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GENETIC DIFFERENTIATION OF INTERGENERIC HYBRIDS OF BLUE FROST FOXES AND THEIR ORIGINAL FORMS BASED ON MICROSATELLITE POLYMORPHISM

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Abstract

The research objective was to determine differences in the genetic structure of the hybrids of Blue Frost foxes and their original forms, i.e. the arctic fox and the silver fox. A multiplex PCR was applied to five loci (FH2054, CPH3, FH2168, FH2164, FH2097). Sequences available from the canine genome enabled the analogous loci to be amplified in both arctic foxes (blue and shadow) and silver foxes as well as in their hybridization crosses – Blue Frost foxes. The number of alleles, the polymorphism information content, and the observed and expected heterozygosity differed between the analysed animal groups. The highest PIC and heterozygosity values were noted for the Blue Frost hybrids. The greatest genetic distance was found between the arctic shadow fox and the silver fox. As expected, Blue Frost foxes turned out to be an intermediate form between parental forms; accordingly, close genetic similarity values were reported between the hybrids and the arctic blue and silver foxes.

Key words: fox hybrid, blue fox, silver fox, microsatellite

Microsatellite sequences have become the most popular source of genetic markers due to high polymorphism information content and uniform distribution in the genome. They are routinely used in the studies on species belonging to the Canidae family. Importantly, microsatellite variability shows evidence of genetic differentiation between the kin-related populations as well as a genetic distance between them (Klukowska et al., 2003). Besides, microsatellite sequence polymorphism is analysed with regard to the productive traits of fur-bearing animals (Jakubczak et al., 2009). Some standard canine microsatellite panels are widely used for genetic identification of the individual dog (Klukowska et al., 2001). Interestingly, microsatellite panels

are very useful not only for individual identification of Canidae species but can be the key tool in the genetic traceability of the silver and arctic foxes and finnraccoon (Jakubczak and Jeżewska, 2008). The broad spectrum of microsatellite sequences application along with the knowledge of their usefulness in parent-offspring analyses provide an excellent source of reliable information in this field.

Currently, fur dealers offer not only pelts obtained from different fox colour types (morphs) of both breeding species but also skins from intergeneric hybrids – the arctic fox (*Vulpes lagopus*) and the silver fox (*Vulpes vulpes*). Although animals resulting from intergeneric hybridization are infertile, they have still been of great interest to breeders due to the unique qualities of fox fur and exterior – a combination of features characteristic of both species. Intergeneric crosses usually show a perfect structure of fur coat in terms of silkiness, softness and fluffiness. Their very dense fur coat occurring on both sides and lower abdomen resembles the arctic fox coat with larger body size characteristic of the silver fox. Hybrids display a wide range of colouration depending on varieties of the species used in hybridization. The most popular crosses prove to be the Blue Frost fox – the hybridized offspring of the female blue arctic fox of a dark colour morph and a male silver fox of medium light fur coat. The hybrids have black fur with interspersed silver-tipped hairs and grey-blue underfur, their underparts are black and the tail has a typical white tip at the end. As for fur industry, any spots at the back parts of the body, brown hair cover as well as light colouring, unclear colour or too short guard hairs are considered highly undesirable features.

The studies on crosses between the silver fox and the arctic fox include the comparative analyses indicating some differences between karyotypes of each animal group (Mäkinen and Gustavsson, 1982). At present, microsatellite polymorphism is used to identify offspring obtained after hybridization between the wild arctic foxes and captive-bred foxes, that is the animals that belong to one species (Norén et al., 2005). The aim of the research was to determine genetic diversity within intergeneric hybrids of Blue Frost foxes and their original forms (arctic fox, silver fox) on the basis of microsatellite variability.

Material and methods

Blood samples were collected from a total of 70 foxes: 11 blue foxes, 11 shadow foxes, 24 silver foxes and 24 Blue Frost hybrids.

