Ann. Anim. Sci., Vol. 9, No. 2 (2009) 133–142

GENETIC VARIATION OF MICROSATELLITE SEQUENCES AND ITS RELATIONSHIP WITH SOME PRODUCTIVE TRAITS OF ARCTIC FOXES

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Abstract

The objective of the study was to determine the polymorphism of microsatellite sequences and its relationship with temperament and mean body weight of arctic foxes. Measurement of body dimensions in all animals was accompanied by 3 behavioural tests performed to determine the temperament of every animal. Blood samples were also collected. For all primer sequences designed for amplification of microsatellite loci in dogs, analogous products were obtained for arctic foxes. The present study confirms the possibility of using canine genetic markers for genetic study of related species (such as the arctic fox) and suggests the need for standardization of behavioural tests used to determine the temperament of fur animals, because the results of individual tests (empathy test, catch test, restraint test) are not conclusive as to the character of an animal. Our study also revealed the presence of alleles characteristic of different weight classes and groups of animals with different temperaments.

Key words: welfare, arctic fox, behavioural test, polymorphism, microsatellite sequences

Breeders are now paying much attention to the improvement of welfare in farm animals, including fur animals. According to a definition proposed in 1965 by the Brambell Committee, welfare is a wide term that embraces both the physical and mental well-being of the animal. Welfare is determined by production (e.g. prolificacy, fertility, growth rate of young animals), physiological (e.g. level of stress hormones and parameters of stress response) and behavioural parameters (e.g. behavioural tests that determine the animal's temperament).

One factor that has the greatest effect on reducing welfare levels in fur animals is stress. All types of stress factors increase the secretion of the pituitary hormone (ACTH), which increases the blood concentration of glucocorticoids. Glucocorticoids are essential for maintaining body homeostasis and for coping with stress, but excessive secretion of this hormone under long-term stress impairs the body's function, resulting in weaker immune function, increased infections and disease, reproductive

disorders, temporary sterility, and changes in reproductive organs. This is detrimental to reproductive performance and productive traits (Kania et al., 2001). A special type of stress is prenatal stress experienced by a pregnant mother (Braastad, 1993). It may be responsible for lower birth weight of pups, lower concentration of adrenalin and reduced gonadal weight (Braastad, 1998). A study with pregnant vixens, performed in 1996, showed that females exposed to stress factors had increased blood cortisol concentrations (Braastad et al., 1998; Osadchuk et al., 2004). It should be noted that 10 out of 11 dead fetuses came from a group of females subjected to a stress factor, i.e. removal from cage (Osadchuk et al., 2003).

Resistance to stress and the animal's temperament determined by it, also have a significant effect on the indicators of innate cellular immunity. A recent study in this area (Kostro et al., 2007) has shown significant differences between selected indicators of nonspecific cellular immunity in female arctic foxes according to behaviour type. The highest capacity for nitroblue tetrazolium reduction and phagocytic and killing activity of neutrophils was found in confident foxes, while the lowest mean values of the analysed parameters were found in the group of fearful foxes.

In recent years, molecular markers have become a useful tool in population and genetic studies of different animal species. Microsatellite markers are one of the most often used markers due to their high polymorphism, codominant inheritance and many other characteristics (Fredholm and Wintero, 1995). It is well known that primers used for PCR amplification are highly suitable for Canidae family (Ślaska and Jeżewska, 2008; Jakubczak and Jeżewska, 2008). Because of many homologies between dog, arctic fox and common fox chromosomes (Graphodatsky et al., 2000), a comparative map of these species can be used to transfer information about mapped genes between these species. In this way, the physical and genetic maps of the common fox can be constructed based on a canine marker map. Klukowska et al. (2002) assigned 8 canine microsatellite loci to 3 linkage groups in arctic and common foxes. Using canine genome data, Ślaska et al. (2008) assigned 17 microsatellite sequences to 5 linkage groups in the raccoon dog. These findings are evidence that canine microsatellite markers can be used to analyse the genome of other Canidae species, including the arctic fox.

The objective of the study was to determine a relationship between the polymorphism of microsatellite sequences and temperament and body weight of arctic foxes. The effect of temperament on the productive traits of animals and the lack of any literature data on the correlation between genetic factors and the temperament and productivity of fur animals have validated our decision to investigate this subject.

