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Gene Mapping as a Method for Verifying Sequence Localization Based on Interspecific Chromosome Painting (ZOO-FISH)*

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The results obtained in the present study made it possible to place selected markers on the physical map of the arctic fox genome. With the use of fluorescence in situ hybridization (FISH) the GHR (3q24) and Il10 (1q21.1-21.2) genes and the FH2537 (5q11.3) microsatellite were localized on arctic fox chromosomes. The results confirmed previously proposed homologies using the ZOO-FISH technique, except for the Il10 gene. This suggests that the gene underwent a rearrangement (an inversion) that changed its localization compared to the dog.

Key words: Gene mapping, ZOO-FISH, arctic fox.

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The *Canidae* family is represented by 13 genera and 36 species, including domestic dogs, wolves, foxes, African wild dogs, raccoon dogs and bat-eared foxes. The members of the *Canidae* family are spread all over the world, except Antarctica. Evolutionary mechanisms such as natural selection and genetic drift have played a key role in the formation of present genera, species and breeds. During their evolution, there have been fixed species-differentiating features, a number of genetic changes responsible for canid features as well as mutations associated with genetic diseases.

The domestic dog (*Canis familiaris*) is a species in which over 400 genetically-based diseases have been described, many similar to human diseases (COTMAN & HEAD 2008). For this reason, the dog has become a model species to study the molecular basis of human hereditary diseases (SEPPÄLÄ *et al*. 2011). As a result, the canine genome is of interest to numerous research centres whose work made it possible to localize many genes as well as microsatellite sequences that serve as molecular markers.

Molecular markers are used in comparative genomics within the *Canidae* family for such species as the red fox, the arctic fox and the Chinese raccoon dog (GRZES *et al*. 2009; SKORCZYK *et al*. 2012). By comparing the genomes of these species, it is possible to extend our knowledge of how the karyotype of this family has evolved. The main chromosomal rearrangements were centric fusions and fissions as well as inversions. Such karyotype rearrangements were analysed using comparative chromosome painting (ZOO-FISH), in which probes specific to one species are hybridized with metaphase chromosomes of another species (YANG *et al*. 1999; FERGUSON-SMITH & TRIFONOV 2007; BUGNO-PONIEWIERSKA *et al*. 2012; PAWLINA & BUGNO-PONIEWIERSKA 2012). ZOO-FISH can be carried out with the use of probes specific to entire chromosomes, their fragments, as well as BAC probes specific to single genes, which at the same

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time enables the physical map of a given species to be extended (RAUDSEPP *et al*. 2001; BUGNO-PONIEWIERSKA *et al*. 2010). Comparative studies generally use genes from gene libraries, such as *GHR* (Growth Hormone Receptor)*, IL-10,* the product of which is interleukin-10 (a cytokine synthesis inhibitor), and the FH2537 microsatellite sequence linked to the *SILV* gene, which determines hair pigmentation (CLARK *et al*. 2006).

The objective of this study was to physically localize *GHR* and *IL10* genes and the microsatellite sequence in the arctic fox (*Alopex lagopus*) genome by ZOO-FISH, using molecular probes obtained from the domestic dog (*Canis familiaris*) genome.

Material and Methods

To obtain metaphase chromosome preparations of the arctic fox (*Alopex lagopus*), peripheral blood lymphocytes were cultured from blood samples collected from 8 animals from private Polish farms. Following cultures were performed by a routine procedure and the obtained cell suspensions were dropped on slides. Then, preparations with metaphase spreads were used for GTG chromosome staining according to WANG & FEDOROFF (1974).

