure 2a and 3 (now figures 10a and b) are presumably close to the final equilibria between drift and migration, which also appears from the graphs. We would like the author to elaborate on the text why these values deviate so much from the values expected from the so called Island model. For example, the model result for 4 migrants/3 years is $F = 0.147$. The theoretical equilibrium value for this scenario is $F = 0.036$ with a generation time of 5 years, and $F = 0.045$ with generation time of 4 years. We understand that the scenario modelled is not the ideal situation of the Island model but the difference is so large, that we think it merits an explanation.

We would also like to comment on the form of the trend curves in Figures 5 and 6 (now Figure 13 and 14). As pointed out in the discussion the *allele frequencies + dispersal* model showed far higher stochastic growth rates even though 1,000 replicates were run. We think that possible explanations to the stochastic behaviour would need to be discussed. For example the blue curve (3 migr/3 yrs) in Fig 6 (now Figure 14) has a significant increase between year 8 and 10 and then from year 10 to 12 you have a significant decrease. For single cases this is expected, but for the mean of 1,000 simulations, it is not intuitive.

Discussion – the population has received only 3 additional genomes through natural immigration up through 2014. However, two additional genomes have been included into the population through translocation of immigrant individuals from northern Sweden in 2013 but this is not mentioned.

Prof. Guillaume Chapron (Swedish Univ. of Agricultural Sciences – Grimsö):

The report is a serious contribution to the difficult issue of the genetic aspect of the viability of the wolf population in Scandinavia. It uses the package VORTEX to address 5 questions asked by the Swedish Environmental Protection Agency. The results are clearly presented and the discussion of the results from the different models is well conducted. The report has well improved from previous intermediate versions. I assume most people will comment on genetic aspects so I will make just one (but important) comment on demographic aspects.

I find very awkward that with the *pedigree + supplementation* model, it is written on page 18 that “the mean final population size was 550 ± 237 (carrying capacity 700)” after 50 years and without immigration. I am sorry but I

think this indicates the model has a problem. The wolf population in Sweden is growing fast (corrected for harvest or not) and assuming an initial population size of 320, the VORTEX simulation would mean a growth rate of 1% per year. Also, with 320 animals, extinction probability should be close to 0 and a mean of 550 cannot be caused by numerous early stochastic extinctions. Scandinavian census data, estimates of population parameters and other population models all have shown a growth around 15%–20% per year. Where is this difference coming from? It is not the first time that I see VORTEX returning unexpected low population growth, could that be a consequence of the way inbreeding depression is modelled? Nevertheless, I believe it is important to understand why the population does not reach its 700-cap after 50 years, because this may simply suggest that the model has a fundamental issue and therefore none of its results can be used.

Prof. Philip W. Hedrick (Arizona State University):

Overall I think that this is an excellent study and meets the expectations of the comments suggested in the previous reviews.

In particular, this report refines the previous model in several ways. (1) First, it examines the effects of the amount of genetic load and the proportion of genetic load due to recessive lethals on both the population size and the inbreeding coefficient. (2) Next, it examines the effect of catastrophes on population size and the inbreeding coefficient. (3) Finally it examines the effect of immigration on genetic variation and inbreeding.

Overall, the study is properly done, and the expectation from the previous reviewers and the governmental agency appears to get precise answers, these results, and any other that would be produced, really should be used to only give general guidelines. In the natural population of Scandinavian wolves, we are dealing with only one replicate and the results here are means of many replicates, that is, they do not really present the variation over replicates. Also, it is assumed that biological details are known. In fact, the impact of inbreeding has great variation across the genome and migrants might change the population dramatically because they are very different from the present population.

- (1) Amount and type of genetic load
- (2) Catastrophes
- (3) Amount of Immigration

Overall these sections were well done and interesting. I have no comments for correction.

Phil Hedrick
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Dr Sean Hoban (University of Tennessee):

I have reviewed the revised final report (January 2015) on viability analysis of Swedish wolves. The main question from SEPA (Swedish Environmental Protection Agency) concerns the following: what immigration rates and/or population sizes are necessary to maintain genetic diversity and prevent inbreeding. The author used the simulation modelling approach Vortex to answer SEPA's main questions. Overall, I approve of the simulation approach and of the tests performed. I think the main conclusion is clear: a necessary minimum size of about 350–400 with 3 effective immigrants per generation. This should maintain currently agreed minimum levels of genetic diversity as well as prevent inbreeding. Specifically, inbreeding can be prevented and allelic diversity maintained only with population sizes ≥ 370 , or by having at least 3 effective immigrants per generation. Regarding whether " ≥ 417 combined with effective immigration of ≥ 3.5 reproducing wolves per generation" is sufficient- the answer seems to be a clear "Yes". I think the report makes a strong contribution and helps to quantify minimum population sizes and migration rates for the Swedish wolf population. This is a good model for other PVAs and a good foundation for further work for this population. In my previous review, I mentioned several points on which the report could be improved, and I now evaluate the revised final report relating to these suggestions.

