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Evaluation of BrdU Influence on Sister Chromatid Exchange in Arctic and Silver Fox

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The sister chromatid exchange (SCE) test is a cytogenetic assay and is known as a biomonitoring test to analyse chromosome damage caused by exogenous factors. The aim of the study was to analyse chromatin instability by determining the number and sites of spontaneous SCE in the karyotype of arctic and silver foxes. Twenty-four animals: 12 arctic foxes (*Vulpes lagopus*) and 12 silver foxes (*Vulpes vulpes*) were investigated. The experiment used peripheral blood lymphocytes following *in vitro* culture. Karyotype evaluation in both fox species was made by RBA staining with modified Giemsa reagent. To analyse SCE, three different bromodeoxyuridine (BrdU) concentrations were added to the culture and the microscopic preparations were stained by the FPG technique. Based on these results, chromosomal polymorphism in the arctic fox and the modal number of B chromosomes in the silver fox were established. Spontaneous SCE in both fox species was observed at a concentration of 0.5 µg/ml BrdU. The number of spontaneous SCE averaged 0.65±0.55 SCE/cell in the arctic fox and 2.33±0.76 SCE/cell in the silver fox. Higher BrdU concentrations induced additional chromatin breaks. The arctic fox was characterized by a greater number of centromeric SCE in relation to the other SCE types. In the silver fox, centromeric and terminal SCE occurred at a similar frequency. No significant effect of the living environment and no effect of sex on increasing SCE frequency were found in either fox species.

Key words: Sister chromatid exchange, mitotic chromosomes, arctic fox, silver fox.

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Found in the cells of all organisms, chromosomes are structures sensitive to ultrastructural damage. Chromatin breaks most often take place after exposure of the genome to mutagenic chemicals or radiation. This results in mutual exchange of the corresponding fragments of chromosome sister chromatids (sister chromatid exchange, SCE). This process occurs spontaneously in every organism, but is also an indicator of the mutagenic potential of various factors. The SCE test is often used to study chromosomal instabilities resulting from the environmental prevalence of many potential mutagens, as exemplified by overused pesticides (ZELJEZIC & GARAJ-VRHOVAC 2002).

Both arctic (*Vulpes lagopus* L. 1758) and silver foxes (*Vulpes vulpes* L. 1758) are mammals in the order Carnivora, family Canidae. They are interesting as an object of scientific study due to the presence of chromosome number polymorphism in their genomes. Chromosomal polymorphism in the arctic fox is due to centric fusions, and in the silver fox it is associated with the presence of additional B chromosomes in the karyotype (BUGNO-PONIEWIERSKA *et al*. 2013). The arctic and silver fox karyotypes vary as a result of chromatin insta-

bility in the genomes of these species, leading to cytogenetic diversity in these animals.

The normal karyotype of the arctic fox is 2n=50 and shows little structural variation of the chromosome complement. In the autosomes, 22 pairs of biarmed chromosomes (NF=88) of meta- (M, m) and submetacentric types (SM, sm), and 2 pairs of acrocentric chromosomes (A) were identified (MAKINEN *et al.* 1985). The X chromosome is metacentric and the acrocentric Y chromosome is the smallest element of the karyotype. A centric fusion (Robertsonian translocation) occurring in the fox karyotype is responsible for variation (polymorphism) in the diploid number of chromosomes (2n), which may vary from 2n=48 (homozygous form), 2n=49 (heterozygous form) to 2n=50 (normal form) among individuals (MAKINEN & GUSTAVSSON 1980; ŚWITOŃSKI 1981; CHRISTENSEN & PEDERSEN 1982). The aberration occurs between acrocentric autosome pairs 23 and 24, resulting in a large biarmed chromosome of metacentric (M) type (CHRISTENSEN & PEDERSEN 1982; MØLLER *et al.* 1985).

The silver fox karyotype is represented by two groups of chromosomes: large and small chromosomes (BASHEVA *et al.* 2010; BUGNO-PONIEWIERSKA *et al.* 2015). The basic chromosome complement of 2n=34 (NF=67) is formed by macrochromosomes, which have a meta- (22 chromosomes) and submetacentric structure (10 chromosomes plus the X sex chromosome). The Y chromosome is a small acrocentric that is difficult to distinguish from B chromosomes without differential staining (MAKINEN 1985). Unlike the constant number of macrochromosomes, the number of B chromosomes was estimated to vary between 0 and 10 (BASHEVA *et al.* 2010). The number of B chromosomes was initially reported to be 0-7 (GRAPHODATSKY *et al.* 2005 after BELIAEV *et al.* 1974) or 0-8 (MAKINEN 1985; ŚWITOŃSKI *et al.* 2003). In breeding animals, between 1 and 3 B chromosomes are most often identified (ŚWITOŃSKI *et al.* 2003). These structures, also known as supernumerary or accessory chromosomes, are recorded as 2n=34+B (0-7). Variation in the number of B chromosomes mainly occurs among individuals, although cases of B chromosome mosaicism are also known. In terms of size and morphology, they are similar to the Y chromosome.

Arctic and silver foxes are used as models in different fields of biological sciences. Due to its living environment, the arctic fox has become a model animal for studying variation in metabolic rate resulting from seasonal availability of food (FUGLEI & ØRISTLAND 1999; 2003). The effect of this factor on leptin and ghrelin levels in arctic foxes was studied by FUGLEI *et al.* (2004) and MUSTONEN *et al.* (2005). Because the arctic fox is part of the terrestrial and aquatic ecosystem, it is also used as an experimental model to investigate the effect of pollution on wild organisms, including the effect of organochlorine compounds on the thyroid (SONNE *et al*. 2009b), bone mineral density (SONNE *et al.* 2009a) and reproduction in foxes (HOEKSTRA *et al*. 2003). CYBULSKI *et al.* (2009) studied the accumulation level of heavy metals, including lead, cadmium and mercury in the kidneys and liver of silver foxes depending on the age of animals and the impact of these factors on reproduction. In turn, VINE *et al.* (2009) analysed different methods for detecting and monitoring the behaviour of wild animal species using the example of the silver fox.

The study was conducted to determine the effect of different concentrations of BrdU on SCE in the karyotypes of arctic and silver foxes. Evaluation of SCE was made with particular consideration of the number and location of the chromosomes of both species.

