Report —2001/2002: Analyses of the captive populations of the Lesser White-fronted Geese .

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Summary

Some genetic characteristics of a group of captive Lesser White- fronted (**LWFG)** *(A. erythropus)* geese from the LWFG farms from Finland was analysed and results were compared with some samples of wild LWFG and Greater White- fronted (G**WFG)**(*Anser albifrons*).
The hypervariable fragment (domain I)of the control region of mt DNA(length 281 bp.) was investigated in 36 captive LWFG and the haplotypes were compared with the original data from wild LWFG (3 samples from Polar Ural) and GWFG (2 samples from Arkhangel sky region, Kanin) and also with data from GenBank. In the group of analysed captive LWFG 9 birds with haplotypes close to mt haplotypes of GWFG were found.
We also carried out the RAPD-PCR analyses with 3 primers (two of 10 bp and 1 of 15 bp) with the DNA from the samples of captive and wild LWFG and GWFG. In general the RAPD-PCR patterns of captive LWFG were much more close to the patterns of wild LWFG than to GWFG. What is interesting that among the analysed LWFG, which has the GWFG mt haplotypes, the RAPD-PCR patterns were close to LWFG type. These results support the prediction that total DNA of the analysed captive LWFGs were close to the wild LWFG DNA.
We also carried out the SSCP analyses of the short fragment of sex chromosome genes, but the preliminary results shown very similar pictures in unisex groups of both species.

Introduction

The problem of finding of the appropriate molecular markers for different species of geese is very important for population ecology and phylogenetics studies and also for conservation and breeding in captivity. Especially it is important for the threatened species such as the Lesser White- fronted geese. White- fronted **GWFG**(*Anser albifrons*) and Lesser White- fronted **LWFG** *(A. erythropus)* geese genetically are very close species. There are some evidences that these two species in nature can create mix pairs (Panov, 1989). It was found that the rate of divergence tested by means of the analyses of the control region (one of the most rapidly evolved regions of the mtDNA) was rather low between them (Ruokonen, 2000). Analyses of the genetic composition of the captive population of the LWfG which was carried out only on

the base of the mtDNA differences has shown that some captive individuals carried the mtDNA of the white-fronted goose suggesting a hybrid origin. It was concluded that in order to clarify the situation nuclear markers should be applied (Ruokonen, 2001). Many-sided study of the genetic composition of captive LWfG populations is very important because the captive stock of LWfG can play an important role of gene banks and act as breeding centers for reintroduction of LWfG to nature.

The aims of the work were:
1 - to study the variation of control region of the mtDNA in a group of captive LWFG from Finland;
2 - to find some molecular markers for total DNA (not only for the mtDNA, which reflects only the maternal lines) which are able to detect differences between the LWFG and GWFG;
3 - using these markers to compare the birds from the LWFG farm with birds from wild populations and with GWFG.

Materials and Methods

We used samples of dry feathers of LWFG collected in nature, muscle and feathers of LWFG from the farm (Finland) samples,also freshly picked feathers of GWFG from Moscow Zoo and feathers from the wild GWFG.The list of samples is in Table1. All the samples of wild LWFG were collected by V.Morozov, of wild GWFG - by K.Litvin and E.Gurtovaia and GWFG from Moscow Zoo by N.Skuratov. Thank you very much all of them.

DNA isolation

Total genomic DNA was isolated by means of use of one of three protocols:
1) Standart methods based on proteinase K and phenol/chloroform extraction (Sambrook et al., 1989);
2) With guanidine thiocyanate and silica (Carter M.J., Milton I.D., 1993;Hoss and Paabo, 1993) with some modifications developed by G. Shaikhaev the producer of the Diatom DNAprep kit (Biokom, Russia);
3) With Instagene (BioRad) or Chelex 100 (Walsh et al., 1991) with some original modifications (Kholodova et al., 2000).

The hypervariable fragment (domain I)of the control region of mt DNA(length 300-282 bp.) was investigated in 25 captive LWFG and the haplotypes were compared with the original data from wild LWFG (3 samples from Polar Ural) and GWFG (1 sample from Arkhangelsky region, Kanin). Also for comparison some sequencies of the wild **LWFG and GWFG** (lenghth about 210 bp) were obtained from Genebank in order to have more vivid picture.

