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Evaluation of the conservation genetic basis of management of grey wolves in Sweden

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Summary

The evaluation panel was asked to assess the scientific basis of conservation genetic recommendations for managing Swedish wolves, more specifically to evaluate views and hypotheses proposed by different researchers. In addition, the panel was asked to provide its own assessment of the population sizes needed for obtaining Favourable Conservation Status, as defined in the EU Habitats Directive, Article 17. Finally, the panel addressed other relevant questions as they emerged during discussions. The main conclusions and recommendations are as follows:

- Scandinavian wolves are highly inbred, there is evidence for deleterious genetic variation that will become expressed during inbreeding, and inbreeding depression must be considered an important problem. The panel supports the view that action should be taken as soon as possible to reduce inbreeding levels.
- The panel does not see a contradiction between the presence of inbreeding depression and the fact that the population has increased. This represents a balance between on the one side a beneficial environment with plenty of prey and a wolf population below carrying capacity favouring population growth, and on the other side inbreeding depression resulting in decreased reproductive performance. At present, the balance is still in favour of population growth, but if inbreeding continues to increase, the situation is likely to reverse.
- The major challenge is to reduce the high inbreeding. There is no other way to achieve this than by introducing new genetic variation, either by natural immigration of grey wolves from Finland or by artificial translocations.
- The panel supports a short-term goal that consists of reducing inbreeding from the current ca. 30% to below 10% over a 20 year period. If the current population in Sweden and Norway is maintained at a maximum of 240 individuals, then 5-10 genetically effective immigrants are required per generation. At this low population size immigration must be continuous. If a larger population size is allowed, then in the longer term fewer immigrants would be required to keep inbreeding below 10%, but the exact numbers would have to be determined analytically.
- The panel finds that obtaining Favourable Conservation Status will require a population size of at least 3,000-5,000 individuals. Wolves throughout Scandinavia, Finland and Karelia-Kola have historically constituted one continuous population, which has since been fragmented and reduced due to human activity. It is therefore most appropriate to focus efforts for obtaining Favourable Conservation Status on this geographical region. The panel consequently recommends transnational collaboration and agreements among Sweden, Norway, Finland and Russia with the purpose of securing a common grey wolf population consisting of 3,000-5,000 individuals. The individual sub-populations of the total population should be connected by migration at a rate to ensure that inbreeding is not a problem. It will require detailed analyses and discussions to determine the specific proportions of the total population to inhabit each country. The relative proportions of suitable habitat found in each country could be a good starting point for the assessment.
- The panel was asked to provide recommendations for a relevant measure of population size. The most relevant measure depends on the question asked. If it concerns the demography of the population, then the most relevant measure is the census population size (N), which is simply the number of individuals. If it concerns conservation genetics questions concerning inbreeding, gene

flow etc., then effective population size (N_e) is the most relevant measure. The ratio between census and effective population size can be used as a conversion factor. However, this ratio is difficult to determine precisely and conversions between census and effective population sizes should therefore be evaluated with caution.

- The panel finds it important to continue close monitoring of Scandinavian wolves. In particular, specific monitoring of potential inbreeding depression is recommended (e.g. physical deformities, changes of life-history parameters) and funding for this purpose should be available. The pedigree established for wild Scandinavian wolves is available on request and is already used in practical management. The panel finds that this is a particularly important tool that should be used for identifying individuals of immigrant ancestry or individuals that in other ways represent particularly valuable portions of the founder genetic diversity. These individuals should subsequently be protected from hunting.
- The panel finds it justified to use offspring of wolves from the captive Fennoscandian wolf population for artificial translocations. There is concern that some introgression with domestic dogs has occurred, but this can be assessed using genetic markers.
- The panel is impressed by the high quality of the science that has been conducted during the monitoring and assessment of the conservation status of Scandinavian wolves. Elucidating the pedigree of a wild carnivore population must be considered a unique achievement, and overall the conservation ecology and genetics research on Scandinavian wolves ranks very highly in the conservation biology sciences.

1. Assignment for the panel

In October 2010 the Large Carnivore Inquiry (Rovdjursutredningen) decided to invite an international panel of Conservation biologists to assess the current genetic status of the Scandinavian wolf population. The purpose of this was to clarify its conservation status in accordance with the Reporting Guidelines under article 17 of the Habitats directive as well as the Guidelines for Population Level Management of Large Carnivores issued by the EC. The background for assigning an international panel was an entrenched disagreement between Swedish research groups regarding this issue, especially over the severity of the inbreeding situation. The panel has had access to peer-reviewed articles with relevance to the topic as well as reports to the Swedish Environmental Protection Agency (Naturvårdsverket) and the Government. The panel members are:

Michael Møller Hansen, Aarhus University, Denmark (Chair)

Liselotte Wesley Andersen, National Environmental Research Institute, Aarhus University, Denmark

Jouni Aspi, University of Oulu, Finland

Richard Fredrickson, University of Montana, Missoula, USA

The panel was given the following questions:

- What are the different hypotheses presented about the required population size for favourable conservation status (FCS) of the wolf? What are the underlying scientific assumptions and prerequisites for each hypothesis? How well supported and documented are they in current science?
- What is the panel's assessment: which hypothesis is the most well-founded?
- What is the panel's own assessment of the population size required to reach and sustain favourable conservation status?
- What would be a relevant measure of population size: number of individuals, number of litters or something else?

The panel read literature and conducted discussions over e-mail through November and December and then met for three days in January 2011, during which time a hearing was held with the research groups involved in the issue: Linda Laikre & Nils Ryman (Stockholm university), Olof Liberg & Håkan Sand (Swedish University of Agricultural Sciences, SLU, Grimsö research station) and Mikael Åkesson (Lund University). Hans Ellegren (Uppsala university) and Pär Forslund (SLU) were unable to participate, but were contacted for specific questions via e-mail.

2. Favourable Conservation Status

"Favourable Conservation Status" (FCS) is a concept defined in the EU Habitats Directive (Council directive 92/43/EEC of 21 May 1992 on the conservation of natural habitats and of wild fauna and flora).

According to Article 1 the conservation status will be taken as 'favourable' when:

- population dynamics data on the species concerned indicate that it is maintaining itself on a long-term basis as a viable component of its natural habitats, and
- the natural range of the species is neither being reduced nor is likely to be reduced for the foreseeable future, and
- there is, and will probably continue to be, a sufficiently large habitat to maintain its populations on a long-term basis;

Grey wolf is listed in Annex IV (species of community interests in need for strict protection) and in Annex II (species of community interest whose conservation requires the designation of special areas of conservation) to the directive. The member states should, according to Article 17, every six years report on the implementation of the measures taken under the Habitats Directive.

