ANNALES
UNIVERSITATIS MARIAE CURIE-SKŁODOWSKA
LUBLIN – POLONIA

VOL. XXIX (4) SECTIO EE 2011

Department of Genetic and Animal Breeding, West Pomeranian University of Technology
in Szczecin, Doktora Judyma 6, 71-460 Szczecin,
email: jskorupski@zut.edu.pl

JAKUB SKORUPSKI, MAREK KMIEĆ

Detection of *PRL*/*Alw*21I polymorphism in ranch American mink (*Neovison vison* Schreb., 1777)

Detekcja polimorfizmu *PRL*/*Alw*21I hodowlanej norki amerykańskiej
(*Neovison vison* Schreb., 1777)

Summary. The aim of the present study was to detect the prolactin gene polymorphism 5978 T/C (*PRL*/*Alw*21I) and to determine the genetic structure of the examined flock, in terms of the analysed SNP. For this purpose, six breeds (Standard, Scanblack, Sapphire, Pearl, Black Cross and Sapphire Cross) of the ranch American mink (*Neovison vison* Schreb., 1777) were examined. Polymorphism 5978 T/C was detected in a fragment of the 3rd intron, by PCR-ACRS-RFLP analysis (*Alw*21I restrictase was used). It was shown that there are two alleles – PRL^T and PRL^C and three genotypes – PRL^TPRL^T, PRL^TPRL^C, PRL^CPRL^C, according to recognized SNP. Genotyping of 165 individuals showed higher frequency (0.87) of the PRL^T allele (highest in Sapphire minks, and lowest in Standard minks) and lower (0.13) of the PRL^C allele. The percentage of individuals with PRL^TPRL^T genotype was equal 75.2%, with PRL^TPRL^C genotype – 26.3%, and with PRL^CPRL^C genotype – 1.2%. The obtained results suggest that the breeding work contributes to the elimination of PRL^C allele from the gene pool of ranch minks and that strong selection pressure on 3rd intron of prolactin gene occurred in Scanblack, Sapphire and Sapphire Cross breeds.

Key words: American mink, ACRS-PCR-RFLP, genetic polymorphism, prolactin

INTRODUCTION

American mink (*Neovison vison* Schreb., 1777) is a popular fur-bearing animal, bred for its high-quality fur. Mink breeding is a good example of a traditional approach to animal husbandry, focusing on statistical analysis of the observed phenotypic variability. This variability is, in traditional approach, a resultant effect of genotype, environment and genotype-environment interactions, which are the basis for assessing the breeding value of the animal, which in turn is a criterion for selection [Nowicki *et al.* 1994, Łukaszewicz 2007]. Although it explains the rules of inheritance of qualitative and quantitative traits, omits the genetic material structure and gene expression mechanisms [Walawski *et al.* 2004, Łukaszewicz 2007].

Modern breeding programs, based on direct analysis of the farm animals genomes, use methods such as cytogenetic description of the chromosomes, gene mapping, comparative genomics, gene cloning and sequencing, transgenesis, and above all the identification of DNA polymorphism with molecular genetic methods [Świtoński 1992]. Ranch minks selective breeding focus primarily on mutant color phases, improvement of the reproductive rates, as well as improvement and equalization of coat characteristics [Filistowicz and Żuk 1995, Sulik and Felska 2000, Bielański *et al.* 2003, Rozempolska-Rucińska *et al.* 2004].

Both traits associated with reproduction, as well as a coat depends in American mink on the impact of prolactin (PRL) – multifunctional peptide hormone, which regulates numerous different biological [Bole-Feysot *et al.*1998]. Prolactin gene polymorphism, demonstrated in other farm animals, is closely connected with quantitative traits important from economic point of view, such as milk yield [Chung *et al.* 1996, Brym *et al.* 2005, Wojdak-Maksymiec *et al.* 2008], fertility [Drogemuller *et al.* 2001, Chu *et al.* 2009], egg yield [Cui *et al.* 2006], woolliness [Lan *et al.* 2009] and, in the light of these reports, the *PRL* gene should be considered as a QTL candidate gene (gene conditioning the quantitative traits) [Cowan *et al.* 1990, Kurył 2000].

The aim of this study was to detect the 5978 T/C (*PRL*/*Alw*21I) SNP in 3rd intron of the American mink *PRL* gene, as well as to determine the frequency of its occurrence.