PCR conditions

The fragment of the control region of the mtDNA was amplified with primers L180 (5 TGGTTATGCATATTCGTGCATAGA 3) and H466 (5 TTTCACGTGAGGAGTACGACTAAT 3). The PCR conditions and

amplification profile were as described by Ruokonen et al (2001) with Taq-DNA polymerase (Sienzim, Russia or Silex, Russia) were used. Amplifications were carried out on thermocycler Terzic (Russia). Oligonucleotides used as primers for RAPD-PCR were: P4 (10 mer) 5 -CCG GCC TTA C-3 (Dolmatova et al., 2000) and P03 (10 mer) 5 -GCG ATC CCC A-3 . Amplification was carried out with primer P03 at 90° C - 2 min.; 95° C - 30 sec., 36 °C - 1 min, 75°C - 2 min, total 40 cycles; 75°C - 10 min.; and with P4 at 95° C - 2 min.; 94° C - 1 min., 40 °C - 1 min, 72°C - 2 min, total 30 cycles; 75°C - 10 min. Amplification reaction were performed in 30 mkl (25 mM MgCl2 , 20 pM primer, 200 mkM of each dNTP).
RAPD-PCR was also carried out with 15 bp primer (GTG)5. For sex chromosomes we used primers P8 (5 -CTC CCA AGG ATG AGR AAY TG, R=A/G, Y=T/C) and P2 (5 -TCT GCA TCG CTA AAT CCT TT) (Griffiths et al., 1998)

Cleaning of the PCR product and contamination control

PCR products were cleaned from agarose gel on QIAGEN colomns.

Contamination control was performed at all stages of work by means of using the negative controls during the DNA extraction and PCR : blank tubes containing all the ingridients except tissues (in extraction) or DNA (in PCR). In all reactions these contamination controls were negative.

Sequencing

Sequencing of the PCR products was performed on automatic ABI-310 (Perkin Elmer) with Big Terminator Kit (Perkin Elmer).

SSCP analyses was carried out as decribed in Orti et al (1997) with some modifications.

Statistical analyses of data and reconstruction of trees

A 300-280 bp fragment of the domain I of control region was aligned and edited manually. Pairwise genetic distances for the haplotypes were estimated using the Kimura s 2-parameter method (Kimura, 1980) and were used to construct a Niebor-joining tree with 500-bootstrap replicates in the MEGA 2 programm (Kumar et al., 2001), for reconstraction of the RAPD-PCR trees the Treecon programm (Van de Peer, Wachter, 1993, 1997). was used.

Table1. Numbers and origin of the samples (* - mt DNA analyses; ~'-RAPD)

# of sample	species	origin	other
L7*'~	LWFG	captive	Finland
L9*~	same	same	same
L10*'~	same	same	same

L11*~	same	same	same
L12*~	same	same	same
L13*~	same	same	same
L14*~	same	same	same
L15*~	same	same	same
L18*~	same	same	same
L19*~	same	same	same
L21*~	same	same	same
L51*~	same	same	same
L52*~	same	same	same
001*~	same	same	same
002*~	same	same	same
003*~	same	same	same
004*~	same	same	same
005*~	same	same	same
006*~	same	same	same
201~	same	same	same
202*~	same	same	same
203*~	same	same	same
204*~	same	same	same
205*~	same	same	same
206*	same	same	same
207*	same	same	same
208*	same	same	same
209*	same	same	same
210~	same	same	same
301*~	same	same	same
302*~	same	same	same
303*~	same	same	same
304*	same	same	same
305*	same	same	same
306*	same	same	same
308*	same	same	same
W1~	same	wild	Polar Ural
2W*~	same	wild	same
W3~	same	same	same
W4~	same	same	same
W6*	same	same	same
DP*	same	same	South Russia
WF23*	GWFG	same	North Russia
WF25*	same	same	same
WF1	same	same	same
WF2~	same	same	same

WF3~	same	same	same
WF6~	same	same	same
WF3z~	same	captive	Moscow Zoo
WF2z~	same	same	same

Results

DNA was successfully extracted from all the samples. For DNA isolation from dry feathers the best results were obtained by means of the guanidine/silica and Instagene methods.

