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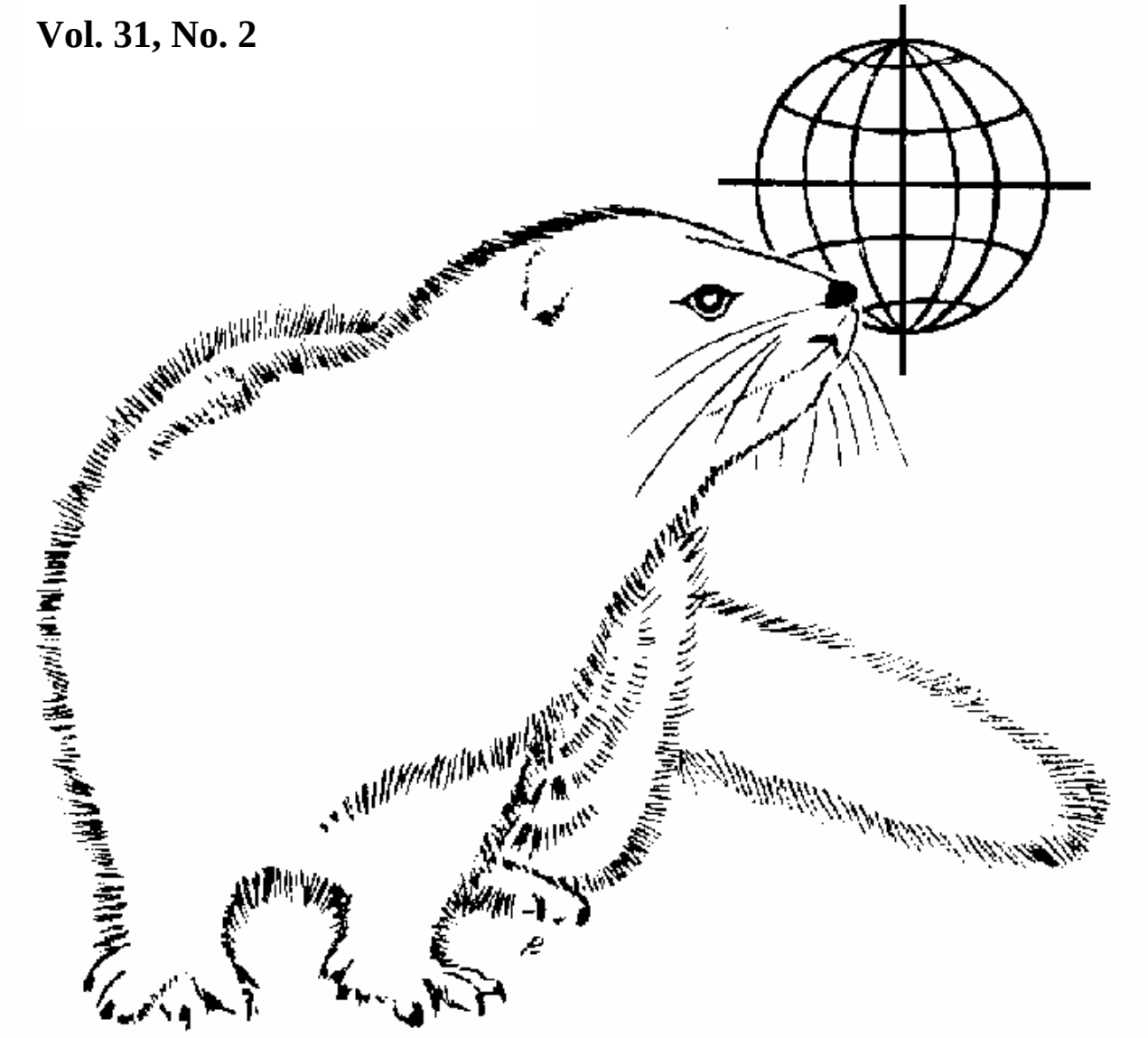

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Notes from the Group of Editors

This issue of Scientifur, Volume 31, No 2, contains one reviewed article, two short communications, as well as a brief mention of a workshop on 'Acidified raw materials and the effect of acids on animal health'.

We take this opportunity to remind you of the IX International Scientific Congress in Fur Animal Production, which will be held in Halifax, Nova Scotia, Canada, 20-23 August, 2008. For more information on the congress please see:

http://www.ifasanet.org/congress/

On behalf of the
Group of Editors

Birthe Damgaard

Morphometric and cytochemical investigation of subcellular structure on sapphire mink leucocytes

L.B. Uzenbaeva, A.G. Golubeva, V.A. Ilukha, N.N. Tyutyunnik and M.G. Nyuppiyeva

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Summary
The morphological and cytochemical specificities of the subcellular structure of leucocytes similar to the Chediak-Higashi syndrome (CHS) were demonstrated in sapphire mink using a computer program for analysis of images. The considerable variety of size and number of cytoplasmic granules in different types of peripheral blood cells and between individuals was established. The abnormality of the subcellular structure containing peroxidase is supposed to be one of the mechanisms to lower the vitality and resistance of sapphire minks to Aleutian disease.

