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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

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Abstract

Rapid development of the canine marker genome map facilitates genome mapping of other Canidae species. In this study we present chromosomal localization of 18 canine-derived cosmid probes containing microsatellites in the arctic fox (*Alopex lagopus*) and Chinese raccoon dog (*Nyctereutes procyonoides procyonoides*) genomes by the use of fluorescence in situ hybridization (FISH). The chromosome localizations in the arctic fox are in general agreement with data obtained from comparative genome maps of the dog and the fox. However, our studies showed that the order of the loci on some chromosomes was changed during karyotype evolution. Therefore, we suggest that small intrachromosomal rearrangements took place.

The arctic fox (*Alopex lagopus*) and the Chinese raccoon dog (*Nyctereutes procyonoides procyonoides*) belong to the Canidae family. In this family a wide range of chromosome diploid numbers is observed (Wayne et al. 1987) and, moreover, an extensive chromosome and karyotype polymorphism in some species has been described. The dog (*Canis familiaris*) has the highest diploid chromosome number ($2n = 78$), with all autosomes being acrocentric. In contrast, the red fox (*Vulpes vulpes*) has the lowest diploid chromosome number ($2n = 34 + B$) with a variable number (0–8) of supernumerary microchromosomes, described as B chromosomes (Mäkinen et al. 1985b). The arctic fox karyotype is characterized by centric fusion polymorphisms, and animals with 50, 49, and 48 chromosomes occur (Mäkinen et al. 1985a). In the karyotype of this species, 10 chromosome pairs have complete heterochromatic arms, for which size polymorphisms were found (Switonski and Gustavsson 1991). There are two subspecies of the raccoon dog: the Chinese raccoon dog ($2n = 54 + B$) (1–4) and the Japanese raccoon dog ($2n = 38 + B$) (2–5). Both subspecies have variable numbers of large B chromosomes (Mäkinen et al. 1986).

The canine marker genome map is very advanced and includes 1,800 genetic markers (Breen et al. 2001), but a recent report describes an extension of this map to 3,400 loci (Andre et al. 2002). It is known that a majority of the primer sequences of canine microsatellites amplify analogous sequences in the red fox (Fredholm and Wintero 1995; Zajac et al. 2000) and arctic fox (Klukowska et al. in press) genomes. It has also been shown that canine-derived cosmid probes can be successfully used in physical mapping of

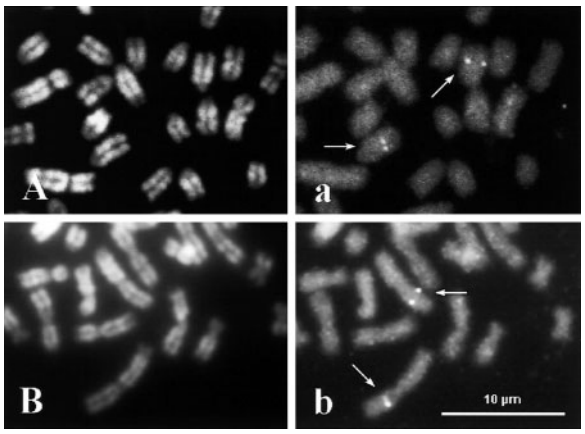

Figure 1. Q-banded partial metaphase spreads of the Chinese raccoon dog **(A)** and the arctic fox **(B)** showing hybridization results with *ZuBeCa5* **(a)** and *ZuBeCa9* **(b)** probes, respectively. The hybridization signals are indicated by arrows.

microsatellite loci in related Canidae species, such as the red fox (Yang et al. 2000) and the arctic fox (Rogalska-Niznik et al. 2000). The aim of the present study was to map a set of 18 microsatellite probes onto the arctic fox and Chinese raccoon dog using fluorescence in situ hybridization (FISH). This study adds new data to the comparative genome map of the dog, red fox, arctic fox, and raccoon dog obtained recently by comparative chromosome painting (Graphodatsky et al. 2001; Yang et al. 1999).

The red fox (known as a farm silver fox), the arctic fox (known as a farm blue fox), and the raccoon dog are fur animals bred in captivity. The fur industry is under pressure by both fashion and animal rights organizations. Therefore studies on housing environment related to improvement of fur animal welfare have become very important (Harri 2000). It is believed that selection on behavior traits may be useful. Therefore development of genome maps may facilitate identification of genes influencing not only fur quality but also behavior traits.

