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Polymorphism of Cytogenetic Markers in Wild and Farm Red Fox (*Vulpes vulpes*) Populations*

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Analysis of the origin of domestic animals is of wide interest and has many practical applications in areas such as agriculture and evolutionary biology. Identification of an ancestor and comparison with the domesticated form allows for an analysis of genetic, physiological, morphological and behavioral effects of domestication. Because fox breeding has been an ongoing process for over a century, differences are expected between farm and wild populations at the chromosomal level. The aim of this work was to analyse polymorphisms at the chromosomal level in foxes raised on farms and those living in the wild. Blood samples and lung tissue served as the experimental material and were obtained after slaughter of 35 foxes, including 28 breeding animals and 7 wild animals. The classical cytogenetic method was used including AgNOR technique, as well as molecular methods such as fluorescence *in situ* hybridization (FISH), and primed *in situ* labeling (PRINS). Analysis of the number of B chromosomes showed the presence of polymorphisms in foxes from both studied populations, but there was no correlation between the number of B chromosomes and the origin and gender of particular animals. An analysis of active nucleolar organizers showed the presence of a large number of polymorphisms and a tendency towards reduction of the number of NORs in the captive-raised population.

Key words: Wild red fox, farm red fox, cytogenetic markers, polymorphism.

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The red fox has the widest geographical range of all carnivorous species naturally occurring in the entire northern hemisphere including North Africa, Europe, Asia and North America (WILSON & REEDER 2005). The fox became a popular animal for fur breeding in North America and Eurasia at the beginning of the twentieth century. Currently, it is one of the most important fur animals both in Poland and worldwide. The initial breeding of foxes was introduced in 1900 in Canada, and in Europe the first fox farm was founded in 1913 in Norway.

The karyotype of red fox (*Vulpes vulpes*) is characterized by the lowest number of chromosomes among all species studied so far in the Canidae family (SWITONSKI *et al.* 2003). The karyotype consists solely of biarmed chromosomes, 16 pairs of metacentric and submetacentric chromosomes and heterochromosomes including the metacentric X chromosome and acrocentric Y chromosome. Apart from the basic set of chromosomes, the red fox karyotype is characterized by the presence of a variable number (0-8) of additional so-called B chromosomes. Variation in the diploid number of red fox chromosomes is associated with the presence of supernumerary B chromosomes. The presence of these structures is rare among mammals. The size of the fox B chromosomes is different in

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relation to other species. B chromosomes of the red fox are very small, comparable in size to the Y chromosome (SWITONSKI *et al.* 2003). Their number varies from 0 to 8, but the most common are 2 or 3 B chromosomes. It has been suggested that B chromosomes of the red fox may be an evolutionary effect of fusion of chromosome fragments. These chromosomes do not have a C band as opposed to the same sized Y chromosome which is characterized by a clear positive CBG region close to the centromere. Studies on the inheritance of B chromosomes showed that their segregation into gametes is random (VOLOBUJEV 1984; YANG *et al.* 1999; PALESTIS *et al.* 2003).

The number of nucleolar organizer regions (NORs) is a characteristic trait of the karyotype of a given species. The red fox has three pairs of autosomes in which NORs are localized: pairs 8, 9 and 13. These regions can be visualized using the silver staining method or *in situ* hybridization of suitable molecular probes. The silver staining method visualizes only transcriptionally active NORs, whereas *in situ* hybridization shows all active and non-active NORs present in the karyotype (PIEŃKOWSKA & ŚWITOŃSKI 1998; SZCZERBAL & ŚWITOŃSKI 2003; SWITONSKI *et al.* 2003).

Fox breeding has been an ongoing process for over a century, therefore differences are expected between farm and wild populations at the chromosomal level. These differences may be the result of evolutionary changes occurring within farm-raised groups, including the fixation of a mutation or a change in the incidence of supernumerary B chromosomes. This information can be especially useful for studies designed to identify regions of the genome associated with the red fox domestication process (STATHAM *et al.* 2011).

The aim of this study was to identify differences at the chromosomal level between farm and wild populations of red fox (*Vulpes vulpes*), using classical and molecular cytogenetic methods in order to determine the putative autonomy of the phylogenetic groups studied.

Material and Methods

The study was approved by the II Local Ethical Committee in Lublin, approval no. 83/2009.

Blood samples and lung tissues were obtained from 35 red foxes after slaughter. 28 breeding animals, including 15 females and 13 males, from fur farms in Chorzelów and 7 wild animals, including 2 females and 5 males from the north and the north-east of Poland, were subjected to detailed cytogenetic analysis.

Preparations of metaphase spreads were obtained from lymphocytes cultured *in vitro* on RPMI 1640 medium with the addition of fetal calf serum, antibiotics and cell division stimulator (pokeweed), according to the method of ARAKAKI & SPARKES (1963). Pulmonary fibroblasts were cultured *in vitro* on DMEM with fetal calf serum and antibiotics. Cultured cells were pelleted and subsequently microscopic preparations were made from them. The primary evaluation of mitotic chromosomes by light microscopy consisted of the calculation of the mitotic index for each slide.

AgNOR banding technique

AgNOR technique was carried out according to the method of HOWELL & BLACK (1980). The mixture for AgNOR selective staining was prepared in a darkened test tube, consisting of gelatin solution and 50% solution of $AgNO_3$ in a 1:2 ratio. The mixture was applied on the surface of a microscopic slide heated at 37ºC. Next, a cover slip was put on the preparation and the slide chamber was inserted into the 37°C incubator for 15 minutes. Then slides were stained with 5% Giemsa solution for 10 seconds at room temperature. Preparations were analyzed under a light microscope (Olympus CHK2-F-GS).

