

## **Betr.: Genetics of the Fennoscandian subpopulation of the Lesser White-fronted Goose.**

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### **1. Population development of the Fennoscandian subpopulation**

The Lesser White-fronted Goose is a monotypic species and formerly had a continuous distribution in the southern tundra between Lapland and Chukotka (ALPHÉRAKI 1904, DEMENTIEV & GLADKOV 1952). Since the first population estimates of the 1950's, a drastic decrease in numbers was recorded from more than 100,000 individuals in the 1950's (USPENSKI 1965) to less than 27,000 birds in the 1990's (DELANY & SCOTT 2002). During the 1930's regularly flocks of c. 50,000 Lesser Whitefronts were recorded in the Western Palearctic (RINGLEBEN 1957) and during the 1950's the Western Palearctic population was still estimated at more than 50.000 birds (USPENSKI 1965), but in the 1990's numbers recorded in this region during midwinter counts never exceeded 10.000 - 13,000 (DELANY & SCOTT 2002, LORENTZEN et al. 1999)

Since the 1980's on most regularly monitored sites the population numbers decreased with about 5% annually, e.g. Hortobagy (Hungary): about 5 %/year since 1985, Bothnian Bay (Oulu, Finland): about 5 %/year since 1985, Valdak Marshes (Norway): about 5 %/year since 1993 etc.. Currently breeding birds merely are found in a minor part of the original breeding range (AARVAK & TIMONEN 2004, AARVAK et al. 1996 & 1997, KALYAKIN 1996, LORENTSEN et al. 1999, MOOIJ 2001, MOROZOV 1996, MOROZOV & KALYAKIN 1997, SYROECHKOVSKI JR. 1996, TOLVANEN et al. 1998, 1999, 2000 & 2001).

This statistically significant decrease of 5% annually means that the regional Fennoscandian subpopulation, current estimate 20-30 breeding pairs (i.e. 100-150 individuals), will be halved in about 10 years time.

According to modern population genetics the critical size for small isolated populations is likely a few hundred individuals. Above this level a population still has the possibility to survive without human help, but below this level such "bottleneck populations" hardly have a chance to recover again without a strong input of new genetic material (BAKER 2004).

With a present population size of estimated 100–150 individuals the Fennoscandian subpopulation of the Lesser White-fronted Goose is clearly far below the critical level. Although genetic diversity is low, until now regular contacts to the local Russian breeding populations could reduce the negative effects of the small population size. But the total Western Palearctic population also shows a dramatic decrease, which could reduce these contacts and genetic diversity on the short term.

In the scope of this critical situation re-enforcement of the Fennoscandian subpopulation by means of re-introduction could be an useful tool to solve this critical situation.

## **2. Is the Fennoscandian subpopulation a distinct genetic unit ?**

Due to the dramatic population decrease the breeding distribution of the species became fragmented during the past century. The more or less isolated breeding range of the small and still decreasing local Fennoscandian subpopulation is the westernmost remnant of this formerly Eurasia-wide breeding distribution.

Based on an analysis of mtDNA RUOKONEN (2001) stated that the Fennoscandian subpopulation could be a distinct genetic unit. At the other hand traditional morphological analysis as well as the results of the genetic tests of nuclear DNA of the wild population of the Lesser White-fronted Goose show no indications at all of the existence of different subpopulations. Besides at least until the middle of the 20<sup>th</sup> century the species had a continuous breeding range between Fennoscandia and Chukotka and even today a part of the Fennoscandian birds uses the same migratory routes as Siberian birds and mixes up with them.

In the scope of recent investigations of the genetic diversity of the mtDNA in Lesser White-fronted Geese, 5 different haplotypes were found in the Fennoscandian subpopulation. The tested sample of the regional Fennoscandian subpopulation was the biggest of all investigated local subpopulations (28 samples of a population of 100-150 individuals, i.e. 19 – 28% of the population!).