FISH mapping

The canine BAC library from the University of Bern, Switzerland was screened by polymerase chain reaction (PCR) using primers designed to amplify parts of the canine GHR and IL10 genes and microsatellite sequence FH2537. Primers were first tested for PCR amplification on whole genomic canine DNA before screening the library. DNA of positive clones (GHR: S006P23F12, IL10: S058P14C09, FH2537: S022P07G03) was extracted from 500 ml overnight cultures of the positive BAC clone using the Qiagen Midi plasmid kit (Qiagen AG, Basel, Switzerland) according to the alkaline lysis protocol for BACs. Direct sequencing of BACs was performed on an ABI 3730 sequencer using BigDye ver. 3.1 chemistry (Applied Biosystems, Rotkreuz, Switzerland). Then 100 ng BAC DNA was labeled with Biotin–16 dUTP with the use of the Nick Translation Kit (Roche Applied Science) and used for FISH on archived G-banded arctic fox metaphases. Arctic fox chromosomes were identified according to the GTG-banded chromosome nomenclature (MAKINEN *et al*. 1985; GRAPHODATSKY *et al*. 2000) using 10-20 metaphase spreads. Then, hybridization was carried out following a standard protocol (PINKEL *et al*. 1986). The slides were DAPI stained and then the analysis of FITC fluorescence signals was performed with the use of a fluorescent microscope coupled with a video camera and an appropriate computer program.

Results and Discussion

The aforementioned experiments allowed for the chromosomal assignment of predicted arctic fox sequences as follows: GHR on ALA3q24 (Fig. 1), Il10 on ALA1q21.1-21.2 (Fig. 2.), microsatellite FH2537 on ALA5q11.3 (Fig. 3.).

Over 4200 markers constitute the last version of the integrated marker fluorescence *in situ* hybridization (FISH)/radiation hybrid (RH) canine map. The markers are distributed over the whole genome with the average distance between adjacent *loci* below 1 Mbp (BREEN *et al*. 2004). The applied probes allowed for the expansion of the cytogenetic map of the arctic fox, which has included 47 markers (genes as well as microsatellites) until now (SZCZERBAL *et al*. 2006; SZCZERBAL *et al*. 2007). Information about the localization of markers on chromosomes is vital for further linkage studies, for example KUKEKOVA *et al*. (2007) constructed the fox map on the basis of 320 microsatelite markers. These markers fulfilled the following criteria: they were dog markers previously optimized for the fox (KUKEKOVA *et al*. 2004) or previously published dog microsatellites (GUYON *et al*. 2003; BREEN *et al*. 2004; CLARK *et al*. 2004) or markers which were available from the Mammalian Genotyping Service of Marshfield Laboratories (Madison, WI).

Studies on karyotype evolution can increase our knowledge about processes which take place during species formation. To this end, a variety of tools can be used such as a marker genome map. On the basis of this type of map, it was discovered that the canid karyotype underwent numerous centric fusions and fissions (GRAPHODATSKY *et al*. 2001; NASH *et al*. 2001). Moreover, it was suggested that whole-arm inversions (conversions from telomere to centromere) might have played a significant role during the formation of the karyotype (NASH *et al*. 2001; ROGALSKA-NIZNIK *et al*. 2003; SZCZERBAL *et al*. 2003). Previous research with the use of BAC probes showed that a different orientation (regarding centromere/telomere position) of the dog chromosomes (in comparison to chromosomes of other canids) is a rather common phenomenon (GRAPHODATSKY *et al*. 2000; SZCZERBAL *et al*. 2003; SZCZERBAL *et al*. 2006). Despite the high degree of conservatism in the pattern of G bands between homologous chromosomes or chromosome arms of the dog and arctic fox, eight centromeres of the dog chromosomes are turned