Material and methods

Twenty-four arctic foxes (including 12 females) from a fur farm in south-eastern Poland were studied on reaching full hair coat maturity in late November/early December. Body weight measurements were accompanied by 3 behavioural tests to determine the temperament of each animal:

1. The empathy test involved putting a stick into the cage and observing the animal's reaction to a novel object (Gacek, 1999).

2. The catch test involved observing the animal's reaction from the time of man's intrusion into the animal's living environment (opening of the cage) to catching.

3. The restraint test involved taking the animal out of the cage and holding it by the neck (using a grasping device) and tail at the same time.

These tests enabled the animals to be classified into three groups differing in temperament, i.e. aggressive (A), indifferent (I) and fearful (F). Animals were classified into temperament groups based on the consistent results of at least 2 behavioural tests. Each test was conducted in 3 replications.

All animals were allotted to 3 weight classes according to body weight. Class 1 included animals weighing above 12 kg, class 2 those weighing 10–12 kg, and class 3 animals lighter than 10 kg. Weight classes were determined based on a study by Jakubczak et al. (1999), who shows a high correlation between the animal's body weight and length, which translates into skin size. Animals heavier than 10.2 kg achieve skin length of over 115 cm, which corresponds to the "000" skin length class.

After the tests, blood was collected from the saphenous vein of each animal into vacuum tubes containing EDTA as anticoagulant. DNA from peripheral blood cells was isolated using the QIAamp DNA Blood Kit (Qiagen). Isolation was followed by preliminary quantitative and qualitative DNA analysis using 1% agarose gel electrophoresis with ethidium bromide as an intercalating dye. Electrophoresis was performed at 80V for 40 min. The concentration and purity of isolated DNA was determined using a spectrophotometer at wavelengths of 260 and 280 nm.

The length polymorphism of 13 microsatellite loci (PEZ1, PEZ3, PEZ5, PEZ6, PEZ8, PEZ12, PEZ20, FHC2010, FHC2079, FH2019, FH2140, FH2168 and FH2054) was analysed by PCR using an MJ Research PTC-225 Tetrad thermocycler. The primer sequences of all amplified microsatellite loci are listed in Table 1.

Table 1. Primer sequences (F and R) used to amplify 13 microsatellite loci

Locus	Primer sequence	
	forward-labelled (5' – 3')	reverse (5' – 3')
PEZ1	GGCTGTCACTTTTCCCTTTC	CACCACAATCTCTCTCATAAATAC
FHC2010	AAATGGAACAGTTGAGCATGC	CCCCTTACAGCTTCATTTTCC
PEZ5	GCTATCTTGTTTCCCACAGC	TCACTGTATACAACATTGTC
PEZ20	CCTAAATTAGAGGTCTAACC	TAAGCGGGAATGTGCTCCTC
PEZ12	GTAGATTAGATCTCAGGCAG	TAGGTCCTGGTAGGGTGTGG
PEZ3	CACTTCTCATACCCAGACTC	CAATATGTCAACTATACTTC
PEZ6	ATGAGCACTGGGTGTTATAC	ACACAATTGCATTGTCAAAC
PEZ8	TATCGACTTTATCACTGTGG	ATGGAGCCTCATGTCTCATC
FHC2079	CAGCCGAGCACATGGTTT	ATGATTCTGATATGCCCAGC
FH2019	ACCCCCCTGATTTGCTTTAC	AGTCCCGGAATCGAGTCC
FH2054	GCCTTATTCATTGCAGTTAGGG	ATGCTGAGTTTTGAACTTTCCC
FH2140	GGGGAAGCCATTTTTAAAGC	TGACCCTCTGGCATCTAGGA
FH2168	GCAAATTACTTACTTCACTATGCC	TTGCAAGACTTCAACATGGC

All PCR products were separated electrophoretically in 4% polyacrylamide gel using an ABI Prism 3100-Avant automated sequencer (Applied Biosystems). The length of microsatellite sequences was determined using Gene Mapper v. 3.5 software. Allele lengths at microsatellite loci were determined using Gene-Scan-500 ROX as internal size standard (Applied Biosystems).