Keywords: Chediak-Higashi syndrome, peroxidase, alkaline phosphatase, glycogen.

Introduction

The leucocytes play an important role in immunological reactions and in the process ensuring the resistance of the organism. Changes in the leucocyte structure and function caused by an adverse environment or genetic abnormalities lead to a weakening of the immunoreactivity (Dell'Angelica et al., 2000). Thus, the leucocyte defect named Chediak-Higashi syndrome can result in a decrease in the resistance found in sapphire mink.

The CHS has been described in man and several animal species such as cattle, cats, rats, mice and some farm bred fur animals (Chediak, 1952; Hammer et al., 2005; Higashi 1954; Fagerland et al., 1987; Ozaki et al., 1994; Padgett et al., 1964; Sjaastad et al., 1990). Giant granules found by light and electron microscopy can serve as a basic morphological characteristic. Formation of abnormally large melanin containing granules causes age-dependant skin, hair and eye depigmentation (Leader et al., 1963; Ozaki et al., 1994; Zvereva et al., 1976).

The diagnosis of CHS is based on the fact of leukocyte dysfunction and presence of large granules in them. Medical treatment is connected with great difficulties as antibiotics can only improve the patient's condition for a short period of time. It has been found that vitamin C promotes the elimination of the leukocyte functional defect. A positive clinical effect was obtained after bone marrow transplantation.

Aleutian mink are the best studied analogue among fur animals and can serve the CHS model (Leader et al., 1963). The disorders were also found in other cage bred fur animals, such as fox and polar fox (Fagerland et al., 1987; Sjaastad et al., 1990). The basic hallmarks of the syndrome in these species are similar to those in man and other animals. The mechanism of the dysfunction has been insufficiently studied to date.

The aim of this investigation was to study the structure and function organization of blood leucocytes in sapphire mink, characterized by a low viability in comparison with other colour mutations.

Materials and methods

Sapphire mink of both sexes bred on the "Praja" Ltd. farm were used. The studies were made in November. Blood was sampled from the tail vein. The leucocyte form and number of abnormal leucocytes were measured using standard method and Pappengheim staining (Berestov, 1971). The cytochemical determination of the leucocyte peroxidase was made after the method of Graham –

Knoll, of the leucocyte alkaline phosphatese after the method of Burstone, and of the glycogen after the method of MaManus and Hotchkiss (Kost, 1975). The morphometric parameters of granules in eosinophils and neutrophils such as number per cell, length, width, square of one granule, total square per cell, factor of circle form, factor of ellipse form, circularity and oblongness were measured using the computer software for image analysis "VideoTest" (VideoTest Ltd, Russia). Factor of circle form (Fcf) characterized the proximity of the figure to the circle and was calculated according to the formula $Fcf=4\pi Sq/Per$, where Sq – square, Per – perimeter. Factor of ellipse form (Fe) characterized the proximity of the figure to the ellipse and was calculated according to the formula $Fe=Sq/\pi d_1 d_2$, where Sq – square, d_1 and d_2 – axes of ellipse. These factors decreased when the perimeter irregularity increased. Circularity (Fc) was calculated as the ratio of square to the maximal diameter ($Fc=4Sq/d^2\pi$, where Sq – square, d – maximal diameter). Oblongness was calculated as the ratio of length to width.

Results and discussion

The specificity of the subcellular structure of leucocytes similar to CHS was found in sapphire mink. This is the formation of abnormal granules whose number, size and intracellular localization significantly differ from standard mink as well as from other mammalian species. Abnormally large granules are detected in eosinophils, neutrophils, basophils and, rarely, in lymphocytes and monocytes. The previous data on defectiveness of leucocytes in mutant mink with homozygous aleutian genes has been confirmed (Leader et al., 1963; Padgett et al., 1964).

Figure 1 shows the leucocytes' differential count and percentage of abnormal cells in sapphire mink peripheral blood. All eosinophils contain large granules of different size abnormally allocated in cell (Fig. 2 A). These changes may be conditioned by a deviation in the membrane structure or in the microtubules system. The degree of granular disturbances was dissimilar in different blood cells and in individual animals. Only single eosinophils were found to have uniformly distributed large granules and looked like normal.