MATERIAL AND METHODS

Prolactin gene polymorphism was examined in 165 individuals, representing six colour breeds (Standard, Scanblack, Sapphire, Pearl, Sapphire Cross and Black Cross) of ranch American mink from one of the Western Pomeranian fur farms. DNA was isolated from muscle tissue using the *High Pure PCR Template Preparation Kit* (Roche). In order to amplificate the target fragment of the prolactin gene 3rd intron, the ACRS-PCR-RFLP (amplification created restriction site-PCR-RFLP) technique was used [Haliassos *et al.* 1989, Sneath and Sokal 1973]. Primer sequences were designed with the *In silico* program [Bikandi *et al.* 2004] to introduce an *Alw*21I recognition site (unpaired base in position 13):

forward – 5'-ACA GCG CTC TGG TCG GAG CA-3',

reverse – 5'-CAG GAG GGC CAG GGA CAA AC-3'.

PCR amplification was performed in final volume of 15,0 μL, with reaction buffer consisting Mg^{2+} (KCl, $(NH_4)_2SO_4$, $MgCl_2$), 0.2 mM of each dNTP, 2.5 mM $MgCl_2$, 10.0 pM of each primer, 1.0 U *DreamTaq* DNA polymerase (Fermentas) and 2.0 μL genomic DNA. Thermal cycling began with an initial denaturation at 95°C for 5 min, followed by 35 cycles of 92°C for 40 s, 55°C for 40 s, 72°C for 40 s, and a final extension at 72°C for 5 min. Restriction fragment length analysis was performed by digestion in the 10.0 mL reaction mixture, which contained 1.0 U of restriction enzyme, reaction buffer (50.0 mM Tris-HCl, 10.0 mM MgCl2, 100.0 mM NaCl, and 0.1 mg/mL BSA, pH 7.5) and 9.0 mL of DNA amplification products of 211 bp. The reaction mixture was incubated overnight at 37°C. The restriction fragments were separated in 2.5% agarose gels in TBE buffer with ethidium bromide (0.5 mg/ml) at a voltage of 90 V, for 40–50 minutes.

The results were analyzed statistically using the computation suite STAT-GEN; genotypes and alleles frequency, as well as observed and expected heterozigocity were determined, as well as a genetic distance between colour breeds.

RESULTS

Conducted restriction analysis of the *PRL* gene fragment allowed to identify the presence of two alleles – PRL^T and PRL^C and three genotypes – PRL^TPRL^T, PRL^TPRL^C, PRL^CPRL^C, according to recognized 5978 T/C (*PRL*/*Alw*21I) SNP (Tab. 1). Genotyping showed higher frequency (0.86) of the PRL^T allele (highest in Sapphire minks) and significant lower (0.13) of the PRL^C allele (lowest in Standard minks). The percentage of individuals with PRL^TPRL^T genotype was equal 75.2% (highest in Sapphire minks), with PRL^TPRL^C genotype – 26.3% (highest in Standard minks), and with PRL^CPRL^C genotype – 1.2% (present only in Standard and Sapphire minks). Significant statistical differences, at $P \leq 0.5$, were found in case of: allele frequency between Scanblack and Standard, Standard and Sapphire, as well as Standard and Sapphire Cross; prevalence of genotypes between Standard and Sapphire, Sapphire and Pearl, as well as Sapphire and Black Cross. It is important to demonstrate a significant difference between percentage of homozygotes (76.4%) and heterozygotes (23.6%) in the analyzed stock. Analysis of the observed and expected number of individuals with homozygous and heterozygous genotype did not demonstrated statistically significant deviation from Hardy-Weinberg equilibrium in examined herd. Most individuals with homozygous genotype were observed in the case of a Sapphire colour breed (95.7%), while most heterozygous individuals were among the Standard (36.4%) and Pearl (34.4%) minks.