To assist member states, guidelines have been developed. The guidelines for reporting under Article 17 (http://circa.europa.eu/Public/irc/env/monnat/library?l=/habitats_reporting/reporting_2001-2007/guidlines_reporting/notesguidelines_2/_EN_1.0_&a=d), prepared by the EC, describes FCS as the overall objective to be reached for all habitat types and species of community interest. It is stressed that the fact that a habitat or species is not threatened, i.e. not faced by any direct extinction risk, does not mean that it is in FCS.

Favourable reference range and favourable reference population are two important concepts in the evaluation of conservation status. The favourable reference range is the historic or potential range, or the area required for population viability including consideration of connectivity and migration issues. The favourable reference range must be at least the range when the Directive came into force, which is 1995 for Sweden. The favourable reference population is the population considered the minimum necessary to ensure the long-term viability of the population/species. The guidelines document mentions that the minimum viable population (MVP) concept may be useful when setting the reference population value, but that MVP can only provide a proxy for the lowest tolerable population size. This means that in practice MVP will be lower than the population level considered at FCS. The favourable reference population must be at least the size of the population when the Habitats Directive came into force..

Another important guidance document is the Guidelines for Population Level Management Plans for Large Carnivores (http://ec.europa.eu/environment/nature/conservation/species/carnivores/docs/guidelines_final2008.pdf), developed by "the Large Carnivore Initiative for Europe" for the EC. In a note to the guidelines (http://ec.europa.eu/environment/nature/conservation/species/carnivores/docs/note_guidelines.pdf) the EC gives support to them, stating that they are not legally binding but do constitute a reference point against which DG Environment will monitor actions taken by the Member States in fulfilment of their obligations under the Habitats Directive.

In the matter of assessing conservation status, the large carnivore guidelines are to a large extent in line with the guidelines for reporting under article 17. However, the large carnivore guidelines put more emphasis on MVP and the use of PVA. They suggest using red list criterion E, which is defined as less than 10 % extinction risk over 100 years based on a PVA . An alternative, in the absence of enough data to conduct a robust PVA, is according to the guidelines to use red list criterion D (predefined thresholds for the red list threat categories).

The large carnivore guidelines also emphasize the importance of maintaining or restoring connectivity. They conclude that the assessment of conservation status should be conducted at the population level, even when the population is transboundary. One consequence of this can be that countries that share a population will be able to achieve FCS at the population level whereas this may not be feasible considering their national segments (sub-populations) in isolation.

According to this, the panel concluded that population size at FCS should be above MVP. The population should have the size and the structure (subpopulations, connectivity) so that problems of small populations are avoided. Finally, the population should also be of a size that would allow for evolutionary potential. By this it is meant that the population should be able to evolve in order to adapt to changes in the environment, e.g. climate change and emergence of new diseases.

How large – in terms of actual numbers – should populations be in order to fulfil these requirements for Favourable Conservation Status? A recent paper analyzed minimum viable population size (MVP) estimates for 212 different species collected from the literature (Traill *et al.* 2007). In order to standardize results from different species they defined MVP as the population sizes required for a 99% probability of persistence over 40 generations. They found a median MVP of 4,169 individuals (95% CI 3,577 – 5,129). The MVP estimates used in the meta-analysis typically accounted for demographic stochasticity as well as some form of environmental stochasticity, and density dependence. Sixty percent of the PVAs also included genetic factors such as inbreeding depression. However, the costs of inbreeding to population growth were routinely underestimated, and "genetic" MVPs included other assumptions that would tend to cause MVP to be underestimated. Traill *et al.* (2007) concluded that minimum viable populations for most species will exceed a few thousands of individuals.

Estimates of "evolutionary" MVP (Traill *et al.* 2010) - the minimum population size required for species to adapt to changing environments through evolution have ranged from effective population sizes (N_e) of 500-1000 (Franklin *et al.* 1980; Franklin & Frankham 1998) to 5,000 (Lynch & Lande 1998). Effective population size of a set of individuals denotes the number of individuals in an "ideal" population that would give rise to the same inbreeding or random genetic drift that occurs in the specific set of individuals in question. Here, an "ideal" population denotes a population with equal sex ratio and a Poisson distribution of offspring among families. A review of genetically effective population sizes among wildlife populations (Frankham 1995) found that "comprehensive" estimates of effective population size were on average 10% of census population sizes, whereas a more recent review found an average ratio of 14% (Palstra & Ruzzante 2008). Two recent studies on wolves found that effective sizes were 29% and 40% of census population sizes (Aspi *et al*. 2006; vonHoldt *et al*. 2008), but the ratio for the Scandinavian wolf population, may be lower than these wolf populations because of its high level of inbreeding accumulation. Hence, "evolutionary" MVPs would be on the order of thousands in terms of census population sizes, and if Franklin's (1980) initial suggestion of a minimum effective population size of 500 is accepted, then this would correspond to 1,250-5,000 individuals. Taken together, demographic and evolutionary MVPs converge on numbers around 3,000-5,000.

3. At which population level should FCS be evaluated (local or transnational)?

According to the reporting guidelines for the EU Habitats Directive Article 17 conservation status should be reported with reference to " current range, potential extent of range taking into account physical and ecological conditions (such as climate, geology, soil, altitude), historic range and causes of change and area required for viability of habitat/species, including considerations of connectivity and migration issues." In the Guidelines for Population Level Management Plans for Large Carnivores, it is also recommended, as above, that FCS be evaluated at the population level even for transboundary populations. From a scientific perspective these considerations about definitions of populations and management units and the need for taking historical distributional ranges and population structure into account are evident and well supported (Crandall *et al.* 2000; Waples & Gaggiotti 2006). Hence, a crucial issue for evaluating the conservation status of Scandinavian grey wolves then concerns the population level and geographical scale at which it should be assessed. Essentially, the general question to answer is: What is a grey wolf population?

It must be acknowledged that grey wolves are highly vagile carnivores. Home ranges can be extensive and individual wolves can migrate hundreds of kilometers within relatively short time spans (Linnell *et al*. 2005, Kojola *et al.* 2006). Moreover, grey wolves can occupy a wide range of ecologically varying habitats (Mech & Boitani 2003). Hence, the historical and natural distribution of grey wolves would be expected to be continuous over large geographical regions, interrupted by landscape features such as seas, oceans and other impassable waterways and mountain ranges, unfavourable environmental conditions etc. A recent study, however, also demonstrates geographical genetic heterogeneity that appears to correlate with ecological factors, such as dietary preferences and availability (Pilot *et al.* 2006). Described in population genetics terms this would mean that the natural population structure would be a continuous population with some degree of isolation-by-distance (i.e. increasing genetic difference with increasing geographical distance) and additional heterogeneity reflecting specific ecological factors. In total, the population would show a considerable degree of genetic and demographic connectivity.