Fluorescence in situ hybridization (FISH)

Whole chromosome painting probe (WCPP) specific for B chromosomes of *Vulpes vulpes* was prepared by chromosome microdissection, DOP-PCR and DOP-labeling with biotin-16-dUTP or digoxigenin-11-dUTP according to BUGNO *et al.* (2009). Additionally commercial painting probes specific to the X (FITC labeled) and Y (Cy3 labeled) chromosomes of the domestic dog (Cambio, UK), were used for fluorescence *in situ* hybridization. A standard FISH protocol (PINKEL *et al.* 1986) with some minor modifications was applied.

After RNAse and pepsin digestion, target metaphase spreads were co-denaturated with probes in a thermal cycler with a slide adapter for 8 minutes at 80°C. Hybridization was carried out for 48 h in a moist chamber at 37°C. The post-hybridization washes were as follows: 0.4 x SSC/0.3 % Tween 20 in 72°C for 2 minutes and in 2xSSC/0.1 % Tween 20 in room temperature for 60 seconds. Chromosomes were dehydrated by washing in 70%, 80% and 95% alcohol series for 1 minute in each concentration at room temperature. Signals were detected and amplified using avidin-fluorescein isothiocyanate and anti-avidin antibodies as well as anti-digoxigenin-rhodamine antiobodies. Metaphase stainings were performed with DAPI (4'-6-diamidino-2-phenylindole). Slides were analysed under a fluorescence microscope (Olympus BX61 with Olympus DP72 camera).

Primed in situ labeling (PRINS) with primers complementary to telomeric sequences

The Starfish Telomeric PRINS Kit (Cambio, UK) was used for visualization of telomeric sequences. A reaction mixture was prepared according to the protocol and subsequently applied to the chromosome preparations. Next, cover slips were put on the slides and they were sealed with rubber gum to avoid evaporation of samples. Slides prepared in this way were inserted into a thermal cycler. Denaturation was carried out for 5 minutes at 94°C and was followed by a 30 minute hybridization step at 58°C using F:5'TTTAGGG-3'7, and R: 5-AAATCCC-3'7 primers. After incubation cover slips were gently removed. The reaction was stopped by two washes in the 'stop' buffer (50 mM EDTA + 50 mM NaCl) (pH 8.0). The first wash was carried out at 58°C for 5 minutes and the second one at room temperature, also for 5 minutes. Before the detection of the signal, chromosomes were dehydrated by washing in 70%, 80% and 95% alcohol series for 3 minutes in each concentration at room temperature. Slides were stained with DAPI and analyzed by fluorescence microscopy (Olympus BX61 with Olympus DP72 camera).

Results

All examined animals presented a normal, typical of the species and gender chromosome set of 2n = 34, XX + B for females and 34, XY + B for males.

AgNOR staining detected active nucleolar organizers on the chromosomes of the examined foxes. Detailed analysis of the distribution of silver deposits on the chromosomes showed large variation between animals or even between different cells of a single animal. The average number of nucleolar organizers in red foxes from the farm population, based on the analysis of 1213 metaphase spreads, was 4.35; statistically most cells (31.66%) contained four silver deposits. The average number of nucleolar organizers in wild red foxes, based on the analysis of 325 metaphase spreads, was 4.67; statistically most cells (37.54%) contained 5 silver deposits.

The individual average number of silver deposits ranged from 3.56 to 5.20 in farm-raised females, whereas in males the average ranged from 4.07 to 4.92. For wild foxes, the average was in the range of 3.95 to 5.71 for males and 4.50 to 4.79 for females. The average number of NORs calculated for all males was slightly higher (4.61) compared to females (4.58).

In the breeding population, the highest number of silver deposits in a single cell was 6. In the wild population of *Vulpes vulpes*, higher variation in number of nucleolar organizers was found as single cells contained even 8 AgNORs (Fig. 1).

In the farm-raised foxes a high frequency of nucleolar organizer region associations was observed. A number of so called 'sticky' associating regions were found in 6 females and 1 male. In addition, in the female L 88, a high number of double and triple associations was detected. (Fig. 2):

– L 81 (34, XX): 24 (20.87%) associations in 115 metaphase spreads;

– L 86 (34, XX): 20 (18.52%) associations in 108 metaphase spreads;