Material and Methods

The study was performed with 12 farmed arctic foxes (*Vulpes lagopus*) and 12 silver foxes (*Vulpes vulpes*), including 6 males and 6 females of each species. The experimental animals originated from one commercial farm in eastern Poland (arctic and silver foxes) and from two commercial farms in western Poland (arctic foxes separately from silver foxes). Animals of the same age (>2 years) were kept according to EUROPEAN COMMISSION – ANIMAL WELFARE requirements (http://ec.europa.eu/agriculture/organic/consumer-trust/animal-welfare/index_en.htm).

The study material consisted of whole peripheral blood drawn from the cephalic vein of the forearm (*Vena cephalica antebrachi*). Blood for analyses was collected during periodic health checks conducted by a veterinarian who routinely visited the farm (Farm Certification).

Cytogenetic analysis was performed on chromosomes from *in vitro* lymphocyte culture under the following conditions: time of culture 72 h, temperature 37.5ºC, constant humidity. Preliminary karyotype evaluation of the analysed species was made using the RBA staining technique (DUTRILLAUX *et al.* 1973), modified with the use of Giemsa reagent. For this purpose, approx. 240 μg/ml of 5-bromo-2'-deoxyuridine (BrdU) was added to part of the *in vitro* lymphocyte culture during the last replication cycle (about 7h before the end of culture). An R-banding pattern was obtained after physicochemical reaction of UV irradiation of chromosomes for 30 min, followed by 1h incubation in 2xSSC buffer (300 mM sodium chloride and 30 mM sodium citrate, pH 7.0) at 65°C. R-bands were detected by staining the preparations with 5% Giemsa in Sorensen's buffer, pH=6.8.

To observe sister chromatid exchanges (SCE) in chromosomes, BrdU was added to the other cultures after 24h (DI BERARDINO *et al.* 1995). Cell cultures for each analysed animal were performed in 3 replications to distinguish BrdU concentrations at which SCE occur spontaneously from those at which SCE are additionally induced in both fox species. Different BrdU concentrations were added to each culture: group I – 0.5 μg/ml; group II – 1.0 μg/ml; group III – 2.5 μg/ml.

A harlequin pattern of chromosomes was generated based on the fluorescence plus Giemsa (FPG) technique according to KIHLMAN & KRONBORG (1975). The FPG staining procedure consisted of two stages: preliminary digestion and staining with Giemsa. In the first stage chromosomes were digested with RNAse solution (10 μg/ml); incubated in Hoechst solution 33258 (0.5 μg/ml); and exposed to UV radiation. The next incubation was carried out at 4°C in darkness for 24h. On the next day, UV irradiation was repeated; preparations were incubated in 0.5xSSC (0.75M sodium chloride + 0.075M sodium citrate, pH=7.0, 58ºC), and finally the preparations were stained with 3% Giemsa (pH=6.8) for 23 min (using our own modification).

Analysis of preparations was performed using a Zeiss microscope coupled to a Nikon DS-Fi1 digital camera. Evaluation of SCE was made using NIS-Elements F2.30 software.

Multi-trait analysis of variance for four traits – interstitial SCE, terminal SCE, centromeric SCE and total SCE – was done using the GLM procedure in SAS (SAS INSTITUTE 2014). At first, the effects of BrdU concentration within species and for both species together were tested to choose the appropriate BrdU level. Afterwards, the effects of factors were examined for BrdU concentration of 0.5 μg/ml. The effects of sex and region were tested within species. The significance of differences between the mean values for the groups was determined by the Tukey-Kramer test. All P values of less than 0.01 were statistically highly significant.

Results

Based on the preliminary cytogenetic characteristics of the arctic and silver fox karyotypes as well as the extent of chromosomal polymorphism, 8 out of the 12 arctic foxes were found to have the heterozygous karyotype (2n=49). The normal karyotype (2n=50) and the homozygous karyotype (2n=48) were each represented by 2 animals (Table 1). A total of 55 metaphases stained by RBG technique were analysed (Fig. 1).

For the establishment of the modal number of B chromosomes in the silver fox, due to a large variation in the number of B chromosomes (2n=34, B [0-3]), 276 chromosome plates were analysed. In the studied silver foxes, the number of B chromosomes found on the metaphase plates varied from 0 (37 plates) to 3 (44 plates). Forty four plates had 3 B chromosomes each. In 102 cells 1 B chromosome was observed and 93 plates had 2 B chromosomes each. Table 2 presents the number of animals and metaphase plates for which the modal number of B chromosomes ranged from 0 to 3. Most foxes were of the 2n=34+1B (5 animals) and 2n=34+2B karyotype (3 animals). The second largest group were animals with the 2n=34+0B and 2n=34+3B chromosome complement. In the examined group, the 2n=34+4B karyotype was observed in only one animal on two plates, but this was not the modal number of B chromosomes.

The analysis of sister chromatid exchanges (SCE) was performed on 317 differentially stained metaphase plates (Fig. 2). In both species, all of the analysed BrdU concentrations enabled differential chromosome staining. The mean number of spontaneous SCE/cell, observed for the lowest BrdU concentration of 0.5 μg/ml, was 0.65±0.55 SCE/cell for the arctic fox and 2.33±0.76 SCE/cell for the silver fox. Higher concentrations induced additional chromatid breaks. For the BrdU concentration of 1.0 μg/ml, the mean values were 1.87±0.67 SCE/cell for the arctic fox and 3.11±0.99 SCE/cell for the silver fox. The mean number of exchanges for the BrdU

Table 1

Cytogenetic characteristics of the arctic fox karyotypes

Chromosome number	Number of	
	Animals	Metaphases
48	2	10
49	8	35
50	2	10
Total	12	55

Table 2

B chromosome polymorphism in the silver fox

Modal number of B chromosomes	Number of	
	Animals	Metaphases
0	2	37
1	5	102
2	3	93
3	2	44
4	0	0
Total	12	276