DNA was isolated with the standard commercial kit QIAamp DNA Blood Mini Kit (QIAGEN) using the protocols recommended by the manufacturer and the QIAcube application. Further analyses included only those samples whose absorption ratio of A260/A280 was found in the 1.7–2.0 range. Whereas the samples that did not satisfy the criterion, had to undergo retreatment before analysis.

Molecular studies involved five microsatellite loci FH2054, CPH3, FH2168, FH2097 and FH2164 (Table 1). A multiplex PCR assay was conducted for all five sequences in the MJ Research PTC-225 Tetrad thermocycler.

Table 1. Primer sequences used for amplification of analysed loci

Locus	Motif	Primer sequences	Source
CPH3	$(GA)_2TA(GA)_{17}$	F: CAGGTTCAAATGATGTTTTCAG R:TTGACTGAAGGAGATGTGGTAA	Fredholm and Winterø, 1995
FH2054	$(GATA)_{16}$	F: GCCTTATTCATTGCAGTTAGGG R: ATGCTGAGTTTTGAACTTTCCC	Fransisco et al., 1996
FH2097	$(GAAA)_{16}$	F: CAATGTCGAATTCCATGGTG R: ATGGAGCAAGATGTGTTTGTG	Fransisco et al., 1996
FH2164	$(GAAA)_{43}$	F: GATTATGACTCGAACCAAAGGC R:TGGAGGAAGTTCATTAAGCAGC	Fransisco et al., 1996
FH2168	$(GAAA)_{20}$	F:GCAAATTACTTACTTCACTATGCC R: TTGCAAGACTTCAACATGGC	Fransisco et al., 1996

The PCR reaction mixture contained AmpliTaq Gold (Applied Biosystems) with the following reagents:

* deionized water – 109 ul
* buffer for PCR assay ×10 – 15 ul
* G/C buffer – 2.8 ul
* Mg2+ – 11 ul
* dNTP* – 7.8 ul
* forward primer – 0.35 ul } for each locus
* reverse primer – 0.35 ul } for each locus
* Gold polymerase – 1 ul

The time-temperature profile for the PCR reaction was established experimentally:

* initial denaturation 95°C – 10 min
* denaturation 95°C – 30 s } 36 cycles
* primer annealing 58°C – 45 s } 36 cycles
* extension of DNA strands 72°C – 60 s } 36 cycles
* final extension 72°C – 20 min
* final hold 4°C – ∞

DNA fragments were electrophoresed in the genetic analyser 3100-Avant. Length of alleles was determined using Gene Mapper software ver.3.5 in relation to the internal lane size standard (Genescan 500 ROX). The microsatellite loci were analysed to estimate the number of alleles, the observed (Ho) and expected heterozygosity (He) and the polymorphism information content (PIC) for each animal group. There was also calculated genetic similarity and genetic distance (Nei, 1978), while the dendrogram was generated on the basis of the unweighted pair group method (UPGM) of analysis.

Results

There were described 5 microsatellite loci in the animals under study. The number of alleles and their size are presented in Table 2. A similar range of allele length was noted for the blue foxes and the silver foxes only in loci CPH3 and FH2097. The lowest number of alleles (3) was recorded in the common silver fox locus CPH3, whereas the highest number in the Blue Frost hybrids, i.e. a maximum of 9 alleles in loci FH2054 and FH2168. Only in locus FH2164 were more alleles found than in hybrids (6) in the common silver fox group (7). In the analysed loci, there occurred alleles characteristic of both the arctic and red fox. Three alleles present only in the common silver fox (141, 189 and 193 bp) were identified in the locus FH2054. There were also determined four arctic fox-specific alleles (165, 173, 177 and 181 bp), with the 177 bp allele being characteristic of the shadow arctic fox. While in the locus CPH3, there were found two alleles pertaining to the arctic fox. These were 148 and 168 bp alleles, and importantly the 148 bp allele was only noted in the shadow fox. Likewise, in the locus FH2097, two arctic fox-specific alleles, 270 and 194 bp, were also noted. The 270 bp allele proved to be characteristic of the shadow variety. The aforementioned locus also comprised one allele (286 bp) characteristic of the common silver fox. The loci FH2168 and FH2164 proved very interesting as their alleles determined for the arctic fox were found within the absolutely different range as compared to those for the common fox. In the locus FH2168 for the common silver fox, there were observed four characteristic alleles – 213, 217, 221 and 225 bp. Whereas in the arctic fox, there occurred specific alleles of the following length: 233, 237, 249, 257, 261, 265 and 269 bp. In addition, the 237 bp allele was reported only in the shadow fox, while 261 and 265 bp alleles in the blue fox alone. In the common silver fox in the locus FH2164, as many as seven alleles were identified, i.e. 265, 269, 273, 281, 285, 289 and 293 bp; it is noteworthy that none of them was found in the arctic fox. This group included alleles of 297, 305, 341, 345, 349 and 365 bp in length. Two of them were characteristic of the shadow variety (305 and 341 bp) and one (349 bp) of the blue variety.