Based on the frequency of alleles in the population, the following parameters of within-population genetic variation were calculated: observed heterozygosity (H_O), expected heterozygosity (H_E) and polymorphic information content (PIC). All calculations were performed using SAS/Genetics™ 9.1.3 statistical software package.

Results

The behavioural tests demonstrated that foxes with an indifferent temperament accounted for the largest group (65%) of all animals tested.

The highest mean body weight (11.89 kg) of all animals was found in aggressive animals and the lowest (11.41 kg) in animals with an indifferent temperament. Fearful foxes were characterized by a mean body weight of 11.79 kg.

The greatest number of animals represented weight class 1 (37.5%), followed by 33.3% animals in class 3 and 29.2% animals in class 2.

For all the primer sequences designed for amplification of canine microsatellite loci, products were also obtained for the arctic fox.

Allele lengths for the loci PEZ5, PEZ20, PEZ12, PEZ3, PEZ6 and PEZ8 in the arctic fox corresponded to allele values in the dog. The allele sizes observed in the arctic fox for the loci PEZ1, FHC2010 and FH2054 were greater than analogous allele sizes in the dog.

As a result of molecular DNA analysis of 24 arctic foxes, a total of 55 alleles belonging to 13 loci were identified (4.23 alleles per locus on average). The number of alleles per locus ranged from 3 (FHC2010, FH2140, FH2168, PEZ6 and PEZ8) to 7 (FHC2079, PEZ12). The lengths of all alleles identified at different microsatellite loci are listed in Table 2.

Table 3 shows the number of alleles identified at different microsatellite loci as well as observed heterozygosity (H_O), expected heterozygosity (H_E) and polymorphic information content (PIC) values determined for the analysed group of arctic foxes.

PIC values for different loci ranged from 0.4285 (FH2054) to 0.7266 (PEZ12). PIC value was greater than 0.5 for 8 out of 13 analysed loci, and exceeded 0.7 for 2 loci (FHC2079, PEZ12).

Differences in PIC values for the analysed markers are probably due to the different numbers of alleles identified at individual microsatellite loci.

The observed heterozygosity ranged from 0.3333 (FH2168) to 0.7895 (PEZ3) according to locus. It was equal or greater than 0.5 for all the analysed loci except FH2054 and FH2168, and equal or greater than 0.6 for 5 out of 13 analysed loci. For 7 out of 13 analysed loci, the observed heterozygosity for different loci was greater than the expected heterozygosity calculated for each locus.

Table 2. Allele lengths for different microsatellite loci (base pairs)

Allele	Locus												
	FHC2010	FHC2079	FH2140	FH2168	FH2054	FH2019	PEZ5	PEZ1	PEZ12	PEZ6	PEZ20	PEZ3	PEZ8
A	425	250	96	228	170	180	98	170	250	169	170	105	234
B	429	254	104	232	174	184	102	174	254	181	174	108	238
C	433	258	108	262	178	188	106	178	258	185	178	111	242
D		262			182	196	110	182	262		182	114	
E		266							266			117	
F		274							274			120	
G		278							278				

Table 3. Parameters estimated for the analysed population at all loci studied

Locus	No. of alleles	PIC	H_o	H_e
PEZ1	4	0.4994	0.555	0.541
PEZ3	6	0.6940	0.789	0.736
PEZ5	4	0.5533	0.550	0.616
PEZ6	3	0.4436	0.647	0.538
PEZ8	3	0.5063	0.600	0.586
PEZ12	7	0.7266	0.600	0.760
PEZ20	4	0.5239	0.588	0.565
FH2019	4	0.4666	0.571	0.559
FH2054	4	0.4285	0.352	0.465
FH2140	3	0.4894	0.590	0.548
FH2168	3	0.5355	0.333	0.611
FHC2010	3	0.5594	0.500	0.635
FHC2079	7	0.7255	0.631	0.761

The expected heterozygosity for different loci ranged from 0.4654 (FH2054) to 0.7618 (FHC2079). The expected heterozygosity for a given locus was greater than 0.5 for 12 out of 13 analysed microsatellite loci and greater than 0.6 for 6 analysed loci.