Materials and Methods

Blood samples were collected from farm arctic foxes and Chinese raccoon dogs, and short-term lymphocyte cultures were established. Chromosome slides with metaphases were QFQ-banded prior to hybridization. International chromosome nomenclature for the arctic fox (Mäkinen et al. 1985a) and a nomenclature for the Chinese raccoon dog proposed by Pienkowska et al. (2002) were applied. Eighteen canine-derived cosmid probes containing microsatellites—*ZuBeCa1*, *ZuBeCa2*, *ZuBeCa3*, *ZuBeCa5*, *ZuBeCa6*, *ZuBeCa7*, *ZuBeCa8*, *ZuBeCa9*, *ZuBeCa10*, *ZuBeCa12*, *ZuBeCa13*, *ZuBeCa16*, *ZuBeCa17*, *ZuBeCa20*, *ZuBeCa25*, *ZuBeCa28*, *ZuBeCa29*, and *ZuBeCa30* (Dolf et al. 2000; Ladon et al. 1998; Schelling et al. 1998a,b, 2000; Schläpfer et al. 1998, 1999; Switonski et al. 1998)—were used in the present study. Cosmid DNA (25 ng) was labeled with biotin-16-dUTP (Boehringer Mannheim) using Prime-a-Gene labeling system (Promega). The slides were denatured in 70% formamide/2× SSC for 2 min at 70°C, plunged immediately into ice-cold 70% ethanol,

Table 1. Comparative localization of 18 microsatellite loci in dog, red fox, arctic fox, and Chinese raccoon dog chromosomes

Microsatellite	Red fox (Yang et al. 2000)	Dog		Chinese raccoon dog (this study)	Arctic fox (this study)
		Yang et al. 2000[a]	Breen et al. 1999[b]		
ZuBeCa1	16q31	10q22	11q25.1-25.3	4q14	5q14
ZuBeCa2	1p32	1q36	1q36	7q26	2q24
ZuBeCa3	2p23	9q21	9q21.3	5q11	12q22
ZuBeCa5	5p22	19q17	19q23	9q23	24q15
ZuBeCa6	12q22	5q14	5q14.3	3q12	10q13-14
ZuBeCa7	5q12	18q18	18q24-25.3	17q12	11q11-12
ZuBeCa8	5p22	19q17	19q23	9q23	24q15
ZuBeCa9	15q21	25q16	30q15.1-15.2[c]	2q12	2p21
ZuBeCa10	1q21	30q16	33q15.3[c]	9q23	1q21
ZuBeCa12	2p24-25	9q11-12	9q11.1-14	5q15	12q23
ZuBeCa13	2p14	9q26	9q26.1-26.2	5q15	12q12
ZuBeCa16	5q12	18q18	18q24-25.3	17q12	11q11
ZuBeCa17	1q23	12q13	12q13	14q13	1q21
ZuBeCa20	2p14	9q26	9q26.1-26.2	5q15	12q12
ZuBeCa25	8q12	17q19	17q24	13q12	5p11
ZuBeCa28	12q13	37q14	35q15[c]	15q13	10p12-13
ZuBeCa29	5q12	18q18	18q24-25.3	17q12	11q11
ZuBeCa30	8q21	17q16	17q15	13q15	5p14

[a] Original FISH localization data.

[b] The localizations following chromosome nomenclature endorsed by the International Society of Animal Genetics (2000).

[c] According to the data on reciprocal comparative chromosome painting, with the use of human and canine probes, canine chromosomes 25, 30, and 37 in Yang's nomenclature correspond to chromosomes 30, 33, and 35, respectively, in Breen's nomenclature (Graphodatsky et al. 2000).