Based on these considerations it must be assumed that the current Scandinavian grey wolves along with grey wolves from Fennoscandia, Karelia and extending further into Russia belong to one single population. Hence, each of these units such as Scandinavian wolves should be considered fragments of a population i.e. sub-populations. In the recent past (until a few hundred years ago) grey wolves throughout the region constituted a largely continuous population connected by gene flow mediated by the extensive dispersal capabilities of the species.

The present situation with different sub-populations throughout Fennoscandia and Karelia is artificial and is a result of human-mediated fragmentation. Essentially, the geographical zones in northern Sweden, Norway and Finland where grey wolves are not tolerated constitute barriers in the landscape through which wolves are unable to disperse. The fact that relatively small but statistically significant genetic differences can be observed among the present sub-populations (Aspi *et al.* 2009) reflects: 1) limited or no gene flow through zones where grey wolves are not tolerated, isolating the sub-populations, 2) random genetic drift, that is random genetic changes caused by low and in the case of Scandinavia very low effective population sizes and founder events and 3) the isolation-by-distance (e.g. Aspi *et al*. 2006) that occurred already in the continuous historical population.

Considering the natural genetic population structure of grey wolves, their distributional range in the recent past and the definitions of reporting units (i.e. the scale at which reporting should be done) in the EU Habitat Directive Article 17 and Guidelines for Population Level Management Plans for Large Carnivores it follows that Favourable Conservation Status should be evaluated and secured at the level of the historical range. In practical terms, the evaluation panel recommends focusing on the current geographically most proximate sub-populations in Fennoscandia and Karelia. The panel notes that there is a knowledge gap concerning population sizes and densities and genetic population structure of the easternmost part of the range that ought to be filled in.

The evaluation panel notes that although inbreeding problems are by far the most severe in the Scandinavian sub-population (Liberg *et al.* 2005), population sizes of the other sub-populations in the historical range are also low, leading to significant conservation concerns (Aspi *et al.* 2006, Aspi *et al*. 2009). Despite the conservation concern for all the sub-populations in the proximate historical geographical range, the evaluation panel finds it a realistic goal to obtain Favourable Conservation Status for the Fennoscandian-Karelian grey wolf population, with effective population sizes (N_e) that secure a potential for future evolution (500 or more). This number corresponds to census population sizes (N) of some thousands (at least 3,000 – 5,000) individuals. Population sizes of these magnitudes would secure long-term sustainability of the population, addressing both genetic and demographic requirements (Frankham *et al.* 2002; Traill *et al.* 2010).

The evaluation panel stresses that the key to obtaining Favourable Conservation Status involves transnational collaboration and agreements involving Norway, Sweden, Finland and Russia. Emphasis must be on 1) securing sufficient connectivity among the present sub-populations, preferably through natural migration or alternatively artificial translocations and 2) securing sufficiently high population sizes within the present fragments (sub-populations) of the historical range. All together this could lead to a total population size fulfilling the requirements for Favourable Conservation Status.

4. Can the Scandinavian population in itself meet requirements for Favourable Conservation Status?

In the proceedings the panel concluded that Favourable Conservation Status should be evaluated and achieved at the level of the historical population range, in practical terms Fennoscandia and Karelia. In practice this can be considered a feasible goal, disregarding political aspects. For arguments sake, however, the panel also briefly considered whether Favourable Conservation Status could be achieved solely within the Scandinavian sub-population, thus disregarding the sub-populations from Karelia and Finland and disregarding the definitions of populations and reporting units put forward in the reporting guidelines for the EU Habitat Directive Article 17.

The panel finds that the most imminent genetic problem with the Scandinavian sub-population is the extremely high inbreeding coefficient of ca. F= 0.3. This level of inbreeding is greater than that found in offspring from matings between full siblings, and substantial inbreeding depression due to a genetic load (harmful alleles that will be expressed under inbreeding) has been demonstrated in Scandinavian wolves (Liberg *et al.* 2005, Räikkonen *et al*. 2006). Inbreeding cannot be reduced by increasing population sizes, although this would have the positive effect of reducing the rate of future inbreeding accumulation. A reduction of inbreeding can only be achieved by introducing new genetic material to the sub-population (Frankham *et al.* 2002). Hence, either natural immigration or artificial translocation is required to reduce inbreeding to an acceptable level. Once this has been achieved, the census population size of the Scandinavian sub-population would have to number in the thousands (3,000-5,000) in order to fulfill the criteria for Favourable Conservation Status. In principle, it would thus be possible to obtain Favourable Conservation Status for the Scandinavian sub-population but 1) this does not address conservation requirements for other remnants of the historical wolf population and 2) although the evaluation panel focuses on biological issues, it is the impression of the panel that a grey wolf population size of this magnitude is not politically acceptable.

5. Meeting requirements for Favourable Conservation Status on a transnational scale.

Given that FCS for the wolf cannot be achieved within the Scandinavian peninsula, what should Scandinavia's contribution to the extended population be? It will be close to impossible to give an exact figure for this, since it depends on a number of factors, for instance the sizes of the Scandinavian, Finnish and Russian subpopulations, the level of gene flow between them and the area of suitable habitat found in the different countries. Considering only the size of the countries/regions (Sweden: 450 000 km^2; Norway: 324 000 km^2; Finland: 338 000 km^2; Kola-Karelia with neighbouring provinces: 840 000 km$^{2)}$, a population of 3000 wolves divided equally would give Sweden 700, Norway and Finland 500 each and Russia 1300 animals. However, taking the amount of suitable habitat, human habitations and infrastructure into account, the available areas would have to be adjusted substantially. But numbers might still need to be considerably higher than today in the Scandinavian peninsula. In short, a number of considerations will have to be taken into account when assessing the contribution of individual countries to a transnational population with Favourable Conservation Status, but the relative proportions of suitable habitat could be a good starting point for the assessment.

6. The different hypotheses presented by the research groups regarding recommended population sizes, immigration rates and urgency of decreasing inbreeding.

The panel was asked to identify and evaluate the different hypotheses presented by research groups concerning recommended population sizes, immigration rates and the urgency of decreasing inbreeding. These opposing views are most clearly expressed in the recommendations provided in a report to Naturvårdsvärket in 2009 (Liberg *et al.* 2009). In this report, two different sets of recommendations are provided. The first set is provided by Liberg, Sand, Forslund, Åkesson and Bensch; in the following, we refer to this group of scientists as the "Liberg *et al.* group". The second set of recommendations is provided by Laikre and Ryman, and in the following they are referred to as the "Laikre & Ryman group".

Despite the disagreements among groups, the evaluation panel also finds it important to stress that it is impressed by the high quality of the science that has been conducted during the monitoring and assessment of the conservation status of Scandinavian wolves. Reconstructing a pedigree of a wild carnivore population must be considered a unique achievement, and overall the conservation ecology and genetics research on Scandinavian wolves ranks very highly in the conservation biology sciences.