Fragment of control region of the mt DNA analyses

Long fragment

The hypervariable fragment (domain I)of the control region of mt DNA(length 281 bp.) was investigated in 36 captive LWFG and the haplotypes were compared with the original data from wild LWFG (3 samples from Polar Ural) and GWFG (2 samples from Arkhangel sky region, Kanin) and also with data from GenBank.

The relationships of the mt haplotypes of studied birds are shown on the neighbor-joining tree in Fig.1.

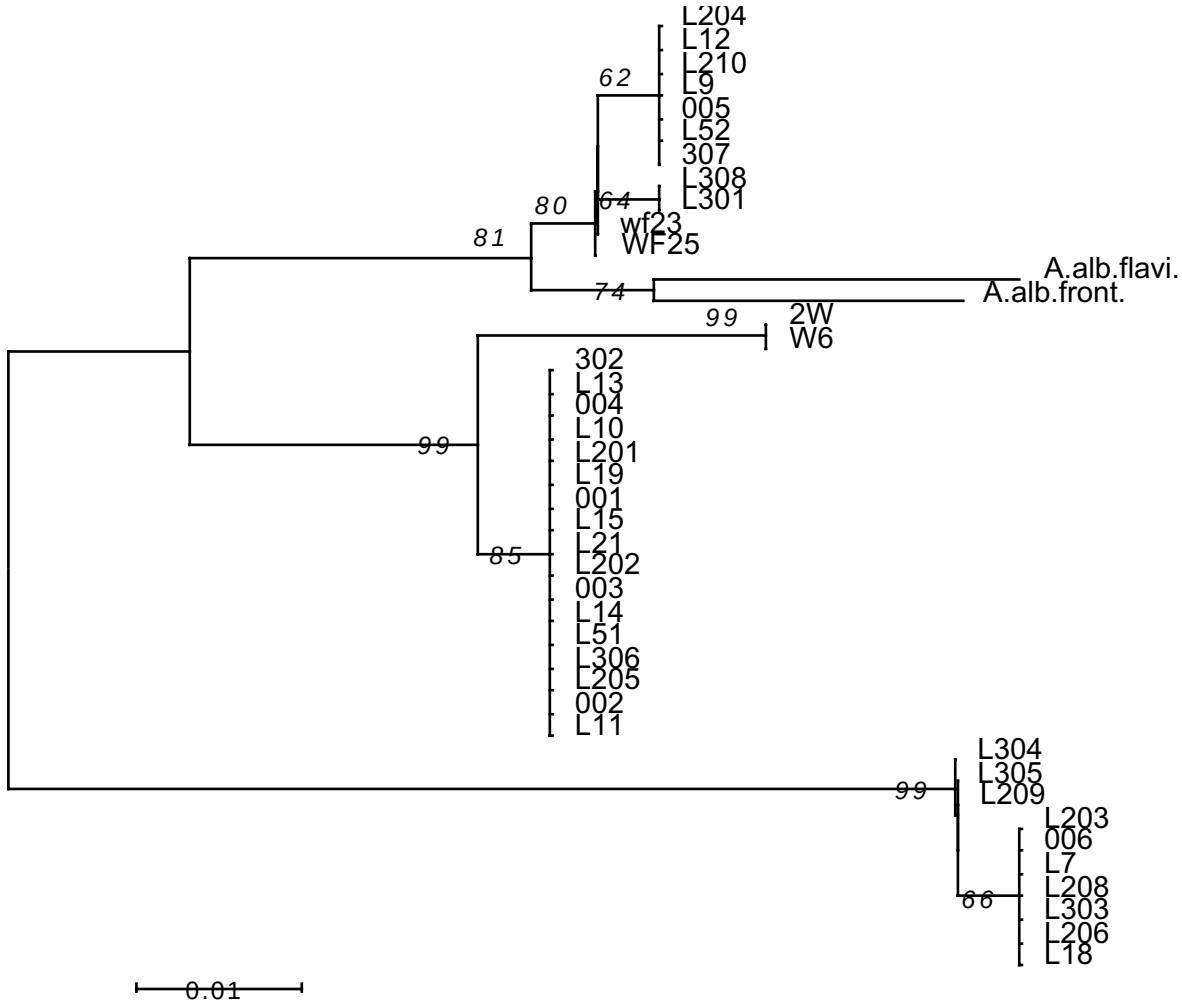

Fig.1

Neighbor-joining tree based on Kimura s 2-parameter distances among mtDNA control haplotypes (281 bp.) of the captive lesser white-fronted geese(all numbers begin with L), wild LWFG (W) and greater white-fronted geese (WF) and A.alb.flavi. and A.alb.front. from GenBank (Paxinos et al., 2002).

