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# The proto-oncogene C-KIT maps to canid B-chromosomes

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## Abstract

Plant and animal karyotypes sometimes contain variable elements, that are referred to as additional or B-chromosomes. It is generally believed that B-chromosomes lack major genes and represent parasitic and selfish elements of a genome. Here we report, for the first time, the localization of a gene to B-chromosomes of mammals: red fox (*Vulpes vulpes*) and two subspecies of raccoon dog (*Nyctereutes procyonoides*). Identification of the proto-oncogene C-KIT on B-chromosomes of two *Canidae* species that diverged from a common ancestor more than 12.5 million years ago argues against the current view of B-chromosomes. Analyses of fox B-chromosomal C-KIT gene from a flow-sorted fox B-chromosome-specific library revealed the presence of intron–exon boundaries and high identity between sequenced regions of canine and fox B-chromosomal C-KIT copies. Identification of C-KIT gene on all B-chromosomes of two canid species provides new insight into the origin and evolution of supernumeraries and their potential role in the genome.

## Introduction

B-chromosomes are small dispensable supernumerary chromosomes found in certain species, in addition to the standard complement of A-chromosomes. The number of B-chromosomes (Bs) per cell may vary among different tissues, individuals, and populations. They do not pair and recombine with any A-chromosomes at meiosis and are often heterochromatic. Although it is generally believed (Jones & Rees 1982, Camacho *et al.* 2000, Dhar *et al.* 2002) that most B-chromosomes lack major genes, this hypothesis has not been rigorously tested (Camacho *et al.* 2000).

*Canidae* species exhibit at least two unusual phenomena of karyotype evolution. First, their chromosomes are extremely rearranged in comparison with other mammals (Yang *et al.* 1999, 2000, Graphodatsky *et al.* 2000, 2001) (only rodent species have more reshuffled karyotypes). Second, within the Carnivora order, only some canids were found to possess supernumerary chromosomes.

B-chromosomes of canids were studied using both conventional and molecular cytogenetic

methods (Beliaev *et al.* 1974, Wurster-Hill *et al.* 1988, Yang *et al.* 1999, Trifonov *et al.* 2002). Fox karyotypes contain from 0 to 7 very small additional elements (Beliaev *et al.* 1974), which mostly consist of fox centromeric heterochromatin (Yang *et al.* 1999). Two subspecies of raccoon dog (Chinese and Japanese raccoon dog) have the biggest acrocentric Bs in the *Canidae* (from 2 to 8 per genome), equal in size to autosome 17. Raccoon dog B-chromosomes were found to have complex molecular structure, comprising two types of heterochromatin, interstitial telomere repeats and inactive ribosomal genes (Wurster-Hill *et al.* 1988, Trifonov *et al.* 2002, Szczerbal & Switonski 2003).

Comparative mapping within the *Canidae* family with canine BAC clones by fluorescence *in-situ* hybridization (FISH) did not reveal any positive signals on B-chromosomes of canids (Switonski *et al.* 2004). In the current study, a BAC clone containing the canine orthologue of the proto-oncogene C-KIT was used to assign this gene to dog, red fox, arctic fox, and raccoon dog chromosomes. The gene was assigned by FISH, as expected, to canid chromosomal regions homologous to human HSA 4q11-q12. Unexpectedly, additional sites of localization for C-KIT on B-chromosomes of the red fox and the raccoon dog were revealed.

The proto-oncogene C-KIT encodes a transmembrane tyrosine kinase, the function of which is critical for proliferation and cell differentiation of haematopoietic, melanoblast and primordial germ cells (Ashman 1999). Most mastocytomas and intestinal stromal tumours detected in human, mouse, dog and rat are caused by C-KIT mutations (Heinrich *et al.* 2002). The pigmentation disorders in a broad range of species, including fish, mouse, pig, goat and human, are associated with different C-KIT mutations (Chabot *et al.* 1988, Fleishman *et al.* 1991, Marklund *et al.* 1998). The human C-KIT gene contains 21 coding exons (Vandenbark *et al.* 1992) and a 1100-bp region of C-KIT mRNA is homologous to the middle part of the Hardy-Zuckerman 4 feline sarcoma virus genome (Besmer *et al.* 1986).