I) The views and recommendations of the **Liberg et al. group** are described in the following.

1. First, they clearly acknowledge that the Scandinavian wolf sub-population is strongly inbred and that there are signs of inbreeding depression. However, they also do not find that the signs of inbreeding depression are that severe. An average litter size of 3.5 is not alarming, although a litter size of 6 is observed from 3 pairs of non-inbred Scandinavian wolves. The population is still growing by 10-15% per year so the loss of genetic diversity is going slower than expected. At the same time, however, the problem of inbreeding and inbreeding depression cannot be ignored, so securing

gene flow into the sub-population is considered a necessary step, but not to the extent that immediate measures should be taken and inbreeding should be drastically decreased.

2. Further, they emphasize a published study (Bensch *et al.* 2006) that seems to suggest a mechanism of general selection for high heterozygosity which should slow down the expected loss of heterozygosity in the population.
3. Given that they do not find the level of inbreeding to be immediately alarming, they recommend an adaptive approach starting with the least controversial management options. They therefore recommend an initial goal of having 2 effective immigrants per generation in order to decrease inbreeding. They further recommend waiting for natural immigration, i.e. over a five year period natural immigration corresponding to 2 effective migrants should occur. If this goal is not achieved, then other measures, notably active translocation of wolves must be considered. The genetic and demographical status of the population should still be followed and the pedigree upgraded and continued.
4. In order to facilitate natural immigration of wolves from Finland, a protected corridor along the Norrland coast is suggested.
5. Concerning the longer-term recommendation by Allendorf & Ryman (2002) that conservation programs should seek to retain 95% of exisiting heterozygosity over a 100 year period, the Liberg *et al.* group suggests lowering the goal to retaining 90% heterozygosity over 100 years. Given the estimated generation time of wolves this corresponds to an effective population size (N_e) of 50. Moreover, assuming a ratio between effective population size (N_e) and census population size (N) of 0.25, this corresponds to a minimum census population size of 200 wolves. However, given the uncertainty of estimating N_e/N ratios a census population size of 300 is recommended.

The evaluation panel's assessment of these issues is provided below, point by point.

1. The panel certainly agrees that the Scandinavian wolf sub-population is strongly inbred and that there are signs of inbreeding depression. An overall inbreeding coefficient of ca. 0.3 must be considered very high and should be taken very seriously. Moreover, it has been found that the genetic load in the sub-population corresponds to ca. 6 lethal equivalents associated with reproduction ("litter-size-reducing equivalents") (Liberg *et al.* 2005) and 5.4 lethal equivalents associated with recruitment to a breeding postion ("breeding-failure equivalents") (Bensch et al. 2006) . This must be considered a high genetic load. There are apparently many detrimental recessive alleles in the population, which should raise significant concerns that inbreeding leads to significant inbreeding depression. The statement that the sub-population has so far grown by 10-15% per year despite inbreeding (and illegal hunting) is correct, but this cannot be taken as evidence that inbreeding depression does not occur (in comparsion, in Finland the average growth of population in 1996–2006 has been has been 21 %; $\lambda = 1.211$). Given that there is an abundance of prey and that the Scandinavian wolf subpopulation is far below carrying capacity, it is not a paradox that the population grows despite inbreeding depression. This can be considered a balance between population growth rate in a beneficial environment and lowered fitness (and thereby population growth) resulting from inbreeding depression, where the effects of the beneficial

environment still overrides the effects of inbreeding depression. With increasing inbreeding in the years to come (unless counteracted by immigration) this balance might turn. Moreover, the overall low genetic diversity due to the extreme founder event of the sub-population means that there is limited genetic variation to respond to sudden events, notably emergence of new diseases, in which case the overall sub-population could be at risk. In total, the evaluation panel finds that introduction of new genetic material into the sub-population should have a high immediate priority.

2. The panel read and discussed the paper by Bensch *et al*. (2006) with much interest. The panel finds it plausible that the observation of maintenance of heterozygosity at the analysed markers is genuine. Indeed, patterns resembling this have also been observed in some inbreeding experiments with model organisms such as *Drosophila* (Demontis *et al.* 2009). However, the panel would dispute the interpretation that this provides evidence for a general mechanism of selection for heterozygosity (a genome-wide effect) as opposed to selection against homozygotes at specific loci with deleterious alleles (a genic effect). Given the extremely low number of founders of the Scandinavian sub-population linkage disequilibrium is expected to be very high. Hence, selection against homozygotes at a specific locus could affect a large region of the chromosome, including the analysed molecular markers. As a likely alternative hypothesis the panel suggests that the observed results actually represent inbreeding depression in action; there is strong selection against deleterious homozygotes at specific loci, and due to the strong linkage disequilibrium hitch-hiking selection extends far along the chromosomes, which includes the studied markers. This interpretation is also consistent with recent population genetic theory. Pamilo and Pálsson (1998) found that selection against homozygous deleterious alleles is expected to cause increased heterozygosity at linked marker loci when there is strong linkage disequilibrium in small populations. They also noted that this effect may generate associations between fitness and heterozygosity at individual markers suggestive of selection for heterozygosity. It should also be noted that a later study of Scandinavian grey wolves using many more markers (250 microsatellite markers as opposed to 31 in Bensch *et al.* 2006) failed to observe a general homozygote excess (Hagenblad *et al.* 2009), although differences in the study designs make it difficult to draw a direct comparison between the two studies. In total, the panel finds that the results suggesting selection for heterozygosity are scientifically interesting, but the interpretation is far from clear-cut. The panel discourages using this result in a practical conservation context as an argument for accepting high inbreeding levels. It should be added that during the panel's discussions with the Liberg *et al.* group there was also a general consensus about interpreting these results cautiously and not using them as a scientific basis for management recommendations.

3. It is not evident why it is recommended that immigration should be at least two effective migrants per generation. Assuming an effective population size of 50 and assuming immigration from an infinitely large population, the results provided by Laikre & Ryman (Fig. 2.a) in the report by Liberg*et al.* (2009) suggest that 2 effective migrants per generation will reduce the inbreeding coefficient from ca. 0.3 to just below 0.2 over 10 generations (ca. 50 years). This inbreeding coefficient must still be considered very high and hence the decrease of inbreeding proceeds slowly. It is however also stated that this is an adaptive strategy; if inbreeding depression becomes

too serious then immigration could be increased, e.g. by translocations of wolves. However, there is no specific target as to which circumstances would prompt further actions.