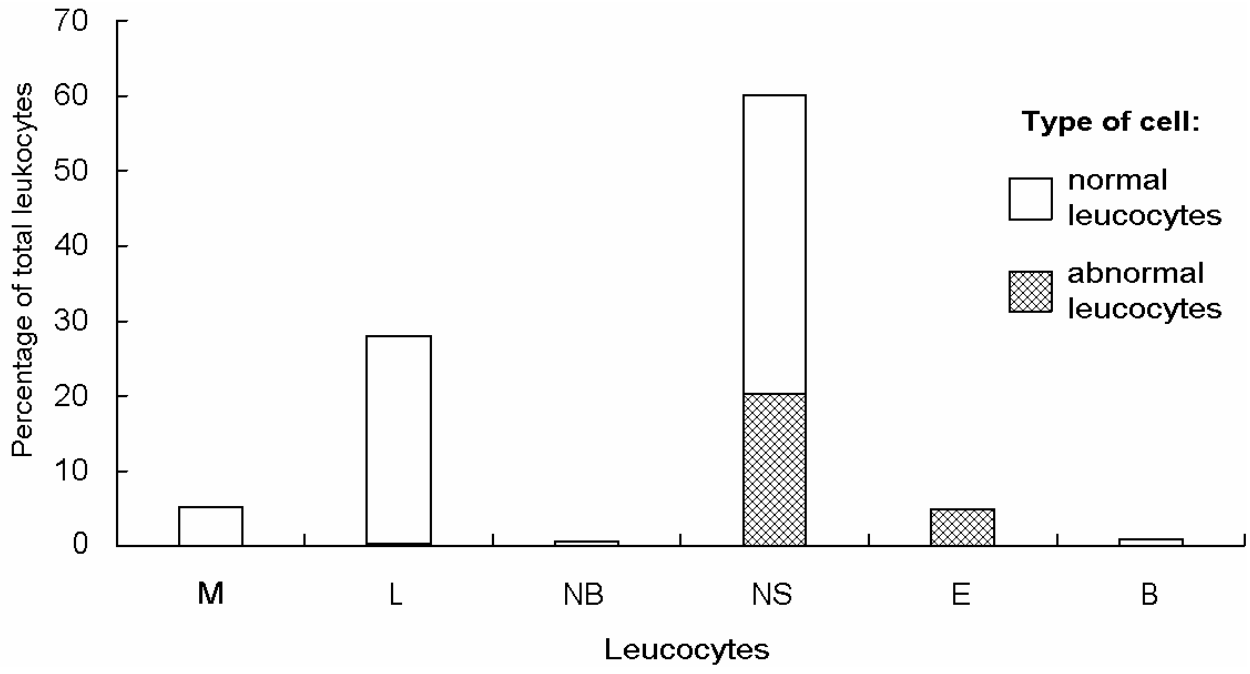

Figure 1. Percentage of total leukocytes in sapphire mink blood.
M – monocytes, L – lymphocytes, NB – neutrophilic band, NS – neutrophilic segmented, E – eosinophiles, B – basophils.

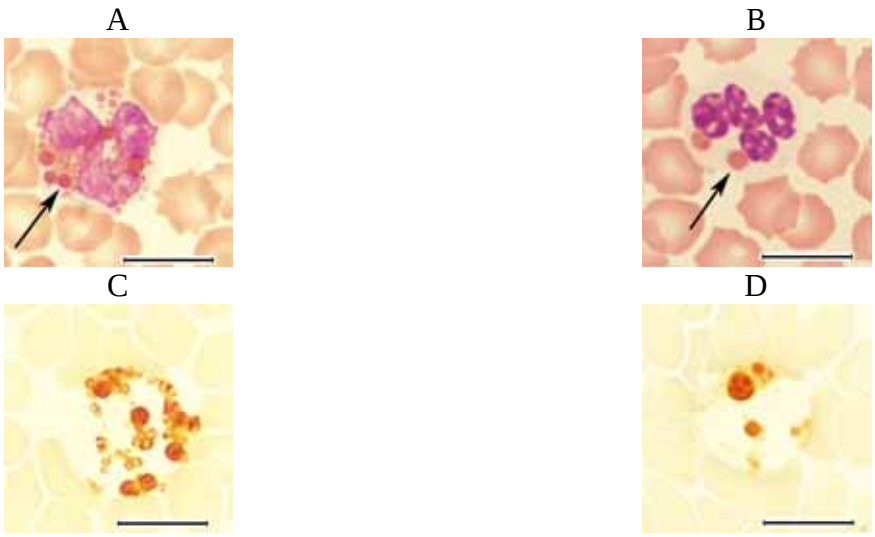

Figure 2. Abnormal granules (arrowhead) in eosinophils (A) and in neutrophiles (B) under Pappenheim staining and peroxidase positive granules in eosinophils (C) and in neutrophiles (D) of sapphire mink blood. Bar = 10 μm.

The intracellular content of granules varied widely – ranged from 1 to 43, with an average of 14.6 per eosinophil (Table 1). On the basis of the results of the computer morphometry, we can draw the conclusion that granules vary greatly in size and form. In particular, the width of granule varies from 0.23 to 3.38 microns and its length may vary from 0.27 to 6.55 microns. Differences in the size are so big that a maximal square of granules is more than 250–fold bigger than a minimal one.