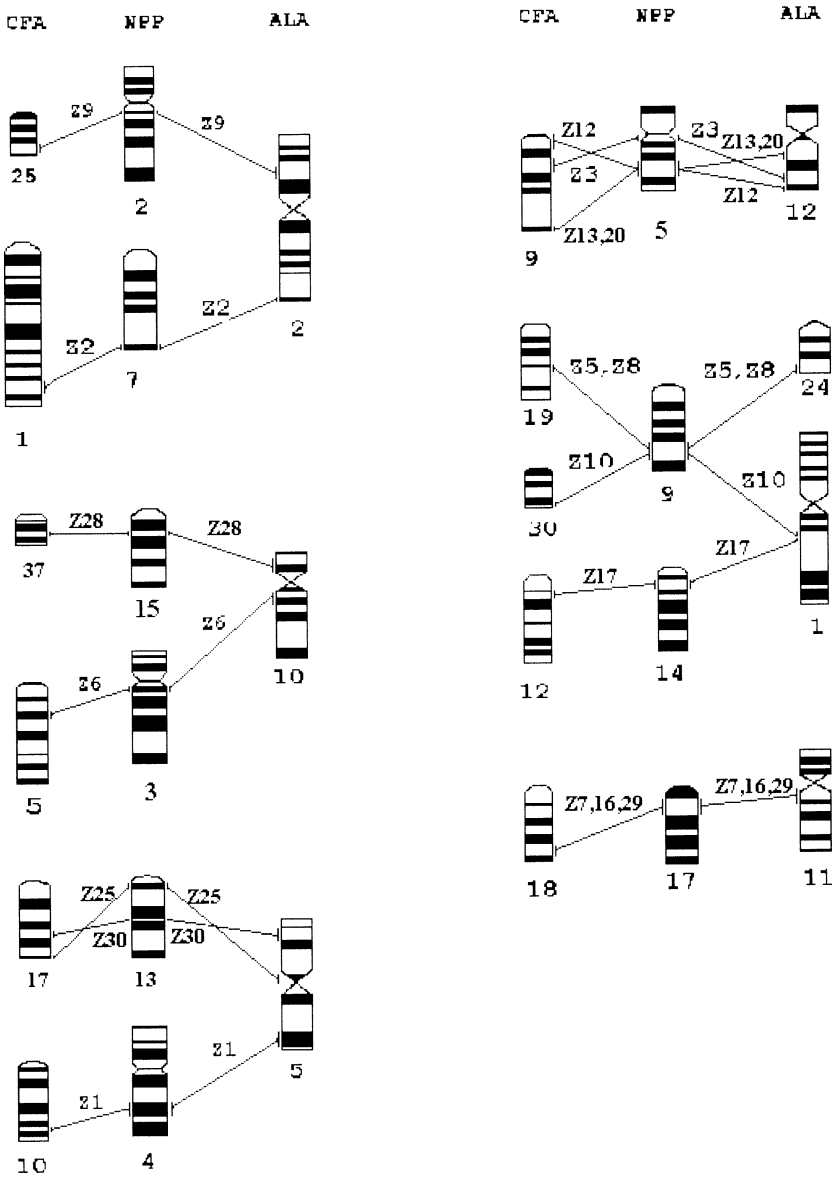

Figure 2. Comparative localization of the canine microsatellites of dog (CFA), Chinese raccoon dog (NPP), and arctic fox (ALA) chromosomes.

dehydrated, and air dried. Biotin-labeled cosmid probes were denatured by heat at 70°C for 10 min, preannealed for 15 min at 37°C, applied onto slides (12 ng DNA per 18 mm × 18 mm), and incubated overnight. Signal detection and amplification, using avidin-FITC and anti-avidin (Vector Laboratories), was applied. Staining was performed with propidium iodide (200 ng/ml). Image capturing and processing were performed with a fluorescence microscope (Nikon E600 Eclipse) equipped with a CCD camera (Hammatsu) driven by computer-aided software (Lucia).

Results and Discussion

Eighteen canine-derived cosmid probes were successfully assigned to the chromosomes of the arctic fox and the Chinese raccoon dog by FISH. Localizations of two cosmid probes (*ZuBeCa5* and *ZuBeCa9*) are shown in Figure 1. A summary of the chromosomal assignments in both investigated species, along with previous localizations of the same probes localized earlier onto chromosomes of the dog and the red fox by Yang et al. (2000), is presented in Table 1. We also show the localizations which follow the chromosome nomenclature of Breen et al. (1999), endorsed by the International Society of Animal Genetics (ISAG) conference (Minneapolis, MN; 2000). Moreover, comparative localization of these microsatellites in the dog, Chinese raccoon dog, and arctic fox chromosomes is shown in Figure 2.