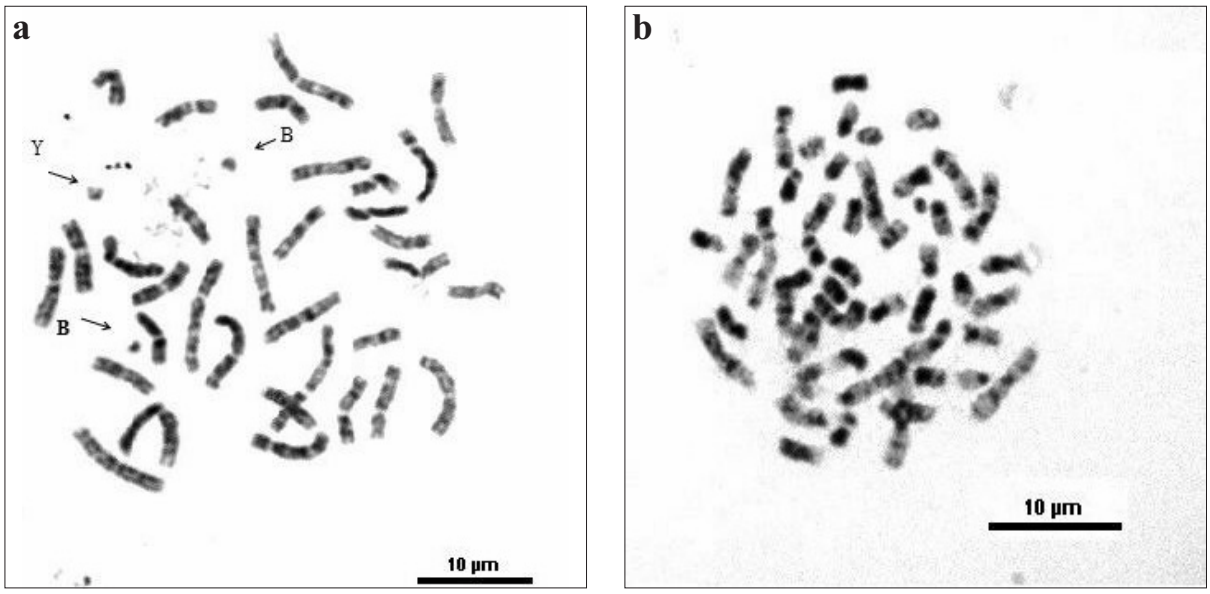

Fig. 1. RBG staining in male foxes: a – arctic fox 2n=34, XY,+2B, b – silver fox 2n=50,XY. In the photo a – arrows indicate Y chromosome and B chromosomes.

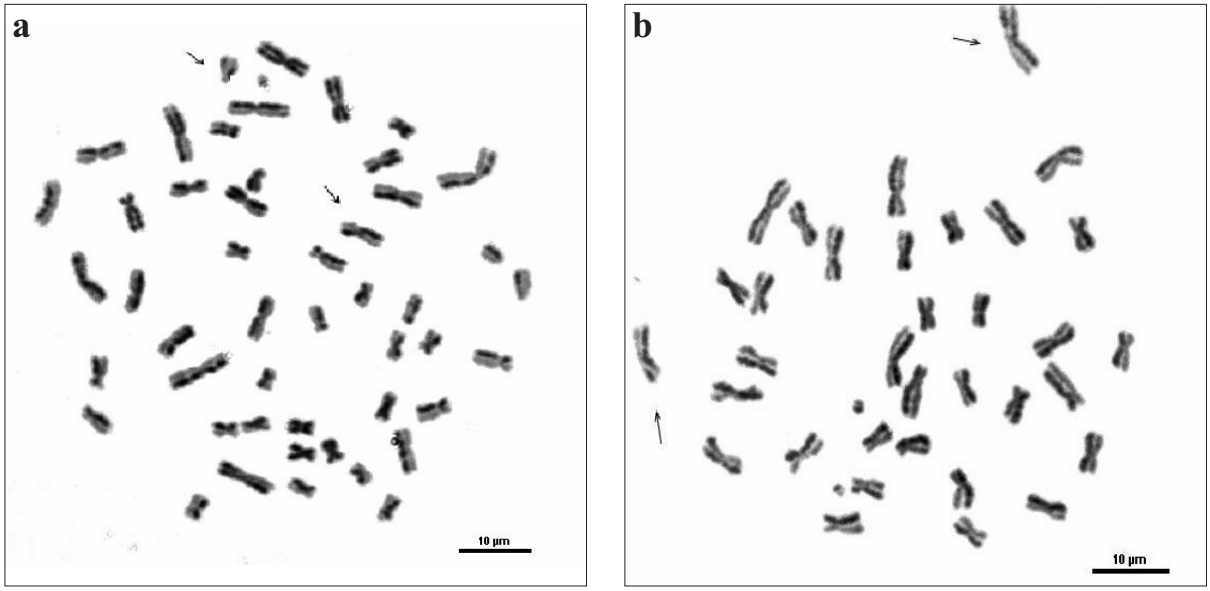

Fig. 2. Sister chromatid exchange in fox: a – arctic fox 2n=49, XY; b – silver fox 2n=34,XX,+2B. Arrows indicate SCEs.

concentration of 2.5 µg/ml BrdU was even higher (3.00±1.64 in the arctic fox and 5.12±1.48 SCE/cell in the silver fox) (Table 3). Highly significant differences were noted in the mean number of SCE between the analysed BrdU concentrations in the case of the arctic fox ($P<0.0001$). In the silver fox, significant differences were found between the mean SCE values for the concentrations of 0.5 and 1.0 µg/ml vs. 2.5 µg/ml BrdU ($P<0.0001$). The BrdU concentration of 0.5 µg/ml was determined to be the dose at which SCE in both fox species occurs spontaneously.

SCE sites were determined on the chromosomes of the studied animals. For all the tested BrdU concentrations in the arctic fox, centromeric exchanges were more often identified than the terminal exchanges, and interstitial SCE were the least frequent (Table 3). For the BrdU concentrations of 0.5 µg/ml and 1.0 µg/ml in the silver fox, centromeric exchanges slightly predominated over terminal SCE. Regardless of the BrdU concentration, interstitial SCE was also the least frequent type of exchange. Differences in the mean number of different exchange types between the analysed concentrations in particular species were highly significant ($P<0.0001$).