Bootstrap values at the nodes are based on 500 replicates, only values above 50 % are shown.

(No. Of Taxa:42
Gaps/Missing data: Pairwise Deletion
Codon Position: Noncoding
Distance method: Nucleotide: Kimura 2-parameter (Pairwise distances)
Tree making method: Neighbor-joining
No. of Sites: 281
No. of Bootstrap Reps=500)

Samples:
All samples are captive LWFGs, except:
W2 and W6 - wild LWFG (collected by V.Morozov)
WF23 and WF25 - wild GWFG(collected by K.Litvin)

*Anser albifrons frontalis ACCESS # AY072579
**Anser albifrons flavirostris" ACCESS # AY072579
(Paxinos et al., 2002)

We found that among the 36 birds of target group of captive LWFG 9 has the haplotype which was very close to the haplotypes of wild GWFG (WF23 and WF25). These were: L9, L12, L52, L204, 210, L301,307, 308 and 005. The sequences of the tested mt DNA region of the other farm samples were clustered with wild LWFG (group L10) from Polar Ural or formed a separate group(L7).
On the Neighbor-joining tree haplotypes of the captive LWFG were separated into three main groups: one of which is very close to the wild LWFGs (in tables I named it LWFG L10), second is rather close to the wild GWFG (in the table I named it hybrids) and the last (it is named LWFG L7) is separate. We created some other types of trees (UPGMA, minimum evolution and others), and all of them were very similar to Neighbor-joining tree.

We calculated the rate of genetic distances between these three groups of haplotypes: the genetic distance between nucleotide group average(Tab.1) and net distance between group average (Tab.2). Both gave same results. It is interesting, that according the rate of genetic distances one cluster of LWFG (L10 , including wild and captive with haplotype) was closer to the group of GWFG and Hybrids than to the other group of captive LWFG (L7).

Table 1.
Genetic distance between group average (3 groups)
(Groups of wild LWFG, GWFG, captive LWFG and questional samples, named as hybrids , were analysed as they were grouped on the neighbor-joining tree)

	[1] #Wild+LWFG(L10)	[2] #Hybrids+GWFG	[3] #LWFG(L7)
[1] #Wild+LWFG(L10)	-	0.012	0.019
[2] #Hybrids+GWFG	**0.053**	-	0.021
[3] #LWFG(L7)	**0.093**	**0.109**	-

Description
No. of Taxa : 42
No. of Groups : 3
Gaps/Missing data : Pairwise Deletion
Codon Positions : Noncoding
Distance method : Nucleotide: Kimura 2-parameter
[Between group average] Standard Error estimated by bootstrap method
(Replications = 500 and random number seed = 49529)
No. of Sites : 281
d: Distance Between group average - under the diagonal (in bold)
S.E : Standard error - above the diagonal

Table 2.
Net distance between group average (3 groups)

	[1] #Wild+LWFG(L10)	[2] #Hybrids+GWFG	[3] #LWFG(L7)
[1] #Wild+LWFG(L10)	-	0.012	0.019
[2] #Hybrids+GWFG	**0.045**	-	0.020
[3] #LWFG(L7)	**0.090**	**0.102**	-

Description
No. of Taxa : 42
No. of Groups : 3
Gaps/Missing data : Pairwise Deletion
Codon Positions : Noncoding
Distance method : Nucleotide: Kimura 2-parameter
[Net between group average] Standard Error estimated by bootstrap method
(Replications = 500 and random number seed = 2918)
No. of Sites : 281
d : Estimate
S.E : Standard error
[1] #Wild+LWFG(L10)
[2] #Hybrids+GWFG
[3] #LWFG(L7)

