**Fra:** Carsten Nowak [mailto:Carsten.Nowak@senckenberg.de]
**Sendt:** 24. februar 2016 17:23
**Til:** Hans Erik Svart
**Emne:** SV: Antw: SV: Wolf genetics contact number

*Dear Hans-Erik,*

***regarding the beavers: are there problems with inbreeding depression or do the beavers not reproduce well or why do you consider the genetic diversity to be a problem in Danish beavers?***

***It is totally fine to present them the text I wrote, just go ahead. My question again just ti reassure myself: You indicated that it is fine to be open regarding the results. In Germany I am contacted now regularly by people who want to know how many wolves we found in Denmark, also the Copenhagen lab asks us. Should I report what we found (4 male individuals) or should I refer to you or what do you recommend?***

*All the very best,*

*Carsten*

*Dr. Carsten Nowak*
*Fachgebiet Naturschutzgenetik - Conservation Genetics Group*
*Forschungsinstitut Senckenberg - Senckenberg Research Institute*
*Standort Gelnhausen - Station Gelnhausen*
*Clamecystrasse 12, D-63571 Gelnhausen*
*tel 06051-61954-3122*
*fax 06051-61954-3118*
*www.senckenberg.de*
*www.bik-f.de*

Senckenberg Gesellschaft für Naturforschung
Rechtsfähiger Verein gemäß § 22 BGB
Senckenberganlage 25
60325 Frankfurt
Direktorium: Prof. Dr. Dr. h.c. Volker Mosbrugger, Prof. Dr. Andreas Mulch, Dr. Johannes Heilmann, Prof. Dr. Katrin Böhning-Gaese, Prof. Dr. Uwe Fritz, PD Dr. Ingrid Kröncke
Präsidentin: Dr. h.c. Beate Heraeus
Aufsichtsbehörde: Magistrat der Stadt Frankfurt am Main (Ordnungsamt)

*>>> Hans Erik Svart <HES@nst.dk> 24.02.2016 15:41 >>>*

Dear Carsten,
Thank you for the article about beaver. It seems that introducing beavers from Bavaria would be a good idea.
Regarding wolf, we have asked DCE a number of questions. However, instead of answering I writing, they would prefer a meeting with us. This will be at the beginning of next week. Most likely we will present the reservations you have to their findings. Is this ok with you?
All the best
Hans Erik

**Hans Erik Svart**
| Nature Protection
+45 93587950 | hes@nst.dk

**Ministry of Environment and Food of Denmark**
Nature Agency | Haraldsgade 53 | 2100 Copenhagen Ø, Denmark | Tel. +45 72 54 30 00 | nst@nst.dk |www.naturstyrelsen.dk

**Fra:** Carsten Nowak [mailto:Carsten.Nowak@senckenberg.de]
**Sendt:** 23. februar 2016 10:32
**Til:** Hans Erik Svart
**Emne:** SV: Antw: SV: Wolf genetics contact number

*Dear Hans Erik,*

***Will the statement be released or will anything become public and when would that probably be?***

***We have intensively studied beaver genetics in Germany (attached as pdf). The diversity in beavers is really low, but I would not be to concerned too much, as he seems to generally cope with this well. Moreover, adding a few beavers, for instance from Bavaria will effectively solve the problem. And brings back a more natural state compared to the conservation of impoversished anthropogenic relict populations as the "Elbebiber". You might read the "management implications section" on page 12 and 13, there I commented on this issue.***

*All the best,*

*Carsten*

*Dr. Carsten Nowak*
*Fachgebiet Naturschutzgenetik - Conservation Genetics Group*
*Forschungsinstitut Senckenberg - Senckenberg Research Institute*
*Standort Gelnhausen - Station Gelnhausen*
*Clamecystrasse 12, D-63571 Gelnhausen*
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Aufsichtsbehörde: Magistrat der Stadt Frankfurt am Main (Ordnungsamt)
*>>> Hans Erik Svart <HES@nst.dk> 23.02.2016 08:50 >>>*
Dear Carsten,

Thank you for clearifying the discrepancy between your results.
We highly appreciate your advice and will now have to consider our next step.
Today I will be at a meeting on beaver where we also will be facing some problems regarding genetic diversity in the future. Our beavers comes from the Elbe river population.
All the best
Hans Erik

**Fra:** Carsten Nowak [mailto:Carsten.Nowak@senckenberg.de]
**Sendt:** 22. februar 2016 16:09
**Til:** Hans Erik Svart
**Emne:** SV: Antw: SV: Wolf genetics contact number