Table 1. Parameters of blood leucocytes granules in sapphire mink

Measured parameters	Eosinophil				Neutrophilic segmented			
	Mean	± Std. Error	Min.	Max.	Mean	± Std. Error	Min.	Max.
Total number	761				379			
Number per cell	14,63	± 1,40	1,00	43,00	2,15	± 0,09	1,00	6,00
Square of one granule, μm^2	1,33	± 0,05	0,04	11,14	0,96	± 0,03	0,16	5,85
Total square per cell, μm^2	19,42	± 1,05	3,89	36,14	2,06	± 0,08	0,32	5,85
Length, μm	1,44	± 0,03	0,27	6,55	1,14	± 0,02	0,46	3,25
Width, μm	1,06	± 0,02	0,23	3,38	0,94	± 0,02	0,36	2,62
Factor of circle form, arb. units	0,93	± 0,00	0,31	1,00	0,99	± 0,00	0,55	1,00
Factor of ellipse form, arb. units	0,98	± 0,00	0,01	1,00	0,99	± 0,00	0,10	1,00
Circularity, arb. units	0,71	± 0,01	0,16	1,00	0,84	± 0,01	0,29	1,00
Oblongness, arb. units	1,36	± 0,01	0,62	4,13	1,22	± 0,01	0,99	3,12

Modified enlarged granules with the affinity to acid dyes and pinkish-orange in colour are found in neutrophils (Fig. 2 B). They stand out against a background of very thin cytoplasm and significantly differ from barely perceptible dust–like grains in standard mink neutrophils. In comparison with eosinophils, the number of granules in neutrophils is not large and varies from 1 to 6 with an average number of 2.2, and their average total square is equal to 2.06 microns.

Less significant changes were found in lymphocytes, where single granules were rarely found. Contrary to other authors, who postulated that all blood cells in Aleutian mink are abnormal (Leader et al., 1963), we did not find syndrome

specific disturbances in monocytes. The small number of basophiles makes it difficult to estimate their dysfunction. The sapphire mink basophiles contain rather large round granules of similar size but with differently coloured granules. The colour of the granules varies from violet to black.

The results of the investigation allow us to conclude that abnormal cytoplasm granules are formed as a result of aggregation and fusion of particles. In some cases the borders between particles are clearly visible but in other cases the borders are absent. Some authors suggest that pathology of intracellular organelles originates as result of abnormal granulogenesis in Golgi complex or in endoplasmic reticulum (Rogovin et al., 1977). The relative cell volume of granules was the same both in the normal and affected foxes, but there were significantly fewer granules per unit area in the affected animals (Fagerland et al., 1987).

Our investigation found that the total square of granules increased as the number of granules per cell increased (Fig. 3 A). The square of a single granule increased as a result of an enlargement in the process of their consolidation and fusion (Fig. 3 B). Cytochemical results support the fact that sapphire mink have a defect in peripheral blood leucocytes. The abnormal peroxydase positive granules in eosinophiles and neutrophiles (Fig. 2 C, D) were interpreted as a violation of the granule forming mechanism. Contrary to the peroxidase, the alkaline phosphatase and the glycogen were located outside the abnormal granules.