Names and sample composition of groups:
[1] #Wild LWFG + farm LWFG (haplotypeL10) (n=19): samples ## 10, 11, 13, 14, 15, 19, 21, 51, 201, 202, 205, 302, 306, 001, 002, 003, 004W2, W6
[2] # Farm LWFG- Hybrids (n=9) + wild GWFG (n=4): samples ## 9, 12, 52,204, 210, 301, 307, 308, 005 andWF23, WF25, A.alb.flavi. , A.alb.front.
[3] #LWFG(haplotype L7) (n=10): samples ## 7, 18, 203, 206, 208, 209, 303, 304, 305, 006

In Table 3 the genetic distance between group average was calculated, when all the samples were divided in 5 small groups: #LWFG L10 (n=17); Hybrids (n=9); #LWFG L7 (n=10); #wild LWFG (n=2) and #GWFG (n=4). In this case the rate of genetic distance from the wild LWFG from min to max was: LWFG L10 ,

LWFG Hybrids , GWFG and LWFG L7 . From the wild GWFG this order was: LWFG Hybrids , LWFG L10 , wild LWFG, and LWFG L7 . So the captive group of LWFG with haplotype L7 was the most distant group both from the wild LWFG and from GWFG.

Table 3.
Genetic distance between group average (5 groups)
*(Groups of wild LWFG and GWFG, captive LWFG and questional samples, named as hybrids , were analysed separately*282 bp

	#LWFG L10 [1]	# Hybrids [2]	#LWFG L7 [3]	#wild LWFG [4]	#GWFG [5]
#LWFG L10 [1]	-	[0.013	0.019	0.009	0.013
# Hybrids [2]	**0.049**	-	[0.022	0.016	0.005
#LWFG L7 [3]	**0.092**	**0.110**	-	0.020	0.020
#wild LWFG [4]	**0.022**	**0.064**	**0.104**	-	0.016
#GWFG [5]	**0.058**	**0.019**	**0.107**	**0.071**	-

Description
No. of Taxa : 42
No. of Groups : 5
Gaps/Missing data : Pairwise Deletion
Codon Positions : Noncoding
Distance method : Nucleotide: Kimura 2-parameter [Between group average]

Standard Error estimated by bootstrap method
(Replications = 500 and random number seed = 40845)
No. of Sites : 281
d: Distance Between group average - under the diagonal (in bold)
S.E : Standard error - above the diagonal

Names and sample composition of groups:
[1] #LWFG (L10) (n=17): samples ## 10, 11, 13, 14, 15, 19, 21, 51, 201, 202, 205, 302, 306, 001, 002, 003, 004
[2] # Hybrids (n=9): samples ## 9, 12, 52, 204, 210, 301, 307, 308, 005
[3] #LWFG(L7) (n=10): samples ## 7, 18, 203, 206, 208, 209, 303, 304, 305, 006
[4] #wild LWFG (n=2): samples ## W2, W6
[5] #GWFG (n=4): samples ## WF23, WF25, A.alb.flavi., A.alb.front..

Short fragment

We also compared our results with some data on wild LWFG (accs.## AF234605, AF234606, AF317910, AF317911), and GWFG (haplotypes alb4,alb8,alb7, A.alb.flavi., A.alb.front.(Ruokonen et al., 2001* and Paxinos et al., 2002**) from GenBank . In this case we have to test the shorter fragment because of the length and position of the sequences from GenBank. The analyses of the results for fragments of 208 bp are shown in
Fig.2.