Table 1. Frequencies of genotypes and alleles of *PRL* gene (*PRL*/*Alw*21I) in examined herd (values in columns marked with the same letter differ statistically, $P \leq 0.05$)
Tabela 1. Frekwencje genotypów i alleli genu prolaktyny (*PRL*/*Alw*21I) w badanym stadzie (wartości w kolumnach oznaczone tą samą literą różnią się między sobą istotnie przy $p < 0,05$)

Colour breed Odmiany barwne	Number of individuals Liczba osobników	Genotypes – Genotypy						Alleles Allele	
		PRL^TPRL^T		PRL^TPRL^C		PRL^CPRL^C			
		N	frequency frekwencja (%)	N	frequency frekwencja (%)	N	frequency frekwencja (%)	PRL^T	PRL^C
Scanblack	29	24	82.8	5	17.2	0	0	0.9138^{a}	0.0862^{a}
Standard	22	13	59.1^{a}	8	36.4^{a}	1	4.5	0.7727^{abc}	0.2273^{abc}
Sapphire	23	21	91.3^{ab}	1	4.3^{abc}	1	4.3	0.9348^{b}	0.0652^{b}
Pearl	32	21	65.6^{b}	11	34.4^{b}	0	0	0.8281	0.1719
Black Cross	30	21	70.0	9	30.0^{c}	0	0	0.8500	0.1500
Sapphire Cross	29	24	82.8	5	17.2	0	0	0.9138^{c}	0.0862^{c}
Total Ogółem	165	124	75.2	39	23.6	2	1.2	0.8697	0.1303

Calculated genetic distance values allowed distinguish two relatively distant genetically (in terms of 5978 T/C polymorphism of prolactin gene) groups of colour breeds – first include Scanblack, Sapphire and Sapphire Cross minks and second Pearl, Black Cross and Standard minks. A strong selection pressure on 3rd intron of the PRL gene seems occurred in the first group.

DISCUSSION AND CONCLUSIONS

Previous studies of Vardy and Farid [2002] from the Nova Scotia Agricultural College showed that the 5978 T/C polymorphism (*PRL*/*Alw*21I) of the *PRL* gene is strongly correlated with 6952 C/G polymorphism, also occurring within the 3rd intron. The results of sequencing of the area encompassing both mentioned SNPs indicates that T at position 5978 has been always accompanied with C at position 6952, while the C at position 5978 has been accompanied with G at position 6952. This observation allows to refer the results of undertaken studies to those obtained by Vardy and Farid [2002]. Namely, according to it, highest frequency of alleles PRL^G (6952 C/G – *PRL*/*Nla*IV SNP) has been demonstrated in wild minks (0.20), while in Standard minks was equal 0.05, in Pastel minks – 0.03, and in Scanblack minks has not been detected. In comparison, the results of present studies showed that frequency of allele PRL^C (5978 T/C) was equal 0.23 in Standard minks and 0.09 in Scanblack minks. In other breeds, this frequency was equal 0.07 for Sapphire, 0.09 for Sapphire Cross, 0.15 for Black Cross and 0.17 for Pearl. Therefore, the results of this study do not support the Farid and Vardy's thesis that the *PRL* gene 3rd intron was under strong selection pressure in farmed minks, but rather shows tend to indicate two groups of colour breeds, differing in the intensity of selection pressure on described DNA fragment.

Obtained results (including Vardy and Farid's findings for wild mink) suggest that the breeding work contributes to the elimination of PRL^C allele from the gene pool of ranch minks and that strong selection pressure on the 3rd intron of prolactin gene occurred in Scanblack, Sapphire and Sapphire Cross breeds. Evidence of this is also calculated homozygosity rate – the highest in Sapphire, Scanblack and Sapphire Cross minks, as well as heterozygosity rate – the highest in Standard, Pearl and Black Cross minks.

Analysis of the colour breed-specific traits, essential for the rearing and breeding of American mink, do not allow to indicate their relationship with the described polymorphism. This applies to both the reproduction performance and the fur performance. Nevertheless, the lack of detailed data on the performance traits of the examined individuals makes it impossible to determine their correlation with analysed polymorphism. Thus, it must be assumed that the results of this study do not allow to determine the usefulness of described prolactin gene fragment as a performance traits marker in the traditional approach to animal breeding, in which only simple production traits markers, clearly associated with a particular phenotype, are considered to be significant. On the other hand, in the context of the modern approach to the breeding process, examination of each genetic variation (especially of the SNP) is extremely valuable, not only in the cognitive, but also application sense – by its potential use in estimation of the genomic breeding value (GBV) [Zabaneh and Mackay 2003, Osten-Sacken 2009, Żukowski *et al.* 2009]. It is very important especially in the case of genes encoding multiple functions hormones,

which undoubtedly is prolactin. Such genes concern a number of traits categorized as performance-significant. In case of the *PRL* gene, it is mainly the effect of hormone on fertility, which is often regarded as a decisive factor for the economic results of the mink production [Socha *et al.* 2002a, Socha *et al.* 2002b].