In spite of the much smaller sample size from other Western Palearctic sites (Bolshezemelskaja Tundra: 14 samples of a population of 3,000-5,000 individuals; Yamal Peninsula: 25 samples of 4,500-6,000 individuals; Taimyr Peninsula: 15 samples of 3,000-4,000 individuals), which means that likely not all haplotypes are found yet, all samples showed 4-5 different haplotypes per site of which at least 3 were shared with the Fennoscandian subpopulation (RUOKONEN et al. 2004).

Analysis of mtDNA haplotypes showed a clear cline from west to east. From the Fennoscandian birds about 85% of the mtDNA haplotypes belonged to the western type, from the birds of the Bolshezemelskaja Tundra and the Yamal Peninsula about 60% and of the Taimyr Peninsula and China about 27% of the haplotypes belonged to this type. The extremely high proportion of one single Western haplotype in the Fennoscandian birds indicates impoverished genetic diversity.

The two most common mtDNA-haplotypes found in the Fennoscandian Lesser White-fronted Geese W1 and E1 (covering almost 90% of all detected haplotypes) were also found on the other Western Palearctic sites: Bolshezemelskaja Tundra (64%), Yamal Peninsula (64%), Taimyr Peninsula (87%) as well as Kazakhstan (82%). (RUOKONEN et al. 2004).

Furthermore recent genetic analysis revealed that a considerable part of the Fennoscandian males (50%) carried mtDNA haplotypes that were found also in individuals outside Fennoscandia, whereas Fennoscandian females only carried the most common Western Palearctic and Fennoscandian mtDNA haplotype (RUOKONEN 2000 & 2001, RUOKONEN & LUMME 1999, RUOKONEN et al. 2004).

These data indicate that the small Fennoscandian breeding population has an impoverished genetic diversity, but still is an integrated part of the Western Palearctic breeding population because it “imports” at least about 50% of its males from the neighbouring Russian breeding population, which at present is likely to reduce the danger of inbreeding.

Genetic tests of nuclear DNA strongly indicated that all tested birds belong to one closed unit (KHOLODOVA 2001).

Fragmentation of the breeding range is rather recent and too short for speciation or subspeciation processes. Moreover studies on marked birds indicate that the remnants of the Fennoscandian breeding population of the Lesser White-fronted Goose have regular contacts to their Russian conspecifics, which likely delays both speciation processes and the process of losing genetic diversity. During autumn migration a part of the Fennoscandian birds flies east as far as the Taimyr Peninsula and uses the same migratory routes as Russian birds, as was shown by telemetry (AARVAK et al. 1997).

These facts do not support the hypothesis of the local Fennoscandian subpopulation being “a genetic distinct unit”.

### 3. Genetic composition of wild Fennoscandian Lesser White-fronted Geese

Between 1989 and 1996 WWF-Finland released 143 Lesser Whitefronts bred in captivity. These birds belonged basically to the same genetic “mixture” as the Swedish captive stock and were released “*near the breeding places of the remaining wild LWfG population, and the released geese were expected to join and follow the wild birds*” (MARKKOLA et al. 1999). Some of them were shot at the Kola Peninsula, others were resighted “*in Southern Sweden, Denmark, The Netherlands, Belgium, Great Britain and even in Spain. Only eight or nine restocked individuals have been reported back in Finnish Lapland during the subsequent summers, and only one (a non-breeding adult female in 1993) has returned to the restocking area. No breeding of the restocked birds has been confirmed in Finland. .... The oldest released LWfG resighted (2 Ind.) have been on their 4<sup>th</sup> calendar year*” (MARKKOLA et al. 1999).

There is a considerable possibility that at least some of these birds will have mixed up with the remnants of the wild Fennoscandian subpopulation during the 1990s. The fact that they were released within the actual breeding area of the Fennoscandian subpopulation makes it even more likely that the original wild birds have interacted with the released ones.

Between 1981 and 1999 the Swedish re-introduction project released almost 350 Lesser White-fronted Geese. As a result the present Swedish re-introduced population was estimated at 100-150 individuals in 2004.