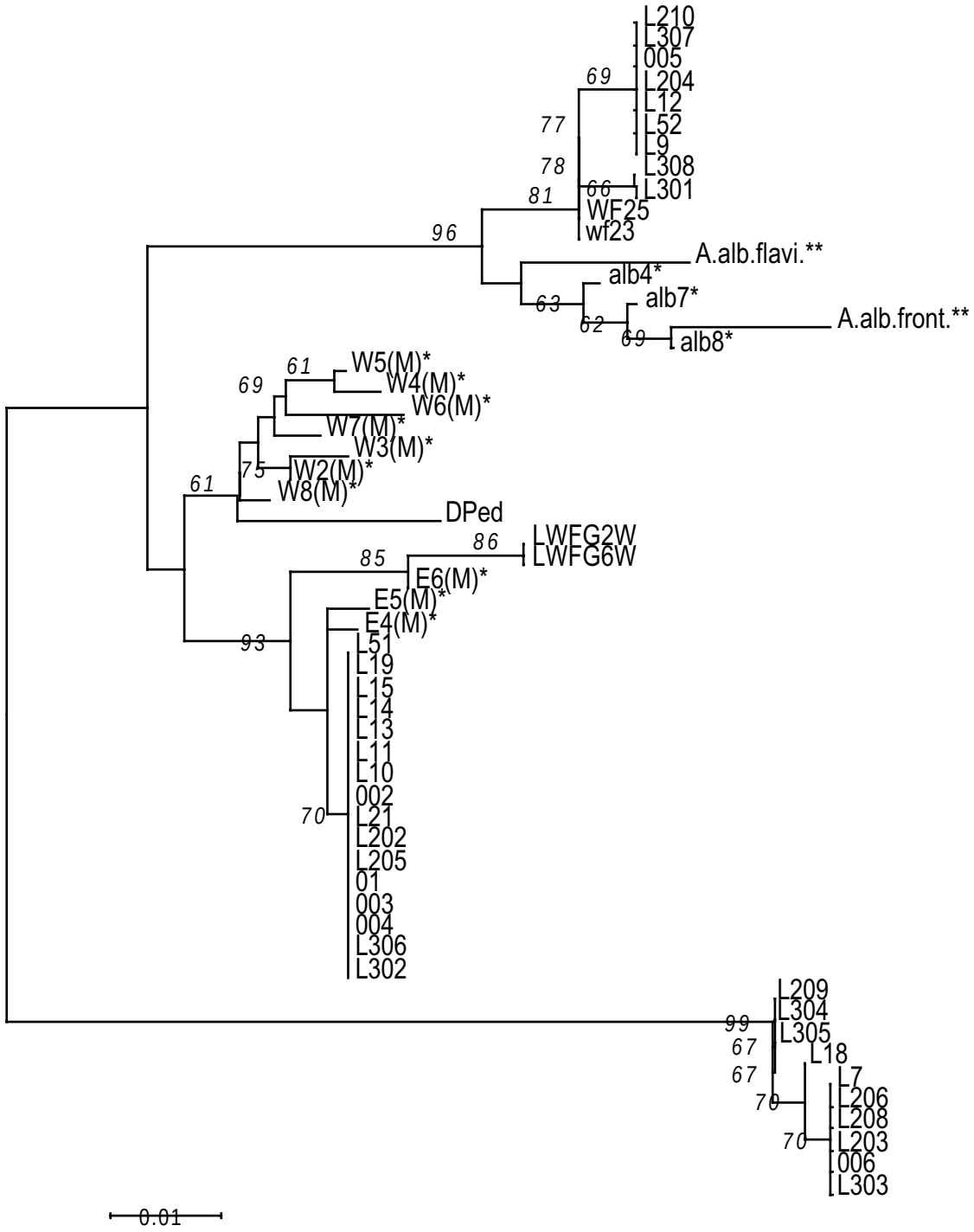

Fig.2

Neighbor-joining tree based on Kimura s 2-parameter distances among mtDNA control haplotypes (208 bp.) of the captive lesser white-fronted geese(all numbers begin with L or with 00), wild LWFG (W, DP and W(M)*) and greater white-fronted geese (WF and alb*). Bootstrap values at the nodes are based on 500 replicates, only values above 50 % are shown.

* and **- sequences from Genbank (Ruokonen et al 2001* and Paxinos et al., 2002**).

In this case we also found the same picture: 9 of the analysed captive LWFG has the haplotypes of the control region of the mtDNA which are close to the wild GWFG. The haplotypes of these birds were also close to the captive LWFG described earlier and supposed to belong to the hybrids (Ruokonen et al., 2001). In the other analysed captive LWFG We found two groups of haplotypes. One group (named haplotype L10) with a high bootstrap support clustered with wild LWFG from the Taimyr and with samples of the E-lineages (Eastern) as discribed by M.Ruokonen (2001). One our sequence (DP) of wild LWFG collected in winter 2001 in the South Russia fall in the cluster with Weatern LWFG haplotypes. And other group of haplotypes of captive LWFG (named as L7) formed a separate cluster.