The observed heterozygosity was higher than the expected heterozygosity for 7 out of 13 microsatellite loci analysed. The greatest difference emerged for the FH2168 locus, for which the observed heterozygosity was 0.3529 and the expected heterozygosity was 0.4654. Slightly smaller differences occurred at the microsatellite loci PEZ12 (difference between observed and expected heterozygosity of 0.16), FHC2010 (0.135), FHC2079 (0.1302) and FH2054 (0.1125). For the other loci, these differences were less pronounced.

The analysis of 13 microsatellite loci revealed the presence of alleles characteristic of different temperaments. Alleles at the PEZ3 locus were characteristic of the aggressive temperament, alleles at the FHC2010, FHC2079, PEZ1, PEZ3 and PEZ12 loci were characteristic of the indifferent temperament, and those at the FHC2079, PEZ5, PEZ6 and PEZ12 loci were typical of fearful animals. Characteristic alleles were also found for each weight class. The characteristic alleles were at the FHC2079, PEZ3 and PEZ12 loci for animals belonging to weight class 1; FHC2079, PEZ5, PEZ6 and PEZ12 for animals belonging to weight class 2; and FHC2010 and PEZ3 for animals representing weight class 3. Detailed information on the alleles characteristic of different temperaments and weight classes (including lengths in base pairs) is listed in Table 4.

Table 4. Allele lengths (in base pairs) with significant effects on temperament (A – aggressive, I – indifferent, F – fearful) and weight class

Trait		Mean body weight (kg)	Locus												
			PEZ1	PEZ3	PEZ5	PEZ6	PEZ8	PEZ12	PEZ20	FH2019	FH2054	FH2140	FH2168	FHC2010	FHC2079
Temperament	A	11.89		108											
	I	11.41	178	105,117				254,258,266						429,433	254,258,266
	F	11.79			102	181		262							262
Weight class	1	13.53	170	105				266	170		178		228		274
	2	10.93			102	181		262							
	3	9.32		108						196			232	429	

Discussion

The results of the empathy, catch and restraint tests were not always consistent. In the case of the catch and restraint tests, it is necessary to open the cage door and catch a fox, thus encroaching on the animal's territory. This leads to stress conditions that normally do not occur, and may lead to behavioural changes and misinterpretation of the animal's temperament. The empathy test is an interesting alternative proposed by Gacek (1999). In this test, there is no need to open the cage door or catch the animal, which eliminates stress related to invasion of the animal's living space and may lead to the display of normal behavioural patterns. Nevertheless, the present results show the need for standardization of behavioural tests used to determine the temperament of fur animals because of the failure to provide a consistent determination of the animal's temperament.

As a result of PCR reaction using all primer sequences developed for the dog (*Canis familiaris*), amplification products were also obtained for the DNA of the arctic foxes studied. Likewise, Ślaska and Jeżewska (2008) obtained amplification products for microsatellite loci and fragments of raccoon dog genes using the primers described in the literature for the domestic dog. Also a study by Jakubczak and Jeżewska (2008) with a parentage verification kit in dogs showed the possibility of using canine primer sequences for product amplification in the arctic fox, common fox and raccoon dog.

Based on analysis of the differences in length between alleles at individual microsatellite loci, it can be assumed that the repeat motif for all microsatellite markers studied in the arctic fox remained consistent with the information provided in the literature data and internet databases on repeat motifs in the analogous microsatellite loci of the dog (Pádár et al., 2001; Völkel, 2005; Hellmann et al., 2006). Our study and those of other authors (Pádar et al., 2001; Völkel, 2005; Hellmann et al., 2006) are in disagreement with Neff et al. (1999) concerning the repeat motif at the PEZ3 locus. Neff et al. (1999) report that the repeat motif of the PEZ3 locus has 4 base pairs, while the literature data and our study point to a triplet repeat motif of the PEZ3 locus.