A key aspect in the rearing of offspring by the mother is the quantity and quality of the milk [Barabasz 1984], while in Polish conditions the cases of the agalactia (inhibition of lactation), resulting in relatively low survival of young minks, are often reported [Krzywoszyński 1983, Bielański *et al.* 2003]. In the future studies on the *PRL* gene polymorphism, special attention should be paid to relationship of high milk production by females with measures required for their vigour and vitality during offspring feeding period. It is important because of the particularly heavy burden of the female body observed in carnivorous fur-bearing animals – during the peak lactation mink females produce nearly 100 g of milk per day, which represents about 10% of their body weight [Barabasz 1984].

REFERENCES

Barabasz B., 1984. Wzrost norcząt w okresie laktacji. Hod. Drobn. Inwent. 4, 7–9.

Bielański P., Zoń A., Piórkowska M., 2003. Wstępne wyniki badań nad poprawą wskaźników odchowu szczeniąt norek. Zesz. Nauk. Przegl. Hod. 68, 71–78.

Bikandi J., San Millán R., Rementeria A., Garaizar J., 2004. *In silico* analysis of complete bacterial genomes: PCR, AFLP-PCR, and endonuclease restriction. Bioinformatics 20, 798–799.

Bole-Feysot C., Goffin V., Edery M., Binart N., Kelly P.A., 1998. Prolactin (PRL) and its receptor: actions, signal transduction pathways and phenotypes observed in PRL receptor knockout mice. Endocr. Rev. 19, 225–268.

Brym P., Kamiński S., Wójcik E., 2005. Nucleotide seguence polymorphism within exon 4 of the bovine prolactin gene and its associations with milk performance traits. J. Appl. Genet. 45, 179–185.

Chu M.X., Wang X.C., Jin M., Di R., Chen H.Q., Zhu G.Q., Fang L., Ma Y.H., Li K., 2009. DNA polymorphism of 5' flanking region of prolactin gene and its association with litter size in sheep. J. Anim. Breed. Genet. 126, 63–68.

Chung E.R., Rhim T.J., Han S.H., 1996. Associations between PCR-RFLP markers of growth hormone and prolactin genes production traits in dairy cattle. Korean J. Anim. Sci. 38, 321–336.

Cowan C.M., Dentine M.R., Ax R.L., Schuler L.A., 1990. Structural variation around prolactin gene linked to quantitative traits in an elite Holstein sire family. Theor. Appl. Genet. 79, 577–582.

Cui J.X., Du H.L., Liang Y., Deng X.M., Li N., Zhang X.Q., 2006. Association of polymorphisms in the promoter region of chicken prolactin with egg production. Poult. Sci. 85, 26–31.

Drogemuller C., Hamann H., Distl O., 2001. Candidate gene markers for litter size in different German pig lines. J. Anim. Sci. 79, 2565–2570.

Filistowicz A., Żuk B., 1995. Zastosowanie programów hodowlanych w doskonaleniu zwierząt futerkowych w Polsce. Zesz. Nauk. Przegl. Hod. 21, 55–68.

Haliassos A., Chomel J.C., Grandjouan S., Kruh J., Kaplan J.C., Kitzis A., 1989. Detection of minority point mutations by modified PCR technique: a new approach for a sensitive diagnosis of tumor-progression markers. Nucleic Acids Res. 20, 8093–8099.

Krzywoszyński W., 1983. Schorzenia gruczołu mlecznego u lisów i norek. Hod. Drobn. Inwent. 4, 12–13.

Kurył J., 2000. Geny cech ilościowych zwierząt gospodarskich – aktualny stan badań. Prace Mat. Zoot. 56, 7–50.