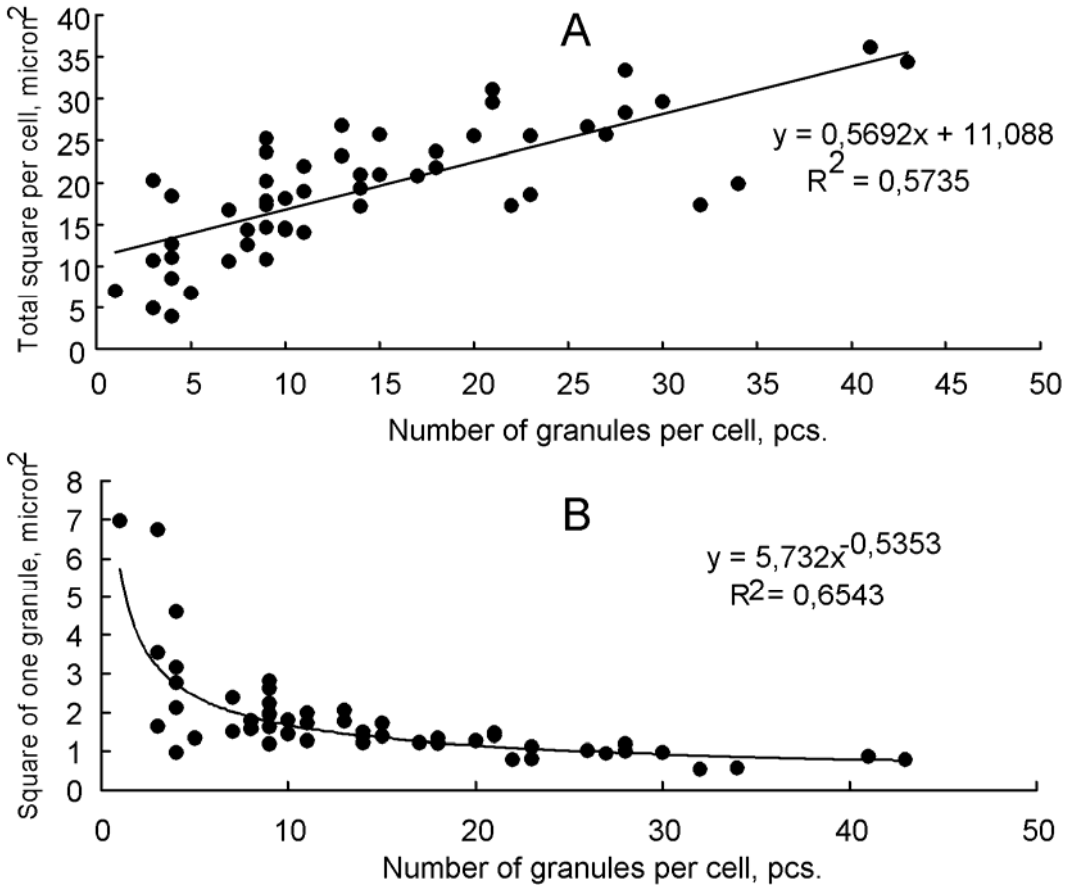

Figure 3. Dependence between number of granules and total square (A) and average square of one granule (B) in eosinophils of sapphire mink blood. The regression equations are presented.

Such morphological and cytochemical investigations demonstrate the defected structure of leucocytes in sapphire mink with the homozygous recessive Aleutian gene. The degree of abnormality varies in some leucocytes and between individuals. Abnormal granulocyte granules contain the peroxidase, but the glycogen and the alkaline phosphatase were not found in them. The abnormal structure of the granules is the basis of the leucocyte

dysfunction and apparently leads to the decrease in resistance to bacterial infection of sapphire mink.

Acknowledgements
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Characterization of microsatellite markers isolated from the American mink (*Mustela vison*) genome

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Abstract

Two size-selected mink genomic libraries were constructed and recombinant colonies (n=6,144) were screened with a pool of probes, containing $(AAAG)_8$, $(AAGG)_8$, $(AGGG)_8$, $(ATAG)_8$ and $(AG)_{15}$ oligonucleotides in equal amounts. A total of 44 colonies were hybridized and confirmed upon replating. Sequencing of the clones revealed 35 microsatellites with unique sequences. There were 19 *di*- and 6 *tetra* nucleotides, and the remaining 10 had complex repeats. Twenty eight (80%) were polymorphic in a panel of 20 mink from two Danish farms.

Key words: American mink, microsatellite, polymorphism

In this study additional mink microsatellite sequences were isolated from two genomic libraries constructed by complete overnight digestion of 30 µg of genomic DNA from one female black mink with either *Sau*3A1 or *Nla*III. Digested fragments were size separated on 1% agarose gels, and fragments of 300 to 1300 bp were recovered from the gels and purified using the QIAquick Gel Extraction kit (Qiagen, Ontario). Size-selected fragments were ligated into *Bam*HI- and *Sph*I-digested dephosphorylated pGEM-7Zf(-) vector (Promega, Madison, USA), respectively. The ligated products were used to transform maximum efficiency competent *E. coli* (DH5α, Invitrogen, Burlington, Ontario) and cells were plated out on LB/ampicillin/IPTG/X-gal medium and incubated overnight. A QpixII colony picker (GENETIX, UK) was used to transfer recombinant colonies into 384-well plates containing LB/ampicilin media with 10% glycerol. Colonies were transferred onto Hybond-N+ nylon membranes (Amersham Biosciences, Piscataway, NJ, USA), using 384-pin plastic replicators, and grown on LB/ampicilin plates overnight. Two positive and two negative colonies were added on each membrane as controls. Colonies were denatured, neutralized and fixed on membranes by baking for 2 h at 80ºC. Cell debris were removed by incubating membranes in a digestion solution (100 µg/ml proteinase K, 50 mM Tris Cl, pH 7.6, 0.1% SDS and 50 mM NaCl) at 37ºC with gentle agitation for at least 6 h, and were rinsed in 2X SSC (Sambrooke and Russell, 2001).

Membranes were hybridized with a pool of oligonucleotide probes labeled with the ECL chemiluminescence DNA detection kit (Amersham, Bioscences) according to the manufacturer's

instructions. The pool contained the same amount of $(AAAG)_8$, $(AAGG)_8$, $(AGGG)_8$, $(ATAG)_8$ and $(AG)_{15}$. Pre-hybridization and hybridization were performed at 42ºC in a rotisserie hybridization oven for 1 h and 3 h, respectively. Membranes were exposed to the Super RX Fuji X-ray films, and positively hybridized colonies were re-plated and re-hybridized for confirmation. A few bacterial cells from each confirmed colony were directly transferred to a PCR cocktail, and the DNA insert was amplified using the pUC/M13 universal forward (5'-CGC CAG GGT TTT CCC AGT CAC GAC) and reverse (5'-TCA CAC AGG AAA CAG CTA TGA C) primers (Promega) and a standard PCR profile with an annealing temperature of 50ºC.

Amplified DNA inserts were cleaned in 96-well Multiscreen PCR cleanup plates (Millipore Bioscience, Cambridge, ON) and bi-directionally sequenced using the BigDye Terminator Reaction Kit, V3.1, and analyzed on an ABI 3130 DNA Sequencer (Applied Biosystems, Foster City, CA, USA). Sequence alignment and editing was performed using the Sequencher software (Gene Codes Corp., Ann Arbor, MI). Primers for the amplification of microsatellite loci were designed using the Oligo Primer Analysis Software, Version 6.0 (Molecular Biology Insight, Cascade, CO).