Comparative chromosome analysis of the dog, red fox, arctic fox, and Japanese raccoon dog by the use of reciprocal chromosome painting suggests that the ancestor of the

Canidae family had a low chromosome diploid number, similar to the Japanese raccoon dog karyotype (Graphodatsky et al. 2001). Until now, three comparative chromosome maps existed: dog versus red fox (Yang et al. 1999), dog versus arctic fox (Graphodatsky et al. 2000), and dog versus Japanese raccoon dog (Graphodatsky et al. 2001). So far the Chinese raccoon dog has not been included in such studies. The Chinese and Japanese raccoon dog karyotypes were compared by banding techniques; however, the results were not concordant (Mäkinen et al. 1986; Ward et al. 1987). Correspondence between karyotypes of both subspecies can be predicted from localization of locus-specific probes (*ZuBeCa* markers) on chromosomes of the dog and the Chinese raccoon dog.

The presented assignments in the arctic fox genome are in agreement with the report on comparative chromosome maps of the dog, red fox, and arctic fox (Graphodatsky et al. 2000), in which 42 conserved autosome segments were identified. However, it should be emphasized that the chromosome painting approach may not detect intrachromosomal rearrangements. An interesting example concerns dog chromosome 9 (CFA9), Chinese raccoon dog 5 (NPP5), and arctic fox chromosome 12 (ALA12). ALA12q is painted by the CFA9 whole chromosome painting probe. Our study suggests that a fragment of NPP5q was inverted in ALA12q. The centromere position of canine CFA9 has been conserved according to the Chinese raccoon dog chromosome, but it is oriented toward the telomeric region of ALA12. Moreover, we found that three closely localized microsatellites on NPP5q (*ZuBeCa12*, *ZuBeCa13*, and *ZuBeCa20*) reside on two different fragments of CFA9 and ALA12. A rearranged order of the loci (*ZuBeCa25* and *ZuBeCa30*) was also observed on CFA17 and ALA5p when compared with NPP13. This also was probably caused by an inversion.

The present study extends the physical genome map of the arctic fox to 21 loci, including those previously mapped by Rogalska-Niznik et al. (2000), and gives the first data about the physical map of the Chinese raccoon dog genome. Our data indicate that in the karyotype evolution of these species, intrachromosomal rearrangements also occurred. Further development of these maps will not only support studies on karyotype evolution in Canidae, but can also be used to identify genes important for selective breeding.

References

Andre C, Guyon R, Kim L, Hitte C, Lorentzen T, Cadieu E, Parker HG, Quignon P, Lowe JK, Renier C, Gelfenbeyn B, Gloux S, Vignaux F, Galibert F, and Ostrander EA, 2002. Mb resolution hybrid map of the canine genome. In: XXVIII International Conference on Animal Genetics, Gottingen, Germany, August 11–15, 2002. Available at http://www.gwdg.de/~bbrenig/ISAG2002.html.

Breen M, Bullerdiek J, and Langford C, 1999. The DAPI banded karyotype of the domestic dog (*Canis familiaris*) generated using chromosome-specific paint probes. Chromosome Res 7:401–406.

Breen M, Jouquand S, Renier C, Mellersh CS, Hitte Ch, Holmes NG, Cheron A, Suter N, Vignaux F, Bristow AE, Priat C, McCann E, Andre C, Boundy S, Gitsham P, Thomas R, Bridge WL, Spriggs HF, Ryder EJ, Curson A, Sampson J, Ostrander EA, Binns MM, and Galibert F, 2001. Chromosome-specific single-locus FISH probes allow anchorage of an 1800-marker integrated radiation-hybrid/linkage map of the domestic dog genome to all chromosomes. Genome Res 11:1784–1795.

Dolf G, Schelling C, Stahlberger-Saitbekova N, Fu B, Schläpfer J, and Yang F, 2000. Seven cosmid-derived canine microsatellites. Anim Genet 31:411–412.

Fredholm M and Wintero AK, 1995. Variation of short tandem repeats within and between species belonging to the Canidae family. Mamm Genome 6:11–18.

Graphodatsky AS, Yang F, O'Brien PCM, Perelman P, Milne BS, Serdukova N, Kawada SI, and Ferguson-Smith MA, 2001. Phylogenetic implications of the 38 putative ancestral chromosome segments for four canid species. Cytogenet Cell Genet 92:243–247.

Graphodatsky AS, Yang F, O'Brien PCM, Serdukova N, Milne BS, Trifonov V, and Ferguson-Smith MA, 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.

Harri M, 2000. Effect of housing environment on fur animal welfare. Scientifur 24:3–10.

