

SUPPLEMENTARY INFORMATION

Publication: Metapopulation effective size and conservation genetic goals for the Fennoscandian wolf (*Canis lupus*) population

Linda Laikre, Fredrik Olsson, Eeva Jansson, Ola Hössjer, and Nils Ryman

Corresponding author e-mail: linda.laikre@popgen.su.se

SUPPLEMENTARY APPENDIX 1

Information on wolf populations in Fennoscandia of relevance for metapopulation effective size analysis

The Scandinavian wolf population (Sweden and Norway)

The Swedish wolf population has been closely monitored by tracking, radio-tracking, and molecular genetic analyses since it was reestablished in the 1980s by a pair most likely originating from Finland/Russia. A more or less complete pedigree of the population is available making computations of inbreeding and kinship coefficients possible (Vilà et al. 2003; Åkesson and Bensch 2010; Laikre et al. 2013; Åkesson et al. 2014; Table 2). The population is shared with Norway and approximately 5-10 percent of the population resides on the Norwegian side of the border; the total Scandinavian population is estimated to be about 400 individuals (Svensson et al. 2014).

Over the period 1985–2014 a total of 18 wolves have been documented to immigrate into the Scandinavian population. Out of these, three (males) have managed to reproduce with wolves of the resident populations; one in 1990 and two in 2008. One female – first observed in 2010 – managed to find a partner but was shot in 2015 and never reproduced. In the winter of 2012/2013 one pair immigrated and was translocated to the area of Tiveden, Sweden. This couple has reproduced twice but the majority of their offspring so far appears to have been killed (Liberg & Sand 2009; Svensson et al. 2014).

Thus, average annual immigration rate is estimated as $18/30=0.6$, and average annual immigration rate that has resulted in reproduction is $5/30=0.17$ (including the pair in Tiveden which has not yet contributed genes to the contemporary population). For the Scandinavian wolf population, generation time has been estimated as c. five 5 years (Forslund 2009; Liberg et al. 2012; Bruford 2015) so the 30 year period corresponds to 6 generations and an empirical rate of about 0.83 reproductively successful immigrants per generation. The severe genetic situation of the Scandinavian wolves has been discussed for decades (Laikre 1995; Laikre & Ryman 1999; Andrén et al. 1999; Räikkönen et al. 2006).

The Finnish wolf population:

The number of wolves in Finland as of January 2015 is estimated as 220–245 (Natural Resources Institute Finland 2015). The population was extinct, or at least nearly so, for over 100 years; intense wolf hunting started in the 1850's and there were hardly any wolves left to hunt in the beginning of the 20th century (Ermala 2003; Bisi et al. 2007, 2010). Despite legal protection since 1973 wolves did not regularly reproduce until 1995 when Finland joined the European Union, and the demand for wolf protection efforts became considerably strengthened as the wolf is a listed species under the EU conservation legislation of the Habitats Directive (available at: <http://ec.europa.eu/environment/nature/legislation/habitatsdirective/>).

Following the stronger protection the population size in Finland quickly boomed and

reached a minimum of 250 individuals and 25 breeding pairs in 2006. Subsequently, the population size quickly decreased again from 250 to 150 individuals (Jansson et al. 2012). Like in the Scandinavian wolf population (Liberg et al. 2012), poaching was generally acknowledged as the primary cause for the observed negative population trends in Finland (Anonymous 2015) affecting the population's genetic status as well (Jansson et al. 2012). Wolf hunting was re-initiated in Finland in 2015 and was continuing during 2016. The argument to allow the hunt is to reduce "problem individuals", conflict situations, and poaching (Anonymous 2015). In total, 17 wolves were killed in this legal hunt during 2015 and 43 during 2016. 10 of these 70 hunted wolves were confirmed pack leaders (so-called alpha individuals; <http://riista.fi/metsastys/saalisiseuranta/kannanhoidollinen-susisaalis/>).

