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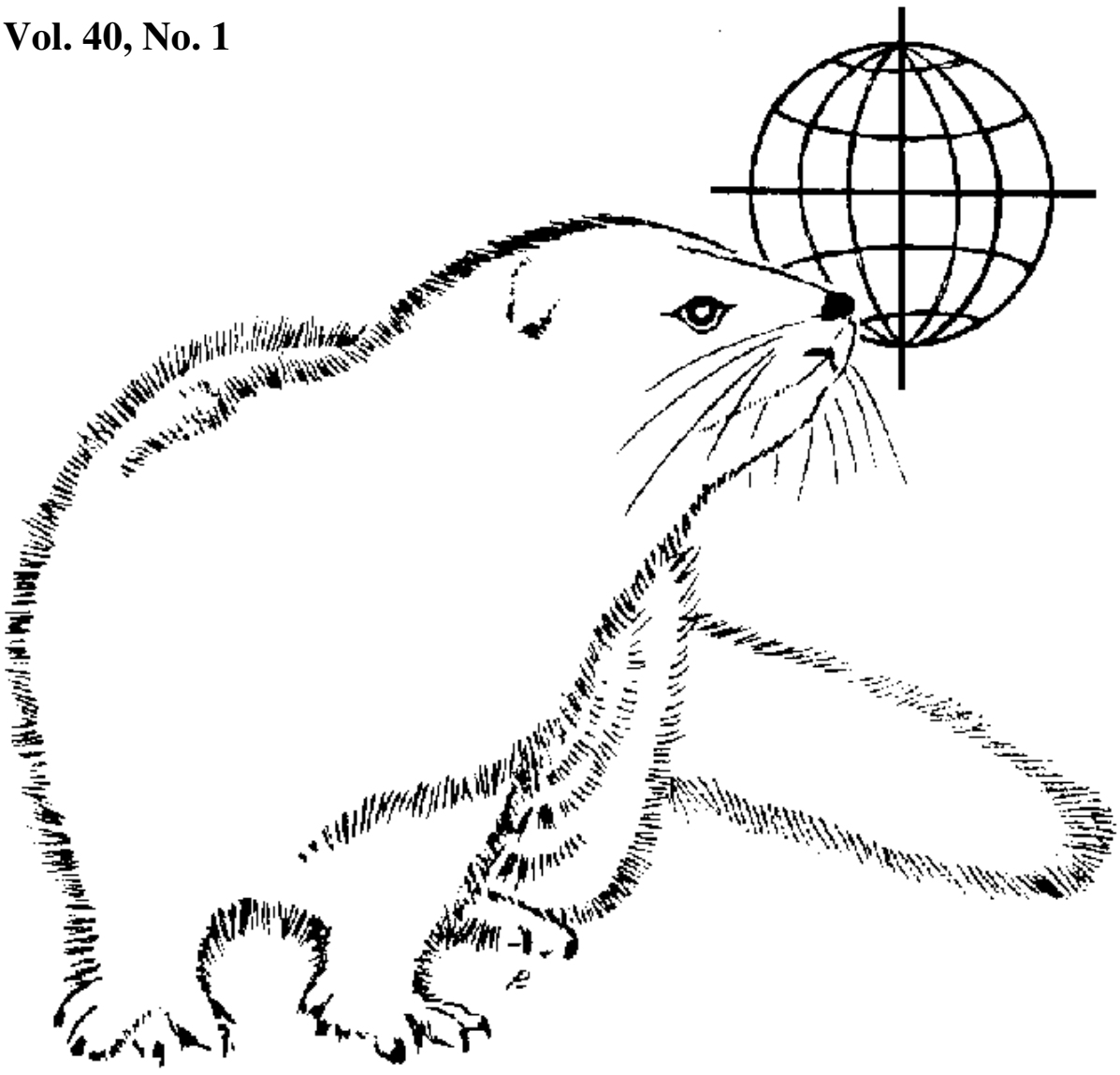

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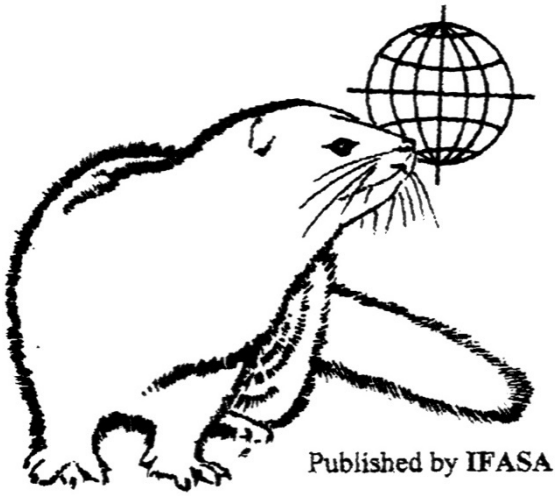

Notes from the Editor

Genomic mapping is advanced in most livestock species as are the use of these tools in e.g. genomic selection. The fur animal scientific community is significantly smaller than the communities in the other farm animal species and the effort in the genomic field has therefore been much less. In this issue of Scientifur, the abstract of a paper describing the results and status of 70 years of genetic research in the American mink is given. With the development of the genomic techniques as well as the related costs we can foresee that fur animal production also will benefit from the use of genomics in the future.

It is a pleasure to present a summary of a Ph.D.-thesis where captive mink are used as a model organism for solitary carnivores. The study was carried out in order to understand mate choice in mink which also may help to improve breeding in conservation programmes e.g. in zoos.

The International Scientific Congress in Fur Animal Production will be held in Helsinki, Finland from 23rd to 28th August 2016. The XIth IFASA Congress is organized by ProFur. The congress covers research topics within fur animal production such as breeding, genetics, and reproduction; nutrition, feeding, and management; behaviour and welfare, and health and diseases. Registration can be performed at the IFASA webpage: http://ifasanet.org/ The last day for congress registration is 25th July 2016.