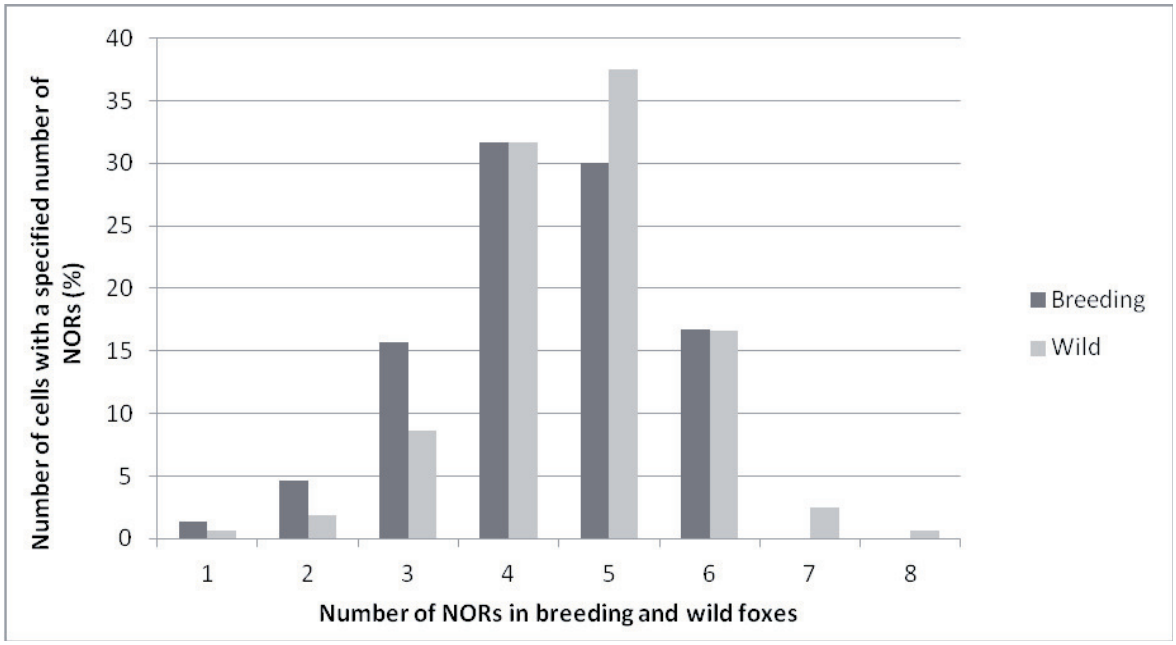

Fig. 1. Frequency of occurrence of AgNOR bands in the breeding and wild population of red foxes.

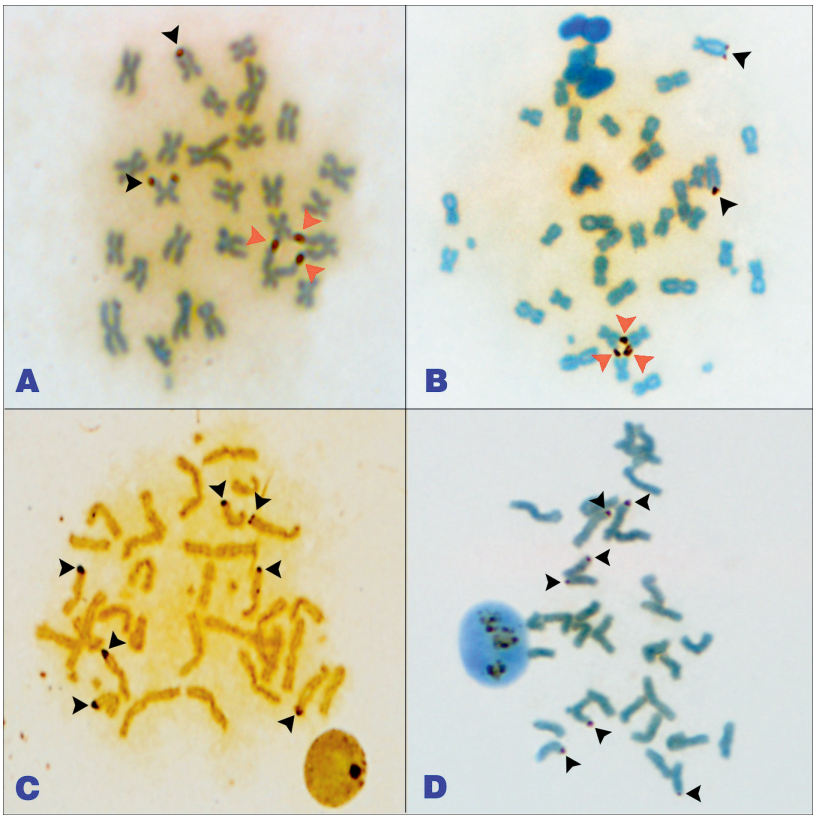

Fig. 2. AgNOR staining of metaphase spreads of *Vulpes vulpes*. Nucleolar organizer regions (NORs) are indicated with arrows. Associated region is marked in red color (A, B). C, D – metaphase spreads of fox from the wild population, characterized by the presence of 7 silver deposits.

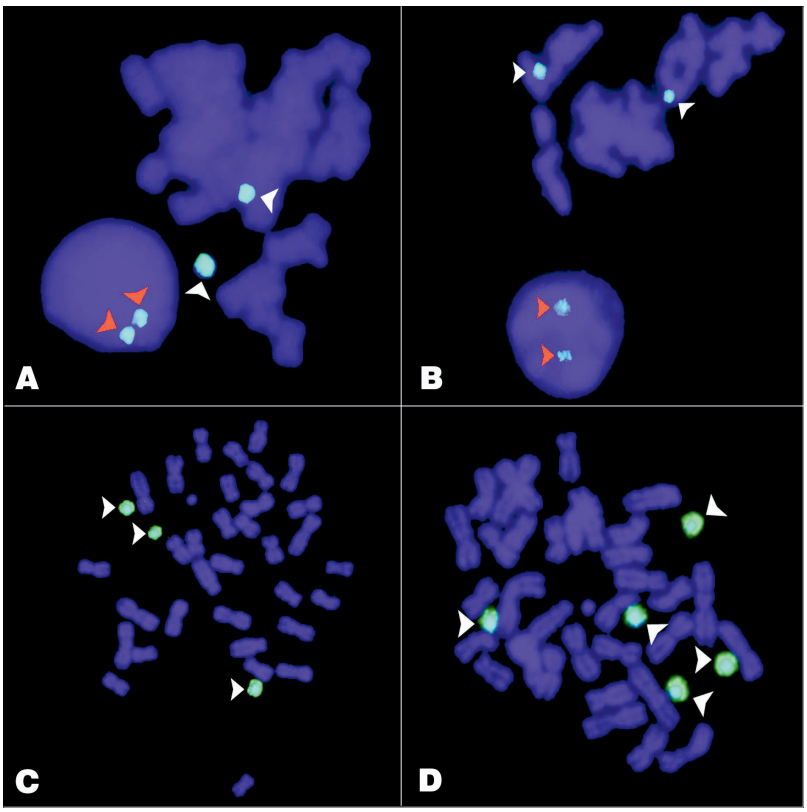

Fig. 3. B chromosomes of *Vulpes vulpes* – localization of molecular probe derived from the red fox genome (FISH). A, B poor quality metaphase spreads showing clear fluorescence signals specific for B chromosomes. White arrows indicate regions of probe hybridization with B chromosomes. Red arrows (A, B) indicate clear signals in the interphase nuclei. A, C – metaphase spreads of two farmed male foxes; B, D – metaphase spreads of two males from the wild population.