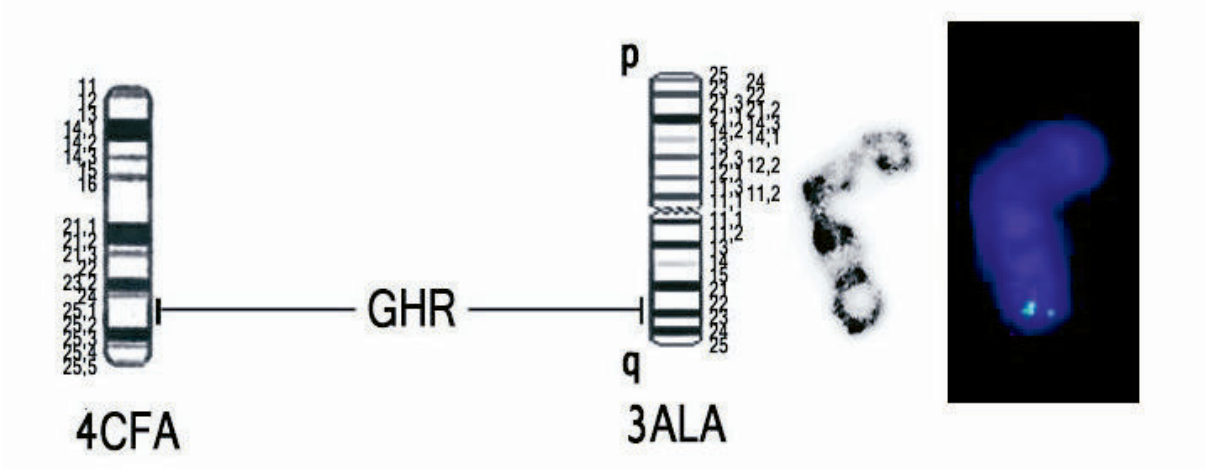

Fig. 1. The localization of GHR gene in the arctic fox.
Left side: idiograms of the dog and arctic fox chromosomes indicating the localization of the GHR gene on dog chromosome (CFA4) and presumed localization on arctic fox chromosome (ALA3q24). Right side: GTG banding and FISH technique confirming the localization of the gene on the arctic fox chromosome.

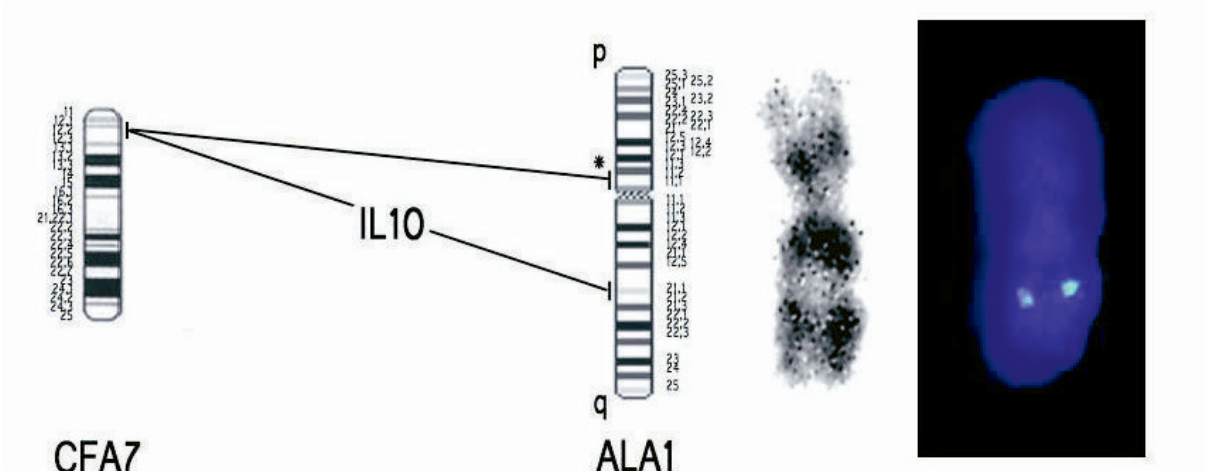

Fig. 2. The localization of IL10 gene in the arctic fox.
Left side: idiograms of the dog and arctic fox chromosomes indicating the localization of IL10 gene on dog chromosome (CFA7) and presumed localization on arctic fox chromosome (ALA1p) on the basis of ZOO-FISH with the use of WCPP probes (the asterix) and the observed localization of the gene after the mapping. Right side: GTG banding and FISH technique on artic fox chromosome with fluorescent signals of the IL10 gene.