For the level of spontaneous SCE (0.5 µg/ml BrdU), the mean number of exchanges was 0.65±0.55 SCE/cell in the arctic fox and 2.33±0.76 SCE/cell in the silver fox (Table 3). In the case of the arctic fox, centromeric SCE were predominant (0.44±0.54 SCE/cell) with interstitial SCE being the least frequent

Table 3

Means and standard deviations (SD) of SCEs depending on BrdU concentration

Species	BrdU concentration (μg/ml)	Number of		Number of SCE							
		Animals	Examined cells	Interstitial		Terminal		Centromeric		Total	
				Mean	SD	Mean	SD	Mean	SD	Mean	SD
Arctic fox	0.5	12	55	0.04^{A}	0.19	0.18^{A}	0.39	0.44^{A}	0.54	0.65^{A}	0.55
	1.0	12	55	0.29^{AB}	0.53	0.58^{AB}	0.81	1.00^{B}	0.69	1.87^{B}	0.67
	2.5	12	60	0.30^{B}	0.50	1.10^{B}	1.54	1.60^{C}	0.91	3.00^{C}	1.64
P value				0.0032		0.0002		<0.0001		<0.0001	
Silver fox	0.5	12	52	0.40^{A}	0.60	0.94^{A}	0.67	0.98^{A}	0.67	2.33^{A}	0.76
	1.0	12	46	0.65^{AB}	0.77	1.22^{A}	0.89	1.24^{AB}	0.99	3.11^{A}	0.99
	2.5	12	49	1.14^{B}	1.00	2.27^{B}	1.35	1.71^{B}	1.10	5.12^{B}	1.48
P value				0.0004		<0.0001		0.0011		<0.0001	

$^{A, B, C}$ values within the same column and species marked by different letters differ highly significantly at P<0.01.

Table 4

Number of animals, examined cells and SCE distribution (mean and standard deviation SD), by species and sex

Species	Sex	Number of		Number of SCE							
		Animals	Examined cells	Interstitial		Terminal		Centromeric		Total	
				Mean	SD	Mean	SD	Mean	SD	Mean	SD
Arctic fox	Female	6	30	0.07	0.25	0.20	0.41	0.47	0.51	0.73	0.45
	Male	6	25	0.00	0.00	0.16	0.37	0.40	0.58	0.56	0.65
P value				0.1489		0.5729		0.6552		0.1781	
Silver fox	Female	6	28	0.43	0.63	1.00	0.72	0.93	0.66	2.36	0.83
	Male	6	24	0.38	0.58	0.88	0.61	1.04	0.69	2.29	0.69
P value				0.7151		0.4706		0.5376		0.7073	

(0.04±0.19). The silver fox was characterized by a similar number of terminal (0.94±0.67) and centromeric exchanges (0.98±0.67) per cell (Table 3). Furthermore, the analysis of R- and SCE-bands demonstrated that in both cases damaged sister chromatids in a given chromosome occurred at the junction of euchromatin and heterochromatin. In addition, B chromosomes stained positively with FPG were evaluated but no SCE were found in these structures.

Table 4 presents the frequency of SCE depending on sex and species. In the arctic fox, total SCE was 0.73±0.45 SCE/cell in females and 0.56±0.65 SCE/cell in males. In the silver fox, the mean number of SCE was 2.36±0.38 SCE/cell in females and 2.29±0.69 SCE/cell in males. In addition, females were characterized by a greater number of exchanges than males. No interstitial exchanges were found in male arctic foxes. No significant differences between sexes were found in either silver (P=0.7073) or arctic foxes (P=0.1781) for the analysed traits (interstitial SCE, terminal SCE, centromeric SCE, total SCE).

Variation was observed in SCE number depending on the region of Poland from which the studied animals originated (Table 5). In the case of arctic foxes, total SCE averaged 0.52±0.51 SCE/cell in animals from eastern Poland and 0.77±0.57 SCE/cell in animals from western Poland. Likewise, silver foxes from eastern Poland had a slightly lower mean number of SCE (2.15±0.72 SCE/cell) compared to animals from the western areas (2.52±0.77 SCE/cell) (Table 5). No interstitial exchanges were observed in arctic foxes from eastern Poland. In both species of foxes, no significant differences (P>0.05) between the regions of origin were found for the four analysed traits.

Table 5

Number of animals, examined cells and SCE distribution (mean and standard deviation SD), by species and region

Species	Region	Number of		Number of SCE							
		Animals	Examined cells	Interstitial		Terminal		Centromeric		Total	
				Mean	SD	Mean	SD	Mean	SD	Mean	SD
Arctic fox	East Poland	6	25	0.00	0.00	0.08	0.28	0.44	0.51	0.52	0.51
	West Poland	6	30	0.07	0.25	0.27	0.45	0.43	0.57	0.77	0.57
P value				0.1487		0.0709		0.9998		0.0745	
Silver fox	East Poland	6	27	0.30	0.61	0.81	0.62	1.04	0.65	2.15	0.72
	West Poland	6	25	0.52	0.59	1.08	0.70	0.92	0.70	2.52	0.77
P value				0.1836		0.1503		0.5237		0.0783	

Discussion

There are several species within the Canidae family that are characterised by polymorphism including both arctic and silver foxes. The first species has a variable diploid number of chromosomes 2n=50,49,48; whereas the second species has additional B chromosomes in its genomes 2n=34+B.

In surveys of the extent of chromosomal polymorphism conducted in fox farms in Poland, ŚWITOŃSKI (1981) found that almost 50% of the population included individuals with the heterozygous karyotype and this number was independent of the animals' sex or farm. However, JASZCZAK *et al.* (1987) stated that the individuals with a karyotype of 2n=48 and 2n=49 chromosomes constituted 75% of the stock. Different conclusions were drawn by MAKINEN and GUSTAVSSON (1980) as they indicated domination of the typical form of karyotypes among foxes from Scandinavian farms. Homozygous animals (2n=48) constituted a small percentage of these populations. However, a distribution of karyotypes (2n=48, 2n=49 and 2n=50) close to a 1:2:1 ratio was obtained by MØLLER *et al.* (1985). In our survey of 12 arctic foxes, up to 8 individuals had the heterozygotic form of the karyotype (2n=49) which is more than 50% of the analysed group. The remaining 4 individuals were characterized by the proper karyotype 2n=50 (2 animals) and its homozygotic form 2n=48 (2 animals).