Of 6,144 recombinant colonies tested, 123 were positively hybridized, of which 44 (35.8%) were confirmed upon re-plating and re-hybridization, of which seven had no repeat; two had similar sequences and the remaining 35 (0.57% of the recombinant colonies) contained a microsatellite sequence. There were 10 complex repeats, 19 *di*- and 6 *tetra*-nucleotides. All 35 sequences were unique when compared with GenBank sequences using BLAST and were submitted to GenBank (accession numbers DQ830767 to DQ830801).

Each locus was PCR amplified using a fluorescently labeled forward primer (6-FAM or HEX). Amplifications were performed in 10 µl total volume containing (final concentration) 1X PCR buffer, 0.2 mM each dNTP, 800 nM each primer, 0.24 unit of Taq polymerase (Amplitaq Gold, Applied Biosystems) and 20 to 50 ng of genomic DNA. Allele sizes were determined with an ABI sequencer equipped with the GeneScan software programs (Applied Biosystems). Twenty eight (80%) of the 35 loci were polymorphic in a panel of 20 mink from two Danish farms. The number of alleles per locus varied between 2 to 8 (Table 1). Data were analyzed using Arlequin (version 3.1), and no evidence for linkage disequilibrium between loci was observed, but significant deviation from Hardy-Weinberg expectations ($P>0.05$) were detected for three of the four markers (Mvi2605, Mvi2620 and Mvi3616).

Of the seven monomorhpic loci, three contained dinucleotide repeats. These were Mvi2604 (DQ830775, $(GA)_{17}$), Mvi2607 (DQ830778, $(GA)_{16}$) and Mvi2618 (DQ830787, $(CT)_6 \ldots (CT)_5$). The first two loci with large number of repeats will likely be polymorphic in more heterogeneous populations. Forward and reverse primers, respectively, for Mvi2604 were 5'-AGA GGG CTG CAT TGT GGT TCC and 5'-GCA TCG GGC TTT CTG CTC AG, and those for Mvi2607 were 5'-CTC AAA CAC GTT TTC CGT TCA and 5'-CCT CCC ACC AGT GGT GC. The remaining four loci contained tetranucleotides; Mvi2617 (DQ830786), Mvi3610 (DQ830797), Mvi3612 (DQ830798) and Mvi3613 (DQ830799).

Acknowledgments

We gratefully acknowledge the financial contribution of Canada Mink Breeders Association, Nova Scotia Fur Institute and Nova Scotia Department of Agriculture (Agri-Focus 2000 program). We also acknowledge the PhD grant awarded to R. Anistoroaei for this project from Danish Fur Breeders Association, Copenhagen Fur Center, and the Faculty of Life Sciences, University of Copenhagen. Technical assistance of Priyanka Rupasinghe and Tennille Crossman is greatly appreciated.

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Table 1. Microsatellite loci identified in the American mink (Mustela vison).