Wolves are monitored by the Finnish Game and Fisheries Research Institute which is part of the Finnish Natural Resources Institute since January 2015. In this work some wolves are radio-collared and examined, biological samples are collected for DNA analysis, and the number of individuals is estimated annually (Kojola et al. 2006; 2009; 2014). Genetic studies have shown that the Finnish wolf population has a large amount of variation (Aspi et al. 2006; Jansson et al. 2012; Niskanen et al. 2014), but that after the initial re-establishment in the mid-1990's, the population has become somewhat genetically isolated from its Eastern source population. Using single-sample estimators based on linkage disequilibrium (LD) methods (Tallmon et al. 2008; Waples & Do 2008), the effective population size (N_e) was estimated to be ~50–70 among wolves born prior 1998 even though the number of breeding pairs was ≤ 5 , and thus likely reflected the N_e of the source population rather than the local N_e (Jansson et al. 2012). In contrast, for wolves born in 2007–2009 (this is the fifth generation because generation time has been estimated as ~3.4 years in the Finnish population; Aspi et al. 2006), N_e was only ~20–30 (and lower than the number of breeding individuals). Due to heavy fluctuations of the population census (N) and effective sizes in 1995–2009, the estimated N_e/N ratio varied a lot (depending also on the estimation method used) from 0.094 to 1.473, but was most of the time ~0.28 (Jansson et al. 2012).

Concordant with the rather small local N_e , genetic drift in the Finnish population was evident - i.e., genetic differentiation measured as F_{ST} between temporal samples grew, and inbreeding has recently increased ($F_{IS} = 0.108$ in 2007–2009). Genetic differentiation between the Finnish and Russian Karelian populations is moderate ($F_{ST} \sim 0.1$) corresponding to 1–3 migrants per generation as evaluated from several different estimation methods (cf. Aspi et al. 2009; Jansson et al. 2012; Niskanen et al. 2014). Recently, the migration rate appears to have decreased, and has been larger from Finland to Russian Karelia than vice versa (Aspi et al. 2009); during 2007–2009, the only confirmed immigrant from Karelia to Finland was shot close to the border in 2008 (Jansson et al. 2012).

Immigration from the Scandinavian population is practically negligible; only three wolves have been confirmed entering Finland from Scandinavia during the past decade. Two of them were shot in the reindeer herding area, and did therefore not contribute to the Finnish gene pool. One of them was shot in 2005, the other one in 2009 (Sand et al. 2007, 2010; Jouni Aspi, personal communication). The third, and latest immigrant from Scandinavia to Finland was a radio-collared young male from southern Norway who quickly passed through norther Finland to Russia in November 2014 but reappeared in the Finnish Lapland one year later (GPS network is not comprehensive in northern Russia and signals were not received while the wolf was on the Russian side of the

border). The current status and location of this wolf is unknown, but it is reported in the media as holding the world record in documented distance of wolf migration (<http://www.aftenposten.no/nyheter/Ulv-fra-Hedmark-satte-verdensrekord-7760325.html>; Jouni Aspi, personal communication). Even though some immigrants may go undetected, reindeer herding areas create a very strong dispersal barrier in the whole northern Fennoscandia (Kojola et al. 2006; 2009). Moreover, typical dispersal distances for wolves in the area are only ~100 km (based on telemetry and genetic studies on Finnish wolves; Kojola et al. 2006; Aspi et al. 2006) suggesting that long-range dispersal might be a rather unusual event.

Genetic studies have shown that today's effective population size is only ~8% of its historical size of ~590 back in the late 19th century (Aspi et al. 2006), and that the Finnish wolf population has lost a significant portion of its former variation (Jansson et al. 2014). These results indicate that, back in time, the Finnish wolf population was part of a much larger, more uniform, and genetically more diverse Fennoscandic population (Jansson 2013).

Wolf populations in the Karelia and Kola parts of Russia:

Rather limited information on wolf populations in Russia is available. Reports from Russia mainly concentrate on census population estimates and hunting statistics (Lomanova et al. 2011; Bragina et al. 2015). According to the latest report by Lomanova et al. (2011), there were almost 50 000 wolves in Russia in 2010, of which ~13 000 west of the Ural Mountains (i.e. in the European side of Russia, see Appendix S3). Of these, approximately 450 are in the Fennoscandic part of Russia: in the Kola Peninsula ~100 (Lomanova et al. 2011), and ~300–350 in the Karelian Republic (Danilov 2005; Jansson 2013; but see Lomanova et al. 2011 giving a higher estimate of 460 in 2010). Hunting pressure in Karelia is high with ~80–100 wolves reported to be killed annually (Lomanova et al. 2011; Jansson 2013). In contrast, only three wolves were reported to be killed in the Kola region in 2008 and 2009 and a stable number of about 100 wolves was reported for 2008–2010 (Lomanova et al. 2011).