Lan X.Y., Pan Ch.Y., Chen H., Lei Ch.Z., Li F.Y., Zhang H.Y., Ni Y.S., 2009. Novel SNP of the goat prolactin gene (PRL) associated with cashmere traits. J. Appl. Genet. 50, 51–54.
Łukaszewicz M., 2007. Genetyka molekularna w hodowli zwierząt. Przegl. Hod. 9, 5–8.
Nowicki B., Pawlina E., Kruszyński W., Łoś P., 1994. Leksykon terminów z zakresu genetyki i hodowli zwierząt. PTZ, Warszawa.
Osten-Sacken A. 2009. Selekcja genomowa – rewolucja w hodowli. Hoduj z Głową, 5–6, 60–63.
Rozempolska-Rucińska I., Jeżewska G., Zięba G., 2004. Effect of genetic factors on the litter size in mink. Anim. Sci. Pap. Rep., 22. Suppl. 2, 121–124.
Sneath P. H. A.. Sokal, R. R., 1973. Numerical Taxonomy. Freeman, San Francisco.
Socha S., Markiewicz D., Wojewódzka A., 2002a. Plenność niektórych odmian barwnych norki hodowlanej (*Mustela vison* Sch.). Zesz. Nauk. Przegl. Hod. 68, 79–85.
Socha S., Markiewicz D., Zięba G., 2002b. Analiza czynników wpływających na użytkowanie rozpłodowe samców norki standardowej (*Mustela vison* Sch.). Zesz. Nauk. Przegl. Hod. 64, 63–70.
Sulik M., Felska L., 2000. Ocena wpływu samca i terminu krycia na plenność i długość ciąży u norek. Zesz. Nauk. Przegl. Hod. 53, 115–121.
Świtoński M., 1992. Analiza genomu zwierząt – istotnym elementem współczesnej hodowli. Zesz. Nauk. Przegl. Hod. 6, 31–47.
Vardy T.L., Farid A., 2002. Polymorphisms at the mink prolactin locus. J. Anim. Sci. 80, Suppl. 1, 379.
Walawski K, Pareek Ch.S., Czarnik U., Zabalewicz T., 2004. Identyfikacja *loci* cech ilościowych (QTLs) użytkowości mlecznej bydła za pomocą skanowania genomu, zgodnie z procedurą „selektywnego łączenia DNA" [In:] Postępy genetyki molekularnej bydła i trzody chlewnej, ed. Świtoński M. Wyd. AR, Poznań, 171–199.
Wojdak-Maksymiec K., Kmieć M., Strzalaka J., 2008. Prolactin Gene Polymorphism and Somatic Cell Count in Dairy Cattle. J. Anim. Vet. Advances. 7, 35–40.
Zabaneh D., Mackay I.J. 2003. Genome-wide linkage scan on estimated breeding values for a quantitative trait. BMC Genet. 31, Suppl. 1, 1–6.
Żukowski K., Suchocki T., Gontarek A., Szyda J. 2009. The impact of single nucleotide polymorphism selection on prediction of genomewide breeding values. BMC Proc. 23, Suppl. 1, 1–5.

*The project was funded by the National Science Center

Streszczenie. Celem niniejszej pracy było wykrycie polimorfizmu genu prolaktyny 5978 T/C (PRL/Alw21I) oraz określenie struktury genetycznej badanych stad w odniesieniu do analizowanych SNP. W tym celu zostało zbadanych sześć odmian barwnych (Standard, Scanblack, Sapphire, Pearl, Black Cross i Sapphire Cross) norki amerykańskiej (*Neovison vison* Schreb., 1777). Wykorzystując metodę PCR-RFLP ACRS, wykryto we fragmencie 3. intronu polimorfizm 5978 T/C, (enzym restrykcyjny Alw21I). Wykazano istnienie dwóch alleli – *PRL*T i *PRLC* i trzech genotypów – *PRL*T*PRL*T, *PRL*T*PRLC*, *PRLCPRLC*. Badania genetyczne 165 osobników wykazały wyższą częstotliwość (0,87) allelu *PRL*T (najwyższy u norek Sapphire, a najniższy u Standard) i niższą (0,13) występowania allelu *PRLC*. Odsetek osobników z genotypem *PRL*T*PRL*T był równy 75,2%, z *PRL*T*PRLC* – 26,3%, a z *PRLCPRLC* – 1,2%. Uzyskane wyniki wskazują, że prace hodowlane przyczyniają się do eliminacji allelu *PRLC* z puli genowej norki i zachodzi silna presja selekcyjna genu prolaktyny w odmianach barwnych Scanblack, Sapphire oraz Sapphire Cross.

Słowa kluczowe: norka amerykańska, ACRS-PCR-RFLP, polimorfizm genetyczny, prolaktyna