RAPD-PCR analyses was used for study the DNA-polymorphism.
RAPD-PCR (Random Amplified Polimorphic DNA) analyses is able to give an information about the total DNA. This method is based on the amplification of genomic DNA by using a single oligonucleotide of random sequence as primer.
The amplification products resolved on agarose or polyacrilamide gel give rise to a complex pattern, specific for a given genome. In comparison with the classic biomolecular methods RAPD does not need previous information regarding the sequence, and it uses a smaller quantity of DNA (Williams et al., 1990; Welsh et al., 1991; Masetti et al., 1996; Novikova et al., 2000). We found that the RAPD-PCR patterns with the DNA extracted from feather and muscles from the same bird were similar, so both types of samples can be used for such work. It is very useful for non-invasive sampling especially for endangered species such as LWFG. The best resolution gave the use of 5 % polyacrilamide gel for 5-6 h., although other concentrations and regimes as well as using the agarose gels are also able to show the differences between two species DNA patterns.

The preliminary results demonstrated that all three RAPD primers were able to show some differences between DNA patterns of LWFG (farm and wild) and GWFG.
The RAPD patterns of LWFG from wild nature and from the farm were very similar with primer P03 (fig.3) and with P4 also. We found that some birds, for example L9, which had the hybrid mt haplotype, were accordingly the RAPD-PCR analyses of the total DNA were close to the captive and wild LWFG.

Fig.3 RAPD pattern with primer P03. Wfth- wild LWFG (feather), Wmscl- wild LWFG (muscle), WF - WFG, L - LWFG from farm.

Wfth	**Wmscl**	**L7**	**L9**	**L10***	**L13**	**L15**	**WF2**	**WF3**
___	___	___	___	___	___	___	___	___
___	___	___	___		___	___		
							___	___
___	___	___	___	___	___	___	___	___
___	___	___	___	___	___	___	___	___
___	___	___	___	___	___	___	___	
___	___	___	___	___	___	___		
								___
___	___	___	___	___	___	___		
							___	
								___
___	___	___	___	___	___	___	___	
___	___	___	___	___	___	___		
							___	
___	___	___	___	___	___	___	___	___

* In general L10 has the close pattern with other LWFG both from the farm and wild nature. The differences in one case of this bird from the other LWFG were connected with the absence of one band, but there were not detected any bands which were usual in the patterns of GWFG.

The results of PCR with different RAPD primers are shown in the Appendix (Fig.1a-12a). Each gel is described separately. For some of them the trees with bootstrap values were constructed. In cases when all the samples have the same pattern we just give the list of the samples.The main purpose why we put the material in this form it is to show that in spite of the known variability of the RAPD patterns in all cases the general picture shows that the RAPD patterns of total DNA of all analysed captive LWFG, including those, which were determined by mtDNA analyses as hybrids ,

are close to each other and much more close to the wild LWFG than to the wild GWFG.

Additionally we carried out the **SSCP** analyses of the short fragment of sex chromosome genes, but the preliminary results shown the same pictures in unisex groups of both species.

Discussion

White fronted (*Anser albifrons*) and Lesser white fronted *(A. erythropus)* geese are very close species: both morphologically and ecologically. It was also found that the rate of divergence tested by means of the analyses of the control region (one of the most rapidly evolved regions of the mtDNA) between these species is rather low (Ruokonen, 2000). The period of divergence from the common ancestral form of LWFG and GWFG is comparatevely low. These species could be defined as young , and it is not a surprise that a lot of similar features could be found between them and the rate of differences is not very high. That is why the problem of hybridization and especially the base of conclusion about the fact of hybridization between these species is very complicated.

We found in the group of captive LWFG two clusters of haplotypes (groups L7 and L10) which are rather different from reach other . Accordingly Garrigan et al.,(2002) this pattern can result from a bottleneck because, when population sizes are reduced, rare allels are easily lost, many of which may be mutational intermediates between the survival haplotypes .