My main concerns were as follows: (1) Confidence intervals were not shown for any of the Figures so it was not possible to assess if any situations differed significantly, and gave the misleading impression to the reader that the displayed values (e.g. inbreeding after 100 generations) are known to exact certainty. (2) The concept of effective immigrants vs. real immigrants was not sufficiently clear. (3) A spatial model with pack structure would be preferred. (4) Hunting loss was not included, and consideration of the large northern European wolf population was not clear.

- (1) The inclusion of confidence intervals on some of the Figures now makes it clear that for a number of scenarios (Figure 1, Figure 4b) the starting and ending values do not differ substantially, while for other scenarios (Figure 5, 6), the outcomes are quite distinct. This has been a useful improvement.
- (2) I think the author has now emphasized to a sufficient degree the distinction between effective and real immigrants (pages 3 and 4), though it is still not clear in all cases whether the recommendations refer to effective or real immigrants (e.g. on page 11 sometimes it is "immigrants" and sometimes "effective immigrants"). I think that to avoid confusion, any time the author means effective immigrants he should write "*effective immigrants*"— although this may be cumbersome it would probably be best for the reader, though this is only my opinion.
- (3) I understand the pack model is difficult, and I hope sometime in the future it can be performed. I think the author has argued sufficiently for this point in the final report.

- (4) Discussion regarding connection to the larger regional population is included. Also included are useful discussions of effective population size and genetic viability.

I have no major further suggestions and hope the report, which is excellent, can be useful.

Prof.s Eeva Jansson, Nils Ryman & Linda Laikre (Stockholm University):

We consider the present approach for exploring the genetic future of the Scandinavian wolf population appropriate and relevant, and we acknowledge the initiative of conducting this work and the efforts needed for completion of the study. The approach is to use currently best available biological information as a basis for computer simulations and a widely appreciated modeling tool, the software Vortex.

We also recognize the problems of this approach, reflected in the fact that software limitations does not allow the simultaneous use of all available information on the genetics of wolves in this area. This is reflected in the modeling of two separate basic scenarios described by the author as the *pedigree + supplementation* vs. the *allele frequencies + dispersal* models.

Key take home messages from this work appear to include the following.

- If left isolated the Scandinavian population is in poor genetic shape; with current population size limits of 300 for Scandinavia (SEPA 2013) the population is predicted to lose 13% heterozygosity, retain less than 50% of the alleles of a well-connected population of similar size, and have an inbreeding level of 0.37 within the next 20 generations.
- Low immigration rates of one genetically effective migrant per 12, 6, or 3 years will not solve the situation – inbreeding is predicted to increase to 0.3 or more, and 25–40% of the alleles maintained by a well-connected population are predicted to be lost. Thus, the empirically observed genetically effective immigration rate during the last 25 years, when 5 immigrant wolves have managed to reproduce in Scandinavia (equivalent to 1 per 5 years = 1 per generation), appears too low for short term conservation.
- A total of 3–6 genetically effective immigrants per 3 year period is needed to prevent further increase of inbreeding and also appears to retain alleles and heterozygosity in the short term perspective of 20 generations (ca. 100 years).
- If the population size is allowed to increase, however, less immigration is needed to prevent further loss of genetic variation and increase of inbreeding. For example, two genetically effective migrants per 3 year period from a large population are needed with a population maximum of 417.
- In neither of the above cases is the genetically effective size even close to the target of an effective size of $N_e = 500$ recommended for long term viability which is implied by FRP (Favourable Reference Population; Evans & Arvela 2011).

The work presented appears to be well conducted, and the results are significant, relevant, and of interest to the large audience concerned with this issue. Some points are important to highlight.

Time frames modeled – long- and short-term conservation

The modeling work focuses exclusively on time frames reflecting short-term perspectives with all the simulations run for only $t = 10$ or 20 generations (50 or 100 years), while several of the questions posed by the SEPA relate to population sizes needed for FRP (Favourable Reference Population). The FRP is understood as a “Population in a given biogeographical region considered the minimum necessary to ensure the long-term viability of the species” (European Commission 2006; Evans & Arvela 2011).

Short-term goals reflect different levels of ambition for management rather than biologically based assessments of the amount of genetic variation necessary for future evolution. For example, applying SEPA’s goal for short-term conservation, i.e. retention of 95% of heterozygosity over 100 years, only requires an effective size of $N_e \approx 195$ (Allendorf & Ryman, 2002) that does not meet the minimum criterion for long-term viability of $N_e \geq 500$. Thus, the long-term perspective is not modeled in this report, and the short-term genetic effects of the migration and population sizes may not apply to time frames reflecting FRP and long-term viability.