– L 88 (34, XX): 11 (20.75%) associations in 53 metaphase spreads;

– L 90 (34, XX): 32 (18.93 %) associations in 169 metaphase spreads;

– L 93 (34, XX): 14 (17.28 %) associations in 81 metaphase spreads;

– L 96 (34, XX): 11 (25.00 %) associations in 44 metaphase spreads;

– L 99 (34, XX): 29 (33.72%) associations in 86 metaphase spreads;

B chromosomes

Using a probe specific for B chromosomes of the red fox, clear signals were detected on metaphases as well as in the interphase nuclei (Fig. 3).

Detailed analysis of the B chromosomes revealed variation between animals as well as between cells of a single animal in the range of 0 to 4 for farm red foxes (most commonly 1, 2 or 3 in 96.12% of cases), and in the range of 0 to 6 for the wild population (most commonly 1, 2 or 3 in 87.43% of cases).

The average number of B chromosomes in red foxes from the farm population based on the analysis of 1032 metaphase spreads was 1.89; statistically most cells (44.1%) contained 2 supernumerary chromosomes. The average number of B chromosomes in wild red foxes based on the analysis of 501 metaphase spreads was 2.26; statistically most cells (47.5%) contained 2 B chromosomes.

The individual average number of B chromosomes ranged from 0.82 to 5.20 in farm females, whereas in males from the average ranged from 1.63 to 2.68. For wild foxes the average was in the range of 1.84 to 3.32 for males and 1.78 to 2.80 for females. The average number of supernumerary chromosomes calculated for all males was slightly higher (2.22) compared to females (2.02).

In the breeding population, the highest number of B chromosomes in a single cell was 4. In the wild population of *Vulpes vulpes*, higher variation in number of B chromosomes was found as single cells contained even 6 of these chromosomes (Fig. 4).

Interspecies hybridization of probes specific for heterochromosomes

FISH hybridization using a probe from the genome of the dog, specific for X and Y sex chromosomes, showed a clear signal on chromosome X or Y (depending on gender). There was observed a conservative pseudoautosomal region of heterosomes, which showed a clear band signal on the X chromosomes. The B chromosome-specific probe derived from the genome of the red fox showed a clear signal on B chromosomes (Fig. 5).

Localization of telomeric sequences – primed in situ DNA synthesis (PRINS)

PRINS with an oligonucleotide probe complementary to telomeric sequences showed strong signals on the telomeres of all chromosomes and in the pericentromeric regions of several chromosomes (Fig. 6).

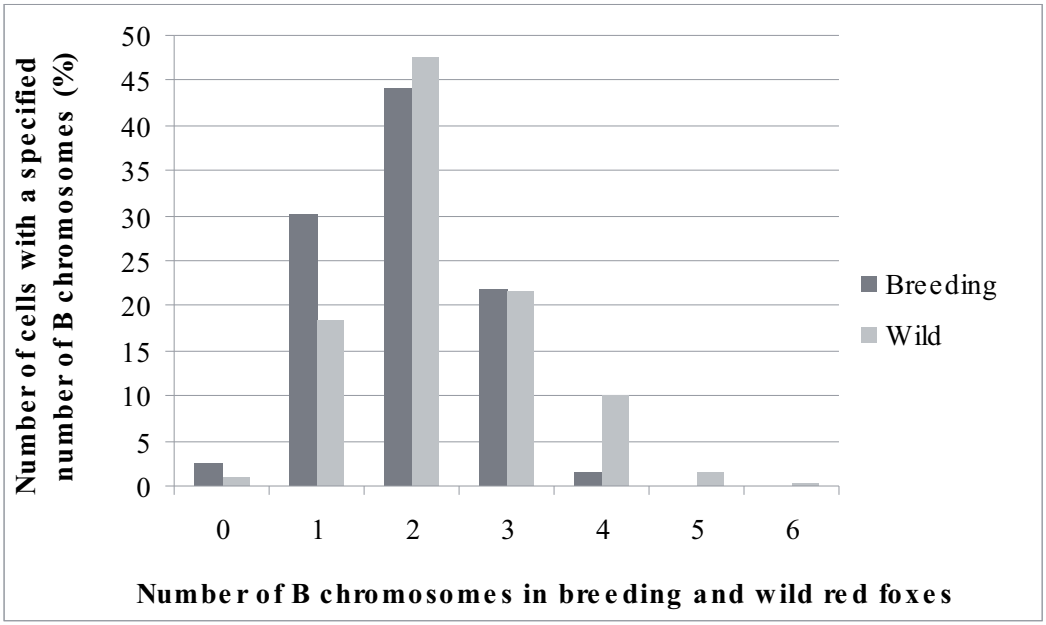

Fig. 4. Frequency of different B chromosome numbers in the breeding and wild population of red foxes.