Please notice that from volume 40, there will be no review procedure for manuscripts submitted for publication in Scientifur and the scientific content of the article will be the sole responsibility of the author(s). Experimental methods used and reported in SCIENTIFUR shall meet ethical standards of animal treatment as previously. As a new feature, addresses of authors of abstracts are given.

Vivi Hunnicke Nielsen

Editor Scientifur

BREEDING, GENETICS AND REPRODUCTION

70 years of research on the American mink (*Neovison vison* SCHREB., 1777) genetics - where are we now?

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The purpose of this review is to present the current state of knowledge about the genetics of the American mink (*Neovison vison* Schreb., 1777) – a species that achieved in the twentieth century an unprecedented ecological success associated with the dynamic development of its economic use. However, despite the large popularity and economic importance of the American mink as a fur animal, and the scale of the problems associated with its introduction beyond the range of natural occurrence, genetic research, particularly molecular genetics and genomics of this species, show relatively little progress. The article contains a comprehensive description of the studies undertaken on the genetics of the species, both in terms of cytogenetics, molecular genetics, genomics, population genetics and phylogenetics. The progress of the genome sequencing project of the American mink is also described as well as its transcriptome annotation. The article also deals with still unexplained and not completed, despite 70 years of genetic research, issues such as the standard karyotype, the precise molecular basis of coat color inheritance and systematic position of the species.

Genetika 2015, 47(1): 357-373

Breeding for better eye health in Finnish blue fox (*Vulpes lagopus*)

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J. Anim. Breed. Genet., 2016:133(1): 51-58. doi: 10.1111/jbg.12170 [E-pub ahead of print]

A large insertion in intron 2 of the TYRP1 gene associated with American Palomino phenotype in American mink

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A number of American mink phenotypes display a range of brownish colours. One of these phenotypes, namely American Palomino (b^{P} b^{P}) (AP) has been found to be associated with the tyrosinase-related protein 1 (TYRP1) gene by genotyping microsatellite markers in one sire family. Trials for amplifying the genomic DNA and cDNA at the beginning of intron 2 of AP TYRP1 revealed the presence of a large insertion of approximately eight kb. The insertion most likely disrupts different elements necessary for the splicing of intron 2 of the TYRP1 gene. In AP RNAseq data indicate, however, the presence of the wild-type (wt) transcript at very low levels and Western blot reveals three products when using an antibody raised against middle part of the TYRP1 protein. One individual from another brown mink phenotype-commercially named Dawn-was also investigated at the molecular level by long-range PCR and the same size insertion appears to be present. By this we suggest that certain modifiers of TYRP1 would induce different brown colour degradation, which results in at least two different phases of brown.

Mamm. Genome., 2016. [E-pub ahead of print]

The Genetics of Deafness in Domestic Animals

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Although deafness can be acquired throughout an animal's life from a variety of causes, hereditary deafness, especially congenital hereditary deafness, is a significant problem in several species. Extensive reviews exist of the genetics of deafness in humans and mice, but not for deafness in domestic animals. Hereditary deafness in many species and breeds is associated with loci for white pigmentation, where the cochlear pathology is cochleo-saccular. In other cases, there is no pigmentation association and the cochlear pathology is neuroepithelial. Late onset hereditary deafness has recently been identified in dogs and may be present but not yet recognized in other species. Few genes responsible for deafness have been identified in animals, but progress has been made for identifying genes responsible for the associated pigmentation phenotypes. Across species, the genes identified with deafness or white pigmentation patterns include MITF, PMEL, KIT, EDNRB, CDH23, TYR, and TRPM1 in dog, cat, horse, cow, pig, sheep, ferret, mink, camelid, and rabbit. Multiple causative genes are present in some species. Significant work remains in many cases to identify specific chromosomal deafness genes so that DNA testing can be used to identify carriers of the mutated genes and thereby reduce deafness prevalence.

Front. Vet. Sci., 2015: 2:29
doi: 10.3389/fvets.2015.00029. eCollection 2015

Comparison of American mink embryonic stem and induced pluripotent stem cell transcriptomes

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Background
Recently fibroblasts of many mammalian species have been reprogrammed to pluripotent state using overexpression of several transcription factors. This technology allows production of induced pluripotent stem (iPS) cells with properties similar to embryonic stem (ES) cells. The completeness of reprogramming process is well studied in such species as mouse and human but there is not enough data on other species. We produced American mink (Neovison vison) ES and iPS cells and compared these cells using transcriptome analysis.

Result
We report the generation of 10 mink ES and 22 iPS cell lines. The majority of the analyzed cell lines had normal diploid chromosome number. The only ES cell line with XX chromosome set had both X-chromosomes in active state that is characteristic of pluripotent cells. The pluripotency of ES and iPS cell lines was confirmed by formation of teratomas with cell types representing all three germ layers. Transcriptome analysis of mink embryonic fibroblasts (EF), two ES and two iPS cell lines allowed us to identify 11831 assembled contigs which were annotated. These led to a number of 6891 unique genes. Of these 3201 were differentially expressed between mink EF and ES cells. We analyzed expression levels of these genes in iPS cell lines. This allowed us to show that 80% of genes were correctly reprogrammed in iPS cells, whereas approximately 6% had an intermediate expression pattern, about 7% were not reprogrammed and about 5% had a "novel" expression pattern. We observed expression of pluripotency marker genes such as Oct4, Sox2 and Rex1 in ES and iPS cell lines with notable exception of Nanog.

Conclusions
We had produced and characterized American mink ES and iPS cells. These cells were pluripotent by a number of criteria and iPS cells exhibited effective reprogramming. Interestingly, we had showed lack of Nanog expression and consider it as a species-specific feature.