We were unable to find any genetic studies concerning wolves in the Kola Peninsula whereas Karelian wolves have been genetically characterized in three scientific studies (that clearly separate Karelian and Finnish wolf samples: Aspi et al. 2009; Jansson et al. 2012; Niskanen et al. 2014). Aspi et al. (2009) used 29 wolf samples from Karelia (1995–2000), which were analyzed jointly with samples from the neighboring Finnish and Arkhangelsk populations. This study showed significant inbreeding (measured as deficiency of heterozygotes giving a F_{IS} of 0.094) in the Karelian population, and a relatively small effective size of ~40–50 together with the already mentioned genetic distinction from the Finnish population. Both populations were also significantly diverged from the Arkhangelsk population further east in Russia (Appendix S3). The Russian populations (Karelia and Arkhangelsk) were genetically closer to each other (F_{ST} = 0.05) than to the Finnish population ($F_{ST}(\text{Finland-Karelia})$ = 0.151, $F_{ST}(\text{Finland-Arkhangelsk})$ = 0.176). Even though a limited number of samples was available for this study, the results clearly indicated a pattern that has been repeatedly found in other studies: despite huge dispersal potential of wolves, their populations tend to be genetically subdivided even within quite short distances (e.g., von Holdt et al. 2010; Scandura et al. 2011; Stronen et al. 2012). A later study including more Karelian samples (n = 37; Jansson et al. 2012) confirmed the low amount of gene flow between the Finnish and Karelian populations. Based on frequencies of private alleles in these populations during 2007–2010 the average number of migrants between populations (Nm) was estimated to 1.2.

Wolf populations southeast of Fennoscandia (Russia)

A general trend of increasing wolf numbers has been reported in Russia since 1991 when the Soviet Union collapsed and wolf control measures were discontinued (Bragina et al. 2015; Appendix S3). Hunting statistics during 2008–2009 indicate large regional variation in hunting quotas (Lomanova et al. 2011), and in some cases the number of wolves killed equals or even exceeds the estimated number of individuals from the same area (Appendix S3). Thus the local anthropogenic pressure can still be heavy even though the total number of wolves in whole Russia is counted in tens of thousands (Bragina et al. 2015; Appendix S2).

Large-scale genetic investigations of European wolf populations including samples from Russia have shown that the genetic differentiation among local populations is correlated with climate, habitat type, and wolf diet composition (Pilot et al. 2006, 2012). However, detailed knowledge of the genetic structure of wolf populations inhabiting Russia is scarce. Sastre et al. (2011) analyzed 47 wolf samples from four interconnected regions in northern European Russia, south-east from Karelia (areas of Tver, Smolensk, Vologda and Kaluga; cf. Appendix S3). They observed a surprisingly small contemporary N_e of a few hundred (138–312.5), and concluded that this could indicate population fragmentation, contrary to what was assumed for Russian wolf populations. Consistent with this, a later study by Pilot et al. (2014) employing a large panel of SNP markers estimated the most recent effective population sizes to be 159 for the north-eastern European Russia, 224 for the southern European Russia/eastern Ukraine, and 239 for the northern Belarus. For the whole Eastern Europe, the global N_e was 1366.

Internal subdivision within the eastern wolf distribution area is further supported by recent genetic studies from other eastern European countries nearby (Hindrikson et al. 2013; Czarnomska et al. 2013; Moura et al. 2014). Limited dispersal and gene flow among and between eastern wolf populations is probably due to a combination of several factors. They include high hunting pressure and historical population bottlenecks (Hindrikson et al. 2013; Moura et al. 2014), but also unfavorable landscape and/or natal-habitat biased dispersal reducing dispersal at longer distances in particular (Czarnomska et al. 2013).

References (not included in the article)

Andrén H, Ebenhard T, Ellegren H, Ryman N, Sæther B-E (1999). Report from the working group of the large carnivore committee appointed by the Swedish Government 1998-1999. Appendices of the final report from the committee to the government; SOU 1999:146. (In Swedish).