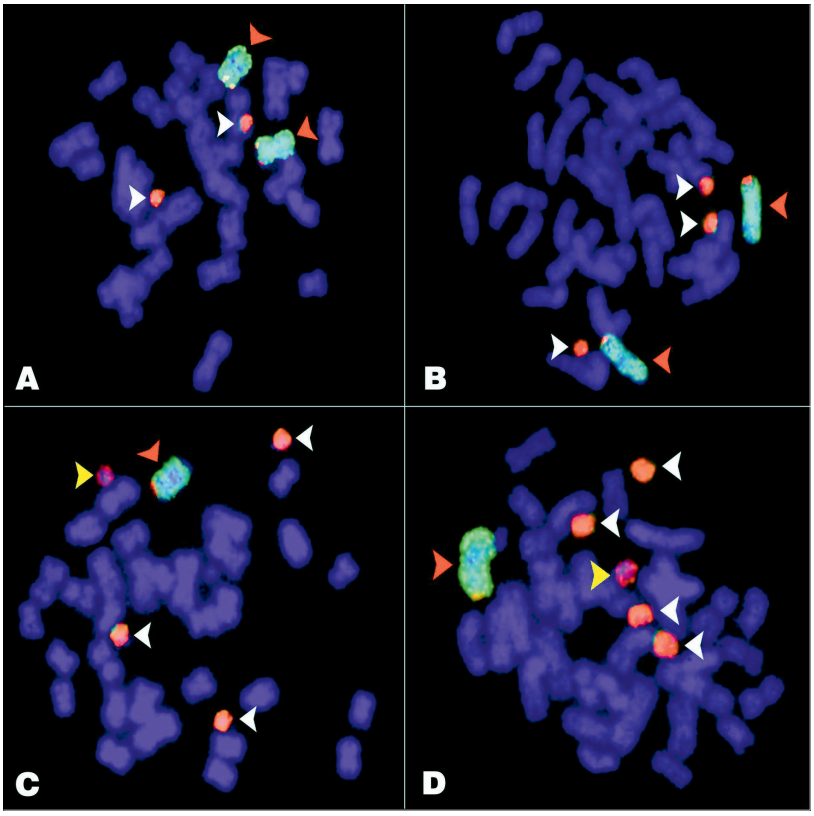

Fig. 5. Localization of molecular probes specific for heterochromosomes and for B chromosomes (FISH). A – female, farm population; B – female, wild population, C – male, farm population, D – male, wild population. B chromosomes are indicated with white arrows, X chromosomes with signals visible in the pseudoautosomal regions are marked with red arrows, and Y chromosomes are indicated with yellow arrows.

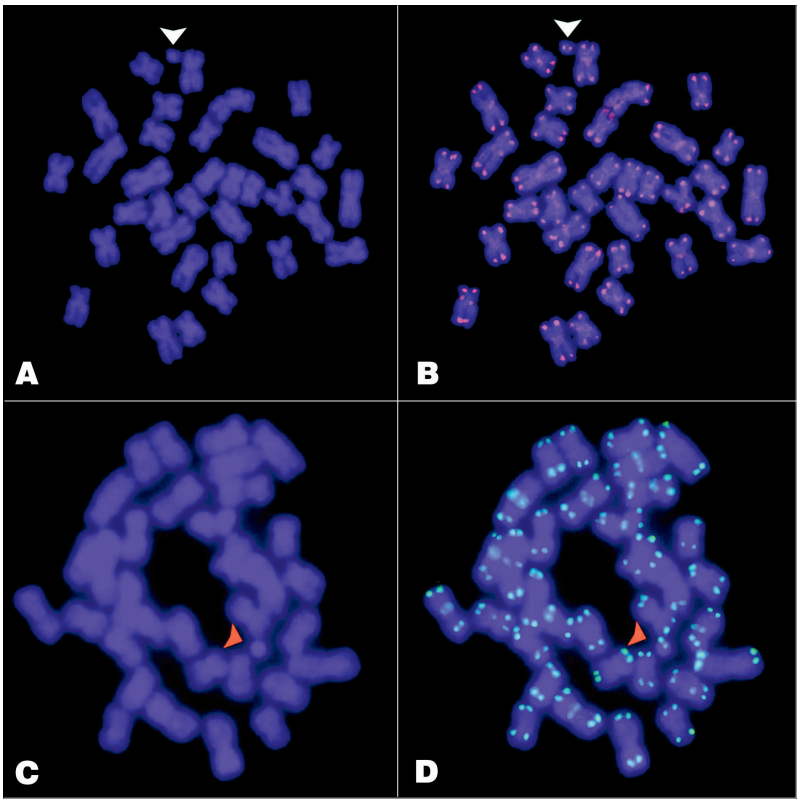

Fig. 6. Localization of signals on telomeric sequences (PRINS). A, B – female, breeding population, direct Cy3 labeling, C, D – male, breeding, indirect biotin labeling. Acrocentric B chromosome presenting a typical distal telomeric signal (A, B), is indicated with white arrows, whereas red arrows point to a single acrocentric chromosome in the karyotype of male red fox, the Y chromosome (C, D).