It is intersting, that the genetic distance between two haplotype groups of LWFG (L7 and L10) was larger than between one of these groups (L10) and the group including GWFG and Hybrids .So accordingly analyses of the fragment of control region of the mtDNA the captive group of LWFG with haplotype L7 was the most distant group both from the wild LWFG and from GWFG. The otherhaplotype group(L10) of captive LWFG was more close to the wild GWFG than to the other haplotype of LWFG.We can suppose that the haplotype L10 is more close to the

ancestral type than haplotype L7 . In general, the picture of divergency between these two species on the molecular rate is very complicated, and there is an opportunity that haplotypes of captive LWFG which we determined as Hybrids (on the base of their simialarity with GWFG mt types) may be just the unique ancestral forms which are common in two related species.

According our preliminary data the RAPD patterns of the total DNA of some analysed LWFG with hybrid mt haplotypes were very close to the patterns of wild LWFG, although the LWFG from nature as well as from the farm were rather different from the GWFG .

So, we name hybrids the mtDNA haplotypes, not birds! These two species - LWFG and GWFG - genetically are very close to each other, and may be that type which we determine as hybrids just an ancient ancestral-like type. It may be close to the ancestral type of the mtDNA and be common in both species.

Why these common mt haplotypes have not been detected in nature in the wild populations of LWFG and GWFG?

There are two answers. The first: during the bottleneck of the LWFG these rare haplotypes were lost by the gene drift. The second: the number of studies wild samples of LWFG and GWFG from different parts of range is still rather low and these haplotypes were not found. The conclusion from the first answer: It is very important to save the birds with these unique haplotypes in captivity. The conclusion from the second answer: The wide study of the wild populations of both related species is very important.

Another scenario is: the questional LWFG, determined by mtDNA as hybrids indeed have some blood of the hybrids between LWFG and GWFG. Is it a sufficient base to kill them?

Hybridization between species is not unique in the wild nature. There are a lot of samples in different groups: amphibians, butterflies, birds, mammals, reptiles, fish. Hybridization between different bird species is not rare in nature (Rising, 1983; Panov,1989). Panov (1989) in his review wrote that more than 5 % of birds species

are involved in hybridization in zones of secondary contacts, and with an account of the occasional sympatric hybridization this per cent increases up to 10 % of the whole number of the world ornithofauna. Hybridization between LWFG and GWFG takes place in the wild nature (Panov,1989).

In some aspects, concerning the conservation of the evolutionary process, the words which were said about the American Red Wolf by Paquet (2001) can be applied to the LWFG:

> The dilemma for conservation is to determine whether such hybridization would have occurred had there been no human disturbance. If the Red wolf is not a valid species, the point is moot. If it is a valid species and hybridization occurs as a natural phenomenon, then it is the process rather than the entity that needs to be protected. On the other hand, if the Red wolf is valid but hybridization is unnatural, then the species should be protected for all the reasons we work to protect biodiversity. The lesson would seem to be that we should give equal consideration to the protection of evolutionary processes as well as to the protection of species. Until now, conservationists have focused only on the protection of species, but that approach will fail in evolutionary time. (Paquet, 2001, p.47).

The main conclusions of this work are:

- The full genetic screening of the farm gees is very important, for it can find out new unique haplotypes which may be lost in nature;

- The questional birds bearing the haplotypes close to the GWFG are not the target of killing but of studying and preserving. As soon as there is a chance that these birds has the rare and ancient type of genotypes which is theoretically can be common with the close species GWFG these birds should be alive. For the decision of the hybrid nature of these birds the wide comparison with both wild LWFG and especially with wild GWFG must be done. On different species of animals it was shown that in most cases the lack of the genetic diversity can lead to declining of the survaval rate. That s why it is necessary to do as much as possible in order save all the genetic types of

endangered species. In the case of the LWFG it is very important.
The captive populations of the LWFG can be used as the reserves of these rare haplotypes which may be absent in nature. This is a very important point to support the geese farms all over the world in order to save the genetic diversity of LWFG.

- From the other hand for the current reintroduction it is better to use the tested birds with typical LWFG haplotypes.

- The similiarity of fragments of the sex chromosomes of LWFG and GWFG in the SSCP analyses shows the close relations of these species.

The group of tested samples in this research was rather limited, so our conclusions are rather preliminary. Further investigations are needed to better define the relationships between these two species and to find some more informative molecular markers which can help in conservation of the LWFG and study of the hybridization problem between different geese species.

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