N_e/N_c ratio

The ratios of effective to census size (N_e/N_c) obtained using N_e -estimates from Vortex with immigration are large (0.53–0.86) and reflect the reduced depletion of heterozygosity obtained with immigration. This heterozygosity change is affected by both genetic drift (in the recipient and donor populations) and immigration. This means that the N_e computed from the observed change of heterozygosity reflects genetic processes of the system as a whole (both populations in this case). Comparing this N_e to census size of the recipient population only appears strange and does not really imply anything about N_e/N_c ratios in general. Rather, it reflects a mix up of the concepts of local (sub-population N_e) and global effective size ($N_{e,T}$; the effective size of a population system as a whole; cf. Hössjer et al. 2014, 2015; Ryman et al. 2014).

When the author computes the ratio N_e/N_c for isolated populations the estimate becomes lower (0.39 for $k = 300$ and 0.32 for $k = 700$, $t = 50$ years in both cases) and in line with the $N_e/N_c \approx 0.35$ of Andrén (2005; Vortex simulations).

We are concerned, however, over the discrepancy between the estimates of N_e generated by Vortex and those obtained when the author uses the formula in the section on Effective size of the Discussion and applies a generation interval of 5 years, which is typically assumed for the Scandinavian wolf population, including the present study. The N_e/N_c estimates of isolated populations then become 0.22 and 0.25. These ratios are in line with the 0.24 estimate reported by Forslund (2009; individual based modeling).

The discrepancy makes us believe that Vortex uses another (shorter) generation interval than five years, possibly the one that Vortex calculates from the demographic input parameters characterizing each particular model. The generation intervals applied in the different simulations are not presented, however. Thus, it is not possible to evaluate whether or not they differ from the assumption that a wolf generation is about five years, or if the difference is “acceptable” when evaluating how “realistic” a particular model appears (cf. below on validation).

Because of the above we think that the estimates of N_e and N_e/N_c generated by Vortex should be interpreted with caution. For example, we feel that the results from the *allele frequencies + dispersal* model are overly optimistic when suggesting that one immigrant per three years and a carrying capacity of $k = 700$ would result in an $N_e = 463$. When using the data in Supplementary Table 4 from this simulation, a generation time of 5 years, and the formula provided in the section on Effective population size of the Discussion we obtain $N_e = 299$.

Validation

The simulation results have not been validated through comparing them with expectations from analytical models (we have noted this in reviews of previous versions of this report). Such validation seems important considering the seemingly unexpected outcomes under some of the models. As noted by the author, for example, inbreeding appears to increase independently of population size under the *pedigree + supplementation* model (Supplementary Table 3), which is not expected under traditional population genetics theory. It is stressed that the results from this model should be treated with caution, and that they are retained in the report for comparative purposes. Perhaps these seemingly erroneous results should not have been included as they can lead to confusion. More important, though, is that this apparent inconsistency was fairly easy to detect from a summary table, whereas other potential errors may be more difficult to recognize in the absence of a systematic attempt to validate key outputs from the different modules of the program.

Similarly, under the *allele frequencies + dispersal* model it appears that inbreeding does not change as expected under a classical island model, and the author suggests that the discrepancy can be an effect of the chosen marker loci. We find it difficult to see, however, how the choice of loci could affect the rate of inbreeding as long as all loci are treated as selectively neutral. A better description of, and an analysis of the reasons for, this inconsistency between observed and expected rates of inbreeding seems warranted, particularly since the *allele frequencies + dispersal* model is considered the most “realistic” one for the present study.

Finnish and Karelian populations

The *allele frequencies + dispersal* model is said to use empirical estimates on allele frequencies in Finland, Karelia, and Scandinavia. However, information

is missing on the frequencies that were used, the number of individuals from which they were estimated, pairwise F_{ST} values between populations, diversity indices at different loci, and information on from where in Finland and Karelia samples originated (as we have pointed out in previous reviews). Further, all modeling assumes that the donor population in Finland/Karelia is large. In reality, however, these populations are fragmented and not very large. The most recent census estimates are for Finland ~ 220–245 (2015; estimate by the Natural Resources Institute, Finland) and for Karelia 300–350 (2013; numbers given in the Finnish media referring to Karelian hunting authorities). Both populations are currently harvested. In Finland wolf hunting was initiated again in 2015 (29 licenses issued, 17 of which were used; Finnish Wildlife Agency 2015; Finnish Ministry of Agriculture and Forestry 2015) e.g. in hope to reduce poaching, and in Karelia killing fee is still paid. In 2013 alone 101 wolves were reported shot in Karelia. Due to hunting and apparent problems of poaching in Finland, it is highly unlikely that these populations will come even close to their carrying capacities any time in the near future.