Anonymous (2015). *Management plan for the Finnish wolf population* (in Finnish). Ministry of Forestry and Agriculture. Available from http://www.mmm.fi/attachments/mmm/julkaisut/julkaisusarja/2015/DTapld7Xv/Susikannan_hoitosuunnitelma.pdf (Accessed May 2015)

Aspi J, Roininen E, Ruokonen M, Kojola I, Vilà C (2006). Genetic diversity, population structure, effective population size and demographic history of the Finnish wolf population. *Mol Ecol* **15**: 1561–1576.

Bisi J, Liukkonen, T Mykrä S, Pohja-Mykrä M, Kurki S (2010). The good bad wolf – wolf evaluation reveals the roots of the Finnish wolf conflict. *Eur J Wildl Res* **56**: 771–779.

Bragina EV, Ives AR, Pidgeon AM, Kuemmerle T, Baskin LM, Gubar YP et al. (2015). Rapid declines of large mammal populations after the collapse of the Soviet Union. *Conserv Biol* **29**: 844–853.

Czarnomska SD, Jedrzejewska B, Borowik T, Niedziałkowska M, Stronen AV, Nowak S et al. (2013). Concordant mitochondrial and microsatellite DNA structuring between Polish lowland and Carpathian Mountain wolves. *Conserv Genet* **14**: 573–588.

Danilov PI (2005). Game animals of Karelia. Ecology, resources, management, protection. Nauka, Moscow 338 pp (in Russian).

Forslund P (2009). Modeling growth and inbreeding in the Scandinavian wolf population. Appendix 1:3 of Suggestions for actions to strengthen the genetic status of the wolf population, Report by the Swedish Environmental Protection Agency to the Swedish Government Dnr 429-8585-08 Nv (In Swedish).

Ermala A (2003). A survey of large predators in Finland during the 19th-20th centuries. *Acta Zool Lituan* **13**: 15–20.

Hindrikson M, Remm J, Männil P, Ozolins J, Tammeleht E, Saarma U (2014). Spatial Genetic Analyses Reveal Cryptic Population Structure and Migration Patterns in a Continuously Harvested Grey Wolf (*Canis lupus*) Population in North-Eastern Europe. *PLoS ONE* **8**(9): e75765.

Jansson E (2013). Past and present genetic diversity and structure of the Finnish wolf population. Doctoral thesis, University of Oulu. Available from: <http://herkules oulu.fi/isbn9789526201160/isbn9789526201160.pdf> (Accessed May 2015).

Jansson E, Harmoinen J, Ruokonen M, Aspi J (2014). Living on the edge: Reconstructing the genetic history of the Finnish wolf population. *BMC Evol Biol* **14**: 64.

Kojola I, Aspi J, Hakala A, Heikkinen S, Ilmoni C, Ronkainen S (2006). Dispersal in expanding wolf population in Finland. *J Mamm* **87**: 81–86.

Kojola I, Kaartinen S, Hakala A, Voipio H-M (2009). Dispersal Behavior and the connectivity Between Wolf Populations in Northern Europe. *J Wildl Manag* **73**: 309–313.

Kojola I, Helle P, Heikkinen S, Lindén H, Paasivaara A, Wikman M (2014). Tracks in snow and population size estimation: the wolf *Canis lupus* in Finland. *Wildl Biol* **20**: 279–284.

Laikre L (1995). Swedish wolves are threatened with inbreeding. *Forskning & Framsteg* 1995: 44-46. (In Swedish).

Laikre L, Ryman N (1999). Genetics and viable large carnivore populations. Pages 29-40 in: Ebenhard, T. and Höggren, M. (eds.), What are viable populations of wolf, wolverine, lynx, brown bear, and golden eagle? Swedish Biodiversity Center, Report 1, 140 pp. Uppsala University & Swedish University of Agricultural Sciences. (In Swedish)

Lomanova, NV (ed.) et al. (2011). Game animals of Russia. Status of game resources in Russian Federation in 2008-2010. Issue 9. Information and analytical methods. FGY Tsentrokhontrol, Moscow (in Russian), pp. 145-152. Available from: eco.sfu-kras.ru/sites/eco.institute.sfu-kras.ru/files/Sost.pdf (accessed May 2015).