Polymorphism information content (Table 3) in both colour morphs of the arctic fox was similar: it approached a 0.5 value or was higher. Only in locus FH2054 did the PIC value of 0.3677 determined in the shadow arctic fox group appear to be markedly lower as compared to the blue fox group and the lowest out of all values established. In the common silver foxes, the PIC values differed from those for the arctic foxes as the information content was the lowest in locus FH2097 and the highest in FH2164. In all loci, except for FH2097, PIC values exceeded 0.5. As for the Blue Frost hybrids group, polymorphism index content reached higher values in all the evaluated loci. In four out of five analysed loci, the content exceeded 0.7 to reach the highest value in locus FH2168 out of all under study. Only in locus CPH3 was the PIC value shown to be slightly lower than in other loci, but it exceeded 0.5.

The values of observed and expected heterozygosity are summarized in Table 4. The highest heterozygosity was found in the Blue Frost crosses which exhibited a Ho level > 0.7 in all loci analysed. The highest value was determined in these animals in locus FH2164 – 1.0000. In the group of common silver foxes, the estimates

of Ho proved to be rather similar in all loci, ranging from 0.4231 in locus FH2097 up to 0.5385 in locus FH2054. In both groups of arctic foxes, low heterozygosity was found in locus FH2164, while in locus FH2054 a difference in heterozygosity between both colour types of the arctic fox appeared to be substantial (shadow foxes – 0.3333, blue foxes – 0.7778). As for blue arctic foxes, in as many as two loci (CPH3 and FH2168) heterozygosity reached 1.0000, which is likely to result from the small size of the population studied.

Table 2. Number of alleles (NA), range of allele size and specific alleles in each studied animal group for analysed sequence

	Shadow fox			Blue fox			Silver fox			Blue Frost fox	
	NA	size range (bp)	specific alleles (bp)	NA	size range (bp)	specific alleles (bp)	NA	size range (bp)	specific alleles (bp)	NA	size range (bp)
FH2054	5	165–181	165 173 177 181	5	165–185	165 173 181	5	141–193	141 189 193	9	141–193
CPH3	5	148–168	148 168	4	152–168	168	3	152–160	-	6	148–160
FH2168	6	229–269	233 237 249 257 269	7	229–269	233 249 257 261 265 269	5	213–229	213 217 221 225	9	213–269
FH2097	4	274–294	294	5	270–294	270 294	4	274–286	286	6	270–294
FH2164	5	297–365	297 305 341 345 365	4	297–365	297 345 349 365	7	265–293	265 269 273 281 285 289 293	6	273–365

Table 3. PIC values determined for analysed loci in each animal group

	Shadow fox	Blue fox	Silver fox	Blue Frost fox
FH2054	0.3677	0.6035	0.5634	0.7370
CPH3	0.6219	0.6571	0.5392	0.5519
FH2168	0.7027	0.7470	0.6412	0.8161
FH2097	0.6490	0.6419	0.4181	0.7021
FH2164	0.4966	0.5392	0.6980	0.7021