Here we report the localization of C-KIT proto-oncogene on B-chromosomes of two canid species – red fox (*Vulpes vulpes*) and raccoon dog (*Nyctereutes procyonoides*) by FISH. It is the first finding of a known autosomal gene on the mammalian additional chromosomes. In addition, we detected the C-KIT presence on all the canid Bs investigated and revealed unexpected conservancy between sequenced regions of canine C-KIT gene and fox B-chromosomal C-KIT copy. These findings argue in favour of a potential role of B-chromosomes in the genome and give new insights into their origin and evolution.

## Materials and methods

### *Slide and probe preparation*

Metaphase chromosomal spreads were prepared from primary fibroblast and peripheral blood lymphocytes cultures and bone marrow of dog (*Canis familiaris*), fox (*Vulpes vulpes*), Arctic fox (*Alopex lagopus*), Chinese raccoon dog (*Nyctereutes procyonoides procyonoides*) and Japanese raccoon dog (*Nyctereutes procyonoides viverrinus*) as described previously (Yang *et al.* 1999, Graphodatsky *et al.* 2000, 2001, Trifonov *et al.* 2002).

Fox and raccoon dog B-chromosome specific probes were obtained by flow sorting and microdissection, as described (Yang *et al.* 1999, Trifonov *et al.* 2002).

### *Canine BAC library screening*

The RPC181 canine 8.1-fold BAC library (http://www.chori.org/bacpac/mcanine81.htm) was screened to identify BAC clones containing canine C-KIT, KDR and PDGFRA genes using canine gene specific probes according to the standard protocol (Kukekova *et al.* 2003).

### *FISH*

Fluorescence *in-situ* hybridization was performed using a standard protocol (Yang *et al.* 1999, Graphodatsky *et al.* 2000). Canine BAC clones containing C-KIT (104D-16, 265L-22 (AC142093)), KDR (67P-13) and PDGFRA (311A-23) were labelled by NICK-translation kit (Invitrogen, CA). PCR fragment (spanning C-KIT intron 3) was amplified with primers KIT5/KIT6 (Table 1A) and labelled in additional PCR cycles.

*PCR amplification and screening flow-sorted fox B-chromosome-specific library*

Fox genomic DNA and flow-sorted fox B-chromosome-specific library (B-chromosome library) were amplified with canine primers: PDGF1/PDGR1, KITBAC1/KITBAC2, and KDRF3/KDRR3 (Table 1 A) using the PCR program: 96°C for 2 min; 30 cycles of 96°C (20 s), 58°C (20 s), and 72°C (20 s); and a final extension at 72°C for 5 min. PCR products were analysed on a 1.2% agarose gel.

A C-KIT exon scan in the fox B-chromosome library was performed using canine C-KIT primers (Table 1B) and the same PCR program. Canine sequences corresponding to C-KIT genomic DNA from BAC clone 265L-22 were identified through the blast search with canine C-KIT cDNA

*Table 1.* Primers used for probe preparation and C-KIT exon scaning.

