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Date

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Executive Secretary Bert Lenten
UN CAMPUS
UNEP/AEWA Secretariat
Herman Ehlers - Str 10
53113 Bonn GERMANY

Subject: Comments on the manuscript: "Genetic analysis of captive Lesser White-fronted Geese in Germany" Pedall *et al.*

Dear Bert Lenten

Please, find attached comments of the Finnish experts concerning above mentioned manuscript.

Yours sincerely,

Matti Osara
Senior Adviser

Comments on the Pedall *et al.* manuscript.

Minna Ruokonen & Juha Merilä

Mitochondrial DNA

In the Table below are the mtDNA haplotype frequencies from the Table 3 in Pedall *et al.* reorganized by the species and populations. The first striking observation is that haplotype “LWFG1” found in both lesser and greater white-fronted geese is present only in the captive population of the lesser white-fronted goose. The fact that haplotype LWFG1 does not exist in the wild lesser white-fronted goose population strongly suggests that its presence in the captive lesser white-fronted goose stock is due to hybridization in captivity, and this is not clearly enough stated in the manuscript.

Table. Haplotype frequencies from Pedall *et al.* Table 3 listed according to species and populations.

	greater white-fronted goose		lesser white-fronted goose		bean goose	greylag goose
	Russia	Germany	captive	Russia	Russia	Germany
GWFG	12	-	-	-	-	-
LWFG1	-	45	42	-	-	-
LWFG2	-	-	2	-	-	-
LWFG3	-	-	10	-	-	-
LWFG4	-	-	10	-	-	-
LWFG6	-	-	2	-	-	-
LWFG5	-	-	-	7	-	-
LWFG7	-	-	-	4	-	-
LWFG8	-	-	-	2	-	-
LWFG9	-	-	-	4	-	-
LWFG10	-	-	-	2	-	-
LWFG11	-	-	-	2	-	-
BG	-	-	-	-	7	-
GLG	-	-	-	-	-	5
N	12	45	66	21	6	5

The second observation is even more striking: the captive and wild populations of the lesser white-fronted goose do not have a single mtDNA haplotype in common. In the captive population haplotypes LWFG1-4 and LWFG6 are found, whereas in the wild population haplotypes LWFG5 and LWFG7-11 are present. This is a very strange finding and the explanation is not obvious. Even if the sample size for the wild lesser white-fronted goose population is small, it is typical that common haplotypes are sampled with a greater probability than the rare ones, and the same fact applies to the captive populations as well. The probability that the captive population carries five lesser white-fronted goose haplotypes that are currently extinct in the wild population is extremely small, especially as it is known from previous work (Ruokonen

et al. 2004) that there are two very common haplotypes (found in 64% of the individuals) present in the wild population. Possibly, this calls for reassessment of the methodological part of the work

Setting the reason aside, the finding has consequences for the interpretation of the results. The purpose here would be to examine the genetic composition of the captive stocks based on the data obtained from the wild population as a reference sample. So, now the results tell that in the captive population there are four unknown haplotypes (LWFG2-4 and LWFG6) and one haplotype (LWFG1) in common with the greater white-fronted goose, the latter of which could suggest that 64% of the German captive lesser white-fronted geese have a hybrid origin. Also, as seen from the Fig. 3 in Pedall *et al.*, the species do not cluster into monophyletic groups and e.g. the bean goose and the greylag goose are more closely related to the “lineage II” than the lineage I and II are to each other suggesting that not enough resolution has been obtained with this marker. Therefore, it is impossible to say, or even to guess, based on the tree topology, to which species some of the haplotypes belong.

Nuclear DNA

In the results for the assignment test (program Structure) the authors do not state the findings clearly enough. When K=3, the groups correspond to 1) captive lesser, 2) wild lesser and 3) greater white-fronted goose + greylag goose, and when K=4, the groups are 1) captive lesser, 2) wild lesser, 3) greater white-fronted goose and 4) greylag goose. So, in both analyses, the program suggests that the captive and wild lesser white-fronted goose belong to different groups, which tells that they are differentiated from each other. A list of alleles shared by or private to the species/populations would have helped to evaluate the performance of Structure analysis.

Concerning the eight putative hybrids found, it would have been essential to know which mtDNA haplotypes these individuals carried. This could have given an additional viewpoint for the analysis and especially for the conclusions. If the German captive lessers carry greater white-fronted goose nuclear alleles, there probably is also heterospecific mtDNA in the captive population.

Implications

The results of Pedall *et al.* do not differ from previous results in such a way that the common decision to not to use the present old captive stocks for reintroduction/population supplementation should be reconsidered. The German stocks were shown to include hybrids, but the manuscript is lacking an effort to try to clarify the situation in depth (e.g. how many captive stocks were sampled and how many of them were affected?). However, it seems that the hybrids come from different farms (M. Wink, pers. comm. to M. Osara), and this implies that there are probably also other birds affected, as the hybrids do not reproduce by themselves. This means that after removing the hybrids found, the captive stock can not be considered pure, contrary to the conclusion by Pedall *et al.*

Other comments:

Introduction: The lesser white-fronted goose has never been wintering in Kazakhstan, France or Hungary.

Introduction: references missing for hybridization in the wild, as well as for the “old migration route” leading to Germany.

Fig. 1 comes out of the blue, is not explained in the material and methods.

Table 3 contains errors, a lot (e.g. nucleotide positions that do not seem to vary or contain only one of the nucleotides and dots: 16, 132, 162, 339, 492, 755, 763). Also, assigning haplotypes based on heteroplasmic nucleotide positions should be justified and the reasoning used should be explained: there are no simple rules for this. One could say that there are only seven “LWFG” haplotypes instead of 11.

3.2. Positive F_{is} values: most likely due to Wahlund effect, considering the sampling strategy.