Locus Name	GenBank Accession Number	Repeat motif (5'-3')†	Primer sequences (5'-3')	T_a Used (^{0}C)	$MgCl_2$ conc. (mM)	No. alleles	Min. alleles (bp)	Max. alleles (bp)	H_O	H_E
Mvi1901	DQ830767	$(TAAA)_{4...}(CA)_{11}$	F: CCT ATT TGT GAT CGC TGT C R: TAAAATGGTGCAGATAGTGTG	60	2.0	4	262	274	0.30	0.41
Mvi1902	DQ830768	$(CA)_{10}$	F: AAA TAA AAA TGG GCA CAT AC R: TTG CCT GTA AAA CTG GTA TAG	64-55	2.0	3	204	210	0.75	0.58
Mvi1903	DQ830769	$(CA)_{13}$	F: AGA CCA AGT TTA AGA AAT TAA R: TAT TAT AGA CTC AAG TGG GA	64-55	2.0	3	168	172	0.70	0.57
Mvi1906	DQ830770	$(CA)_{17}$	F: GAT AAA GAA GAT GTG GGG TAT R: CTC ATT TCG CTA AGC ATA AT	60	2.0	4	137	149	0.45	0.47
Mvi1909	DQ830771	$(GT)_{18}$	F: TGG AAT GAA ACA TAT GCA A R: AGG TAA CCA TTA TTT CCC TTA	64-55	2.0	3	149	153	0.75	0.61
Mvi1910	DQ830772	$(GT)_{10}$	F: GGG GTA CGA TGC AAA GTT TAC R: TGG ACC AGC GTC TCT ATC TCT	60	2.0	3	263	267	0.35	0.44
Mvi1911	DQ830773	$(AC)_{15}$	F: CTG GTT GTG GGT AGG TCC R: CAG ATT TAG CAG CAG TCC ATC	60	2.0	4	187	195	0.55	0.60
Mvi1912	DQ830774	$(CA)_{14}$	F: TTA CCC GGA AAA CCC AAT ACT R: TGT GCA TGT GCG TCA GAG	60	2.0	5	104	112	0.60	0.69
Mvi2605	DQ830776	$(ATTT)_{3}...(AG)_{9}$	F: GGA GTA CAT CTC ATG CTT TCA R: GCT CAG CAG GGA GCC T	64-55	3.5	3	251	265	0.20	0.51
Mvi2606	DQ830777	$(ATTT)_{5}T(GA)_{17}$	F: TCA GAT GCT CCA TCA ACT GAG R: GGA GAG TCT GCT TGG GAT TCT	64-55	3.5	7	132	166	0.65	0.59
Mvi2608	DQ830779	$(GA)_{12}$	F: GAG GCC ACA CAG CTA GGT CAC R: CTG CTG CTC ATG CTC GC	64-55	3.5	3	159	165	0.20	0.19
Mvi2609	DQ830780	$(AG)_{13}$	F: GTC TGC GGA CTT TTC TAG TTG R: GCT CAG TGG GGA CCC T	60	3.5	4	210	226	0.55	0.68
Mvi2610	DQ830781	$(CT)_{12}GTC(AAAT)_{3}$	F: CCT CTG CTG TTG CTT CA R: AAT TCT CAG GCC AAG TAA ACT	64-55	3.5	6	148	160	0.45	0.50
Mvi2611	DQ830782	$(AG)_{10}$	F: CTC ATC CGT GAA ATT TAA GAA R: CCC CGT CAT CTA TCC C	60	3.5	2	147	151	0.20	0.18
Mvi2612	DQ830783	$(AG)_{12}$	F: TCT AGA AAA TGG GTG TTA TTC R: CAG TTA TGT GCT TGC TTG	64-55	3.5	5	140	154	0.85	0.79
Mvi2614	DQ830784	$(GA)_{16}$	F: GCA TGC TGC TAC AAC TTG ATA R: CTG GGC TGT GCA TCA G	64-55	3.5	3	144	154	0.65	0.63
Mvi2616	DQ830785	$(AG)_{6}$	F: GAG TCA AAC AAG CTG TAG AAA R: CTC CCC TGA GTG TAA AGA	64-55	3.5	2	235	237	0.15	0.15

Table 1. Continued

Locus Name	GenBank Accession Number	Repeat motif (5'-3')†	Primer sequences (5'-3')	T_a Used (^{0}C)	$MgCl_2$ conc. (mM)	No. alleles	Min. alleles (bp)	Max. alleles (bp)	H_O	H_E
Mvi2619	DQ830788	$(CT)_{15}(AT)_{10}$ TCCA$(ATTT)_3$	F: CCC TCT CTG CTT GCC TCT C R: AAT CCA GCC GAA TCG TTA AAA	64-55	3.5	3	215	219	0.35	0.39
Mvi2620	DQ830789	$(CT)_{17}(AT)_{12}$	F: CTC TTT ATC TTC CCC TAC C R: TGG CCA TCT CTA TAT CTA TTC	64-55	3.5	5	168	178	0.25	0.74
Mvi2621	DQ830790	$(GA)_{12}$	F: AAG GTG ATT GGT CAA AGG TTT R: CCC TCC CCA CTG CTA AT	64-55	3.5	4	105	111	0.20	0.41
Mvi2622	DQ830791	$(GA)_{13}$	F: TGC CCT TCC CTA CTT GTC R: TCT CAC TAA CTG TCG AAC AGC	64-55	3.5	5	142	150	0.25	0.38
Mvi2623	DQ830792	$(CCT)_4$…$(TC)_{20}$	F: CTC TTG GCC GGT CCC T R: CGA AGC AGC CTC TAG CAT CTA	64-55	3.5	7	186	202	0.65	0.78
Mvi2624	DQ830793	$(TC)_{16}$	F: GGG AGT CTG CTT GAG GAT T R: AGA GAA GGA AAG AGA GGG TCT	64-55	3.5	8	117	141	0.80	0.78
Mvi2626	DQ830794	$(ATTT)_3(GA)_{15}$	F: AAT GGT AGG AGA AAA CTT TCA R: GGC TCA GTC TGT TAA GTG TC	64-55	3.5	5	235	245	0.45	0.42
Mvi2627	DQ830795	$(TC)_{14}$	F: CAA GGT TGC CAC AAT AGT CT R: ATG AGG AAG GTG CAT AAT CTG	64-55	3.5	4	149	163	0.30	0.34
Mvi3603	DQ830796	$(CAAA)_6$	F: TAC CGT CAG GAA AAT AAA CAA R: CAC AAT CCT ACA CAA TCC TCA	64-55	2.0	4	137	149	0.40	0.45
Mvi3615	DQ830800	$(TTCC)_4$…$(TTCC)_6$…$(CTTT)_3$	F: CCG TAA TGT CAG ATG TCA AAT R: GCT CAG TGG GGT ATG GAG	64-55	3.5	5	269	279	0.75	0.65
Mvi3616	DQ830801	$(TTCC)_4CT$ $(CCTT)_5$	F: CCA AAA CTC CCT CTA CTC TAC R: GCT AAA AGA AGC CAA TCT C	64-55	3.5	2	212	216	0.10	0.42