ŚWITOŃSKI *et al.* (2003) concluded that 2 or 3 B chromosomes occur most often in the karyotype of the silver foxes. In farmed foxes, BUGNO-PONIEWIERSKA *et al*. (2013) determined from 0 to 4 B chromosomes which is consistent with the results of this study. The highest proportion in the group tested by BUGNO-PONIEWIERSKA *et al.* (2013) was represented by karyotypes with 2 B chromosomes (almost 45%), followed by 1 supernumerary chromosome (30%) and 3 B chromosomes (more than 20%). The silver foxes have a variable number of supernumerary B chromosomes (ŚWITOŃSKI *et al.* 2003). In the tested group, their number among individuals varied from 0 to 4. No foxes with a modal number of B chromosomes equal to 4 were observed. The modal numbers of karyotypes 2n=34+1B and 2n=34+2B were observed in 5 and 3 foxes, respectively, without intra-individual variation. The additional modal number of chromosomes was not observed in two animals (16%). 3 B chromosomes constituted the modal value in two foxes (16%).

The applied SCE test allows for an evaluation of the impact of physical and chemical factors with potential genotoxic and mutagenic properties on living organisms (KUCHTA-GŁADYSZ *et al.* 2016). SCE occurs as a result of single-strand or double-strand breaks in the DNA strand (SZELESZCZUK *et al.* 2014). Several factors able to induce this type of damage to genetic material are commonly found in the environment. Among the strong inducers of SCE are UV and X radiation and also substances used in veterinary medicine (such as chloramphenicol, mitomycin C) or agricultural bactericides and fungicides (including thymol) (WÓJCIK *et al.* 2004; BUYUKLEYLA & RENCUZOGULLARI 2009). Factors that increase SCE number in animal and human cells also include benzo(α)pyrene, carbon particulates, combustion gas molecules, benzene and nicotine. These compounds are common air pollutants in urban and suburban areas (AHMED *et al.* 1998). Another factor that induces SCE is bromodeoxyuridine which is used to visualize sister chromatid exchanges. Added to *in vitro* culture as a thymidine analogue, BrdU is incorporated into DNA and can be used to assess chromatin instability. This is because BrdU in the DNA chain is dehalogenated to uracil through the elimination of the bromide ion from the bromodeoxyuridine molecule by means of uracil-DNA glycosylase, and then uracil is embedded in the damaged single DNA strand. The increase in SCE may result from the increased number of strand

breaks during the incorporation of uracil into DNA (WILSON & THOMPSON 2007; WÓJCIK & OBE 2007). The SCE test was used in our study to determine the frequency of SCE as well as to try to specify the karyotype stability of the tested species.

Due to the inducing effects of BrdU, spontaneous SCE should be estimated using the lowest possible dose enabling the observation of this phenomenon. The concentration should be adjusted individually for each species due to different sensitivities of the organisms to this factor. Researchers have used a wide range of BrdU doses to study the level of spontaneous SCE. According to LEINBENGUTH and THIEL (1986), the optimum BrdU concentration is in the range of 15-120 μg/ml, whereas MURALI and PANNEERSELVAM (2011) used 5/10/15/20 μg/ml BrdU. In turn, a concentration of 10 μg/ml was used by DI MEO *et al*. (1993), AHMED *et al*. (1998), CIOTOLA *et al*. (2005), PERETTI *et al.* (2008) and WÓJCIK *et al*. (2011). WILSON & THOMPSON (2007) believed that spontaneous SCE occurs when BrdU concentrations are low or close to zero. A similar view was held by DI BERARDINO *et al.* (1995; 1996; 1997) who used BrdU in concentrations of 0.1/0.25/0.5/1.0/2.5/5.0 μg/ml and showed that spontaneous SCE occurs with 0.1 μg/ml in cattle and sheep, and with 0.25 μg/ml BrdU in goats. Analysing spontaneous SCE in cats, SZELESZCZUK *et al*. (2014) used 5 concentrations of BrdU (0.25/0.5/1.0/2.5/5.0 μg/ml) and considered the 0.5 μg/ml concentration as sufficient for analysing spontaneous SCE in this species. The same doses were used in tests of chinchillas by KUCHTA-GŁADYSZ *et al.* (2015) who reported that spontaneous SCE in the chinchilla occurred with the dose 0.5 μg/ml.

Because of the negative result in differential dyeing of SCE by means of FPG method using the concentration of 0.25 μg/ml of by SZELESZCZUK *et al.* (2014) and KUCHTA-GŁADYSZ *et al.* (2015), we applied 3 different doses of BrdU (0.5/1.0/2.5 μg/ml) to identify the concentration of the level of spontaneous SCE in arctic and silver foxes. It was revealed that spontaneous SCE occured in both fox species at a dose of 0.5 μg/ml.