Discussion

Nucleolar organizer regions (NORs) containing sequences of rDNA genes were detected in *Vulpes vulpes* on the telomeric parts of q arms of chromosomes 8, 9 and 13. (MÄKINEN 1985). Experiments carried out herein using differentiating AgNOR staining identified a wide range of individual variability among the studied animals in both the farm population and the wild population. The analysis of nucleolar organizers, based on a minimal number of 20 metaphase spreads per animal, showed that in red foxes from the breeding population most cells (31.66%) contained 4 silver deposits, whereas in the wild population most cells (37.54%) contained 5 silver deposits. Farm raised *Vulpes vulpes* tended to have fewer NORs, while the largest number of silver deposits (8) was found in a wild male fox. Rare cases of additional NORs (in comparison to the number determined for a species) have been reported so far in other species, e.g. the horse (LOGINOVA *et al.* 1996; DERJUSHEVA *et al.* 1997). Known mechanisms do not always clearly explain the observed variability of nucleolar organizer regions at the chromosomal, cellular, individual or population levels. Moreover, the literature provides little information on these mechanisms in farm animals. The identification of potential mechanisms which alter the level of transcriptional activity of nucleolar organizer regions seems to be particularly interesting in the context of chromosomal polymorphism and the evaluation of AgNOR size as chromosomal markers. Research on the repression of transcriptional activity of nucleolar organizer regions suggests that there are at least three mechanisms resulting in the inactivation of nucleolar regions. These include the elimination of rDNA, DNA methylation and gene silencing (position effect within heterochromatin or telomeres) (DOBIGNY *et al.* 2002; WNUK *et al.* 2006). The obtained results with respect to the karyotype of *Vulpes vulpes* are comparable with prior observations of PIEŃKOWSKA & ŚWITOŃSKI (1998).

The structure of B chromosomes has been the subject of numerous studies in recent years. A commonly known and widely accepted explanation is that B chromosomes are derived from A chromosomes. From this perspective, they are a by-product of the evolution of the standard karyotype (CAMACHO *et al.* 2000). B chromosomes can reach very high frequencies in natural populations, depending on the degree of tolerance of the species towards these additional elements, as well as the mechanism of their accumulation in cells, if there are any. B chromosome occurrence is often stable for several years within the same population. In the past this has led many authors to conclude that the polymorphism is in equilibrium and the frequency of occurrence is the result of two opposing forces: (i) the accumulation of B chromosomes which tend to occur with greater frequency, (ii) adverse effects of B chromosomes on the fitness of individuals.

Most authors suggest that the B chromosomes of mammals are probably the remains of structural rearrangements occurring during the evolution of karyotypes in ancestral forms. VOLOBUJEV (1984) suggested that each different type of chromosome has its own path of origin.

The role of B chromosomes in the evolution of eukaryotic genomes seems negligible. Given that their presence is not required for the survival and reproduction of the individual, the chromosomes would seem to be an unnecessary feature of the genome compared to the basic genome (CAMACHO *et al.* 2000). They are characterized by mitotic and meiotic instability, and consequently a non-Mendelian pattern of inheritance, which leads to their accumulation in one cell, and elimination in the other. For this reason, they are considered to be 'selfish' or even 'parasitic' genetic elements (EICKBUSH *et al.* 1992; PERFECTTI *et al.* 2004; BASHEVA 2010).

Studies in the 60's and 70's of the last century conducted by MOORE & ELDER (1965), GUSTAVSSON & SUNDT (1967) and BUCKTON & CUNNINGHAM (1971) showed that the variable number of acrocentric B chromosomes in the red fox ranges between 0 and 6, being a complement to the basic A set. On the basis of these studies it was concluded that chromosome polymorphism has no significant effect on the phenotype, and that they consist mainly of heterochromatin, similarly to supernumerary chromosomes occurring in certain species of plants and invertebrates (ELLENTON & BASRUR 1981).

Our application of fluorescence *in situ* hybridization (FISH) with probes specific for B chromosomes derived from the genome of the red fox made it possible to determine the exact number of these chromosomes, even in poor quality preparations. The analysis showed the presence of polymorphism among animals and within cells of individuals. The analysis of B chromosomes on the basis of a minimal number of 40-50 metaphase spreads from each animal determined that their number in the farm population ranged from 0 to 4, and statistically most cells (44.1%) contained two extra chromosomes. In the case of animals from the wild population, the number of B chromosomes ranged from 0 to 6 and statistically most cells (47.5%) contained 2 supernumerary chromosomes. In both cases, there was no gender-dependent difference in the distribution of B chromosomes. Similar findings were reported by

ELLENTON & BASRUR (1981) and ŚWITOŃSKI *et al.* (2003) which confirm the results obtained in this study. In addition, this study determined that a greater range of the number of B chromosomes is observed in a wild population of red foxes.

YANG *et al.* (1999) used a molecular probe derived from a single B chromosome and obtained signals on all B chromosomes present in the cell, which means that the probe complementary to one of the chromosomes hybridized with all other B chromosomes. On this basis, it can be assumed that the same repetitive sequences are present on all B chromosomes of *Vulpes vulpes*. The same correlation is also observed in other animal species (KARAMYSHEVA *et al.* 2002; TRIFONOV *et al.* 2002; SZCZERBAL & ŚWITOŃSKI 2003; MATSUBARA *et al.* 2008).

The results of this work specifying a pattern in the variable number of B chromosomes in the red fox correspond to ŚWITOŃSKI *et al.* (1987) as well as to work carried out in other species such as the raccoon dog (SHI *et al.* 1988) or East-Asiatic mouse (KOLOMIETS *et al.* 1988).

All these species show supernumerary chromosome mitotic instability and mosaicism of their number in the germ line. It has not yet been established whether a variable number of chromosomes affects the fitness of animals, but it is an interesting issue that pertains to many species of wild animals. Despite numerous studies, data on the genetic role of B chromosomes of *Vulpes vulpes* are scarce. A series of studies carried out by many groups to date has not provided detailed information on their function (VOLOBUJEV & RADJABLI 1974; VOLOBUJEV *et al.* 1976; BASHEVA *et al.* 2010).