Moreover, studies by Aspi et al. (2009) and Jansson et al. (2012) showed that the Finnish and Karelian populations are genetically diverged ($F_{ST} \sim 0.1$) and can currently not be considered to represent one panmictic population. Gene flow between them is restricted. Minimum effective sizes for these populations have been estimated (in contrast to what is stated in the answer to SEPA's questions 3 & 5) to be in the range $N_e = 20$ –67 for Finland (with a declining trend; Jansson et al. 2012) and $N_e = 40$ –47 for Karelia (Aspi et al. 2009). In the Finnish population the ratio N_e/N_c was relatively stable during a population growth in 1995–2006 and approximately 0.28 (Jansson et al. 2012). It is noteworthy, however, that this ratio is not necessarily stable over longer periods: during the following population decline the ratio dropped to 0.097 in the Finnish wolf population. Similar observation of a surprisingly low N_e/N_c ratio in wolf has recently been reported from Bulgaria (0.12; Moura et al. 2014). It is possible that excessive hunting – whether being legal or not – can disrupt the complex social dynamics of wolf packs weakening the genetic status of a population. This can be especially likely if and when breeding alpha wolves are killed (Jansson et al. 2012; Moura et al. 2014).

Taken these facts together, it is clear that the Swedish wolf population, despite its current problems with high inbreeding, represents a highly important component of the total Fennoscandic metapopulation. This is even more so when the anthropogenic pressure towards wolves stays at such high levels in its neighboring countries and genetic connectivity between the populations is weak.

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Dr. Torbjörn Nilsson (County Administrative Board of Värmland, Sweden):

1. The design of the basic biological model is excellent. The author uses the best available and updated empirical estimates of most parameters, and to the most uncertain parameters, the author applies a clever and reasonable approach by exploring the effects of various combinations, comparing these to the known development of the population, and choosing the combination giving results best resembling the known population trajectory.
2. I agree with the author that for long-term survival and to conform to FRP status, the wolf in Sweden needs to become part of a sufficiently connected metapopulation or system of populations, stretching over several countries, which together achieve a genetically effective population size (N_e) of at least 500 (which likely corresponds to an actual population of a few thousand animals).

Which countries that should be accounted for when setting the FRP value is not self-evident, and this is a matter both of biology and of interpretation of the EU legislation. The author here follows Møller Hansen et al (2011) in suggesting that such a system of populations might be shared between Sweden, Norway, Finland and Russian Karelia. However, there are three relevant arguments for not including Russia or part of Russia in this context: that there is little knowledge about how continuous or fragmented the Russian wolf population is; that recent studies on wolves in Finland indicate reduced gene flow from Russia (Jansson et al 2012); and that Russia has not signed the Bern Convention on the Conservation of European Wildlife and Natural Habitats.

3. An interesting aspect of the results is the rather moderate effects of immigration on genetic viability parameters, which was found in simulations where the donor population was limited and had allele frequencies based on empirical data. Although the donor population was large (starting at 1,000 animals and capped at 2,000 animals) relative to the current Finnish population, more than one genetically effective immigrant per year was needed to make inbreeding coefficient decrease. In previously published attempts at modeling the viability of the Scandinavian wolf population while including immigration, the immigrants have usually been assumed to be completely unrelated, implying an infinite source population. The results of the present study clearly illustrate that such an approach will give over-optimistic results.

4. As mentioned by the author, estimating N_e for populations receiving immigrants can be complex. This deserves some further elaboration.

Genetically effective population size, N_e , is principally a measure of how fast genetic changes, e.g. drift, take place in a population. N_e is determined by many properties of the population, e.g. its actual population size (N_c), sex ratio, what proportion of the animals that reproduce, how reproductive success varies between the reproducing individuals, overlapping generations, and population fluctuations. The ratio N_e/N_c is a summarizing measure of how all population properties except its census size affect the rate of genetic changes.

For a closed population, the ratio N_e/N_c can be estimated by measuring (in a simulation or by repeated field sampling) how much genetic drift reduces its genetic variation per generation, expressing this drift rate in terms of N_e , and dividing by the total actual size of the population.

If the population is panmictic, we may sample only a geographic subset of it (e.g., the wolf population of Värmland as a subset of the Scandinavian population), and the rate of drift in the sampled subset will still be determined by the size and other properties of the whole population, so the N_e value found should then be divided by N_c for the whole population.

In both cases, we may use the estimated ratio N_e/N_c to calculate what N_c is required to meet a certain viability criterion expressed in N_e .

However, when the rate of loss of heterozygosity is measured in simulated populations which repeatedly receive immigrants from a donor population, the heterozygosity loss measured and hence the N_e calculated are determined by both the size and other properties of the recipient population, the size and other properties of the donor population, and the frequency of immigration from the donor population to the recipient population. This is in a sense an intermediate situation between the two described above, but in this intermediate case it is not self-evident to what extent N_e of the whole system should be used when calculating N_e/N_c . *Since the N_e calculated is then affected by the size and properties of the donor population, it may be misleading to divide that N_e value by the actual size N_c of only the recipient population*, as has been done in the present study. Therefore, I doubt if the strikingly high N_e/N_c estimated in that way (ranging from 0.53 to 0.86) can be interpreted in a meaningful way.