T^a, annealing temperature for primers; $MgCl_2$ conc., magnesium chloride for primer pairs. Forward primers were fluorescently labelled with either 6-FAM or HEX.
PCR profiles.
Touchdown PCR program, 95^{0}C for 15 min; then 95^{0}C for 15 s, 64-55^{0}C (dropping 1^{0}C every one cycle) for 30 s; 72^{0}C for 1 min; followed by 72^{0}C for 10 min.
Few loci were amplified using a PCR program: 95^{0}C for 15 min; and 35 cycles of 94^{0}C for 30 s; 60^{0}C for 30 s; 72^{0}C for 2 min. followed by final extension at 72^{0}C for 10 min. Primers were fluorescently labelled and optimized for use on an ABI 3100 sequencer.

Independent inheritance of *Stardust* and *Cross* colour types in the North American mink

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During a tour to Danish mink farms to obtain family material with segregation of the classical colour genes to the mink DNA project at our faculty, I have observed a new segregation type that I want to describe. The animals are present on a farm owned by Kennet Møller, Hovedvejen 35, Vejrup, Bramming.

Stardust is a comparatively new colour variant in the mink. It can be seen in the heterozygote being a mixture of dark and white hairs, with a uniform distribution all over the body. The homozygote *Stardust* is much lighter in colour than heterozygote. *Cross* (Black cross) is a well known mutation being present in the heterozygote with a dark stripe down the back and dark areas over the shoulders. It is getting lighter down towards the belly. The homozygote form is nearly white all over the body with a few darker hairs on back of the head.

As both phenotypes have dominant inheritance and are characterized by a mixture of hair with different colours the data given below can determine if they originate from the same locus.

In Table 1 is given the segregation pattern in four litters with the combination of *Wild mink* bitches and a sire having the phenotype *Stardust* and *Cross*, As the male had been pelted a sister to the male with the same phenotype is shown in Figure 1a. As given in Table 1, four different colour types in the litters were observed.

Table 1. Segregation ratios in four litters after Wild mink bitches and a Stardust/Cross male.

	Wild mink	*Cross*	*Stardust*	*Stardust /Cross*
Litter 1	0	3	3	2
Litter 2	3	1	2	1
Litter 3	1	2	1	1
Litter 4	2	3	2	2
Total	6	9	7	6

From the results shown in Table 1 it can be concluded that:

The two genes have independent inheritance. If they were alleles in the same locus, the *Wild mink* colour type could not have occurred. It is independent inheritance of the two loci with a 1:1:1:1 segregation ratio in the four classes. This is in very good agreement with the ratio of 6:9:7:6 found in the 28 pups. All four types occurred in all litters except litter 1, where *Wild mink* was not represented.

The *Cross* allele and the *Stardust* allele modify the effect of one another, as the *Stardust/Cross* phenotype easily can be put in a separate light class, see picture below, Figure1 b. Notice the dark tail on one of the *Stardust/Cross* pups.

a. 

b. 

Figure 1. a) A bitch which has the phenotype *Stardust/Cross*. b) Segregation in a litter after a *Wild mink* females and a male of the type *Stardust/Cross*.

Workshop on

'Acidified raw materials and the effect of acids on animal health'

Årslev Kro, Aarhus, Denmark, 6-7 March 2007

The workshop, which was attended by well over 30 fur industry people and scientists from Denmark, Finland, the Netherlands, Norway, and Sweden, was divided into the following three sessions:

1) Effect of different acids on the animals

Presentations given:

Acid-base chemistry and physiology, *Søren Wamberg*

Effect of formic acid and benzoic acid on animal health, *Ilpo Pölönen*

Effect of mineral acids on animal health, *Søren Wamberg*

Acid preservation of raw materials intended for fur animal feed, *Tor Mikael Lassén*

Treatment of lower urinary tract diseases with different kinds of acids, *Tove N. Clausen*

2) Experiences from the use of acidified raw materials in feed for fur animals

Presentations given:

Experiences with acidified raw materials and acids in feed in Denmark, *Tor Mikael Lassén*

Experiences with acidified raw materials and acids in feed in Norway, *Øystein Ahlstrøm*

Experiences of the use of acidified raw materials in mink feed in Sweden, *Eva Aldén*

Experiences with acidified raw materials and acids in fur animal feed in Finland – Formic acid in blue fox feed, long term effects 2004-2006, *Nita Koskinen*

3) How large amounts of acidified raw material can be used in fur animal feed

This session consisted of the following four workshops:

I) Principles for preservation and estimation of the quality of acidified raw materials,

II) Effect of acids on animal health,

III) Acidified raw materials in feed for fur animals, and

IV) Use of acidified raw materials to prevent lower urinary tract diseases.

The workshop was organized by Tor Mikael Lassén, tml@landscentret.dk, Danish Agricultural Advisory Service, National Centre, Fur Animals, Udkærsvej 15, Skejby, DK-8200 Århus N, Tel: +45 87 40 50 00 Fax: +45 87 40 50 10

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