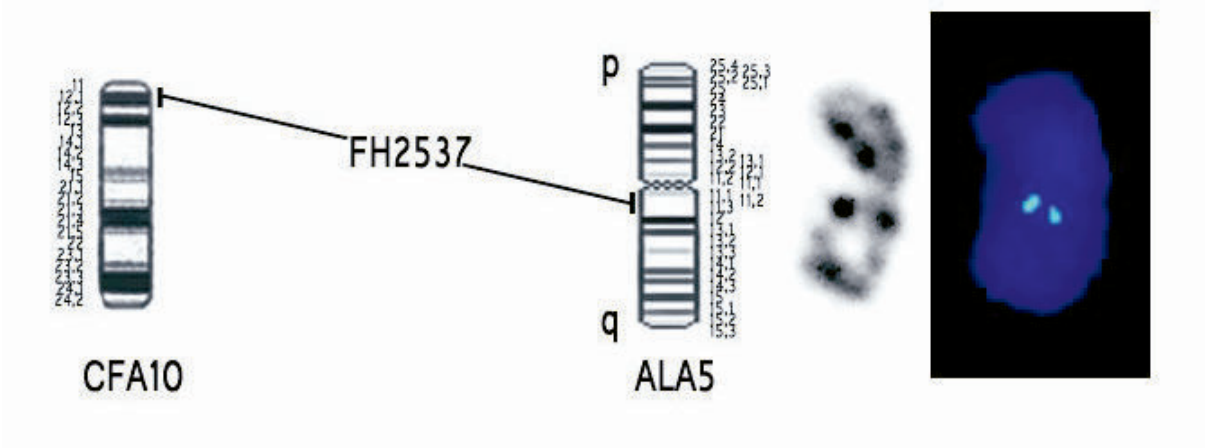

Fig. 3. The localization of a microsatellite sequence in the arctic fox.
Left side: idiograms of the dog and arctic fox chromosomes indicating the localization of microsatellite sequence FH2537 on dog chromosome (CFA10) and presumed localization on arctic fox chromosome (ALA5q11.3). Right side: GTG banding and FISH technique confirming the localization of the sequence.

towards the telomeres of the arctic fox chromosomes (GRAPHODATSKY *et al*. 2000).

Two genes and one microsatellite were located on the physical map of *Alopex lagopus*. The loci of these genes were first identified in humans and later in dogs (BARTON *et al*. 1989; ESKDALE *et al*. 1997). Comparison of their localization in the species of *Canidae* family makes it possible to detect similarities and differences, and thus to infer possible rearrangements that might have occurred during their evolution.

Canine chromosome 4 (CFA4), which contains the *GHR* gene locus, corresponds to the long arm (q) of arctic fox chromosome 3 (ALA3) and this is where the *GHR* gene was localized. Canine chromosome 10 (CFA10), on which the FH2537 microsatellite sequence was identified, corresponds to the long arm (q) of chromosome 5 of the arctic fox and in this case the localization of the gene agrees with the previously determined homology (ALA5) (GRAPHODATSKY *et al*. 2000).

An attempt was also made to locate the *IL10* gene, which is found on chromosome 7 of the domestic dog. According to the comparative map of the *Canidae* family, the expected localization of this gene in the arctic fox would cover the proximal region (in close proximity to the centromere) of the short arm (p) of chromosome 1. However, this gene was localized in the pericentromeric region of the long arm (q) of chromosome 1.

The discrepancy between the results obtained by ZOO-FISH may be due to the fact that this technique shows homology of larger segments without providing any information about the organization of smaller units like genes, so small rearrangements may go undetected. Presumably, an inversion occurred during karyotype evolution of the arctic fox relocalizing the *IL10* gene. This should be confirmed by additional experiments and research, e.g. by constructing a BAC contig map, a radiation hybrid (RH) map or by sequencing selected fragments of the arctic fox genome.

However, a scenario accounting for a rearrangement of the inversion type is highly probable because many cases of inversion were described within the canid family. For example, the canine ATP2A gene was localized on chromosome 26 in the proximal region. Despite the homology of canine chromosome 26 to the long arm (q) of chromosome 14 in the arctic fox, this gene was localized in the telomeric region of the same chromosome. The same gene was also located in the red fox (*Vulpes vulpes*) on the short arm (p) of chromosome 10 in the telomeric region. A centromere-telomere inversion has contributed to this phenomenon (NOWACKA-WOSZUK & SWITONSKI 2010). Another example is the localization of the HTR2A and TYRP2 genes. Both were identified on the short arm of chromosome 6 in the red fox. This arm is homologous to chromosome 22 of the domestic dog. The former gene was located within the telomere, the latter gene in the proximal position. The situation is reverse in the domestic dog, with TYRP2 found in the telomeric part and HTR2A in the pericentromeric region. This suggests that a centromere-telomere inversion occurred during the evolution of the canid lineage (KUKEKOVA *et al*. 2009).