4. The evaluation panel finds that the suggestion to create a protected migration corridor along the Bay of Bothnia coast could be a major step towards connecting the wolf sub-populations in Finland and Scandinavia. This could greatly increase the prospects for obtaining Favourable Conservation Status for the total Fennoscandian-Karelian wolf population. The panel is unable to provide detailed comments on the expected efficiency of a corridor of the size and location suggested.

5. The panel discussed and evaluated the guidelines by Allendorf & Ryman (2002) stating that the recommended effective population size should fulfil the criterion that 95% of heterozygosity be retained over a 100 year period. The panel also discussed this issue with one of the authors (Ryman). On one side, the panel finds it important to have a specific and well-defined goal for how much loss of genetic diversity that can be accepted. On the other side, there is not really a strong biological argument as to whether the target should be 95%, 90% or 99% for that matter. In general, the panel finds that it is not a fruitful discussion whether the target should be 90 or 95%. The key issue for the Scandinavian grey wolves is the demographic history and its genetic consequences. The extremely low number of founders and the lack of gene flow have led to very high inbreeding coefficients. Hence, the panel finds that the target should be to bring the inbreeding down to an acceptable level using the combination of the two parameters immigration and population size that will fulfil that target within a reasonable time frame.

II) The views and recommendations of the **Laikre & Ryman group** are described in the following, based on their contributions to the same report (Liberg *et al.* 2009).

1. Laikre and Ryman suggest an international metapopulation of N_e= 500-5000 to sustain evolutionary potential. If N_e /N = 0.25, then this corresponds to a census population size of 2000-20000. This goal should be achieved by international collaboration with the neighbouring countries.

2. If short term viability is considered, then the recommendations of Allendorf & Ryman (2002) should be followed (retention of 95% of existing heterozygosity for 100 years) This would correspond to N_e= 200. It is discussed which among a range of suggested N_e /N that should be assumed. However, if it is assumed that N_e /N are 0.20 - 0.25, then this would correspond to a recommended population size of 800-1000 individuals (as opposed to 300 recommended by the Liberg et al. group).

3. If only around 200 wolves are allowed to persist, then the goal of retaining 95% heterozygosity can only be achieved by immigration corresponding to at least 4 effective migrants per generation.

4. It is argued that inbreeding should be reduced by immigration by taking immediate measures (natural gene flow or translocations). This is due to the fact that the inbreeding coefficient already is very high, that there is a considerable genetic load in the population and that significant inbreeding depression already occurs. A goal of reducing the current inbreeding coefficient to < 0.1 could be achieved over 5 to 20 years by immigration corresponding to 5 to 10 effective migrants

per generation. This depends to a significant extend on the effective population size of the donor population (e.g. Finland); the lower the N_e in the donor population, the more difficult will it be to decrease inbreeding.

5. It is argued that inbreeding depression is probably underestimated as apart from litter size it is not specifically monitored. Observed defects such as cryptorchism are likely to be heritable and reflect recessive deleterious alleles. In this case, a frequency of observed cryptorchism of 0.08 would correspond to a frequency of 0.27 of the deleterious recessive allele. Simulations suggest that because deleterious alleles have already reached such high frequencies, very high effective population sizes would be required (N_e of ca. 200) in order to avoid further increase of recessive allele frequencies.
6. Pedigree information could be used more efficiently, e.g. by using the relative kinship estimates of the individuals to identify the genetically important individuals that should be protected from hunting.

The evaluation panel's assessment of these issues is provided below, point by point.

1. The panel agrees with the recommendation of aiming towards an international "metapopulation" with an effective population size of at least 500. The panel reached a similar conclusion when evaluating the options for obtaining Favourable Conservation Status (see above). However, as a small note the panel would prefer not to use the term "metapopulation" in this context, as in many definitions this concept involves extinction-recolonization dynamics among sub-populations, but there is no evidence of these dynamics in undisturbed grey wolf populations.
2. As explained in the panel's evaluation of point 5 regarding the Liberg *et al*. group (see above), the panel on one side finds it important to have a stated conservation goal, but on the other side also finds it somewhat arbitrary that the goal should be to retain at least 95% heterozygosity over 100 years. However, the real issue concerns the high level of inbreeding in Scandinavian grey wolves and how to reduce it.
3. The panel agrees with the rationale *per se*, but again finds that emphasis should be on reducing the level of inbreeding.
4. The panel agrees that the level of inbreeding is very high and even without further evidence of physical defects caused by inbreeding depression, the high genetic load already demonstrated corresponding to six lethal equivalents for litter size and 5.4 lethal equivalents for recruitment to breeding status should raise significant concerns (Liberg *et al.* 2005; Bensch *et al*. 2006). It can always be argued to which level inbreeding should be reduced; reducing it to below 0.1 over a time scale of 20 years appears to be a realistic goal. In any case, considering the genetic load and very high inbreeding coefficient in Scandinavian grey wolves the panel finds it important to take steps to introduce new genetic material as soon as possible.

As a matter of fact, the panel has some concerns that the recommendations for number of effective migrants may be on the optimistic side. These recommendations are made on the basis of Fig. 2.a in Laikre & Ryman's contribution to the report, which specifically assumes immigration from an INFINITELY large donor population into a Scandinavian sub-population with N_e of 50. However, the nearest donor population (the Finnish sub-population) is far from infinite, and N_e has been estimated at ca. 40 (Aspi *et al.* 2006). This would suggest that Fig. 2.b provides a more realistic scenario, where both the donor population and the Scandinavian subpopulation have an N_e of 50. Fig. 2.b shows that under these circumstances it will be difficult to obtain a permanent decrease of inbreeding coefficient. However, Fig. 2.b may also not be realistic, if gene flow occurs from presumably larger sub-populations (notably Karelia-Kola) into the Finnish sub-population, or if the effective gene flow is substantially greater than that expected from neutral expectations – as has been observed in several cases of "genetic rescue" (e.g. Ebert *et al*. 2002; Saccheri & Brakefield 2002)..The true picture is expected to lie somewhere between Fig. 2.a and 2.b. The panel would recommend further analyses, for instance using individual based modelling as conducted by Forslund in another part of the report (Liberg *et al.* 2009), which should specifically take the costs of inbreeding into account. These simulations could involve immigration into a Scandinavian subpopulation of N_e = 50 from a Finnish subpopulation of N_e = 40 which again is connected by gene flow involving a larger Karelian-Kola population of N_e = 100. This type of approach might provide the most realistic tool for determining the number of effective migrants required for reducing the inbreeding coefficient below 0.1.