BMC Genomics, 2015: 16 Suppl 13: S6. doi: 10.1186/1471-2164-16-S13-S6. [E-pub ahead of print]

Aging influences steroid hormone release by mink ovaries and their response to leptin and IGF-I

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Biol. Open., 2016: 5(2): 174-177
doi: 10.1242/bio.016436

Fecal progestin concentrations as an indicator of reproductive success in American Mink

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Anim. Reprod. Sci., 2016: 165: 11-16
doi: 10.1016/j.anireprosci.2015.11.023. [E-pub ahead of print]

Comparative studies on testicular and epididymal morphology, and serum hormone concentrations in foxes and the hybrids during the breeding season

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Anim. Reprod. Sci., 2016: pii: S0378-4320(16)30067-7 doi: 10.1016/j.anireprosci.2016.02.027. [E-pub ahead of print]

NUTRITION, FEEDING AND MANAGEMENT

Interactions between retinol, α-tocopherol and cholecalciferol need consideration in diets for farmed mink (*Mustela vison*)

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A sufficient but balanced vitamin supplementation is a prerequisite for a satisfactory growth pattern and an effective immune system in mink and all other species. The fat-soluble vitamins are very sensitive to over- or under-supply because they interact with each other with respect to dose-response and chemical form. The purpose of the present study was to investigate the effect of increasing the amount of retinol in combination with RRR-α-tocopherol or all-rac-α-tocopherol in the feed given to growing mink on their retinol, cholecalciferol and α-tocopherol concentrations in plasma and selected organs. The results showed that the mink met their retinol requirements from the basal diet, but there were no negative effects of supplying various amounts of retinol on their plasma α-tocopherol concentrations. On the other hand, the study showed that the cholecalciferol status in plasma, assessed as the 25-hydroxycholecalciferol concentration, was low when retinol was supplemented in the feed at high levels. In addition, supplementation with RRR-α-tocopherol in

the feed negatively affected the plasma concentration of 25-hydroxycholecalciferol compared with supplementation with all-rac-α-tocopherol. In general, female mink had higher concentrations of fat-soluble vitamins in plasma than male mink.

Br. J. Nutr., 2016: 115(5): 751-758
doi: 10.1017/S0007114515005206. [E-pub ahead of print]

Retention of solute and particle markers in the digestive tract of chinchillas (*Chinchilla laniger*)

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J. Anim. Physiol. Anim. Nutr. (Berl), 2016.
doi: 10.1111/jpn.12441. [E-pub ahead of print]

The Large-Scale Removal of Mammalian Invasive Alien Species in Northern Europe

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Pest Manag. Sci., 2016. doi: 10.1002/ps.4224. [E-pub ahead of print]

HEALTH AND DISEASE

Anatomical distribution and gross pathology of wounds in necropsied farmed mink (*Neovison vison*) from June and October

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Background
Wounds are regarded as an indicator of reduced welfare in mink production; however, information on the occurrence and significance of wounds is sparse. To provide a basis for assessment and classification of wounds in farmed mink, the distribution pattern and characteristics of wounds in farmed mink in June and October, respectively, is described. A total of 791 and 660 minks from 6 to 12 Danish mink farms, respectively, were examined. The minks were either found dead or were euthanized due to injury or other disease. Mink included from June were kits in the pre-weaning and weaning period (1-2 months old). Mink included from October were juveniles in the late growth period (approximately 5-6 months old) or older. Macroscopic pathology and wound location was systematically recorded.

Result
There was considerable variation in morphology as well as location of wounds between June and October. Wounds were primarily located on the front parts of the body and in the head in June (1-2 month old kits) and mainly on the rear parts of the body and on the tail in October (5-6 month old kits and older). Moreover, there were significantly more females than males with wounds for most wound types, and significant differences in occurrence of ear and tail base wounds between certain colour types.

Conclusions
Wounds varied significantly from June to October with respect to morphology and anatomical location. Wounds in June were primarily located on the front parts of the body and in the head, while wounds in October were mainly present on the hind parts of the body and on the tail. The majority of the wounds were found in specific well defined skin areas and could therefore be grouped into categories according to anatomical location.

Acta. Vet. Scand., 2016: 58(1): 6. doi: 10.1186/s13028-016-0187-6

Development of an Enzyme-Linked Immunosorbent Assay Based on Fusion VP2332-452 Antigen for Detecting Antibodies against Aleutian Mink Disease Virus

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doi: 10.1128/JCM.02625-15. [E-pub ahead of print]

Comparison of biological and genomic characteristics between a newly isolated mink enteritis parvovirus MEV-LHV and an attenuated strain MEV-L

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A virus isolated from mink showing clinical signs of enteritis was identified as a high virulent mink enteritis parvovirus (MEV) based on its biological characteristics in vivo and in vitro. Mink, challenged with this strain named MEV-LHV, exhibited severe pathological lesions as compared to those challenged with attenuated strain MEV-L. MEV-LHV also showed higher infection and replication efficiencies in vitro than MEV-L. Sequence of the complete genome of MEV-LHV was determined and analyzed in comparison with those in GenBank, which revealed that MEV-LHV shared high homology with virulent strain MEV SD12/01, whereas MEV-L was closely related to Abashiri and vaccine strain MEVB, and belonged to a different branch of the phylogenetic tree. The genomes of the two strains differed by insertions and deletions in their palindromic termini and specific unique mutations (especially VP2 300) in coding sequences which may be involved in viral replication and pathogenicity. The results of this study provide a better understanding of the biological and genomic characteristics of MEV and identify certain regions and sites that may be involved in viral replication and pathogenicity.