Table 4. Observed (Ho) and expected heterozygosity (He) in analysed loci for each animal group

	FH2054		CPH3		FH2168		FH2097		FH2164	
	Ho	He	Ho	He	Ho	He	Ho	He	Ho	He
Shadow fox	0.3333	0.4052	0.7778	0.7190	0.7778	0.7843	0.7778	0.7386	0.4444	0.5556
Blue fox	0.7778	0.6928	1.0000	0.7516	1.0000	0.8235	0.6667	0.7320	0.3333	0.6340
Silver fox	0.5385	0.6320	0.4231	0.6222	0.4615	0.7104	0.4231	0.4653	0.5200	0.7527
Blue Frost fox	0.9048	0.7875	0.7143	0.5947	0.9524	0.8548	0.8571	0.7642	1.0000	0.7538

Table 5. Nei's genetic similarity and genetic distance between shadow foxes, blue foxes, silver foxes and intergeneric hybrids

	Shadow fox	Blue fox	Blue Frost fox	Silver fox
Shadow fox	***	0.9088	0.6937	0.2440
Blue fox	0.0956	***	0.7029	0.3842
Blue Frost fox	0.3657	0.3526	***	0.6731
Silver fox	1.4105	0.9565	0.3959	***

The highest genetic similarity (0.9088) and therefore the smallest genetic distance (0.0956) was found between the shadow arctic fox and the blue arctic fox (Table 5). This provides evidence that both colour morphs are very close as regards genetics. The highest genetic distance (1.4105) was recorded between the common silver fox and the shadow arctic fox. The distance between the common silver fox and the blue arctic fox was substantially smaller (0.9565). The Blue Frost hybrids obtained from intergeneric hybridization showed a close similarity to both the common silver fox (0.6731) and the blue arctic fox (0.7029). The graphic illustration of these relations is the phylogenetic tree (Figure 1). The distance between particular clades corresponds with the values of genetic similarity and genetic distance noted between individual groups.

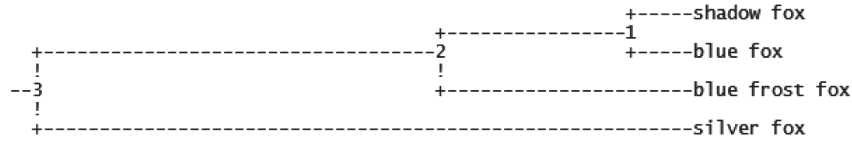

Figure 1. UPGM dendrogram based on Nei's genetic distance

Discussion

The present study aimed at characterizing the genetic structure of the discussed animal groups (shadow and blue arctic foxes, silver foxes and Blue Frost crosses) as well as determining the usefulness of microsatellite markers. The highest polymorphism was recorded in locus FH2168, where the highest number of alleles was found in both colour morphs of the arctic fox (6 and 7) and in hybrids (9). The allele length was found to be in the 213–229 bp range for the common silver foxes and 229–269 bp for both colour types of the arctic fox. Analogous differences in allele length ranges established for the common and arctic fox were also found in locus FH2164. The alleles ranged from 265 to 293 bp for the common fox and from 297 to 365 bp for the arctic fox. In both loci the highest number of alleles differentiating each colour morph of the arctic fox was stated.

The PIC values determined in locus FH2168 were among the highest and ranged from 0.6412 in the common silver foxes, through 0.7027 and 0.7470 in the arctic foxes up to 0.8161 in the Blue Frost foxes. Heterozygosity in the analysed locus proved high (0.7778; 1.0000; 0.9524) in all the groups except for the common silver foxes (0.4615). Similar research results were reported by other authors. For the same locus, Klukowska et al. (2003) reported 5 alleles of the common fox to range from 217 to 233 bp and 9 alleles of the arctic fox to range from 233 to 273 bp. They found PIC values to be 0.62 in the red fox and 0.75 in the arctic fox. Jakubczak et al. (2009) also described locus FH2168 in arctic foxes. These authors obtained 3 alleles of 228, 232 and 262 bp in length and the PIC value of 0.5355. Only the observed heterozygosity was far lower at 0.333. The other loci were also characterized by high polymorphism. The exception was locus FH2054 in the shadow common foxes group, for which both the lowest PIC (0.3677) and observed heterozygosity values (0.3333) were found. Likewise, Jakubczak et al. (2009) reported low PIC value of 0.4285 for locus FH2054 of the arctic fox and the observed heterozygosity of 0.352.