5. It is interesting to compare the N_e/N_c ratios calculated from the allele frequency model without immigration, ranging between 0.224 and 0.246, to the value estimated by Forslund (2009) with a wolf-specific model designed in MatLab, which gave the ratio 0.24. Forslund's model probably represents the life history of wolves more realistically than is possible with Vortex; at the same time, a weakness in Forslund's study was that effects of environmental stochasticity were ignored. Anyhow, the close similarity between the results of the present study and those of Forslund, although they were reached with very different modeling approaches, seems to me to strengthen the conclusion that N_e/N_c for the Scandinavian wolf population is in the vicinity of 0.22–0.25.

6. A general limitation when working with Vortex is that the source code is not published or directly readable, so the modeler cannot see exactly what the program is doing with the input data. As long as the resulting output is in line with theory, with other modeling efforts, and/or with empirical findings, we use to rely on the program being well established and thoroughly tested, and therefore credible. However, if unexpected results appear, and especially if some newly developed feature of the program has been used, we cannot exclude the possibility that there may be some error in the program.

As the author points out, there is reason to be concerned about the fact that when pedigree data were linked to Vortex, predicted inbreeding coefficient was completely unaffected by population size – even in the absence of supplementation. This is clearly seen in the first seven rows of Supplementary Table 3: for $k = 200$ and $k = 700$, inbreeding coefficient reached the identical value of 0.317, and for five intermediate k -values the resulting inbreeding coefficient varied randomly between 0.312 and 0.318. This absence of covariation between population size and final inbreeding coefficient seems to contradict all previous theory and modeling experience within the field.

Since there is no plausible biological explanation for this result, I fear the most likely explanation is that there may be some serious error in how Vortex handles the pedigree data. Therefore, at present I think no conclusions should be drawn from the “*pedigree + supplementation*” model.

7. Finally, in discussing the ratio between genetically effective immigration and total immigration, the author provides empirical figures for the years 2008–2012. It should be noted that this period is arbitrarily delimited, and that if more years are included, the ratio between genetically effective and total immigration will be even lower.

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Prof. Pekka Pamilo (University of Helsinki):

The reports include a combined demographic and genetic analysis of the Scandinavian wolf population using a Vortex simulation program. The program uses the available demographic parameters and stochastically explores the trajectories of population size and genetic diversity over generations. This

is a useful approach to predict the fate of endangered populations, including predictions on population extinction. The outcome of the simulations naturally depends on the input values, such as reproductive parameters, mortality and deleterious gene forms. Dr Bruford uses the available data published in earlier literature and suggested by the leading researchers in the field. When the information is uncertain (hard to get) or the results are sensitive to variation in the input parameters, a range of possible values have been used (e.g. concerning the proportion of recessive lethals contributing to genetic problems). This is a valid approach and the parameter values used in the models seem realistic to me and cover possible variation in an appropriate manner. The supplementary report complements the results of the final report by incorporating observational data from Scandinavian wolves into the simulations. One alternative model uses the known pedigree structure of the Swedish wolf population but assumes that the potential immigrants are genetically unique (carry unique alleles). The second alternative is based on the observed allele frequencies in the Swedish wolf population and assumes that the immigrants are drawn from a population which is characterized by the allele frequencies observed in Finland and Karelia. The use of observational data increases the realism of the models, even though it unfortunately is not currently possible to combine the two data sets (pedigree, allele frequency estimates) in a single model in Vortex. Yet, the models give good and useful insights on the effects of the model parameters (population size, number of immigrants). As the pedigree-based model assumes that the immigrants are genetically unique, the model overestimates the role of immigration especially as regards the level of genetic diversity. The outcome from “the *allele frequencies + dispersal* model”, suggesting that higher immigration levels are required to maintain a genetically diverse population, is therefore particularly important. As pointed out in the supplementary report, the results refer to genetically effective immigration which is much less than the actual number of immigrating individuals.

The reports discuss the limitations of the models, depending on the options available in the used Vortex software. The limitations are partly overcome by comparing results from alternative models and from different sets of parameter values. It is therefore justified to conclude that the obtained simulation results describe well the likely trajectories of demography and genetics of the Scandinavian wolf.

As shown by the results in the final report, the population trajectories depend strongly on the assumptions on harmful genetic effects. The total effect of such effects is summarized as the number of lethal equivalents, but it is not really known to what extent they include genetic variants with mildly harmful effects in a large number of loci or major effects in few loci. Genes with major effects should be purged effectively in small populations and this is shown by the simulation results. Realistic parameter values are evaluated simulating the past history of the Scandinavian wolf population with different genetic assumptions. The trajectories best agreeing with the realized population growth are those where only a moderate fraction of harmful effects are

based on recessive lethals. This makes sense and I find the conclusions reliable. Even though the simulation results show population growth when the carrying capacity is large, it is interesting to see that the extinction probability remains very high.