The average frequency of occurrence of SCE was analysed in various species of animals and amounted to 3.38 in domestic cats (SZELESZCZUK *et al*. 2014), 3.40 in chinchillas (KUCHTA-GŁADYSZ *et al*. 2015), 2.69 in rabbits and 1.41 in coypus (KUCHTA-GŁADYSZ *et al.* 2016), 5.99 in cattle (CIOTOLA *et al*. 2005), from 2.73 to 6.62 in goats (WÓJCIK & SMALEC 2012; DI MEO *et al*. 1993), 5.14 in horses (WÓJCIK *et al.* 2011) and 7.86 SCE/cell in sheep (SIVIKOVA & DIANOVSKY 1995). Visible differences in the amount of spontaneous SCE were observed by DI BERARDINO *et al*. (1997) who compared the results of their work conducted on different animal species including cattle, goats, sheep and buffalo. SCE occured at the level of 2.48 SCE/cell in cattle (DI BERARDINO *et al.* 1995), 3.28 for goats (DI BERARDINO *et al.* 1996) 4.08 in sheep (DI BERARDINO *et al*. 1997) and 6.66 SCE/cell in buffalo. The greater frequency of exchanges in sheep and buffalo in comparison to goats and cattle could result from properties of the species karyotype and from the various numbers of double-stranded chromosomes. Among the analysed species, the number of biarmed chromosomes varied from 10 in buffalo to 6 in sheep whereas cattle and goats had no biarmed chromosomes (DI BERARDINO *et al.* 1997). On the basis of the tests performed on foxes, it was revealed that the average value was at the level of 0.65 SCE/cell in the arctic fox and 2.33 SCE/cell in the silver fox. These differences can result from the influence of supernumerary B chromosomes in the cells of the silver fox. The unconventional behaviour of B chromosomes during cell division and their non-Mendelian inheritence (CAMACHO *et al*. 2000; BUGNO-PONIEWIERSKA *et al*. 2013b) could cause a greater frequency of exchanges with the lowest tested concentration of BrdU. In contrast, the centric translocation in arctic foxes does not cause mitosis disorders and is inherited according to Mendelian principles (MØLLER *et al*. 1985). However, because of the presence of this aberration, a higher SCE level in the arctic fox may be expected. On the other hand, the presence of B chromosomes in the karyotype of the silver fox can significantly decrease the genetic stability of the genome of this species (MAKUNIN *et al*. 2014). There are studies revealing that B chromosomes impact chromosomal segregation during meiotic division (VUJOSEVIC & BLAGOJEVIC 2004; BASHEVA *et al*. 2010). However, their role is not fully known. The increase of SCE level in the cells of the tested species depending on the dose seems of interest. In the case of the silver fox, the increase of SCE frequency by 1/3 (3.11) at the level of 1.0 μg/ml BrdU and over 4.5-times (5.12) higher value with the highest dose of BrdU 2.5 μg/ml were found and the differences are statistically significant ($P<0.0001$). In case of the arctic fox, the differences in exchange frequency depending on BrdU concentration were highly significant ($P<0.0001$) with every used dose as their increase was 3-times higher with 1.0 μg/ml of BrdU and over 4.5-times higher for 2.5 μg/ml of BrdU. Such a rapid increase may be connected with the presence of the greater amount of biarmed chromosomes as DI BERARDINO *et al.* (1997) suggest in their surveys. Arctic foxes in comparison with silver foxes have 45-47 biarmed chromosomes in their karyotype whereas the silver fox has only 33 such chromosomes (MAKINEN 1985; BUGNO-PONIEWIERSKA *et al.* 2015).

The site of SCE occurrence within a chromosome was examined. In the arctic fox, 68% centromeric exchanges (0.44 SCE/cell) were found and less than 28% (0.18 SCE/cell) were terminal exchanges. The significant saturation with heterochromatin (on 10 pairs of chromosomes on the strands (MAKINEN *et al*. 1985)) of the arctic fox's karyotype is mainly responsible for the placement of SCE sites next to centromeric regions. However, the silver fox was characterized by approximate SCE frequency in centromeric and terminal regions which was 42% (0.98 SCE/cell) and 40% (0.94 SCE/cell), respectively, and SCE placement was synchronized with the junction of eu- and heterochromatin in this karyotype. Exchanges on B chromosomes were not revealed in the silver fox. KUCHTA-GŁADYSZ *et al*. (2015) found a predominance of SCE in regions next to centromeres (59%) in chinchillas. The exchange frequency in the distal part was determined at the level of 39%. Telomeric exchanges dominated in cats and comprised 65% of SCE (SZELESZCZUK *et al.* 2014). The occurrence of SCE on the junction of euchromatin and heterochromatin was described by WÓJCIK and SMALEC (2012) as well as KUCHTA-GŁADYSZ *et al*. (2015).

One of important factors that impact the amount of SCE is the sex of an individual. We did not find significant differences between sexes of either fox species (P>0.05) after the analyses of interstitial, terminal, centromeric and total SCE. Similar observations concerned horses (WÓJCIK *et al.* 2011), goats (DI MEO *et al*. 1993; WÓJCIK & SMALEC 2012) and chinchillas (KUCHTA-GŁADYSZ *et al.* 2015).

The foxes originated from the area of eastern and western Poland. The region of Poland had no significant effect (P>0.05) on the frequency of SCE phenomenon. However, the average number of SCE in animals from eastern Poland was 0.52 SCE/cell in arctic foxes and 2.15 SCE/cell in silver foxes. On the other hand, the numbers of 0.77 SCE/cell in the arctic foxes and 2.52 SCE/cell in the silver foxes was found in the case of animals from western Poland (Table 5). The differences that occurred in the amount of SCE in both fox species also result from a different location of farms. The farm in eastern Poland is situated in the vicinity of forests and small farms with ecological agriculture. The farms from the area of western Poland are located in areas with high intensity agriculture and close to urban agglomerations. However, the surveys did not include the tests from the range of influence of a specific environmental factor. SIVIKOVA and DIANOVSKY (1995) tested the impact of the mixture of metals which usually occur in the polluted area. They revealed significant differences between the control group (7.86 SCE/cell) and the polluted area (9.17 and 9.28 SCE/cell). AHMED *et al.* (1998) analysed the frequency of SCE phenomenon in animals from agricultural areas and areas where the animals are exposed to pollutants. The average number of SCE for the animals from agricultural areas was 8.3 SCE/cell and from polluted areas it was 11.8 SCE/cell with significant differences between these groups. The high SCE level in buffalo from the polluted regions could result from the exposure of these animals to air pollutants.

The surveys proved that various concentrations of BrdU as a genotoxicant significantly influence the induction of SCE in karyotypes of arctic and silver foxes. This process is a normal phenomenon in karyotypes but in determined methodical conditions. In the case of arctic and silver foxes, SCE is of spontaneous character when the concentration of BrdU is 0.5 μg/ml and a higher concentration additionally evoked SCE and thus this substance cannot be used as an indicator in the SCE test. Thereby, testing the impact of BrdU on the amount sites of SCE in karyotypes of selected species of Canidae such as the arctic and silver foxes, methodical conditions of SCE test for further surveys of biomonitoring of the environment for these species were also determined. Moreover, a further feature of karyotypes, the number of spontaneous SCEs, was indicated.

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References

AHMED S., MAHROUS K., EL-SOBHY H. 1998. Cytogenetic study of buffalo under pollution of environmental conditions. Mutation Res. **419**: 21-26.

BASHEVA E.A., TORGASHEVA A.A., SAKAEVA G.R., BIDAU C., BORODIN P.M. 2010. A- and B- chromosome pairing and recombination in male meiosis of the silver fox (*Vulpes vulpes* L., 1758, Carnivora, Canidae). Chromosome Res. **18**: 689-696.