| Primer name | Primer sequence | Primer position on canine genomic DNA | Reference canine sequence |
|---|---|---|---|
| **A. Primers used for probe preparation and amplification of PDGFRA and KDR genes** | | | |
| KITBAC1 | TCTGTTGATGGAGGGGTGACTTG | 42498–42520 | AC142093 |
| KITBAC2 | GCCACTGTCACCTACTGCTTTTCC | 43468–43491 | -//- |
| KITF2 | GTATGACATCATGAAGACGTGCT | 83216–83238 | -//- |
| KITR2 | GAATTGATCCGCACGGAATGGTC | 84430–84452 | -//- |
| KIT5 | TCTGTTGATGGAGGGGTGACTTG | 42193–42216 | -//- |
| KIT6 | GCCACTGTCACCTACTGCTTTTCC | 43503–43527 | -//- |
| PDGF1 | CAACCACAGTCTCGTTGGTCTGT | 156–178 | Trace: 283822970 G630P678773RA1 |
| PDGR1 | AGCATTGCAAGAGGCAACACTGA | 554–576 | -//- |
| KDRF3 | TTACAGTCATGGTTCCCAGCATCT | 196–219 | Trace: 302341894 G630P85743FC11 |
| KDRR3 | TGAATACCCACATATCTGTCCTGA | 578–601 | -//- |
| BGLF1 | CAATCTGCTTGCAGCAGCAGAGA | 177825–177847 | AC127475 |
| BGLR1 | CAGTGAAGCTCACTGAGCTTAGCA | 177324–177347 | -//- |
| **B. Primers used for C-KIT exon scan from fox B chromosome specific library** | | | |
| KIT4F2 | AAAGCAGTAGGTGACAATGGCA | 43471–43492 | AC142093 |
| KIT4R2 | AGACAGCCCCACCTCAGACAGA | 43796–43817 | -//- |
| KIT5LF3 | GTAACACATGAACACTCCAGAATCA | 48084–48107 | -//- |
| KIT5LR3 | GAAGTGTTCCAATGACAGACTTGT | 48353–48377 | -//- |
| KIT5MLF1 | TAGCTGGCATCATGGTGACTT | 48284–48304 | -//- |
| KIT5MLR2 | ACAACTTCAGTTACAAGTTTCCAC | 48646–48669 | -//- |
| KIT6LF2 | ACCTGACCCCAGTCCGCTATCCT | 52232–52254 | -//- |
| KIT6LR2 | GCTCCGGTTTGGGATATGCCTCA | 52420–52442 | -//- |
| KIT6MLF1 | TGGAGAGAATGTGGATCTGA | 52390–52409 | -//- |
| KIT6MLR1 | CCTCGTATGCCAGGTGTCCT | 52617–52636 | -//- |
| KIT7F2 | CTGTGGACAAGATGCGCACCCGA | 55524–55546 | -//- |
| KIT7R2 | AGCTGTTCCAAGCACGAGCCACA | 55768–55790 | -//- |
| KIT8F2 | TGGTGAGGTGTTCCAGCAGTCT | 69688–69709 | -//- |
| KIT8R2 | TCTGAAGTTCAACTACCCTGCT | 69901–69922 | -//- |
| KIT11F1 | CTTACCTACAAGTATCTACAGGTA | 73072–73095 | -//- |
| KIT11R1 | CCTAAAGTCATTGTTACACGTACA | 73351–73374 | -//- |
| KIT12F1 | TGATAGATTCCTCATAAAGCACCT | 73446–73469 | -//- |
| KIT12R1 | TGAGGGCATGCAGCCACCTGCAGA | 73710–73733 | -//- |
| KIT17F1 | GTGCACCGAAGAGTTTCAAGGCA | 78782–78804 | -//- |
| KIT17R1 | GGTACTCACGTTTCCTTTGACCA | 79034–79056 | -//- |
| KIT18F1 | CAGCTGGAGCGATGTCTGTAGGA | 82434–82456 | -//- |
| KIT18R1 | CTGCTTCCTGAGGCACAAACGGT | 82747–82769 | -//- |
| KIT21F1 | GTGATCCTGAGAATGCAGATGT | 84293–84314 | -//- |
| KIT21R1 | TCCTCCCTTGCTCCAAGTCTGT | 84764–84785 | -//- |

(AF0044249). To define the exon–intron structure of canine C-KIT gene, two sequences were aligned using Cornell server (http://ser-loopp.tc.cornell.edu/cbsu/align2genome.htm). C-KIT primers were designed based on canine intron and exon sequences. Sequencing of PCR products amplified on fox DNA was done using ABI3730 Genetic Analyzer (Applied Biosystems Inc., CA) with ABI Big Dye kit using a standard protocol.