Evaluating the relevant parameter values has certainly been the most time-consuming part of the project, but the real aim was to predict the future fate of the population as explained in the five points on the first page of the report. If the future population size is controlled and set to a given value (ranging from 170 to 570 in the simulations), it is possible to predict the rate of loss of genetic diversity. Naturally, diversity is lost fastest in small populations and at the same time inbreeding level rises fast. The simulations demonstrate clearly that with the natural rate of immigration, the loss of genetic diversity and the increase of inbreeding level exceed the rates that are considered tolerable for natural populations. Such harmful genetic consequences can be compensated by supplementing the population with immigrants at a rate much higher than natural. The need for a high immigration rate becomes evident when one considers the results from the realistic “*allele frequencies + dispersal* model” and the difference between genetically effective and actual immigration. The alternative scenarios need to be considered properly as the simulations showed that the population growth strongly depends on genetic effects. If the population size is too small, the level of immigration required to maintain a viable and healthy population becomes too large to be practical and the goal of maintaining the population cannot be reached.

This is a very good study and highly important for planning practical management actions.

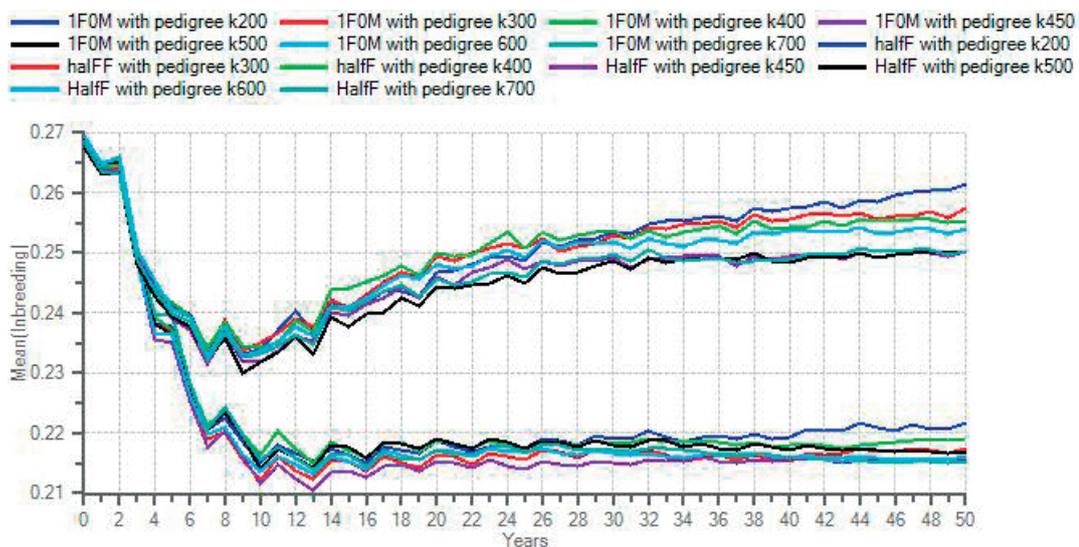
Supplementary material

Supplementary Table 1. The effect of modest numbers of migrants per generation on key mean genetic parameters after 100 years in simulations with monogamy and long-term monogamy (in parentheses \pm SD, 1,000 simulations).

	Proportion of gene diversity retained	Increase in mean inbreeding coefficient	Allelic variation
170 1 mig per gen	0.835 (0.836 \pm 0.055)	0.154 (0.151 \pm 0.063)	12.95 (12.86 \pm 2.45)
270 1 mig per gen	0.878 (0.878 \pm 0.032)	0.115 (0.115 \pm 0.041)	16.95 (16.85 \pm 2.68)
370 1 mig per gen	0.904 (0.904 \pm 0.022)	0.089 (0.089 \pm 0.023)	20.91 (21.02 \pm 2.94)
170 2 mig per gen	0.86 (0.856 \pm 0.047)	0.128 (0.132 \pm 0.056)	16.27 (16.10 \pm 2.83)
270 2 mig per gen	0.889 (0.889 \pm 0.031)	0.104 (0.104 \pm 0.037)	19.84 (19.98 \pm 3.08)
370 2 mig per gen	0.911 (0.910 \pm 0.022)	0.082 (0.084 \pm 0.029)	24.02 (24.05 \pm 3.31)

Supplementary Table 2. Vortex 9.99 output for the above model (Long-term monogamy) versus Vortex 10 output (parenthesis \pm SD, 1,000 simulations)