Since a few years ago this population seems to have spread beyond the borders of the former re-introduction area. Intermixing with the remnants of the original Fennoscandian breeding population cannot be excluded. Moreover, because birds of the re-introduced population locally share staging areas with birds of the original Fennoscandian subpopulation, most likely mixed breeding pairs exist (ANDERSSON 2004 & pers.comm., RUOKONEN 2001, VON ESSEN 1991 & 1999, VON ESSEN et al. 2000).

In a number of staging areas it is very likely that Lesser Whitefronts of different origins occurred: descendants of re-introduced Finnish and Swedish re-introduction projects as well as birds of the original Fennoscandian and Russian subpopulations (MOOIJ & HEINICKE in prep., PERSSON 2004). Because pair bonds in geese are made during migration or wintering the chance that birds of different origin mixed up during their stay on these sites is considerable.

As long as the nuclear DNA of these birds is not screened for a good panel of 10 or more polymorphic microsatellites or their genomes are scanned with AFLPs we can not be sure that they do not have any Greater White-fronted Goose genes in their nuclear DNA complement.

The ancestors of the European captive breeding stocks of Lesser White-fronted Geese without doubt originate from different Eurasian breeding sites. Three out of 15 investigated birds (20%) have been shown to have the most common Western haplotype and eight (53%) the most common eastern haplotype (RUOKONEN 2001). Both haplotypes were covering 73% in captive and 89% in the wild Fennoscandian subpopulation, which shows a great similarity in the mtDNA composition of both groups (RUOKONEN 2001, RUOKONEN et al. 2004).

The results of the analysis of nuclear DNA by KHOLODOVA (2001) confirmed this great similarity between captive and wild birds. Because of this great genetic similarity it might be almost impossible to separate the free-living descendants of captive birds from the original wild birds in a natural situation.

Four of 15 investigated captive Lesser Whitefronts (27%) from Finnish breeding stocks showed a haplotype extremely close to a haplotype found in White-fronted geese *Anser albifrons* (RUOKONEN 2001). In the Swedish breeding stocks a comparably high frequency of this haplotype was found (ANDERSSON, pers.comm.). Therefore there is a considerable risk that some birds with this questionable haplotype were released in the course of the Finnish and Swedish re-introduction projects in the past and will have reproduced in subsequent years. As long as it is not possible to decide if this shared mtDNA-haplotype is a result of hybridisation between both species or of the retention of an ancient shared haplotype of their recent common ancestor, such birds cannot be welcomed in the endangered population.

It hardly will be possible to recover birds with this questionable genetic composition or their offspring in the wild. Therefore the only possibility to “neutralise” these possibly “unwelcome” birds would be to release a high number of genetically “clean” birds, to minimise the fraction of birds with a possibly “not desirable” genetic composition. Therefore the unintentional “mistake” of the Finnish and Swedish re-introduction programmes to release birds with a possibly questionable genetic composition should not be used as an argument for the prolongation of a re-introduction moratorium, but instead should be a strong argument to restart re-introduction with genetically “clean” birds on a large scale.

#### **4. Possible hybridisation in the captive breeding stocks.**

Studies on mtDNA-diversity showed that some captive Lesser White-fronted geese do carry a haplotype which is extremely similar to a haplotype found in Greater Whitefronts (RUOKONEN 2000 & 2001, RUOKONEN & LUMME 1999).

According to several geneticists (e.g. FUNK & OMLAND 2003, POWELL 1991) certain alleles in one species may appear more closely related to alleles from different species than to other conspecific alleles. Such deviations from species-level monophyly seem to indicate mtDNA flow between species, but can have a variety of causes and easily could lead to erroneous evolutionary interpretations. The common mtDNA-haplotype shared by Lesser and Greater White-fronted Goose could be a result of hybridisation between both species or of the retention of an ancient shared haplotype of their recent common ancestor. Because it is not possible to resolve this question by means of the present genetic data, no geese with this questionable mtDNA-haplotype should be released by re-introduction projects.

Therefore in reaction on the results of RUOKONEN (RUOKONEN 2000 & 2001, RUOKONEN & LUMME 1999) the Swedish re-introduction programme was stopped and all birds were checked on their genetic composition. Also the birds of the Finnish breeding stock were tested.