5. The panel agrees that inbreeding depression is likely to have been underestimated as it has not been specifically monitored. It is possible (though not proven) that the observed physical defects could reflect inbreeding depression due to homozygosity for deleterious recessive alleles. If this is indeed the case, then it is correct that physical deformities of a relatively low frequency (e.g. 0.08) corresponds to a high frequency (e.g. 0.27) of the deleterious recessive allele. It is also correct that if deleterious recessive alleles have been allowed to reach such high frequencies then there is a non-negligible risk that they may spread further due to random genetic drift. The simulations by Laikre & Ryman illustrate this point. However, the conclusion that N_e should be at least 200 in order to avoid further spread depends on the specific allele frequencies and the assumed selection coefficients. The panel therefore does not regard an N_e of 200 as a specific management recommendation, but rather consider these simulations as illustrating the seriousness of the inbreeding that has accumulated and the need for reducing inbreeding before it increases further.

6. The panel agrees that the pedigree represents a unique resource that should be utilized as much as possible in practical management. This issue was discussed with the Liberg *et al.* group, who expressed a high awareness of this issue. The panel was informed that the pedigree was immediately available on request, to scientists and the general public alike. There was a general consensus that the pedigree should be used to identify individuals that were particularly genetically valuable to the sub-population, e.g. by representing an important part of the founder genetic diversity or showing particularly low kinship with other individuals. These individuals should then be protected from hunting. However, the viewpoint was also expressed that "micro-management" would not be feasible, where particular individuals with high kinship and inbreeding were targeted

for hunting, based on the pedigree information. The panel finds that the pedigree is particularly valuable for monitoring inbreeding depression, which would also necessitate further detailed recording of emerging physical defects or negative developments in important life-history parameters. The Liberg *et al.* group pointed out that it was in fact difficult to obtain funding for this type of monitoring. The panel finds that monitoring of possible inbreeding depression is such an important and fundamental part of the scientific basis of the management of Scandinavian wolves that funding for this purpose should be secured. To sum up the discussion with both groups and internally within the panel, it is particularly recommended to make use of the pedigree for

- identifying individuals to be protected from hunting, first and foremost immigrants, F1s, F2s, backcross wolves, but also other individuals that may represent a particularly high proportion of the founder genetic diversity. Several measures can be calculated for quantifying the "genetic value" of individuals, such as mean kinship coefficient, and there are several empirical examples in the literature of how to use such approaches in practical conservation (Cunningham *et al.* 2001; Goncalves da Silva *et al.* 2010).
- assessing the degree to which hunting affects the inbreeding coefficient.
- identifying packs for cross-fostering of introduced pups, if such a strategy is to be implemented, for increasing gene flow.
- monitoring the future development of inbreeding depression by monitoring physical deformities and changes in life-history parameters and associating them with the pedigree. The panel reiterates its view that funding for this type of monitoring is important and should be available.

III) In a subsequent report (Laikre & Ryman 2010) the Laikre & Ryman group evaluates the genetic consequences if the number of wolves in Sweden should be kept at a maximum of 210 and additionally 30 wolves are allowed in Norway, corresponding to a total number of 240 individuals. Moreover, they evaluate the consequences if at most 20 "foreign" individuals are introduced into Sweden. The conclusions regarding these issues are as follows:

1. If the population size of the Scandinavian grey wolf is kept at 240 individuals and no gene flow occurs (either natural or via translocations), then the inbreeding coefficient will increase to 45% after 20 generations. If an N_e/N ratio of ca. 0.2 is assumed, then this will correspond to an N_e of around 50 and a rate of inbreeding, ΔF, of > 1%. This is higher than the recommended rates of inbreeding when a time scale of more than a few generations is considered and may lead to further inbreeding depression. Genetic variation will also be lost quickly and there is a high risk that, some harmful alleles will increase in frequency.

2. The inbreeding coefficient will be reduced to just above 10 % if 20 genetically effective immigrants are introduced. Inbreeding will, however, increase again to the same level after 20 generations if no further immigration takes place and N is kept at 240, corresponding to N_e of ca. 50. The higher the population size, the slower the increase of inbreeding.

3. It is suggested that the goal should be to reduce the inbreeding coefficient to below 10% and keep it there. Over a time frame of 20 generations (100 years) this can only be achieved if immigration is continued after the introduction of 20 genetically effective individuals.

4. The discussion concerning the distribution of deleterious alleles addressed in the previous report (Liberg *et al.* 2009) is taken up again. The specific distribution of deleterious alleles is difficult to assess. It is reiterated that detrimental/harmful recessive alleles are present in Scandinavian wolves and that the low number of founders have led to a situation where deleterious recessive alleles from the onset occur at high frequencies. It is also pointed out that when the effective population size is low as in Scandinavian wolves, then selection cannot act properly to eliminate deleterious alleles (genetic drift overrides selection). Finally, it is pointed out that a recent study of grey wolves on Isle Royal suggests extensive inbreeding depression, expressed as physical deformities (Raikkonen *et al.* 2009). The case of the highly inbred Isle Royale wolf population has previously been used to argue that severe inbreeding can occur in wild populations without associated inbreeding depression, but the new study disproves this conclusion.

5. It is important to establish an international collaboration with the aim of connecting the Northern European grey wolf population genetically. The aim should be to create a population network facilitating natural genetic exchange among sub-populations to conserve genetic variation and keep the levels of inbreeding at an acceptable level. This point was also raised in the previous report (Liberg *et al.* 2009).

The panel's assessment of these points is as follows:

1. The panel agrees. This is in line with conservation genetics principles.

2. The panel also agrees with this. This is in accordance with conservation genetics principles.

3. The panel also agrees with this (see also the response to point 4 above related to Laike & Ryman's conclusions in the Liberg *et al.* (2009) report)..

4. The panel agrees with these considerations (see also the response to point 5 above related to Laike & Ryman's conclusions in the Liberg *et al.* (2009) report).

5. The panel agrees that the key to a long-term solution involves transnational collaboration. This is further described above in the sections concerning Favourable Conservation Status.

IV) Finally, the panel has assessed the population viability analysis and individual based modelling conducted by Pär Forslund in the report by Liberg *et al.* (2009). Some of the results and the analyses conducted by Laikre & Ryman that assume mainland-island or finite island models can be seen as different alternative methods. The panel does not see major discrepancies between the methods, and from a general point of view the integration of ecological and population genetic parameters in Forslund's simulations are endorsed. In some important aspects, notably the modelling of inbreeding depression, the panel concurs with the view that this has likely been underestimated, as only reduction of litter size is considered (indeed, it would be highly relevant to include the lethal equivalents reported by Bensch *et al.* (2006) in the model as well). Also, it is assumed that environmental variation does not influence the demographic parameters, which is unlikely to be the case under real circumstances. However, the results are discussed and interpreted cautiously taking these potential shortcomings into account.