	Proportion of gene diversity retained	Increase in mean inbreeding coefficient	Allelic variation
170 1 mig per gen	0.836 (0.835 \pm 0.054)	0.151 (0.152 \pm 0.062)	12.86 (12.97 \pm 2.40)
270 1 mig per gen	0.878 (0.877 \pm 0.036)	0.115 (0.115 \pm 0.044)	16.85 (16.99 \pm 2.73)
370 1 mig per gen	0.904 (0.903 \pm 0.023)	0.089 (0.092 \pm 0.03)	21.02 (21.02 \pm 2.98)
170 2 mig per gen	0.856 (0.857 \pm 0.046)	0.132 (0.130 \pm 0.055)	16.10 (15.93 \pm 2.98)
270 2 mig per gen	0.889 (0.890 \pm 0.029)	0.104 (0.103 \pm 0.037)	19.98 (19.98 \pm 3.06)
370 2 mig per gen	0.910 (0.911 \pm 0.022)	0.084 (0.083 \pm 0.028)	24.05 (24.22 \pm 3.36)



Supplementary Figure 1. Inbreeding coefficient in the Scandinavian wolf population starting from 2012 pedigree values using carrying capacities of 200–700 and the P+S model with population supplementation of one female per three (1FOM) and six (HalfF) years. Means of 1,000 simulations are presented.

Supplementary Table 3. Key model results at 50 years for the Scandinavian wolf population starting from 2012 pedigree values with carrying capacities of 200–700 with population supplementation.

Scenario	Stochastic r	SD (r)	Mean Pop size	SD Pop size	Gene diversity	SD (GD)	Percent GD retained	Inbreed coeff
No supplementation k200	0.047	0.233	170.27	53.67	0.63	0.07	87.0	0.317
No supplementation k300	0.047	0.23	249.78	85.67	0.636	0.072	87.8	0.318
No supplementation k400	0.051	0.227	332.52	118.54	0.642	0.069	88.7	0.316
No supplementation k450	0.051	0.226	367.2	139.84	0.64	0.077	88.4	0.315
No supplementation k500	0.048	0.231	402.78	158.89	0.644	0.074	88.9	0.315
No supplementation k600	0.056	0.226	481.84	193.12	0.648	0.072	89.5	0.312
No supplementation k700	0.053	0.229	550.33	236.6	0.642	0.075	88.7	0.317
One female/12 years k700	0.094	0.228	627.88	169.9	0.711	0.058	98.2	0.272
One female/6 years k200	0.112	0.231	190.25	28.36	0.73	0.049	101.0	0.251
One female/6 years k300	0.112	0.231	284.38	46.86	0.736	0.047	101.7	0.25
One female/6 years k400	0.113	0.231	375.09	72.01	0.74	0.049	102.2	0.25
One female/6 years k450	0.113	0.231	422.04	81.48	0.744	0.047	102.8	0.246
One female/6 years k500	0.115	0.228	473.75	81.53	0.746	0.044	103.0	0.246
One female/6 years k600	0.113	0.23	565.83	101.74	0.742	0.046	102.5	0.259
One female/6 years k700	0.116	0.227	651.06	133.42	0.746	0.048	103.0	0.247
One female/3 years k200	0.139	0.23	192.86	23.14	0.769	0.04	106.2	0.223
One female/3 years k300	0.14	0.233	292.45	30.26	0.776	0.04	107.2	0.22
One female/3 years k400	0.139	0.233	389.65	41.06	0.776	0.038	107.2	0.223
One female/3 years k450	0.141	0.232	435.94	51.66	0.78	0.038	107.7	0.22
One female/3 years k500	0.14	0.232	483.75	63.64	0.779	0.039	107.6	0.222
One female/3 years 600	0.143	0.23	582.37	72.94	0.781	0.038	107.9	0.221
One female/3 years k700	0.142	0.23	679.06	85.92	0.782	0.037	108.0	0.221
One pair/3 years k200	0.171	0.241	196.01	19.31	0.818	0.032	113.0	0.184
One pair/3 years k300	0.173	0.24	292.35	31.99	0.821	0.032	113.4	0.184
One pair/3 years k400	0.176	0.231	390.87	42.65	0.823	0.03	113.7	0.185
One pair/3 years k450	0.176	0.235	438.02	49.65	0.823	0.031	113.7	0.186

Supplementary Table 3 continued.