The present study follows the current trend in the development of cytogenetic research in which genetic maps are constructed for those animal species that have received little research and interest (RAUDSEPP & CHOWDHARY 2001). The results obtained will further broaden our knowledge of genome organization in the *Canidae*, thus enabling better understanding of the evolutionary process in this mammalian family. In addition, the results will extend the physical map of the red fox, which will allow for identification and determination of new loci of genes important from the viewpoint of fox farming. Moreover, the identification of genes that control susceptibility to specific diseases is important because *Canidae* are model animals for studying human diseases, including cancer.

References

BARTON D.E., FOELLMER B.E., WOOD W.I. FRANCKE U. 1989. Chromosome mapping of the growth hormone receptor gene in man and mouse. Cytogenet. Cell Genet. **50**: 137-141.

BREEN M., HITTE C., LORENTZEN T., THOMAS R., CADIEU E., SABACAN L., SCOTT A., EVANNO G., PARKER H., KIRKNESS E., HUDSON R., GUYON R., MAHAIRAS G., GELFENBEYN B., FRASER C., ANDRE C., GALIBERT F., OSTRANDER E. 2004. An integrated 4249 marker FISH/RH map of the canine genome. BMC Genomics **5**: 65.

BUGNO-PONIEWIERSKA M., PAWLINA K., DARDZIŃSKA A., ZĄBEK T., SŁOTA E. KLUKOWSKA-RÖTZLER J. 2010. FISH mapping of six genes responsible for development of the nervous and skeletal systems on donkey (*Equus asinus*) chromosomes. Hereditas **147**: 132-135.

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

CLARK L.A., FAMULA T.R., MURPHY K.E. 2004. Evaluation of a rapid single multiplex microsatellite-based assay for use in forensic genetic investigations in dogs. Am. J. Vet. Res. **65**: 1446-50.

COTMAN C.W., HEAD E. 2008. The canine (dog) model of human aging and disease: dietary, environmental and immunotherapy approaches. J. Alzheimers Dis. **15**: 685-707.

ESKDALE J., KUBE D., TESCH H. et al. 1997. Mapping of the human IL10 gene and further characterization of the 5-prime flanking sequence. Immunogenetics **46**: 120-128.

FERGUSON-SMITH M.A., TRIFONOV V. 2007. Mammalian karyotype evolution. Nature Rev. Genet. **8**: 950-962.

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.

GRAPHODATSKY A.S., YANG F., O'BRIEN P.C., PERELMAN P., MILNE B.S., SERDUKOVA N., KAWADA S.I., FERGUSON--SMITH M.A. 2001. Phylogenetic implications of the 38 putative ancestral chromosome segments for four canid species. Cytogenet Cell Genet. **92**: 243-7.

GRZES M., NOWACKA-WOSZUK J., SZCZERBAL I., CZERWINSKA J., GRACZ J., SWITONSKI M. 2009. A comparison of coding sequence and cytogenetic localization of the myostatin gene in the dog, red fox, arctic fox and Chinese raccoon dog. Cytogenet. Genome Res. **126**: 173-179.

GUYON R., LORENTZEN T.D., HITTE C., KIM L., CADIEU E., PARKER H.G., QUIGNON P., LOWE J.K., RENIER C., GELFENBEYN B., VIGNAUX F., DEFRANCE H.B., GLOUX S., MAHAIRAS G.G., ANDRE C., GALIBERT F., OSTRANDER E.A. 2003. A 1-Mb resolution radiation hybrid map of the canine genome. Proc. Natl. Acad. Sci. U S A **100**: 5296-5301.

KUKEKOVA A.V., TRUT L.N., OSKINA I.N., KHARLAMOVA A.V., SHIKHEVICH S.G., KIRKNESS E.F., AGUIRRE G.D., ACLAND G.M. 2004. A marker set for construction of a genetic map of the silver fox (*Vulpes vulpes*). J. Hered. **95**: 185-94.