Virus. Genes., 2016. [E-pub ahead of print]

The phosphorylation of Ser221 in VP2 of mink enteritis virus and its roles in virus amplification

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Virus. Res., 2016: 217: 76-84
doi: 10.1016/j.virusres.2016.03.004. [E-pub ahead of print]

Factors associated with usage of antimicrobials in commercial mink (*Neovison vison*) production in Denmark

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Betulinic acid enhances TGF-β signaling by altering TGF-β receptors partitioning between lipid-raft/caveolae and non-caveolae membrane micro-domains in mink lung epithelial cells

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Background
TGF-β is a key modulator in the regulation of cell proliferation and migration, and is also involved in the process of cancer development and progression. Previous studies have indicated that TGF-β responsiveness is determined by TGF-β receptor partitioning between lipid raft/caveolae-mediated and clathrin-mediated endocytosis. Lipid raft/caveolae-mediated endocytosis facilitates TGF-β degradation and thus suppressing TGF-β responsiveness. By contrast, clathrin-mediated endocytosis results in Smad2/3-dependent endosomal signaling, thereby promoting TGF-β responsiveness. Because betulinic acid shares a similar chemical structure with cholesterol and has been reported to insert into the plasma membrane, we speculate that betulinic acid changes the fluidity of the plasma membrane and modulates the signaling pathway associated with

membrane microdomains. We propose that betulinic acid modulates TGF-β responsiveness by changing the partitioning of TGF-β receptor between lipid-raft/caveolae and non-caveolae microdomain on plasma membrane.

Methods
We employed sucrose-density gradient ultra-centrifugation and confocal microscopy to determine membrane localization of TGF-β receptors and used a luciferase assay to examine the effects of betulinic acid in TGF-β-stimulated promoter activation. In addition, we perform western blotting to test TGF-β-induced Smad2 phosphorylation and fibronectin production.

Results and conclusions
Betulinic acid induces translocation of TGF-β receptors from lipid raft/caveolae to non-caveolae microdomains without changing total level of TGF-β receptors. The betulinic acid-induced TGF-β receptors translocation is rapid and correlate with the TGF-β-induced PAI-1 reporter gene activation and growth inhibition in Mv1Lu cells.

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Partitioning and kinetics of methylmercury among organs in captive mink (*Neovison vison*): A stable isotope tracer study

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The mink dam motivation for maternal care decrease with both litter age and the number of kits

J. Malmkvist, D.D. Sørensen, T. Larsen, R. Palme & S.W. Hansen

We investigated effects of separating the dam from the litter using brown first-parity farm mink dams (n = 374) taken away from the litter either day 49 ± 1 (7 weeks, n = 185) or day 56 ± 1 (8 weeks, n = 189) after birth. The two treatment groups had an equal (P = 0.76) litter size at the time of separation (5.5 ± 0.17; range 1-11 kits). Likewise, there was no significant difference in dam body weight (P = 0.43). However, the litter size negatively influenced both the dam weight and body condition (P < 0.001) regardless of the separation age. Stereotypies D0-D1 were influenced by group (8 weeks > 7 weeks) and increased with number of kits (P < 0.01), indicative of dam hunger/metabolic burden in the preceding period. We found no signs of nipple/inflammation problems, evaluated visually and by Infrared Thermography (IRT) measuring surface temperatures of active teats (83% out of 2042 nipples). Dams separated at litter age 7 weeks had higher concentrations of cortisol metabolites during the first week after removal; i.e. day of separation, D0: 18.8%, D1: 34.5%, D7: 36.9% higher FCM than in 8 weeks dams (P = 0.014). Likewise, the dam calls increased on the separation day, peaking on the first day after separation (D1). The proportion of dams with calls was higher in the 7-week group (P= 0.024). We interpret these results as a higher maternal motivation in dams at 7 weeks than at 8 weeks after birth. Additionally, the separation-induced calling in dams decreased with increasing litter size (P= 0.022). Thus in addition to litter age, the size of the litter is important for the maternal motivation. These factors should, therefore, be taken into account for determining the optimal separation time on mink farms.

Annual Report 2015, 7-14. Kopenhagen Research, Agro Food Park 15, DK-8200 Aarhus N, Denmark

It is possible to take a representative sample of animals based on the number of cages in use in each shed

A.F. Marsbøll, B.I.F. Henriksen & S.H. Møller

The welfare may vary between groups of animals within the same farm due to management or biological reasons. The welfare assessment in WelFur-Mink is therefore based on a sample that is representative for the animals on the farm in relation to a number of factors. The present sampling method requires a lot of information about the animals and their environment, which it is not always possible to get beforehand. We therefore developed a new sampling method, which is only based on the number of cages in use in each shed on the farm. The purpose of this study was to examine whether the new method provides a sample that is representative of the animals on the farm, and the importance of the order in which sheds are summarised in. This was examined based on a farm with almost 42,000 cages, where the sampling of 300 samples using the new method was simulated. The results showed that it is possible to use the new method to select a sample that reflects the actual distribution on the farm. The order of the sheds is expected to have only a limited impact if similar sheds are summarised consecutively.

Annual Report 2015, 15-23. Kopenhagen Research, Agro Food Park 15, DK-8200 Aarhus N, Denmark

Development of a method to screen the urine of female mink to determine pregnancy

M.S. Hedemann

The aim of the present study was to investigate whether it is possible to identify barren female mink on basis of a non-targeted metabolomics analysis of urine samples. Urine samples were collected on a commercial farm on March 24 and April 8 and 15, 2015. The samples were collected during what was expected to be early pregnancy. A total of 97 samples were collected from 58 female mink. The urine samples were analyzed with liquid chromatography-mass spectrometry and data were analyzed using pattern recognition methods (principal components analysis). There was a clear separation between collection days, however, only 5 female mink were barren and these didn't form a group clearly separated from the pregnant females. The separation between the sampling days showed that there were changes in the amount or composition of the metabolites excreted in the urine during the period investigated. Identification of a part of the metabolites showed that the oxidation of fatty acids as well as protein and/or amino acid metabolism was

changed. Furthermore, changes in the excretion of some vitamins were observed.