In both cases not only maternally inherited mtDNA but also the biparentally inherited nuclear DNA was tested. The analysis of nuclear DNA of Finnish captive Lesser White-fronted Geese brought no indications of hybridisation between Greater and Lesser White-fronted Geese. Besides the analysed Lesser White-fronted geese (wild and captive birds) were clearly delimited as a unit separated from the tested Greater White-fronted geese.

Furthermore, the results indicated that total DNA of the analysed captive Lesser Whitefronts was close to the wild Lesser Whitefront DNA. These facts indicate that there is only a minimal risk of negative influences on the remnants of the wild Fennoscandian LWfG subpopulation caused by “hybridisation” (KHOLODOVA 2001). Still all birds with the common mtDNA-haplotype shared by both Lesser and Greater White-fronts were removed from the breeding stocks.

In April 2004 the Swedish, Finnish and German re-introduction groups agreed on a close co-operation in breeding Lesser Whitefronts and on a common method to test the genetic composition of these birds. This test programme will be supervised by a group of genetic experts (Prof. Dr. Allan Baker, Dr. Marina Kholodova, Prof. Dr. Michael Wink) and Finnish population ecology expert Prof. Dr. Martti Soikkeli.

Allan Baker wrote to the common method:

*“The issue of the common haplotype shared by LWF and GWF geese being due to hybridization or retention of a shared haplotype in their recent common ancestor is difficult to resolve with the present genetic data.”..... “Those birds in the captive flock that have the shared haplotype should not be used for breeding of a flock for reintroduction. Additionally, the birds that lack this haplotype should be screened for a good panel of 10 or more polymorphic microsatellites or their genomes scanned with AFLPs to make sure they do not have any GWF genes in their nuclear DNA complement. The three RAPD primers that were used to do this in the past are insufficient to ensure that introgression has not occurred. Then this “pure” stock could be used to breed birds in captivity and to reintroduce them in the wild.*

*I would not necessarily destroy any putative hybrid birds, as about six or more generations of backcrossing of these birds with “pure” LWF geese would almost eliminate any transpecific nuclear genes from GWF geese. By not breeding any females with the shared mtDNA haplotype the transfer of this “wrong” organelle DNA could be prevented.” (BAKER, pers. comm.).*

According to this agreement samples of the German and Finnish captive breeding stocks will be sent to the genetic laboratory of the University of Heidelberg for genetic analysis. Only birds that show to be genetically “clean” will be used for breeding and re-introduction. Therefore on the basis of the results of these genetic tests, only genetically “clean” birds will be selected for future breeding in captivity and re-introduction.

## **5. Reduced viability of captive birds**

The results of the Swedish reintroduction programme indicate that these descendants of captive birds are quite viable in nature. The breeding birds of the population show an average broodsize (c. 3.0 juv./pair) which is comparable to that of the natural Norwegian population (3.2 juv./pair, according to AARVAK & ØIEN 2001 in TOLVANEN et al., 2001), and these reintroduced birds show high survival rates.

Besides the high reproductive success as well as the population increase (in spite of the release moratorium) of the Swedish re-introduced population indicate a viability comparable to or even higher than the viability of the remnant wild population. This fact should dispel the concern that the captive birds could cause an “outbreeding depression” in the wild population because of “reduced fitness”. Because of the richer genetic diversity of the re-introduced birds, the remnants of the wild population rather could benefit from interactions between both groups by escaping the genetic bottleneck.

## **6. Genetic problems of the re-introduced Swedish Lesser White-fronted Goose population**

Compared to other arctic goose species the annual reproductive rates of Lesser White-fronted Geese seem to be on a comparable level, but annual mortality rates seem to be abnormally high, mainly caused by (illegal) hunting. Therefore besides habitat destruction (illegal) hunting seems to be one of the main reasons for the dramatic population decrease (LORENTSEN et al., 1998 & 1999; TOLVANEN et al., 1998, 1999, 2000 & 2001).