KUKEKOVA A.V., TRUT L.N., OSKINA I.N., JOHNSON J.L., TEMNYKH S.V., KHARLAMOVA A.V., SHEPELEVA D.V., GULIEVICH R.G., SHIKHEVICH S.G., GRAPHODATSKY A.S., AGUIRRE G.D., ACLAND G.M. 2007. A meiotic linkage map of the silver fox, aligned and compared to the canine genome. Genome Res. **17**: 387-99.

KUKEKOVA A.V., VOROBIEVA N.V., BEKLEMISHEVA V.R. et al. 2009. Chromosomal mapping of canine-derived BAC clones to the red fox and American mink genomes. J. Hered. **100 Suppl 1**: S42-53.

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

PAWLINA K., BUGNO-PONIEWIERSKA M. 2012. The application of Zoo-FISH technique for analysis of chromosomal rearrangements in the Equidae family. Ann. Anim. Sci. **1**: 5-13.

NASH W.G., MENNINGER J.C., WIENBERG J., PADILLA--NASH H.M., O'BRIEN S.J. 2001. The pattern of phylogenomic evolution of the Canidae. Cytogenet. Cell Genet. **95**: 210-24.

NOWACKA-WOSZUK J., SWITONSKI M. 2010. Comparative cytogenetic mapping of three genes involved in sex determination in four species of the family Canidae. J. Anim. Feed Sci. **19**: 5-12.

PINKEL D., LANDEGENT J., COLLINS C., FUSCOET J., SEGRAVES R., LUCAS J., GRAY J. 1986. Fluorescence in situ hybridization with human chromosome-specific libraries: Detection of trisomy 21 and translocations of chromosome 4. Proc. Nati. Acad. Sci. USA **85**: 9138-9142.

RAUDSEPP T., CHOWDHARY B.P. 2001. Correspondence of human chromosomes 9, 12, 15, 16, 19 and 20 with donkey chromosomes refines homology between horse and donkey karyotypes. Chromosome Res. **9**: 623-629.

ROGALSKA-NIZNIK N., SZCZERBAL I., DOLF G., SCHLAPFER J., SCHELLING C., SWITONSKI M. 2003. Canine-derived cosmid probes containing microsatellites can be used in physical mapping of Arctic fox (*Alopex lagopus*) and Chinese raccoon dog (*Nyctereutes procyonoides procyonoides*) genomes. J. Hered. **94**: 89-93.

SEPPÄLÄ E.H., JOKINEN T.S., FUKATA M., FUKATA Y., WEBSTER M.T. et al. 2011. LGI2 Truncation Causes a Remitting Focal Epilepsy in Dogs. PLoS Genet. **7**: e1002194. doi:10.1371/journal.pgen.1002194.

SKORCZYK A., FLISIKOWSKI K., SWITONSKI M. 2012. A Comparative Analysis of MC4R Gene Sequence, Polymorphism, and Chromosomal Localization in Chinese Raccoon Dog and Arctic Fox. DNA Cell Biol. **31**: 732-738.

SZCZERBAL I., ROGALSKA-NIZNIK N., SCHELLING C., SCHLAPFER J., DOLF G., SWITONSKI M. 2003. Development of a cytogenetic map for the Chinese raccoon dog (*Nyctereutes procyonoides procyonoides*) and the arctic fox (*Alopex lagopus*) genomes, using canine-derived microsatellite probes. Cytogenet. Genome Res. **102**: 267-71.

SZCZERBAL I., KLUKOWSKA-ROETZLER J., DOLF G., SCHELLING C., SWITONSKI M. 2006. FISH mapping of 10 canine BAC clones harbouring genes and microsatellites in the arctic fox and the Chinese raccoon dog genomes. J. Anim. Breed. Genet. **123**: 337-42.

SZCZERBAL I., NOWACKA-WOSZUK J., RACKA M., KLUKOWSKA-ROETZLER J., SCHELLING C., DOLF G., SWITONSKI M. 2007. Cytogenetic mapping and STR polymorphism of two candidate genes (DRD2 and HTR1D) for behavior traits in four canids. Arch. Tierzucht **50**: 412-417.

WANG H. C., FEDOROFF S. 1972. Banding in human chromosomes treated with trypsin. Nat. New Biol. **235**: 52-54.

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.