Annual Report 2015, 25-31. Kopenhagen Research, Agro Food Park 15, DK-8200 Aarhus N, Denmark

Feeding brown mink in the lactation period

T.N. Clausen & P.F. Larsen

The purpose of the investigation was to see if different feeding principles of females and kits during the lactation period had beneficial effect on milk production and kit growth. We used three groups of 300 brown mink females and their litters. The control group was feed a basic feed from birth to weaning and from day 28 the females were feed on the nest box together with the kits. One of the investigation groups was given the same feed as the control group, but from day 28 we continued to feed the females on top of the cage and the kits on the nest box. To the last investigation group, we added 5 percent of soya oil to the basic feed from birth and during the whole lactation period, but from day 28 we fed the basic feed for the kits on the nest box.
The results showed that females fed on the cage throughout the whole lactation period had a better weight day 49 than females fed together with the kits on the nest box from day 28 onwards. Moreover, there were higher weight of females in the lactation period in the group fed on the cage with high energy feed compared to the control group, and weight of male and female kits day 28 were greater in this group than in the other groups.

Annual Report 2015, 33-40. Kopenhagen Research, Agro Food Park 15, DK-8200 Aarhus N, Denmark

Lard or soya oil to mink in the lactation period

T.N. Clausen & P.F. Larsen

To investigate whether soya oil or lard is preferable for the growth of mink kits in the lactation period we used two groups of each 300 brown females. Soya oil or lard was used in the period April 20 to dag 49 after birth. Besides fat from soya oil and lard there were an equal amount of fat from the other raw materials in the feed in both groups.
The results showed no difference in kits or females body weight regardless of whether the added fat was lard or soya oil.

Annual Report 2015, 41-46. Kopenhagen Research, Agro Food Park 15, DK-8200 Aarhus N, Denmark

Feeding one or three times daily in the growth period

T.N. Clausen & P.F. Larsen

To investigate whether mink grow bigger if they a feed several times daily compared to once a day, two groups of black mink and two groups of brown mink were fed once a day or three times a day from weaning to October and once or twice a day from October to pelting.
The results showed that black mink fed three times daily ate more, were heavier at pelting and there was a tendency to longer skins, whereas brown mink ate the same in the two groups and reached equal size. There was no difference in health between groups.

Annual Report 2015, 47-51. Kopenhagen Research, Agro Food Park 15, DK-8200 Aarhus N, Denmark

Organic minerals in the growth period for mink

T.N. Clausen & P.F. Larsen

During the last years some feed kitchens in Denmark have started to use more organic minerals in mink feed because of higher bioavailability than the normally used in organic forms. In a growth experiment we compared performance of organic minerals and inorganic minerals in different levels ranging from current recommendation to no extra supplementation on skin traits in brown mink. Results showed no significant difference in weight at pelting, skin length, skin quality, colour or purity, Moreover the study demonstrated sufficient levels of most minerals in Danish mink feed to fulfill minks needs with use of current raw materials.

Annual Report 2015, 53-60. Kopenhagen Research, Agro Food Park 15, DK-8200 Aarhus N, Denmark

Reduced protein to mink kits in the growing-furring period

T.N. Clausen & P.F. Larsen

In attempt to lower protein content in mink feed it is necessary continuously to investigate the need of protein and amino acids in the growing and pelting period. It is also necessary to find out whether black and brown mink has the same need of protein.
In this investigation we used three groups of black mink with 150 males and females and three groups of brown mink with 135 male and female mink. Protein content was varied from 32 to 24 MEp. One of the groups corresponded to what was advised to the Danish feed kitchens in 2014.
The groups with the advised amount of protein in the feed was not significantly different from the control group. The lowest protein content resulted in lower weight at pelting and a lighter color in the black group. The analyzed protein digestibility was lower than planed and therefore the protein content was actually at a very low level in this study.

Annual Report 2015, 61-68. Kopenhagen Research, Agro Food Park 15, DK-8200 Aarhus N, Denmark

Selection of mink that perform well on a low protein feed - Status for growing-furring period 2014 and breeding period 2015

T.N. Clausen & P.F. Larsen

At the start of the growing season 2011 two selection groups was created. A control group assigned to feed with protein content similar to the average level at the feed kitchens in 2009, and a selection group assigned to feed with a 15% reduction in protein content compared to the level in 2009.
Fourth growth period show a little shorter skin in the selection group, but a steady increase in skin quality compared to the control group. The quality development over the years suggests that we can select animals with good quality at a low protein level but there was slightly higher mortality in the selection group and there were some kits with reduced growth. Fourth lactation period showed an increasing number of kits in both groups compared to previous years. There was a tendency for the lowest litter size at birth in the selection group compared to the control group, probably due to better control of the body condition of control females during the winter period. There were fewer kits in the litter day 28 in the selection group but larger kit weights.

Annual Report 2015, 69-78, Kopenhagen Research, Agro Food Park 15, Aarhus N DK-8200, Denmark

Mink´s requirement for vitamin E from weaning to pelting

D. Clausen, S.K. Jensen, T.M. Lassén, T.N. Clausen & P.F. Larsen

A well-balanced supplementation of vitamins is a prerequisite for satisfying weight gain and welfare of mink. Natural vitamin E is more expensive than synthetic vitamin E, however studies indicates that mink utilizes the natural form better than the synthetic form. An experiment with 1080 Brown male and female mink (*Neovison vison*) was performed in the growth period 2014 to study the utilization and requirement of natural and synthetic vitamin E on pelt quality, weight gain and health. No differences in pelt quality and weight gain was found in mink fed 40 or 80 mg synthetic vitamin E per kg diet, or 20 or 40 mg natural vitamin E per kg diet. However, there were indications of a better utilization and depositing of natural vitamin E. The prevalence of dead animals and hepatic lipidosis were not affected by form or dose.