Klukowska J, Strabel T, and Switonski M, in press. Microsatellite polymorphism and genetic distances between the dog, red fox and arctic fox. J Anim Breed Genet.

Ladon D, Schelling C, Dolf G, Switonski M, and Schläpfer J, 1998. The highly polymorphic canine microsatellite ZuBeCa6 is localized on canine chromosome 5q12-q13. Anim Genet 29:466–467.

Mäkinen A, Gustavsson I, Switonski M, and Takagi N, 1985a. The standard karyotype of the blue fox (*Alopex lagopus* L.). Hereditas 103:33–38.

Mäkinen A, Kuokkanen MT, Isakova GK, Takagi N, and Gustavsson I, 1985b. The standard karyotype of the silver fox (*Vulpes fulvus* Desm.). Hereditas 103:171–176.

Mäkinen A, Kuokkanen MT, and Valtonen M, 1986. A chromosome-banding study in the Finnish and the Japanese raccoon dog. Hereditas 105:97–105.

Pienkowska A, Szczerbal I, Mäkinen A, and Switonski M, 2002. G/Q-banded chromosome nomenclature of the Chinese raccoon dog (*Nyctereutes procyonoides procyonoides*). Hereditas 137:75–78.

Rogalska-Niznik N, Schelling C, Dolf G, Schlapfer J, Pienkowska A, and Switonski M, 2000. Localisation of three canine microsatellites on the arctic fox (*Alopex lagopus*) chromosomes. Med Vet Czech 45:314–316.

Schelling C, Dolf G, Schläpfer J, Switonski M, and Ladon D, 1998a. Assignment of the canine microsatellite ZuBeCa5 to canine chromosome 19q7. Anim Genet 29:409–410.

Schelling C, Schläpfer J, Vennos C, Stahlberger-Saitbekova N, and Dolf G, 2000. Seven novel cosmid-derived canine microsatellites. Anim Genet 31:77.

Schelling C, Stranzinger G, Dolf G, Schläpfer J, and Switonski M, 1998b. Assignment of the canine microsatellite ZuBeCa1 to canine chromosome 10q22-q24. Anim Genet 29:398.

Schläpfer J, Dolf G, Saitbekova N, and Schelling C, 1999. Twelve novel cosmid-derived canine microsatellites. Anim Genet 30:387–388.

Schläpfer J, Switonski M, Schelling C, Stranzinger G, Gaillard C, and Dolf G, 1998. The highly polymorphic canine microsatellite ZuBeCa2 is localized on canine chromosome 1q210-211. Anim Genet 29:329–330.

Switonski M and Gustavsson I, 1991. Centric-fusion translocation and whole-arm heterochromatin in the karyotype of the blue fox (*Alopex lagopus* L.): synaptonemal complex analysis. Cytogenet Cell Genet 57:1–8.

Switonski M, Schelling C, Dolf G, Stranzinger G, and Schläpfer J, 1998. Assignment of the canine microsatellite ZuBeCa3 to canine chromosome 9q21-Q22. Anim Genet 29:326–327.

Ward OG, Wurster-Hill DH, Ratty FJ, and Song Y, 1987. Comparative cytogenetics of Chinese and Japanese raccoon dogs, *Nyctereutes procyonoides*. Cytogenet Cell Genet 45:177–186.

Wayne RK, Nash WG, and O'Brien SJ, 1987. Chromosomal evolution of the Canidae. I. Species with high diploid numbers. Cytogenet Cell Genet 44:123–133.

Yang F, Milne BS, Schelling C, Dolf G, Schläpfer J, Switonski M, Ladon D, Pienkowska A, Bosma AA, Sargan DR, and Ferguson-Smith MA, 2000. Chromosome identification and assignment of dog DNA clones in the dog using a red fox and dog comparative map. Chromosome Res 8:93–100.

Yang F, O'Brien PCM, Milne BS, Graphodatsky AS, Solanky N, Trifonov V, Rens W, Sargan D, and Ferguson-Smith MA, 1999. A complete comparative chromosome map for the dog, red fox, and human and its integration with canine genetic maps. Genomics 62:189–202.

Zajac M, Klukowska J, Slomski R, and Switonski M, 2000. Polymorphism of nine canine-derived microsatellites in farm silver foxes (*Vulpes fulvus*). J Appl Genet 41:43–50.

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