BELIAEV D.K., VOLOBUEV V.T., RADZHABLI S.I., TRUT L.N. 1974. Supernumary chromosome polymorphism and mosaicism in silver foxes. Genetica (Rus) **10**: 58-67.

BUGNO-PONIEWIERSKA M., SOŁEK P., POTOCKI L., PAWLINA K., WNUK M., JEŻEWSKA-WITKOWSKA G., SŁOTA E. 2013. Polymorphism of cytogenetic markers in Wild and Farm red fox (*Vulpes vulpes*) populations. Folia Biol. (Kraków) **61**: 155-163.

BUGNO-PONIEWIERSKA M., PAWLINA K., ORSZULAK-WOLNY N., WOŹNIAK B., WNUK M., JAKUBCZAK A., JEŻEWSKA-WITKOWSKA G. 2015. Cytogenetic characterization of the genome of interspecies hybrids (*Alopex-Vulpes*). Ann. Anim. Sci. **15**: 81-91.

BUYUKLEYLA M., RENCUZOGULLARI E. 2009. The effects of thymol on sister chromatid exchange, chromosome aberration and micronucleus in human lymphocytes. Ecotoxicol. Environ. Saf. **72**: 943-947.

CAMACHO J.P.M., SHARBEL T.F., BEUKEBOOM L.W. 2000. B-chromosome evolution. Phil. Trans. R. Soc. Lond. B. **355**: 163-178.

CHRISTENSEN K., PEDERSEN H. 1982. Variation in chromosome number in the blue fox (*Alopex lagopus*) and its effect on fertility. Hereditas **97**: 211-215.

CIOTOLA F., PERETTI V., DI MEO G.P., PERUCATTI A., IANNUZZI L., BARBIERI V. 2005. Sister Chromatid Exchanges (SCE) in the Agerolese Cattle Population. Vet. Res. Commun. **29**: 359-361.

CYBULSKI W., CHAŁABIS-MAZUREK A., JAKUBCZAK A., JAROSZ Ł., KOSTRO K., KURSA K. 2009. Content of lead, cadmium, and merkury in the liver and kidneys of silver foxes (*Vulpes vulpes*) in relation to age reproduction disorders. Bull. Vet. Inst. Pulawy **53**: 65-69.

DI BERARDINO D., LIOI M.B., SCARFI M.R., JOVINO V., MARGILIANO P. 1995. Spontaneous sister chromatid exchanges in mitotic chromosomes of cattle (*Bos taurus* L.). Genet. Sel. Evol. **27**: 385-393.

DI BERARDINO D., JOVINO V., LIOI M.B., SCARFI M.R., BURGUETE I. 1996. Spontaneous rate of sister chromatid exchanges (SCEs) and BrdU dose-response relationships in mitotic chromosomes of goat (*Capra hircus* L.). Hereditas **124**: 137-143.

DI BERARDINO D., RAMUNNO L., JOVINO V., PACELLI C., LIOI M.B., SCARI M.R., BURGUETE I. 1997. Spontaneous rate of sister chromatid exchanges (SCEs) in mitotic chromosomes of sheep (*Ovis aries* L.) and comparison with cattle (*Bos taurus* L.), goat (*Capra hircus* L.) and river buffalo (*Bubalus bubalis* L.). Hereditas **127**: 231-238.

DI MEO G.P., IANNUZZI L., PERUCATTI A., FERRARA L., PIZZILLO M., RUBINO R. 1993. Sister chromatid exchange in the goat (*Capra hircus*). Hereditas **118**: 35-38.

DUTRILLAUX B., LAURENT C., COUTURIER J., LEJEUNE J. 1973. Coloration par l'arcidine orange de chromosomes prealablement traites par le 5-bromodeoxyuridine (BudR). C. R. Acad. Sci. **276**: 31-79.

EUROPEAN COMMISSION – ANIMAL WELFARE (internet). (updated 2016 Apr 12); Available from: http://ec.europa.eu/agriculture/organic/consumer-trust/animal-welfare/index_en.htm.

FUGLEI E., ØRISTLAND N.A. 1999. Seasonal trends in body mass, food intake and resting metabolic rate, and induction of metabolic depression in arctic foxes (*Alopex lagopus*) at Svalbard. J. Comp. Physiol. B. **169**: 361-369.

FUGLEI E., ØRISTLAND N.A. 2003. Energy cost of running in an arctic fox, *Alopex lagopus*. Can Field-Nat. **117**: 430-435.

FUGLEI E., MUSTONEN A.-M., NIEMINEN P. 2004. Effects of season, food deprivation and re-feeding on leptin, ghrelin and growth hormone in arctic foxes (*Alopex lagopus*) on Svalbard, Norway. J. Comp. Physiol. B. **174**: 157-162.

GRAPHODATSKY A.S., KUKEKOVA A.V., YUDKIN D.V., TRIFONOV V.A., VOROBIEVA N.V., BEKLEMISHEVA V.R., PERELMAN P.L., GRAPHODATSKAYA D.A., TRUT L.N., YANG F., FERGUSON-SMITH M.A., ACLAND G.M., AGUIRRE G.D. 2005. The proto-oncogene C-KIT maps to canid B-chromosomes. Chromosome Res. **13**: 113-122.

HOEKSTRA P.F., BRAUNE B.M., O'HARA T.M., ELKIN B., SOLOMON K.R., MUIR D.C.G. 2003. Organochlorine contaminant and stable isotope profiles in Arctic fox (*Alopex lagopus*) from the Alaskan and Canadian Arctic. Environ. Pollut. **122**: 423-433.

JASZCZAK K., PARADA R., KOSIOR I. 1987. Karyotype distribution in chosen herd of the Arctic fox (*Aloppex lagopus*). Genet. Pol. **28**: 145-149.

KIHLMAN B.A., KRONBORG D. 1975. Sister chromatyd exchange in *Vicia faba* I. Demonstration by a Modified Fluorescent Plus Giemsa (FPG) Techniques. Chromosoma **51**: 1-10.

KUCHTA-GŁADYSZ M., WÓJCIK E., SZELESZCZUK O., NIEDBAŁA P., TYBLEWSKA K. 2015. Spontaneous sister chromatid exchange in mitotic chromosomes of the chinchilla (*Chinchilla lanigera*). Can. J. Anim. Sci. **95**: 543-550.