Annual Report 2015, 79-84, Kopenhagen Fur Research, Agro Food Park 15, DK-Aarhus N, Denmark

Protein requirements in mink before and after implantation

C.F. Matthiesen & A.H. Tauson

The protein requirements in mink are not fully known in all periods throughout the production cycle out of which the gestation is one. The objective of this study was to determine the protein requirements in mink before and after the implantation in order to support a high implantation and fetal survival rate. A total of 106 females were fed different protein levels before (10, 15, 20, 25, 30, 35, 40, and 45% of metabolizable energy -ME- from protein) and after (20, 25, 30, 35, 40, and 45% of OE from protein) implantation. The females were euthanized in mid-April in the study "before implantation" or in late April in the study

"after implantation". Our results showed that the number of implantation sites not was affected by the protein level in the diet before implantation. However, a tendency towards an effect of protein provision on fetal survival rate was noted. This resulted in significantly lower fetal survival rates in females fed 15% of OE from protein compared to females fed 25-45%. The fetal survival rates were not affected by a protein level of 20-45% of the OE from protein after implantation. However, the fetal survival rate in females fed 20% of ME from protein was numerically lower than females fed 25-45% of OE from protein. In conclusion, implantation occurs even when protein provision is low, but fetal survival can be compromised. A protein provision of 20-45% of ME after implantation did not affect the fetal survival rate which indicates that the protein requirements were fulfilled.

Annual Report 2015, 85-89. Kopenhagen Research, Agro Food Park 15, DK-8200 Aarhus N, Denmark

Transmission of Aleutian Mink Disease Virus with fleas

C.M. Hartby, T.H. Jensen, K.S. Larsen, M.S. Hansen, M. Chriél, L.E. Larsen, T. Struve & C.K. Hjulsager

We aimed to investigate if Aleutian mink disease virus (AMDV) could be transferred with fleas from mink to mink and thereby from farm to farm. Six mink females were injected with the virus strain Saeby/DEN/799.1/05 and AMDV-negative fleas were added in the pelt of the mink. The fleas were collected again and transferred to an AMDV-negative recipient group of six mink females housed separately from the infected mink. Fleas, blood samples, oronasal swabs, fecal samples, and tissue samples were tested by PCR for the presence of virus-DNA. The blood samples were tested for antibodies using the CIEP method. Six out of six recipient mink were positive for AMDV DNA in at least one tissue sample after transfer of fleas from virus-positive mink. None of the recipient mink seroconverted or were found positive for virus DNA in the blood before the study was terminated. Our results show, that it is possible for fleas to transfer AMDV from infected mink to non-infected mink.

Annual Report 2015, 91-94, Kopenhagen Research, Agro Food Park 15, DK-8200 Aarhus N, Denmark

***Arcanobacterium phocae* infections in Danish mink**

B. Nonnemann, M. Chriél, G. Larsen, M.S. Hansen, E. Holm & K. Pedersen

We here report the first outbreaks of infections caused by *Arcanobacterium phocae* in mink farms in Denmark. The outbreaks have affected at least twelve farms. Main clinical findings included necrotizing pododermatitis and in some animals dermatitis located to other body sites. The bacterium could be isolated from affected skin samples and, in some animals also from internal organs, such as the liver, indicating a systemic spread. The origin of the infection has not been traced.

Annual Report 2015, 95-99. Kopenhagen Research, Agro Food Park 15, DK-8200 Aarhus N, Denmark.

Improved diagnostics of mink enteritis virus (MEV)

L.K. Kvisgaard, E. Holm, M. Chriél, L.E. Larsen & C.K. Hjulsager

Virus enteritis in mink is caused by infection with a parvovirus called mink enteritis virus (MEV). Until Marts 1st 2015, virus enteritis was diagnosed by detection of virus in feces and intestinal content samples from mink using an antigen ELISA at the National Veterinary Institute in Denmark, DTU. In the present study, a new method for detecting MEV based on real-time PCR was developed. The MEV real-time PCR was shown to be much more sensitive compared to the antigen ELISA. Since Marts 1st 2015 the real-time PCR assay has been used as routine diagnostic test to detect MEV at the National Veterinary Institute, DTU.

Annual Report 2015, 101-104, Kopenhagen Research, Agro Food Park 15, DK-8200 Aarhus N, Denmark

Outbreak of *Clostridium septicum* in Danish mink

G. Larsen, B. Nonnemann, E. Holm, K. Pedersen & M. Chriél

For the first time ever an outbreak with *Clostridium septicum* is detected in Danish mink kits. Late in June

2014 *C. septicum* was detected in 18 male mink kits from 6 different farms. All 6 mink farms received feed from the same feed producer but no samples were examined.
Pathologically all 18 mink kits had swollen heads and bloody discharge from the nose and mouth and gas production was seen in various organs and under the skin. None of the 18 mink kits had skin lesions or stomach/intestine ulcers so route of entry was most probably eruption of the permanent teeth and the deciduous teeth inducing irritated gums and small lesions in the mouth.

Annual Report 2015, 105-108. Kopenhagen Research, Agro Food Park 15, DK-8200 Aarhus N, Denmark

Subtyping of influenza in Danish mink farms in 2014

C.K. Hjulsager, J.S. Krog, M. Chriél, G. Larsen & L.E. Larsen

In the autumn 2014, influenza A virus was detected in 30 submissions of mink from Danish mink farms. In most cases, the virus was subtyped as "pandemic" influenza A virus H1N1 (H1N1pdm09), which has been circulating in Danish pigs since primo 2010, and were also circulating in the human population during the influenza season 2014/2015. In two cases, viruses were the normal Danish swine influenza viruses H1N1 and H1N2. Such viruses have never been detected in humans in Denmark, therefore pigs are the most likely origin of the viruses. Twenty-seven of the positive farms received feed from the same feed producer, thus viruses could have been transferred by the feed, the source being byproducts of untreated swine offal in the feed. Another possibility is that the minks were infected directly by human contact, e.g. by staff or guests infected with influenza when visiting the farms. Neither feed nor human contact as a source of the outbreak has been demonstrated.