To support the remnants of the natural population, the late Dr. Lambart von Essen started a reintroduction programme in Sweden in 1981. To avoid the main threats he decided to create a new safe migration route to safe wintering grounds. He used semi-domestic Barnacle Geese (*Branta leucopsis*) as foster parents for Lesser Whitefront goslings, which learned from their foster parents to migrate to safe wintering grounds in Western Europe. The eggs of semi-domestic Barnacle Geese, breeding in nature and migrating to wintering sites in the Netherlands, were replaced by eggs of a captive Lesser Whitefront breeding stock. After hatching the mixed family was caught and kept in captivity. Shortly before fledging the families were transported to Swedish Lapland, where they were released. In autumn the young Lesser Whitefronts were led by their foster parents to the Netherlands to winter. In spring the mixed families returned to Sweden and separated. The Barnacle Geese stayed in their traditional breeding range in Middle-Sweden, whereas the young Lesser Whitefronts returned to the site where they were released, just as intended.

With this method the Swedish re-introduction project founded the only expanding Lesser White-fronted Goose population worldwide.

Although this project has been very successful, there are some genetic problems.

Besides the described problem that an unknown part of the re-introduced birds possibly possess a mtDNA-haplotype shared by both Lesser and Greater Whitefronts, which could indicate hybridisation between both species, there are strong indications of hybridisation between these Lesser White-fronted and Barnacle Geese in some birds.

First marked birds of the Swedish re-introduced Lesser White-fronted Goose population were observed along the German Waddensea coast in 1991. Most of these birds were found associated with Barnacle Geese and in some of these groups also hybrids between Lesser White-fronted and Barnacle geese were observed (MOOIJ & HEINICKE in prep.). These hybrids most likely are the result of a mistake during the imprinting phase, which makes a part of the juvenile Lesser Whitefronts identify themselves as Barnacle Geese. This imprinting problem is a method-immanent risk, because these young Lesser Whitefronts hatch and grow up in close contact to their Barnacle foster parents and winter in Barnacle flocks.

The re-introduction of young Lesser White-fronted Geese by help of microlight aeroplane would exclude this interspecific hybridisation risk.

## 7. Summary

Based on current data, it can be stated that

- In spite of all measurements in the past decades the local Fennoscandian subpopulation is still decreasing with about 5% annually and will be halved in about 10 years time. According to modern population genetics the critical size for small isolated populations is likely a few hundred individuals. At present the population size is clearly below this critical level and the population hardly has a chance to recover again without a strong input of new genetic material.
- There are hardly any facts to support the hypothesis of the local Fennoscandian subpopulation being “a genetically distinct unit”. Moreover there are strong indications that the Fennoscandian subpopulation is an integrated part of the Western Palearctic breeding population.
- Studies on mtDNA-diversity showed that some captive Lesser White-fronted geese do carry a common mtDNA-haplotype shared by Lesser and Greater White-fronted Goose, which could indicate hybridisation. The current re-introduction projects agreed on a method to test the genetics of the project birds (mtDNA and nuclear DNA) to guarantee that only genetically “clean” birds will be selected for future breeding in captivity and re-introduction.
- Between 1981 and 1996 in Sweden and Finland almost 500 Lesser Whitefronts bred in captivity were released. These birds belonged basically to the same genetic “mixture” as the captive stock. It is very likely that the original wild birds have interacted with the released birds. This unintentional “mistake” of the Finnish and Swedish re-introduction programmes to release birds with a possibly questionable genetic composition could be “repaired” by the re-introduction of genetically “clean” birds on a large scale.
- The high reproductive success as well as the population increase of the Swedish re-introduced population (in spite of the release moratorium) indicates a viability of these birds comparable to or even higher than the viability of the birds of the remnant wild population.
- The Swedish method to re-introduce Lesser White-fronted Geese by help of Barnacle Geese as foster parents is very successful but carries a high risk of hybridisation between Lesser White-fronted and Barnacle Geese. The re-introduction of young Lesser White-fronted Geese by help of microlight aeroplane showed to be comparably successful as the Swedish method but would exclude this hybridisation risk.

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