V) To sum up this long presentation and evaluation of the presented results and hypotheses:

a) In many aspects there is agreement between the Liberg *et al.* group and the Laikre & Ryman group regarding the conservation situation and the management recommendations.

b) The groups disagree on the numbers of genetically effective immigrants required if the census population size is 200. The Liberg *et al.* group finds that 2 genetically effective migrants per generation would be sufficient, at least for the next 10-15 years. The Ryman & Laikre group concludes that at least 5-10 genetically effective migrants are required per generation. *The evaluation panel finds that the inbreeding coefficient of 0.3, the genetic load (deleterious alleles) present in Scandinavian wolves and the demographic history of the sub-population with an extremely low founder number calls for high levels of gene flow in order to decrease the inbreeding coefficient. The panel recommends stating a clear goal for reducing the inbreeding coefficient within a short time span, such as reducing the inbreeding coefficient to 0.1 over 20 years, which according to Laikre & Ryman's analyses will indeed require 5-10 genetically effective migrants per generation.*

c) There is disagreement regarding the urgency of taking action. The Ryman & Laikre group finds that imminent actions should be taken to reduce inbreeding, whereas the Liberg *et al.* group finds that it would be justified to wait for 5 years, evaluate the natural immigration that occurs and at the same time take measures that could benefit the probability of natural immigration. If 2 genetically effective immigrants have not occurred within the 5 year period, measures should be taken to conduct artificial translocations. *As stated above, the panel finds convincing arguments that there should be more than 2 genetically effective migrants per generation; the number should be on the order of 5-10 effective immigrants. Given the very high inbreeding coefficient and the genetic load in Scandinavian wolves the panel finds that management actions to reduce inbreeding should be implemented and that it would not be justified to wait another 5 years. In practice, however, the panel finds that the two strategies could be combined. For instance, immediate action could be taken by using pups from captive populations (zoos) for a first round of artificial translocations. Given the low number of founders of the relevant captive populations this option will only be justified for at most a couple of years. However, at the same time measures could be taken to increase natural immigration of wolves. If this does indeed result in more spontaneous immigration at the necessary rate, then artificial translocation could be discontinued.*

d) There is disagreement regarding the required effective population size if immigration continues to be low. The Laikre & Ryman group argues that for maintaining 95% heterozygosity over a 100 year period, the effective population size should be at least 200. Conversely, the Liberg *et al.* group finds that retaining 90% heterozygosity over 100 years would be sufficient, corresponding to an effective population size of 50. *The evaluation panel has been unable to identify really strong arguments as to whether retainment of 90% heterozygosity is sufficient or if 95% should be the minimum target,*

> *although obviously from a conservation perspective it would be better to retain 95%. Importantly, however, the panel finds that this discussion also misses the point. The key issue concerns the very high inbreeding coefficient and how to reduce it. Immigration is essential in order to reduce inbreeding, and even allowing an effective population size of 200 without having immigration would result in an inbreeding coefficient >= 0.3, which would not be desirable from a conservation perspective.*

VI) Finally, the evaluation panel makes the following recommendations for a short-term and a longer-term conservation goal.

In the **short term**, the main priority should be to reduce inbreeding. A reasonable goal would be to reduce the inbreeding coefficient to below 0.1 over a 20 year period (4 generations) and – importantly – keep it at this low level. This goal can be fulfilled by different combinations of effective population size and numbers of effective immigrants. Based on Fig. 2.a by Laikre & Ryman in the report by Liberg *et al.* (2009), this could be obtained by constant immigration corresponding to 5-10 genetically effective migrants per generation, if the effective population size is 50. If the effective population size could be higher than 50, then lower immigration would be required, but the specific effective population sizes and immigration rates should be determined analytically. There would furthermore be benefits by obtaining a higher effective population size than 50, as this would reduce further spread of deleterious recessive alleles that are already present in the population.

In the **longer term**, the panel recommends transnational collaboration and agreements involving Sweden, Norway, Finland and Russia in order to secure a total population of at least 3000-5000 individuals (or an effective population size of at least 500), where the individual sub-populations, including that in Scandinavia, are connected by gene flow. This could secure Favourable Conservation Status of the total population.

7. What would be a relevant measure of population size?

The panel was asked to provide recommendations for a relevant measure of population size. In short, the most relevant measure depends on the question asked. If it concerns the demography of the population, then the most relevant measure is the census population size (N), which is simply the number of individuals. If the question concerns genetic processes, such as minimizing inbreeding and assessing the minimum immigration required, then effective population size (N_e) is the most relevant measure. Molecular markers may be used to estimate the effective population size through a variety of statistical methods; for instance Aspi *et al.* (2006) estimated the effective size of the Finnish population to be ca. 40 based on analysis of molecular markers. The effective population size is nearly always much lower than the census population size, but if the ratio between the two (the N_e/N ratio) is known, then this can be used as a conversion factor. However, N_e/N ratios should be treated with caution, as they can depend on the situation and the specific species. The mean N_e/N ratio for a large number of species has been found to be 0.1 (Frankham 1995), but with considerable variation among organisms. The suggested N_e/N ratios in grey wolves (Scandinavia and elsewhere) range from ca. 0.2 – 0.4 (Aspi *et al.* 2006; Liberg *et al.* 2009; Laikre & Ryman 2010; vonHoldt et al. 2008) and will be complicated to estimate precisely. The best approach therefore is to take a cautionary approach and assume a range of possible values, as is also done in most of the publications assessed by the panel.

The panel would discourage use of alternative measures of population size, such as numbers of litters. They do not have a rigorous theoretical definition, which would preclude their “translation” into census and effective population sizes.

8. Priority research if management should encompass a total transnational population involving Fennoscandia and Karelia-Kola.

If the Scandinavian, Finnish and Russian Kola-Karelian wolf sub-populations are to be treated as one transnational management unit, there is urgent need for increased co-operation between researchers and management authorities within these countries. There are already good databases with genetic profiles for individual wolves in Sweden and Finland. To allow better genetic identification of individuals and comparison between populations, standardization of the applied genetic markers is necessary. There is already some co-operation between Finnish and Russian large carnivore researchers, but broader international co-operation should be established. There should also be closer co-operation between management authorities from the countries. It could be particularly useful if the action of the SKANDULV working group could be expanded to include Finnish and Russian managers and researchers, or some other organization should be established.

The status and genetic structure of the Swedish and Finnish wolf populations are well known. Less is known about northwestern Russian wolf populations, and there is urgent need to investigate the present status and genetic connectivity of these sub-populations. The Russian Karelian and Archangelsk sub-populations and their immigration between Finland and Russia have been studied (Aspi *et al.* 2009). There are small genetic differences between the populations, but they are probably due to isolation by distance. However, migration in recent years between the populations seems to be low. Genetic diversity of the Finnish population has been decreasing during the last fifteen years (Roininen et al., unpublished). This is partly due to lowered population size, but probably also reflects lowered migration from Russian populations.