Annual Report 2015, 109-113. Kopenhagen Research, Agro Food Park 15, DK-8200 Aarhus N, Denmark

Paralysis in mink due to diskospondylitis

G. Larsen, B. Nonnemann, L. Buelund, E. Holm, T.K. Jensen & M. Chriél

For the first time diskospondylitis and ostemyelitis has been detected as cause of paresis/paralysis in Danish farmed juvenile mink. Pathology showed palpable swelling of the spine in the thoracic region in 6 of 11 examined mink. X-ray examination and histopathology showed chronic diskospondylitis/ osteomyelitis in 8 of 11 mink. The paresis/paralysis was caused by compression of the spinal cord due to the inflammatory reaction. Bacteriology from the involved vertebral bone and liver showed *Streptococcus* spp. and *Staphylococcus* spp. Most of the mink had tail bites and this may serve as the route of infection followed by hematogenic spread to the disks.

Annual Report 2015, 115-117. Kopenhagen Research, Agro Food Park 15, DK-8200 Aarhus N, Denmark

Metabolomics as a tool for identification of markers for the wound healing process in mink

M.S. Hedemann, A. Jespersen & A.S.V. Hammer

The purpose of this investigation was to use metabolomics as a tool to find markers for the wound healing process in mink. The samples analyzed in this study where obtained from three experimental wound model studies in mink (a. Pilot experiment, b. Protein content in the feed, c. Test of topical treatment in an experimental wound model) performed at University of Copenhagen. The results of the pilot and the protein experiment, where all animals had an experimental wound, showed that the use of anesthetics, when the wounds were made, had a major, long-lasting influence on the metabolite pattern in blood, which made it impossible to see an effect of the wound. In the experiment with topical treatment of wounds, a control group without wound was included. In this experiment it was possible to detect a difference between animals without and with a wound 14 days after the wound had been made. A preliminary identification of markers for the wound healing process showed that the metabolites, 3-Cresotinic acid, thromboxane B2 and prostaglandin were involved and these are all directly related to the wound healing process.

Annual Report 2015, 119-125. Kopenhagen Research, Agro Food Park 15, DK-8200 Aarhus N, Denmark

Preliminary results of investigations of gut microbiota of farm mink (*Neovison vison*)

A.S. Hammer, L. Andresen, T.N. Clausen, A. Jacobsen & M.I. Bahl

Currently there is very limited data available on the bacterial composition in the mink intestinal tract (gut microbiota). This makes it difficult to evaluate the significance of bacterial findings in mink. Using a cross-disciplinary approach, high throughput sequencing and pathological methods were applied for analysis of the mink gut microbiota. Preliminary analysis of samples from mink gut and mink feed clearly shows that the gut microbiota is very different from the composition of the microbiota in mink feed. Similar to reports on the microbiota in other carnivores and humans, mink intestinal microbiota was found to be dominated by anaerobic bacteria belonging to the phylum Firmicutes. Individual variability in the bacterial composition was found among the examined mink, which is consistent with what is described in humans and other animal species. The preliminary results presented in this report are part of a project aimed at mapping the microbial composition in the mink gut microbiota and to evaluate associations between gut microbiota and mink health. The project was initiated as a corporation between Copenhagen University, DTU National Food Institute, and Kopenhagen Research.

Annual Report 2015, 127-131. Kopenhagen Research, Agro Food Park 15, DK-8200 Aarhus N, Denmark

Application of processing maps for collection of data from mink farms with an outbreak of diarrhea in the pre-weaning period

C. Dahlin, J.M. Birch, J.F. Agger, H.E. Jensen, T. Struve & A.S. Hammer

In 2015 a case-control study was conducted on mink farms with outbreak of diarrhea in the pre-weaning period and control farms with low or no occurrence of diarrhea in the pre-weaning period. A treatment card was designed and applied for systematic registration of data concerning litter treatment and including date of birth, coat color and litter size. This report presents data concerning treatment for diarrhea in the pre-weaning period. Data was included for 2440 litters on 8 farms (4 case farms and 4 control farms), where the farmers were asked to record data on litters in antimicrobial treatment with treatment cards. The majority (73.6%) of the treated litters was initially big litters (defined as a litter size of 7 or more kits when litter size was initially recorded) and the majority (83.5%) was born before 3rd of May. The cards were found to be a useful tool for registration of litter data and treatment data during field studies of diarrhea in the pre-weaning period. The cards may also be applied for data registration in clinical trials evaluating the effect of measures taken for disease prevention or treatment.

Annual Report 2015, 133-140. Kopenhagen Research, Agro Food Park 15, DK-8200 Aarhus N, Denmark

Additional water for mink kits in the lactation period

T.N. Clausen & P.F. Larsen

A water system to mink kits in the lactation period were the nipple is placed just outside the nest box was tested. To the investigation we used three groups of each 176 black mink females. In the control group females and kits were housed in traditional cages with the water nipple at the end of the cage, in the two investigation groups we installed a watering system where the nipple is placed in the fourth mask in front of the nest box. In the control group and one of the investigation groups we used partial weaning of the biggest kits day 42 after birth, in the other investigation group all kits stayed with the female until day 56 and thereafter weaned.
There was no significant effect of allocating additional water close to the nest box on kit growth, kit loss or litters with bite problems in this investigation. Generally, there was a tendency that in both groups were big kits were partially divided day 42 after birth, fewer litters with bite were present and the female body weight were higher at day 49 compared to the group were the kits were not divided day 42.

Annual Report 2015, 141-145. Kopenhagen Research, Agro Food Park 15, DK-8200 Aarhus N, Denmark

Reproduction in second year old females who was kept alone or with a male kit in the previous growth period

T.N. Clausen & P.F. Larsen

The purpose of the investigation was to see how the feeding of breeding females in the growing period affects next year's reproduction. We used 768 brown first-year females, half of them were placed alone after weaning and half were placed with a male kit. After pelting the females were fed and handled equally.

It was not possible to see any effect on reproduction results depending on whether the female had been alone in a cage in the growing period or together with a male kit.