As a result of cytogenetic studies using fluorescence *in situ* hybridization (FISH) with a molecular probe derived from the dog genome specific for sex chromosomes X and Y, clear signals were detected on the two female X chromosomes, while in males, one signal on the X and Y chromosomes, respectively. In addition, on the X chromosomes, specific hybridization signals for the Y chromosome were observed in the pseudoautosomal region, showing homology between the sex chromosomes. Studies by several authors have shown that the X and Y chromosomes of the dog genome show a high degree of homology with heterochromosomes of *Vulpes vulpes* and other species within the Canidae family, such as silver fox or raccoon dog. This may suggest that the structure of sex chromosomes has not changed significantly during canine karyotype evolution (GRAPHODATSKY *et al.* 2000; BUGNO-PONIEWIERSKA *et al.* 2012).

Telomeres are the physical ends of the chromosomes of most eukaryotic organisms. They consist of repetitive, highly conserved DNA sequences. They are localized at the ends of chromosome arms and in pericentromeric areas.

Additional information was provided by a detailed molecular characterization of B chromosomes using fluorescence *in situ* hybridization (FISH). Application of a telomeric probe has determined that B chromosomes of *Vulpes vulpes* have typical distal signals (CAMACHO 2000).

Our studies designed to localize telomeric sequences on chromosomes of *Vulpes vulpes* using PRINS technique identified clear signals corresponding to telomeric sequences on both arms of all chromosomes and in several cases also in the pericentromeric regions. Typical telomeric signals were also detected on B chromosomes. The results confirm this notion and are supported by ŚWITOŃSKI *et al.* (2003). Moreover ŚWITOŃSKI *et al.* (2003) and WURSTER-HILL *et al.* (1998) performed parallel studies in other species of the family Canidae and obtained similar results. However, in the raccoon dog the signals corresponding to telomeric sequences were dispersed, giving even signals over the entire length of the chromosomes. The authors suggested that in both subspecies of raccoon dogs, B chromosomes are rich in telomeric and telomeric-like sequences.

Summary

This study determined that a wild population of red foxes had higher variation in the number of B chromosomes compared to breeding animals. However, statistically most cells carried 2 supernumerary chromosomes in both populations. Despite the presence of considerable polymorphism, there was no change in the distribution of B chromosomes dependent on gender or the origin of red foxes. However, high variation in the number of active nucleolar organizer regions (AgNOR) was found within both populations. In the case of farm animals, a tendency towards NOR reduction was noted when compared to red foxes living in the wild, which might be the effect of domestication.

References

ARAKAKI D.T., SPARKES R.S. 1963. Microtechnique for culturing leukocytes from whole blood. Cytogenetics **85**: 57-60.

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.

BUCKTON K.E., CUNNINGHAM C. 1971. Variations of the chromosome number in the red fox (*Vulpes vulpes*). Chromosoma **33**: 268-272.

BUGNO M., SŁOTA E., PIEŃKOWSKA-SHELLING A., SCHELLING C. 2009. Identification of chromosome abnormalities in the horse using a panel of chromosome – specific painting probes generated by microdissection. Acta Vet. Hung. **57**: 369-381.

BUGNO-PONIEWIERSKA M., SOJECKA A., PAWLINA K., JAKUBCZAK A., JEZEWSKA-WITKOWSKA G. 2012. Comparative cytogenetic analysis of sex chromosomes in several Canidae species using zoo-FISH. Folia biol. **60**: 11-16.

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

DERJUSHEVA S.E., LOGINOVA J.A., PARADA R., CHIRIAEVA O.G., SMIRNOV A.F., JASZCZAK K. 1998. The comperative analysis of NOR polymorphism detected by FISH and Ag-staining on horse chromosomes. Caryologica **51**: 1-11.

DOBIGNY G., OZOUF-COSTAZ C., BONILLO C., VOLOBOUEV V. 2002. "Ag-NORs" are not always true NORs: new evidence in mammals. Cytogenet. Genome Res. **98**: 75-77.

EICKBUSH D.G., EICKBUSH T.H., WERREN J.H. 1992. Molecular characterization of repetitive DNA sequences from a B chromosome. Chromosoma **101**: 575-583.

ELLENTON J.A., BASRUR P.K. 1981. Microchromosomes of the Ontario Red Fox (*Vulpes vulpes*): Distribution of chromosome numbers and relationship with physical characteristics. Genetica **1**: 13-19.

GRAPHODATSKY A.S., YANG F., O'BRIEN P.C., SERDUKOVA N., MILNE B.S., TRIFONOV V., FERGUSON-SMITH M.A. 2000. A comparative chromosome map of the Arctic fox, red fox and dog defined by chromosome painting and high resolution G-banding. Chromosome Res. **8**: 253-263.

GUSTAVSSON I., SUNDT C.O. 1967. Chromosome elimination in the evolution of the silver fox. J. Heredity **58**: 75-78.

HOWELL W.M., BLACK D.A. 1980. Controlled silver-staining of nucleolus organizer regions with a protective colloidal developer: a 1-step method. Experientia **36**: 1014-1015.

KARAMYSHEVA T.V., ANDREENKOVA O.V., BOCHKAEREV M.N., BORISSOV Y.M., BOGDANCHIKOVA N., BORODIN P.M., RUBTSOV N.B. 2002. B chromosomes of Korean field mouse *Apodemus peninsulae* (Rodentia, Murinae) analysed by microdissection and FISH. Cytogenet. Genome Res. **96**: 154-160.