KUCHTA-GŁADYSZ M., WÓJCIK E., SZELESZCZUK O., NIEDBAŁA P., WOJNAR T. 2016. The effect of Bromodeoxyuridine on Spontaneous Sister Chromatid Exchange Frequency in Rabbit (*Oryctolagus cuniculus*) and Coypu (*Myocastor coypu*) Chromosomes. Folia Biol. (Kraków) **64**: 89-96.

LEINBENGUTH F., THIEL G. 1986. BrdU and EMS dependent sister chromatid exchange and chromosomal breaks in cattle. Arch. Zootec. **35**: 301-308.

MAKINEN A. 1985. The standard karyotype of the silver fox (*Vulpes vulpes* Desm.). Hereditas **103**: 171-176.

MAKINEN A., GUSTAVSSON I. 1980. Chromosome variability in the blue fox. Scientifur **4**: 6-9.

MAKINEN A., GUSTAVSSON I., ŚWITOŃSKI M., TAKAGI N. 1985. The standard karyotype of the blue fox (*Alopex lagopus* L.). Hereditas **103**: 33-38.

MAKUNIN A., DEMENTYEVA P., GRAPHODATSKY A., VOLOBOUEV V., KUKEKOVA A., TRIFONOV V. 2014. Genes on B chromosomes of vertebrates. Mol. Cytogenet. **7**: 99.

MØLLER O.M., NES N.N., SYED M., FOUGNER J.A., NORHEIM K., SMITH A.J. 1985. Chromosomal polymorphism in blue fox (*Alopex lagopus*) and its effect on fertility. Hereditas **102**: 159-164.

MURALI N., PANNEERSELVAM S. 2011. Effect of concentration of bromodeoxyuridine on sister chromatid exchange frequency in crossbred (*Bos taurus* x *Bos indicus*) cattle chromosomes. TNJVAS **7**: 71-78.

MUSTONEN A.-M., PYYKÖNEN T., ASIKAINEN J., HÄNNINEN S., MONONEN J., NNIEMINEN P. 2005. Circannual leptin and ghrelin levels of the blue fox (*Alopex lagopus*) in reference to seasonal rhythms of body mass, adiposity and food intake. J. Exp. Zool. **303A**: 26-36.

PERETTI V., CIOTOLA F., ALBARELLA S., RESTUCCI B., MEOMARTINO L., FERRETTI L., BARBIERI V., IANNUZZI L. 2008. Increased SCE levels in Mediterranean Italian buffaloes affected by limb malformation (Transversal hemimelia). Cytogenet. Genome Res. **120**: 183-187.

SAS INSTITUTE. 2014. SAS/STAT 13.2 User's Guide. SAS Institute Inc. Cary, NC.

SIVIKOVA K., DIANOVSKY J. 1995. Sister-chromatid exchange after exposure to metal-containing emission. Mutation Res. **327**: 17-22.

SONNE C., WOLKERS H., RIGÉT F.F., JENSEN J.-E.B., TEILMANN J., JENSSEN B.M., FUGLEI E., AHLSTRØM Ø., DIETZ R., MUIR D.C.G., JØRGENSEN E.H. 2009a. Mineral density and biomechanical properties of bone tissue from male Arctic foxes (*Vulpes lagopus*) exposed to organochlorine contaminants and emaciation. Comp. Biochem. Physiol. **149** C: 97-103.

SONNE C., WOLKERS H., LEIFSSON P.S., IBURG T., JENSSEN B.M., FUGLEI E., AHLSTRØM Ø., DIETZ R., KIRKEGAARD M., MUIR D.C.G., JØRGENSEN E.H.: 2009b. Chronic dietary exposure to environmental organochlorine contaminants induces thyroid gland lesions in Arctic foxes (*Vulpes lagopus*). Environ. Res. **109**: 702-711.

SZELESZCZUK O., KUCHTA-GŁADYSZ M., WÓJCIK E., OTWINOWSKA-MINDUR A., WOJNAR T., NIEDBAŁA P., GODYNIA B. 2014. Sister chromatid exchange analysis in cats (*Felis catus*). Arch. Tierzucht. **57**: 1-10.

ŚWITOŃSKI M. 1981. Robertsonian translocation in the blue fox (*Alopex lagopus*) and its effect on fertility. Genet. Pol. **22**: 465-474.

ŚWITOŃSKI M., ROGALSKA-NIZNIK N., SZCZERBAL I., BAER M. 2003. Chromosome polymorphism and karyotype evolution of four canids: the dog, red fox, arctic fox and raccoon dog. Caryologia **56**: 375-385.

VINE S.J., CROWTHER M.S., LAPIDGE S.J., DICKMAN C.R., MOONEY N., PIGGOTT M.P., ENGLISH A.W. 2009. Comparison of methods to detect rare and cryptic species a case study using the red fox (*Vulpes vulpes*). Wildlife Res. **36**: 436-446.

VUJOSEVIC M., BLAGOJEVIC J. 2004. B chromosomes in populations of mammals. Cytogenet. Genome Res. **106**: 247-256.

WILSON D.M., THOMPSON L.H. 2007. Molecular mechanisms of sister-chromatid exchange. Mutation Res. **616**: 11-23.

WÓJCIK A., BRUCKMANN E., OBE G. 2004. Insights into the mechanisms of sister chromatid exchange formation. Cytogenet. Genome Res. **104**: 304-309.

WÓJCIK A., OBE G. 2007. Sister-Chromatid Exchange (In: OBE G., VIJAYALAXMI eds. Chromosomal Alternations: Methods, Results and Importance in Human Health. Springer-Verlag, Berlin): 271-283.

WÓJCIK E., SMALEC E., DANIELEWICZ A. 2011. Sister chromatid exchange in selected horse breeds (*Equus caballus*). Arch. Tierzucht. **54**: 107-114.

WÓJCIK E., SMALEC E. 2012. Sister chromatid exchange in Polish White Improved Goats (*Capra hircus*). Folia Biol. (Kraków) **60**: 141-146.

ZELJEZIC D., GARAJ-VRHOVAC V. 2002. Sister chromatid exchange and proliferative rate index in the longitudinal risk assessment of occupational exposure to pesticides. Chemosphere **46**: 295-303.