Moura AE, Tsingarska E, Dabrowski MJ, Czarnomska SD, Jedrzejewska B, Pilot M. (2014). Unregulated hunting and genetic recovery from a severe population decline: the cautionary case of Bulgarian wolves. *Conserv Genet* **15**: 405–417.

Natural Resources Institute Finland. 2015. Accessed at in May 2015 at: <http://www.luke.fi/tiedote/susien-maara-lisaantyi-reilusti-viime-vuodesta/> (in Finnish).

- Niskanen A, Kennedy L, Ruokonen M, Kojola I, Lohi H, Isomursu M *et al.* (2014). Balancing selection and heterozygote advantage in MHC loci of the bottlenecked Finnish wolf population. *Mol Ecol* **23**: 875–889.
- Pilot M, Jędrzejewski W, Branicki W, Sidorovich VE, Jędrzejewska B, Stachura K *et al.* (2006). Ecological factors influence population genetic structure of European grey wolves. *Mol Ecol* **15**: 4533–4553.
- Pilot M, Jędrzejewski W, Sidorovich VE, Meier-Augenstein W, Hoelzel R. (2012). Dietary differentiation and evolution of population genetic structure in a highly mobile carnivore. *PLoS ONE* **7**(6):e39341.
- Pilot M, Greco C, vonHoldt BM, Jędrzejewska B, Randi E, Jędrzejewski W *et al.* (2014). Genome-wide signatures of population bottlenecks and diversifying selection in European wolves. *Heredity* **112**: 428–442.
- Räikkönen, J., Bignert, A., Mortensen, P., Fernholm, B (2006). Congenital defects in a highly inbred wild wolf population (*Canis lupus*). *Mamm. Biol.* **71**: 65–73.
- Sand H, Liberg O, Aronson Å, Forslund P, Pedersen HC, Wabakken P *et al.* (2007). Vargen – Artfakta en sammanställning av data från det skandinaviska argforskningsprojektet SKANDULV 2007 på uppdrag av utredningen om de stora rovdjuren. (In Swedish). Available at: www.slu.se/skandulv.
- Sand H, Liberg O, Aronson Å, Forslund P, Pedersen HC, Wabakken P *et al.* (2010). Den Skandinaviska Vargen en sammanställning av kunskapsläget 1998 – 2010 från det skandinaviska vargforskningsprojektet SKANDULV, Grimsö forskningsstation, SLU. Rapport till Direktoratet for Naturforvaltning, Trondheim, Norge. (In Swedish). Available at: www.slu.se/skandulv.
- Sastre N, Vilà C, Salinas M, Bologov VV, Urios V, Sánchez A *et al.* (2011). Signatures of demographic bottlenecks in European wolf populations. *Conserv Genet* **12**: 701–712.
- Scandura M, Iacolina L, Capitani C, Gazzola A, Mattioli L, Apollonio M (2011). Fine-scale genetic structure suggests low levels of short-range gene flow in a wolf population of the Italian Apennines. *Eur J Wildl Res* **57**: 949–958.
- Svensson L, Wabakken P, Kojola I, Maartmann E, Strømseth, Åkesson M, Flagstad Ø (2014). The wolf in Scandinavia and Finland: Final report from wolf monitoring in the 2013-2014 winter. Report no 12-2014, Norwegian Institute for Nature Research (NINA) and the Swedish Environmental Protection Agency. In Swedish, English summary.
- Stronen AV, Forbes GJ, Paquet PC, Goulet G, Sallows T, Musiani M (2012). Dispersal in a plain landscape: short-distance genetic differentiation in southwestern Manitoba wolves, Canada. *Conserv Genet* **13**: 359–371.
- Tallmon DA, Koyuk A, Luikart G, Beaumont MA (2008). ONeSAMP: a program to estimate effective population size using approximate Bayesian computation. *Mol Ecol Res* **8**: 299–301.
- vonHoldt BM, Stahler D, Bangs EE, Smith DW, Jimenez MD, Mack CM *et al.* (2010). A novel assessment of population structure and gene flow in grey wolf populations of the Northern Rocky Mountains of the United States. *Mol Ecol* **19**: 4412–4427.
- Waples RS, Do C (2008). LDNe: a program for estimating effective population size based on linkage disequilibrium. *Mol Ecol Res* **8**: 753–756.