A study with 14 breeds of dogs (Völkel, 2005) revealed clear differences in PIC values between the different breeds. Mean PIC values for all the breeds were PIC=0.453 for FHC2010, PIC=0.520 for PEZ1, PIC=0.366 for PEZ5, PIC=0.544 for PEZ12, PIC=0.47 for PEZ20, PIC=0.708 for PEZ6, PIC=0.617 for PEZ8, and PIC=0.397 for FHC2079. Despite the smaller number of arctic foxes examined in our study compared to the number of dogs investigated by Völkel (2005), PIC values for the FHC2010, PEZ5, PEZ12, PEZ20 and FHC2079 loci were higher in foxes than in dogs. The greatest difference in PIC values emerged for the FHC2079 locus. It was 0.397 in dogs and 0.7255 in arctic foxes.

In a study with dogs by Lüpke and Distl (2005), PIC value was 0.55 for FH2054, 0.64 for FH2140, and 0.46 for FH2168. In our study, PIC values for analogous loci were lower, possibly due to the smaller number of animals subjected to both analyses.

In a study with 14 breeds of dogs by Völkel (2005), the mean observed heterozygosity (which showed major differences between dog breeds) for individual microsatellite loci was H_O=0.563 for FHC 2010, H_O=0.381 for FHC2079, H_O=0.499 for PEZ1,

H_O=0.451 for PEZ5, H_O=0.544 for PEZ12, H_O=0.531 for PEZ20, H_O=0.736 for PEZ6, and H_O=0.680 for PEZ8. As regards the PEZ1, PEZ5, PEZ12, PEZ20 and FHC2079 loci, the observed heterozygosity for the arctic fox presented in our study were higher than the mean H_O values observed for the analogous loci in the dog.

In a study with dogs (Lüpke and Distl, 2005), the observed heterozygosity was 0.65 for FH2054, 0.66 for FH2140 and 0.57 for FH2168, being in all cases higher than that found in our study. These differences may result from the number of animals analysed.

The expected heterozygosity values reported by Lüpke and Distl (2005) for dogs were 0.62 for FH2054, 0.69 for FH2140 and 0.52 for FH268. The differences in expected heterogyzosity values between the study of Lüpke and Distl (2005) and our study may be due to the more uniform genetic structure of the arctic fox.

In the current study, we showed the presence of alleles characteristic of animals with different weight classes and temperaments. This seems particularly important during selection for domestication of different fur animal species. Selection of indifferent animals, which are preferred in farm breeding, can thus be based on the genotype analysis of individual animals. Animals retained in breeding on the basis of this selection may prove more valuable with regard to some genotypic and character traits. Based on the alleles characteristic of aggressive animals, it is also possible to carry out negative selection that eliminates aggressive animals from further breeding. Likewise, alleles characteristic of different weight classes can serve as a basis for selection of animals for the highest body weight. Unfortunately, the available literature contains no studies on the relationship between microsatellite loci and the temperament and body weight of arctic foxes.

The promising results of our study show that more research is needed with a larger population of arctic foxes.

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Accepted for printing 9 V 2009

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Zmienność genetyczna sekwencji mikrosatelitarnych i jej związek z wybranymi cechami użytkowymi lisów polarnych

STRESZCZENIE

Celem badań było określenie polimorfizmu sekwencji mikrosatelitarnych i jego związku z temperamentem oraz średnią masą ciała lisów polarnych. Wraz z pomiarami masy ciała wszystkich osobników przeprowadzono 3 testy behawioralne, w celu ustalenia temperamentu każdego zwierzęcia oraz dodatkowo pobrano krew.

W przypadku wszystkich sekwencji starterowych przeznaczonych do amplifikacji loci mikrosatelitarnych u psów, analogiczne produkty otrzymano również w przypadku lisów polarnych. Niniejsze badania potwierdzają możliwość wykorzystania markerów genetycznych pochodzących od psa w badaniach genetycznych gatunków z nim spokrewnionych, m.in. lisa polarnego. Wskazują na potrzebę unifikacji testów behawioralnych wykorzystywanych w określaniu temperamentu zwierząt futerkowych, gdyż wyniki poszczególnych testów, tj. empatycznego, chwytania i trzymania „na chwytce" niejednoznacznie określają charakter danego osobnika. Powyższe badania ujawniły również występowanie alleli charakterystycznych dla poszczególnych klas wagowych oraz grup zwierząt o określonym temperamencie.