Scenario	Stochastic r	SD (r)	Mean Pop size	SD Pop size	Gene diversity	SD (GD)	Percent GD retained	Inbreed coeff
One pair/3 years k500	0.173	0.237	487.42	56.13	0.822	0.031	113.5	0.186
One pair/3 years k600	0.176	0.233	589.18	53.41	0.825	0.031	114.0	0.184
One pair/3 years k700	0.175	0.233	684.94	71.11	0.823	0.033	113.7	0.186
Two females and one male/3 years k200	0.199	0.239	195.55	20.81	0.844	0.027	116.6	0.16
Two females and one male/3 years k300	0.2	0.237	294.53	27.93	0.847	0.027	117.0	0.162
Two females and one male/3 years k400	0.201	0.237	394.27	31.83	0.849	0.026	117.3	0.161
Two females and one male/3 years k450	0.202	0.236	440.84	44.71	0.849	0.027	117.3	0.161
Two females and one male/3 years k500	0.2	0.235	493.33	39.58	0.849	0.026	117.3	0.161
Two females and one male/3 years k600	0.2	0.239	587.2	61.33	0.849	0.026	117.3	0.161
Two females and one male/3 years k700	0.203	0.234	687.52	67.56	0.85	0.026	117.4	0.161
Two females and two males/3 years k200	0.214	0.245	195.63	20.54	0.863	0.023	119.2	0.143
Two females and two males/3 years k300	0.215	0.238	294.15	28.73	0.864	0.023	119.3	0.144
Two females and two males/3 years k400	0.215	0.236	392.6	34.67	0.865	0.022	119.5	0.146
Two females and two males/3 years k500	0.215	0.238	491.36	46.32	0.867	0.023	119.8	0.145
Two females and two males/3 years k450	0.216	0.237	442.93	38.2	0.868	0.024	119.9	0.144
Two females and two males/3 years k600	0.217	0.235	592.2	43.28	0.866	0.024	119.6	0.146
Two females and two males/3 years k700	0.216	0.236	689.84	55.48	0.865	0.024	119.5	0.147

Supplementary Table 4. Key parameter results at 50 years for the Scandinavian wolf population with the allele frequencies + dispersal model for carrying capacities 700, 500 and 300.

Scenario	Stochastic r	Mean Pop size	Gene diversity	SD (GD)	Percent GD retained	Inbreed coeff	Mean final Alleles
Six immigrants /3 years k700	0.367	691.41	0.728	0.013	101.0	0.271	24.75
Three immigrants /3 years k700	0.363	683.74	0.718	0.014	99.4	0.280	20.99
Two immigrants /3 years k700	0.366	677.76	0.717	0.015	99.3	0.281	19.93
One immigrant /3 years k700	0.368	696.72	0.710	0.014	98.3	0.287	18.68
One immigrant /6 years k700	0.366	693.61	0.709	0.014	98.2	0.288	17.64
One immigrant /12 years k700	0.367	689.5	0.706	0.017	97.8	0.291	16.11
Six immigrants /3 years k500	0.363	496.72	0.729	0.0157	101.0	0.267	21.4
Three immigrants /3 years k500	0.363	496.83	0.716	0.0154	99.2	0.28	17.95
Two immigrants /3 years k500	0.36	498.38	0.713	0.017	98.8	0.282	16.9
One immigrant /3 years k500	0.36	494.57	0.710	0.017	98.3	0.287	15.76
One immigrant /6 years k500	0.359	492.04	0.706	0.021	97.8	0.289	14.59
One immigrant /12 years k500	0.362	497.49	0.697	0.018	96.5	0.298	12.95
Six immigrants /3 years k300	0.36	299.69	0.728	0.015	101.0	0.268	17.82
Three immigrants /3 years k300	0.35	292.08	0.715	0.021	99.0	0.279	14.58
Two immigrants /3 years k300	0.352	298.48	0.710	0.017	98.3	0.283	13.82
One immigrant /3 years k300	0.352	298.85	0.702	0.022	97.2	0.291	12.72
One immigrant /6 years k300	0.351	296.84	0.694	0.023	96.1	0.301	11.34
One immigrant /12 years k300	0.343	292.94	0.69	0.024	95.6	0.303	10.04

Additional Population Viability Analysis of the Scandinavian Wolf Population

REPORT 6639

SWEDISH EPA
ISBN 978-91-620-6639-0
ISSN 0282-7298

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Rapporten är skriven på engelska med en svensk sammanfattning. Genom att använda simuleringsprogrammet VORTEX undersöker författaren hur den skandinaviska vargstammens genetiska variation och inavelssituation förändras över tid givet antal invandrande vargar från öster vid olika populationsstorlekar. Flera modeller används med ett nytt tillvägagångssätt att anpassa modellerna för att återge den redan observerade tillväxten och inavelssituationen. Resultaten från simuleringarna diskuteras i relation till frågor ställda av Naturvårdsverket och till vetenskapliga kriterier för minsta livskraftiga population (Minimum Viable Population) samt referensvärdet Favourable Reference Population. Rapporten utgör en del av ett underlag i Naturvårdsverkets rapportering av regeringens *Uppdrag att utreda gynnsam bevarandestatus för varg*.

The authors assume sole responsibility for the contents of this report, which therefore cannot be cited as representing the views of the Swedish EPA.

How is the Scandinavian wolf population's genetic variability and inbreeding situation affected by its size and various rates of immigration from a neighbouring eastern population? This was examined in a Population Viability Analysis project, based on updated demographic and genetic data and using more or less customised VORTEX models, that is reported here. The results are discussed in relation to questions asked by the Swedish Environmental Protection Agency and to criteria for Minimum Viable Population size and Favourable Reference Population. The report is part of the scientific material presented by the SEPA within the framework of a government commission in 2015.