*Dear Hans Erik,*

*it is true that we rely most oftenly on relatively fresh materials, and this is the way pretty much all colleagues go. You simply cannot differetiate individuals based on such old samples under central European climates.*
*Aarhus methods are slightly modified compared to ours and should in theory be more sensitive, but they are also prone to higher risk of cross-contamination, which is the main obstacle when doing these analyses. And again. This is not a dispute about the sensitivity of methods, this is about how to interpret data obtained from low-quality samples. Aarhus looked very closely at all data and replicates, picked every genetic signal they could find, irrespective of its intensity and consistency among replicates to assemble genotypes. This is a procedure that necessarily leads to strongly biased outcomes when aiming at identifying individuals. Commonly agreed standards exist for nearly 20 years now stating that your genetic data needs to be successfully replicated in order to be regarded as valid. And we simply do not see this in the datasets delivered to us.*

*All problems would be solved if Aarhus would follow our protocols; these protocols are also followed by the other partners in the CEwolf consortium. And they could still work on developing novel methods. We agreed that we are interested in the Aarhus method in order to test where this discrepancy derives from. We also developed a novel method for individual genotyping of wolves, which Aarhus successfully tested already. The lab is well capable of performing all wolf analyses, without any doubt. But they had no experience in handling these samples and the data resulting from them and where too enthusiastic about their "sensational" findings, then there was no way out anymore. And I understand that they will defend the results. And it will be difficult to convince them they have to change everything from the scratch.*

*All the best,*

*Carsten*

*Dr. Carsten Nowak*
*Fachgebiet Naturschutzgenetik - Conservation Genetics Group*
*Forschungsinstitut Senckenberg - Senckenberg Research Institute*
*Standort Gelnhausen - Station Gelnhausen*
*Clamecystrasse 12, D-63571 Gelnhausen*
*tel 06051-61954-3122*
*fax 06051-61954-3118*
*www.senckenberg.de*
*www.bik-f.de*

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60325 Frankfurt
Direktorium: Prof. Dr. Dr. h.c. Volker Mosbrugger, Prof. Dr. Andreas Mulch, Dr. Johannes Heilmann, Prof. Dr. Katrin Böhning-Gaese, Prof. Dr. Uwe Fritz, PD Dr. Ingrid Kröncke
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Aufsichtsbehörde: Magistrat der Stadt Frankfurt am Main (Ordnungsamt)

*>>> Hans Erik Svart <HES@nst.dk> 22.02.2016 15:01 >>>*

Dear Carsten,

Thank you very much for your fast and detailed response.

It has been our understanding from our communication with University of Aarhus that you *at Senckenberg are in dialogue with them about the methods University of Aarhus use for analyzing DNA from biological material. Do you have a time frame for this work?*

*Aarhus University have informed that the reason for using different methods is that you in Germany most often have relatively fresh samples which contain a lot of DNA while the Danish material they analyze is old and with a small amount of DNA.*

We will most likely have to ask our adviser, Aarhus University, to comment on the information you have provided.

All best

Hans Erik

**Hans Erik Svart**
| Nature Protection
+45 93587950 | hes@nst.dk

**Ministry of Environment and Food of Denmark**
Nature Agency | Haraldsgade 53 | 2100 Copenhagen Ø, Denmark | Tel. +45 72 54 30 00 | nst@nst.dk |www.naturstyrelsen.dk

**Fra:** Carsten Nowak [mailto:Carsten.Nowak@senckenberg.de]
**Sendt:** 22. februar 2016 11:22
**Til:** Hans Erik Svart
**Cc:** Annette Samuelsen; Cristina Nissen; Lasse Jensen
**Emne:** Antw: SV: Wolf genetics contact number