Annual Report 2015, 147-149. Kopenhagen Research, Agro Food Park 15, DK-8200 Aarhus N, Denmark

Animal Personality and Mate Preference in American Mink
- improving breeding success in captive solitary species

PhD thesis by Christina Lemkuhl Noer

Industrial PhD Project

between

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and

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2015

Conservation-breeding programs in zoos are essential for the management of threatened species. By moving animals amongst institutions for prescribed breeding, these programs aim to promote demographic stability and preserve genetic variation in the captive populations. Yet many breeding programs are unsustainable because prescribed pairs of animals are reproductively unsuccessful. A solution to this problem may be to investigate, which male cues and signals females of a species of concern use to assess mate quality before performing their mate choice, and then introduce biological material containing these cues and signals from different potential male partners to the female to test her preference prior to introduction of a mate. Females of many species have the ability to determine the quality of a male and also the male to which they are genetically better matched among available options, and studies of the outcomes of mate choice suggest that choosy females benefit from increased fecundity, litter size, and offspring survival. Thus, providing females with the opportunity to choose from several males might improve the sustainability of captive populations.

This thesis used captive mink as a model organism for solitary carnivores. We developed and tested a method to assess female preference when presented to male urine and faeces and the males themselves. Successively we investigated if these preferences translated into actual mate choice, i.e. paternity. Due to their biological relevance, and because research has proved their importance in other species' mate assessment, we focused on three signals or cues: olfactory (urine and faeces), size, and behaviour. The latter may represent aspects of animal personality.
We were able to measure a female preference for urine and faeces from one of two males in a pairwise preference test as well as for one of the males. However, these preferences were not necessarily correlated with each other or with paternity. This indicates that mate preference and actual choice as shown by paternity are highly complex and rely on multiple cues. Animal personality and how this affects animal behaviour may be one such important cue. Prior to the preference tests we therefore investigated and found that individual mink did in fact show consistency in shyness-boldness measures across different novel contexts and across time and therefore have different personalities. These differences in personality, however, did not correlate with any of the preference measures, nor with paternity.

In the first study we present a novel study on aspects of animal personality in the American mink, by revealing, for the first time, consistency in shyness-boldness measures across different novelty tests. Interestingly, this study shows that consistency in shyness-boldness measures differs across non-social and social contexts, as well as across stages in the approach towards novel objects. This reveals that different aspects of shyness-boldness exist in farmed American mink. These findings highlight the importance of carefully considering the context as well as the limitations of using the shyness-boldness continuum for describing animal personality traits.

The second study adds to the knowledge of animal personality described in the first study, by revealing aspects of the shyness-boldness continuum across seasons in the farmed American mink. This study confirms the existence of personality in mink by showing consistency over time for tests in both non-social and social contexts, and it confirms the different aspects of shyness-boldness. Interestingly, a general shift in the positioning of individuals from shy to more bold was evident when comparing the shyness-boldness measures in the non-breeding season in December to the early breeding season measures in February.

The third study is a free choice study on female preference for two different males and their signals and cues and the resulting paternity in farmed American mink. The study focuses on three types of signals or cues: olfactory (urine and faeces), stationary-visual (size), and variable-visual (behaviour). We were able to measure a female preference for male stimuli (urine and faeces) in all mink. As expected, this study also reveals that mate preference and actual choice as shown by paternity are highly complex and rely on multiple cues. The tendencies in this study suggest that we might be able to use olfactory cues to measure initial female preference for mates prior to introduction, which could improve pair compatibility and breeding success if applied to endangered zoo species.

It is important to realise that females in general use multiple cues to assess males (Jennions & Petrie, 1997). This thesis highlights the importance of investigating female preference in more detail in order to improve breeding success in captive species. A lot of studies still need to be carried out in order to understand mate choice, but this may take years and some endangered species might not be around that

long. We cannot afford losing more and more of the valuable gene pool of these threatened species, because of mate incompatibility resulting in poor breeding outcomes. Therefore, I suggest focusing on specific species or a group of animals with similar life history, like the solitary carnivores, in order to develop methods for including multiple biologically relevant cues for females to assess mates on prior to introduction. This thesis suggests that olfactory cues from urine and faeces and behaviour such as aspects of animal personality might be relevant for testing mate compatibility and should be included in future studies of female preference. The elaboration, complexity, and sexual dimorphism of olfactory signals is difficult for humans to perceive and quantify but according to Clutton-Brock & McAuliffe, 2009 it is likely to match the elaboration of a peacock's tail or the complexity of the sedge warbler's song. This accentuates the importance of more studies on the role of olfaction in sexual selection, mate preference, and mate choice. Natural selection has shaped the female senses to assess the better male among available options and thus the female should be able to make better choices than breeding coordinators consulting studbook data.

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Text. The text should normally be divided into: Introduction, Material and Methods, Results, Discussion, Acknowledgements and References and follow internationally accepted rules. Double documentation in both figures and tables will not be accepted.

Illustrations. All graphs, photos and pictures are considered as figures. All drawings have to be professionally drafted (photocopies are not an acceptable standard). The illustrations should be JPG-, GIF- or TIF-files. Any halftones must exhibit high contrast and text and other details must be large enough to retain the readability even after reduction of figure size to single column (width 80 mm). The width of 170 mm can also be accepted. Colour illustrations can be included in SCIENTIFUR.

Tables. Each table should be typed on a separate page. Tables must be numbered consecutively with Arabic numerals, and have a self-explanatory title. Tables should be planned to fit a final width of 80 or 170 mm.

References. References in the text should be made according to the following examples: Nielsen, 1992; Hansen & Berg, 1993; Bakken *et al*., 1999.
The list of references should be arranged in alphabetic order according to the name of the first author and the year of publication within the names. The year of publication should be written between the name(s) and the title:

Nielsen, V.H., Møller, S.H., Hansen, B.K. & Berg, P. (2007). Genotype - environment interaction in mink. *Scientifur*, 31 (3): 89.

Shirali, M., Nielsen, V.H., Møller S.H. & Jensen, J. (2015**).** Longitudinal analysis of residual feed intake and BW in mink using random regression with heterogeneous residual variance. *Animal,* 8 (10): 1597-1604.