KOLOMIETS O.L., BORBIEV T.E., SAFRONOVA L.D., BORISOV Y.M., BOGDANOV Y.F. 1988. Synaptonemal complex analysis of B-chromosome behavior in meiotic prophase I in the East-Asiatic mouse *Apodemus peninsulae* (Muridae, Rodentia). Cytogenet. Cell Genet. **48**: 183-187.

LOGINOVA J., DERJUSHEVA S., JASZCZAK K. 1996. Some cases of NOR instability in horse chromosomes. Arch. Zootec. **45**: 275-279.

MÄKINEN A. 1985. The standard karyotype of the silver for (*Vulpes fulvus Desm.*). Committee for the standard karyotype of *Vulpes fulvus Desm*. Hereditas **103**: 171-176.

MATSUBARA K., YAMADA K., UMEMOTO S., TSUCHIYA K., IKEDA N., NISHIDA C., CHIJIWA T., MORIWAKI K., MATSUDA Y. 2008. Molecular cloning and characterization of the repetitive DNA sequences that comprise the constitutive heterochromatin of the A and B chromosomes of the Korean field mouse (*Apodemus peninsulae*, Muridae, Rodentia). Chromosome Res. **16**: 1013-1026.

MOORE W.J., ELDER R.L. 1965. Chromosomes of the fox. J. Heredity **56**: 142-143.

PALESTIS B.G., BURT A., JONES R.N., TRIVERS R. 2003. B chromosomes are more frequent in mammals with acrocentric karyotypes: support for the theory of centromeric drive. Proc. R. Soc. Lond. B **271**: 22-24.

PERFECTTI F., CORRAL J.M., MESA J.A., CABRERO J., BAKKALI M., LÓPEZ-LEÓN M.D., CAMACHO J.P. 2004. Rapid suppression of drive for a parasitic B chromosome. Cytogenet. Genome Res. **106**: 338-343.

PIEŃKOWSKA A., ŚWITOŃSKI M. 1998. Chromosomal localization and activity of nucleolar organizer regions in the dog (*Canis familiaris*). Genet. Sel. Evol. **30**: 79-84.

PINKEL D., GRAY J.W., TRASK B., VAN DEN ENGH G., FUSCOE J., VAN DEKKEN H. 1986. Cytogenetic analysis by *in situ* hybridization with fluorescently labeled nucleic acid probes. Cold Spring Harbor Symposia on Quantitative Biology **51**: 151-157.

SHI L., TANG L., MA K., MA C. 1988. Synaptonemal complex formation among supernumerary B chromosomes: an electron microscopic study on spermatocytes of Chinese raccoon dogs. Chromosoma **97**: 178-183.

STATHAM M.J., TRUT L.N., SACKS B.N., KHARLAMOVA A.V., OSKINA I.N., GULEVICH R.G., JOHNSON J.L., TEMNYKH S.V., ACLAND G.M., KUKEKOVA A.V. 2011. On the origin of a domesticated species: Identifying the parent population of Russian silver foxes (*Vulpes vulpes*). Biol. J. Linn. Soc. **103**: 168-175.

ŚWITOŃSKI M., GUSTAVSSON I., HÖJER K., PLÖEN L. 1987. Synaptonemal complex analysis of the B-chromosomes in spermatocytes of the silver fox (*Vulpes fulvus Desm.*). Cytogenet. Genome Res. **45**: 84-92.

SWITONSKI 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 **4**: 375- 385.

SZCZERBAL I., ŚWITOŃSKI M. 2003. B chromosomes of the Chinese raccoon dog (*Nyctereutes procyonoides procyonoides* Gray) contain inactive NOR-like sequences. Caryologia **56**: 209-212.

TRIFONOV V.A., PERELMAN P.L., KAWADA S.I., IWASA M.A., ODA S.I., GRAPHODATSKY A.S. 2002. Complex structure of B-chromosomes in two mammalian species: *Apodemus peninsulae* (Rodentia) and *Nyctereutes procyonoides* (Carnivora). Chromosome Res. **10**: 109-116.

VOLOBUJEV V.T. 1984. The B-chromosome system of mammals. Genetica **52/53**: 333-337.

VOLOBUJEV V.T., RADJABLI S.I. 1974. Investigation of the nature and the role of the additional chromosomes in silver foxes. I. Comparative analysis of additional chromosomes in different tissues and type preparations and at different seasons. Genetika **10**: 77-82.

VOLOBUJEV V.T., RADJABLI S.I., BELYAEVA E.S. 1976. Investigation of the nature and the role of the additional chromosomes in silver foxes. Ill. Replication pattern in additional chromosomes. Genetika **12**: 311-334.

WILSON E.O., REEDER M. 2005. Mammal Species of the World: A Taxonomic and Geographic Reference, 3rd ed. The Johns Hopkins University Press, Baltimore.

WNUK M., SLOTA E., KOTYLAK Z., BUGNO M. 2006. Factors responsible for modulation of ribosomal RNA transcription. Folia Biol. (Kraków) **54**: 1-8.

WURSTER-HILL D.H., WARD O.G., DAVIS B.H., PARK J.P., MOYZIS R.K., MEYNE J. 1988. Fragile sites, telomeric DNA sequences, B chromosomes, and DNA content in raccoon dogs, *Nyctereutes procyonoides*, with comparative notes on foxes, coyote, wolf, and raccoon. Cytogenet. Cell Genet. **49**: 278-281.

YANG F., O'BRIEN P.C., MILNE B.S., GRAPHODATSKY A.S., SOLANKY N., TRIFONOV V., RENS W., SARGAN D., FERGUSON-SMITH M.A. 1999. A complete comparative chromosome map for the dog, red fox, and human and its integration with canine genetic maps. Genomics **62**: 189-202.