*Dear Hans Erik,*

*As requested by you, I am happy to provide some more detailed information concerning our reanalysis and the conclusions drawn by my team with regard to the number of wolves in Denmark and the status of wolf genetics in your country.*
*We were first contacted by Liselotte W. Andersen, University of Aarhus, late 2012 in order to cooperate on the question of the Thy wolf origin. Since then we have been in regular contact and Dr. Andersen visited our lab repeatedly in order to crosscheck her genetic data with us. In the first meetings we were shown final genotypes and just some raw data of canid individuals, which we could identify as wolves, mostly with unknown origin. As we did not saw the entire set of raw data for most samples, we could not judge the reliability of the data. Also, our marker systems were not entirely standardized at this early stage of collaboration.*
*In the frame of the newly founded CEwolf consortium all partners, including Aarhus, agreed on harmonizing their marker systems for wolf analysis to our system, as it has shown to provide robust genetic information proven by the successful reconstruction of the German wolf pedigree based on >4000 analyses of mostly noninvasively collected samples. By exchanging samples and subsequent analysis, it was proven that data harmonization was successful between Denmark and Germany.*
*In autumn 2015 Dr. Andersen asked us for consultancy again in order to crosscheck recent findings of a surprisingly large number of wolves recently detected on basis of wolf scats found in Denmark.*
*Based on our experience in Germany and other countries, it was hard to believe to us that large numbers of wolves might persist in or at least temporarily visit Denmark, leaving few traces of their existence. Dr. Andersen and us agreed that we reanalyze a selection of 50 scat samples at Senckenberg for which Dr. Andersen found a unique wolf individual. The samples arrived on November 9th and were analyzed by our lab technician Michel Schleenbecker and our scientific assistant Anne Jarausch, who routinely perform all genetic analyses in the course of the German genetic wolf monitoring at Senckenberg. We analyzed the samples with four different marker sets:*
*1. A mitochondrial control region fragment which amplifies mammal DNA and is suitable for species discrimination (primers L15995/H16498; detect wolf, dog, fox & prey species)*
*2. A mitochondrial control region fragment specific for the Canis genus (primers WDloopL/WDloopH254; specific for wolves and dogs)*
*3. A set of routinely applied 14 microsatellite markers for individual discrimination (specific for dogs and wolves, with some markers working for foxes as well)*
*4. Two markers for sex discrimination (specific for canids)*
*The results were sent to Dr. Andersen on December 16th. We could confirm the presence of wolves in six samples, three of them showed a sufficiently high quality for individual discrimination. These male individuals have all been detected earlier in Germany (see attached results file). In the other cases, wolf detection was only successful using the mtDNA markers, which are often more sensitive due to the higher copy number of mitochondrial DNA in cells. In three samples we found dog DNA while 35 samples likely derive from the fox or at least show traces of fox DNA. This finding confirms our initial morphological inspection of the scats, where several samples resembled fox scats more closely than wolf scats. Interestingly, the fox was also detected in two of the samples in which wolf DNA was discovered. This finding is, however, not unusual, given the high density of foxes in the landscape and the resulting high chance of fox contamination in environmental samples. Four samples showed no genetic signals, two other samples had not been included in the analysis due to a lack of genetic material.*
*Because it was not possible to explain these contrasting findings between Aarhus and Senckenberg it was decided that Dr. Andersen provides her raw data to us for data reanalysis in our laboratory. The reanalysis of microsatellite data from the Aarhus laboratory performed by Anne Jarausch resulted in the detection of only two wolf individuals based on our*

*standards for analyzing genetic data obtained for noninvasively collected material. The two wolves comprised the dead Thy wolf and his half-brother also deriving from the Milkel pack in Saxony.*
*Combined with the analysis of the 50 more recent wolf samples, this results in four genetically identified individuals, based on the data and samples provided by Dr. Andersen (one individual was found both in the reanalyzed older data and in the set of 50 more recent samples analyzed in our lab). We are aware that it is highly likely that several more samples not considered by us to fulfill the quality criteria for genetic data might show wolf DNA signals and the fact that Dr. Andersen successfully generated consensus genotypes that were initially confirmed by us as wolves in earlier analyses indicates the presence of a higher number of wolf samples and probably also wolf individuals than we accepted following strict scientific standards. However, based on our extensive experience in performing and interpreting genetic data obtained from analysis based on scats and other noninvasively collected wildlife samples we know that it is absolutely mandatory to follow at least the most basic rules of forensic wildlife analysis, such as counting alleles only when they are repeatedly and consistently detected among replicates and if genetic signal intensity does not fall below a certain threshold. Based on our reanalysis, these minimal standards have not been considered in the analyses performed by the Aarhus laboratory. We conclude that most of the microsatellite alleles scored by the Aarhus laboratory likely derive from foxes, leading to a high number of "wolves" with unique genotypes.*
***In conclusion, we see hard genetic evidence for the presence of four wolves in Denmark since 2012. All wolves are males and have been detected earlier in Germany or could be assigned to a German pack. We found no evidence for the presence of wolf packs or female wolves.*** *One individual consists of the dead Thy wolf, another one, found repeatedly in the old, reanalyzed data as well as in our reanalyzed scat samples, is his half-brother deriving from the same pack (Milkel). The two other wolves have been earlier detected in Schleswig-Holstein, making their appearance in Denmark likely. Given the lack of a coordinated wolf monitoring in Denmark and the fact that we likely did not reanalyze the entire genetic dataset, we cannot exclude the presence of packs or females or a higher number of wolves in Denmark.*
*It is by no means my intention to interfere with the Danish wolf monitoring. Given the high public and scientific interest in the currently observed rapid spread of the wolf in Central Europe, however, I strongly recommend to work towards a systematic wolf monitoring in Denmark, which includes both professionally organized sample collection and other field work as well as the conduction of genetic analyses fulfilling international scientific standards for data handling, documentation, analysis and interpretation. Noninvasive wildlife genetics based on the use of environmental samples is not a trivial task and requires a high grade of specialization and experience as well as extraordinary care when interpreting data. I strongly recommend training experienced lab persons in internationally recognized laboratories specialized on forensic and noninvasive wildlife genetics or to rely on already existing laboratories having this experience. In the short term the involvement of our laboratory or a similarly suited institution to back up genetic wolf monitoring in Denmark could be a reasonable solution, while building up professional systems within the country. In my opinion this could be successfully done either in Aarhus or any other well-qualified Danish institution.*
*Given the suitability of habitats and high prey densities it is at least very likely that wolf packs will establish in Denmark within the next few years. While this is great news, it will also result in human-wildlife conflicts (consider the situation elsewhere in Scandinavia or in Germany), which can only be solved on the foundation of a solid wolf monitoring.*