Wolf populations in Eastern Europe have been studied (Pilot *et al.* 2006), and it has been shown that there is non-random spatial genetic structure even in the absence of obvious physical barriers to movement. It was found that the genetic differentiation among local populations was correlated with climate, habitat types, and wolf diet composition. Accordingly, it seems that ecological processes may also have a significant influence on the amount of gene flow among wolf populations. Pilot *et al.*’s (2006) study did not involve samples from Russian Karelia. Hence, genetic differences between Russian Karelian and other eastern European wolf populations have not been quantified. Connectivity between Russian Karelian and adjacent southern and eastern wolf populations should therefore be investigated. However, given that estimated migration rates from Karelian to Finnish wolves have been estimated to be less than 0.03 (Aspi *et al*. 2009), connectivity between Finnish wolves and subpopulations further apart is expected to be very low.

Recently several cases of alleged wolf-dog hybridization in Finland near the Russian border has been reported. Alleles typical to some dog-breeds have also been reported in supposed wolf migrants from

Russia (Roininen et al. unpublished). Possible hybridization between dogs and wolves should be surveyed on a larger scale, and the wolves potentially to be translocated should be genotyped to avoid possible introgression.

9. Captive populations – how useful are they for reintroduction?

The captive Nordic population may constitute a source of genetic material to reduce inbreeding in the Scandinavian wolf sub-population. There are nine founder individuals in the captive Scandinavian-Finnish wolf population. Five of these originated from Scandinavia or Finland, two from Estonia, one from Latvia and one from Russia. There is a possibility that there is some introgression from domestic dogs into the Estonian founders (Laikre & Ryman 2010). The average inbreeding level is around 0.1, which is much lower than that of the wild population (around 0.3).

The evaluation panel finds that the captive wolves could be a useful source of genetic material in the short term for reducing the inbreeding in Scandinavian wolves (in practise, this would involve cross-fostering of pups). It would be important first to verify whether or not introgression from domestic dogs has occurred, which could be done using molecular markers (Verardi *et al.* 2006). It could also be debated, if captive wolves even after a few generations could have experienced some degree of adaptation to the captive environment. Although this possibility is difficult to rule out entirely, the panel finds that this potential concern would be counterbalanced by the need for reducing inbreeding in Scandinavian wolves.

It must be emphasized that using genetic material from the captive populations would only be a short term solution (a few years) due to the low number of founders. In the longer term genetic material from wild populations should be introduced, preferably by natural immigration or alternatively by translocations.

10. What is the POTENTIAL natural immigration into Sweden if wolves were allowed to pass through Northern Sweden?

The potential for natural immigration into the Scandinavian wolf population is a crucial point for conservation management. If gene flow is sufficiently high, a smaller population may be viable in the longer term. At present, the reindeer herding areas of Northern Finland and Sweden are kept virtually wolf-free, meaning that individuals found to attack livestock are promptly removed or shot. The wolves that are able to pass this area without detection are few and far between: in recent years, 1-2 individuals per year have reached the Southern wolf population in Scandinavia. This depends greatly on the size of the Finnish population, which is currently declining. Of these migrants, on average 50% have bred successfully, adding genetic variation. However, statistical models using longer time series suggest that there is only a 60% probability that 1 genetically effective migrant reaches the South per generation (5 years). That is quite insufficient to maintain variation. But if all migrating wolves were allowed/assisted to pass through Finnish and Swedish Lapland, the gene flow would be substantially higher, even more so if illegal killings could be avoided. A suggested figure in this scenario is 2-3 effective migrants (breeding individuals) per generation. If the Scandinavian sub-population is kept at a constant effective population size of 50 (corresponding to the presently allowed census population size of 240), this will not be sufficient to reach a goal of reducing inbreeding to below 0.1 (see Laikre & Ryman's Fig. 2.a in Liberg *et al.* 2009). However, if a larger population could be tolerated and/or if the Finnish subpopulation increased, thereby increasing immigration into Scandinavia, a more beneficial situation could occur. The combinations of migrants and effective population sizes required should be derived after careful analysis.

11. Issues regarding diseases and quarantine.

It has been suggested that translocated wolves should be screened for diseases like rabies, distemper and parvo, as well as internal and external parasites, and should be de-wormed, treated for ecto-parasites and vaccinated against rabies. After the treatments the translocated animals should be in quarantined in captivity, or should remain in the wild equipped with gps-transmitters for 120 days. If wolves to be translocated are taken from captive population or from established Finnish wolf packs, the probability of spreading diseases which do not occur in Sweden will be very low.

Wild animals with rabies in Finland have been found most recently in 1989, and it has never been found in wolves. To prevent the spread of infection from Russia to Finland bait vaccines are annually spread into the forests at the south-eastern border. The prevalence of *Echinococcus granulosus* infection among Finnish wolves is 10-15%, but endemic foci of *E. granulosus* have also been known to occur in northern Norway and Sweden (even though they have not been found during recent years). *Echinococcus multilocularis* has not so far been found in Finland, even though it is known to occur in Russian Karelia in voles (pers. comm., prof. Antti Oksanen, Finnish Food Safety AutorityEvira). Some sporadic cases of parvovirus have been found in raccoon dogs in Finland, but not in wolves.

Single de-worming is able to remove all endoparasites, and keeping animals in quarantine for 120 days is more than enough to prove that there are no rabies symptoms. Keeping the animals in the wild equipped with gps-transmitters for 120 day would be difficult to organize. The Finnish authorities have expressed their willingness to vacate Finnish wolves mainly from the reindeer herding area, and keeping wolves alive there for120 days could be difficult. Moreover, wolves in Finland have been mainly captured for gps-collaring with snow mobiles, and the seasonal time window for that practice is definitely shorter than 120 days (only possible with thick snow cover). However, wolves have been very recently caught with the help of helicopters. The evaluation panel finds it puzzling that the vaccinations and de-worming is not required if immigrant wolves appear spontaneously into southern Sweden, whereas these procedures are required for animals to be translocated.

12. Monitoring the effects of introduced individuals.

The evaluation panel emphasizes the need for continued monitoring of the Scandinavian wolf sub-population. In particular, it would be important to monitor the effect of introduced individuals through continued pedigreeing. The ratio between GENETICALLY EFFECTIVE migrants and the census number of migrants might be similar to the N_e/N ratio, but it is also possible that the two ratios differ. On one side, translocated individuals could exhibit reduced reproductive performance due to effects of handling or, in the case of offspring of captive wolves, adaptation to a captive environment. On the other side, it is also likely that introduced non-inbred individuals and their outcrossed offspring would exhibit higher reproductive success (Ebert *et al*. 2002; Saccheri & Brakefield 2002). This would have the beneficial effect of increasing the ratio between effective migrants / census number of migrants. In other words, it could turn out that a lower census number of migrants would be needed than originally assumed.

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