The present study allowed determining genetic similarity and distance between different fox varieties. The smallest distance (0.0956) was established between the blue arctic fox and the shadow arctic fox. This situation is correlated to the fact that these are two colour morphs of the same animal species. Whereas the distance stated between the common silver fox and the arctic fox was much greater and differed for both colour types of the arctic fox. A larger distance was established between the common silver fox and the shadow arctic fox (1.4105) compared to the blue arctic fox (0.9565). The Blue Frost hybrids are an intermediate form between the common fox and the arctic fox, and the genetic similarity values obtained for them proved close in both the silver common fox (0.6731) and the blue arctic fox (0.7029). The genetic distance values between the red fox, the arctic fox and the dog were determined on the basis of microsatellite sequence polymorphism by Klukowska et al. (2003). In the case of Nei's Ds formula, the estimated distance between the arctic fox and the red fox was 0.481. The distance calculated using Da formula was 0.414 between the red fox and the arctic fox. In turn, genetic distance $(\delta\mu)^2$ between the red fox and the arctic fox was 255. Zatoń-Dobrowolska and Filistowicz (2003) estimated the genetic distance between the arctic fox and the common silver fox on the basis

of polymorphism in transferrin locus. The genetic distance values obtained were the following: 0.948 (using Sokal and Sneath formula), 1.232 (using Nei's standard distance), 0.450 (Nei's minimum distance) and 1.579 (Nei's maximum distance). It is evident that they clearly differed depending on the statistical method applied.

The available literature lacks information on differences in the genetic structure of different fox colour morphs and intergeneric hybrids. The present results from the initial phase of molecular investigations need further research. The microsatellite sequences used have proven useful to determine the genetic distance between the two fox populations under study. The analysis performed on a greater number of animals could give strong evidence for the occurrence of specific alleles and thus indicate the potential for differentiation of each fox colour morph on the grounds of the presence of alleles of characteristic length.

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Zróżnicowanie genetyczne mieszańców międzygatunkowych lisów Blue Frost oraz ich form wyjściowych na podstawie sekwencji mikrosatelitarnych

STRESZCZENIE

Celem badań było określenie różnic w strukturze genetycznej grupy mieszańców Blue Frost oraz ich form wyjściowych – lisa polarnego i lisa pospolitego. Reakcję PCR multipleks przeprowadzono dla pięciu loci (FH2054, CPH3, FH2168, FH2164, FH2097). Sekwencje zaczerpnięte z genomu psa pozwoliły na amplifikację analogicznych loci zarówno u lisów polarnych (niebieskich i cienistych) oraz pospolitych srebrzystych, jak również u pochodzących z ich krzyżowania mieszańców – lisów Blue Frost. Otrzymane liczby alleli, wartości indeksu stopnia polimorfizmu, heterozygotyczności obserwowanej i oczekiwanej różniły się w poszczególnych grupach analizowanych zwierząt. Najwyższe wartości PIC i heterozygotyczności odnotowano u mieszańców Blue Frost. Największy dystans genetyczny stwierdzono pomiędzy lisem polarnym cienistym i lisem pospolitym srebrzystym. Zgodnie z oczekiwaniami lisy Blue Frost okazały się formą pośrednią pomiędzy formami rodzicielskimi, stąd zbliżone wartości podobieństwa genetycznego pomiędzy mieszańcami a lisami polarnymi niebieskimi i lisami pospolitymi srebrzystymi.