*With kind regards,*

*Carsten Nowak*
*Senckenberg Wildlife Genetics Laboratory &*
*National Reference Center for Large Carnivore Genetics*

*Dr. Carsten Nowak*
*Fachgebiet Naturschutzgenetik - Conservation Genetics Group*
*Forschungsinstitut Senckenberg - Senckenberg Research Institute*
*Standort Gelnhausen - Station Gelnhausen*
*Clamecystrasse 12, D-63571 Gelnhausen*
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*fax 06051-61954-3118*
*www.senckenberg.de*
*www.bik-f.de*

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Aufsichtsbehörde: Magistrat der Stadt Frankfurt am Main (Ordnungsamt)

*>>> Hans Erik Svart <HES@nst.dk> 19.02.2016 15:25 >>>*

Dear Carsten,

Thank you for your email below. The downscaling of the number of wolves in Denmark was done and published by Aarhus University, not the Danish Nature Agency. Aarhus University is our consultants regarding wolves. We have a contract with Aarhus University, and they perform analysis of farm animals supposedly killed by wolves. These analysis are from wounds on the killed farm animal. Furthermore the university have got private funding for analysis of faeces found by the public or university researchers. Aarhus University combines all results and publicize the total number of wolves registered in Denmark. As the university act as our consultant we use information provided by them in our communication.

Now, University of Copenhagen has analyzed 200 samples for wolf-DNA. They have just (today) published a press release saying that 1 of these 200 samples was definitely wolf. University of Copenhagen has got almost all material from a small group of wolf-interested citizens. This material is not the same as the material analyzed by Aarhus University, and has not been analyzed by other laboratories. Their assessment is that only "a handful" of wolfes are/have been visiting Denmark.

As you know, in November last year Aarhus University sent 50 samples to *Senckenberg Research Institute for verification. Aarhus University has informed us that out of this you identified two wolfes by using one method and six wolves by using another method out of these 50 samples (species not individuals we presume?). This is in contrast to Aarhus University who found 47 wolves in the same samples. We have been informed by the university that you at Senckenberg are in dialogue with them about further development of the method used by Aarhus University. We would be interested in your assessment of differences and adequacy of the methods for analyzing wolf-DNA applied by Aarhus University and by the CEWOLF consortium.*

*Our main interest in the Danish Nature Agency is to have reliable information on the number of wolves that has been registered in Denmark. Therefore, we are not very pleased with a situation where there is an open disagreement between two scientific institutions on the number of wolves in Denmark. At the Nature Agency (and at both universities) Senckenberg is considered to be the leading institute regarding wolf DNA-analysis.*

Therefore we would also be grateful for your advice as to how we could provide an answer on how many wolves we have registered in Denmark? Furthermore we would like to hear your views as to how we can establish a procedure for furture verification of wolf-DNA analysis.

In general, we are interested in co-operation with you and other "wolf countries" in Northern Europe as we, at the Danish Nature Agency consider the "Danish wolves" as part of the Central European wolf population.

Looking forward seeing you in Schleswig-Holstein, March 9

All best

Hans Erik

**Hans Erik Svart**
| Nature Protection
+45 93587950 | hes@nst.dk

**Ministry of Environment and Food of Denmark**
Nature Agency | Haraldsgade 53 | 2100 Copenhagen Ø, Denmark | Tel. +45 72 54 30 00 | nst@nst.dk |www.naturstyrelsen.dk