*Southern and dot blot hybridization*

DNA of fox, dog and Chinese raccoon dog were isolated from spleen by standard procedures (Sambrook *et al.* 1989).

Dot-hybridization of fox, dog and raccoon dog DNA was performed on membrane Hybond-N (Amersham Pharmacia Biotech, UK). A dog C-KIT fragment was amplified with primers KIT5/KIT6, labelled with [$\alpha$-$^{32}$P] dATP and used as a probe.

For Southern analysis, fox and dog DNA was digested with restriction enzymes, EcoRI and HindIII, 10 μg of each DNA sample was electrophoresed on a 1.0% agarose gel and transferred to nylon membrane Hybond N. Hybridization was performed at 42°C with $10^6$ cpm/ml [$\alpha$-$^{32}$P] dCTP-labelled canine C-KIT probe obtained by PCR amplification with primers KITF2/KITR2 (Table 1A). Hybridization of the Southern blot with a control β-globin probe obtained by PCR amplification with primers BGLF1/BGLR1 (Table 1A) was performed under the same conditions. Southern images were scanned with a Fuji Bio-Imaging Analyzer and scored by MacBAS software.

*Semiquantitative PCR analyses*

The modified method of the semiquantitative PCR (Marone *et al.* 2001) was used. Equal amounts of DNA (80 ng) isolated from one dog and three foxes (with different numbers of Bs) were amplified with C-KIT (KITBAC1/KITBAC2) and gamma tubulin (AKGTF2/AKGTR2) primer pairs using the same PCR program as before. PCR aliquots were collected after the 20th, 25th, 30th and 35th cycles and analysed in 6% polyacrylamide gel. The intensity of the C-KIT band (437 bp) and the internal control (gamma tubulin, 288 bp) between different samples was scored by eye.

## Results and discussion

*Localization of C-KIT on the chromosomes of* Canidae

BAC clones 104D-16 and 265L-22, containing partial and full sequence of the canine C-KIT gene was used to localize this gene on dog and fox chromosomes. BAC 265L-22 sequence (Accession no. AC142093) includes five ordered pieces which span 146806 bp. BLAST search with canine C-KIT cDNA (AF044249) revealed the position of the C-KIT gene in 265L-22 (3255–84696 bp). BLAST of the sequences surrounding the C-KIT gene did not reveal any other known genes in this BAC. C-KIT was assigned by FISH, as expected, to homologous regions on canine chromosome 13q22–23 and red fox chromosome 2p12 (Figure 1a, b). Unexpectedly, FISH also revealed additional signals on B-chromosomes of the red fox (Figure 2a, b, c). Forty other canine BAC clones used in the current study were assigned only to fox autosomes and did not reveal any signals on Bs (manuscript in preparation).

To investigate C-KIT localization in genomes of other canids with and without Bs, we hybridized the C-KIT probe onto chromosomes of Chinese raccoon dog, Japanese raccoon dog and arctic fox (another canid species that lacks Bs). The hybridization results demonstrate that the C-KIT probe was localized to the proximal part of the long arm of Chinese raccoon dog chromosome 6, the proximal part of Japanese raccoon dog chromosome 2q and arctic fox chromosome 11p13 (Figure 1c, d), i.e. regions homologous to human 4q11–12, where C-KIT has been previously located (Yarden *et al.* 1987). In both Chinese and Japanese raccoon dog, signals were also revealed on the distal part of all Bs. In Chinese raccoon dog, multiple signals were detected in several B-chromosomal segments (Figure 2c, d, e).

Metaphase chromosomal spreads from different individuals of red fox and raccoon dog with the variable number of Bs were hybridized with the

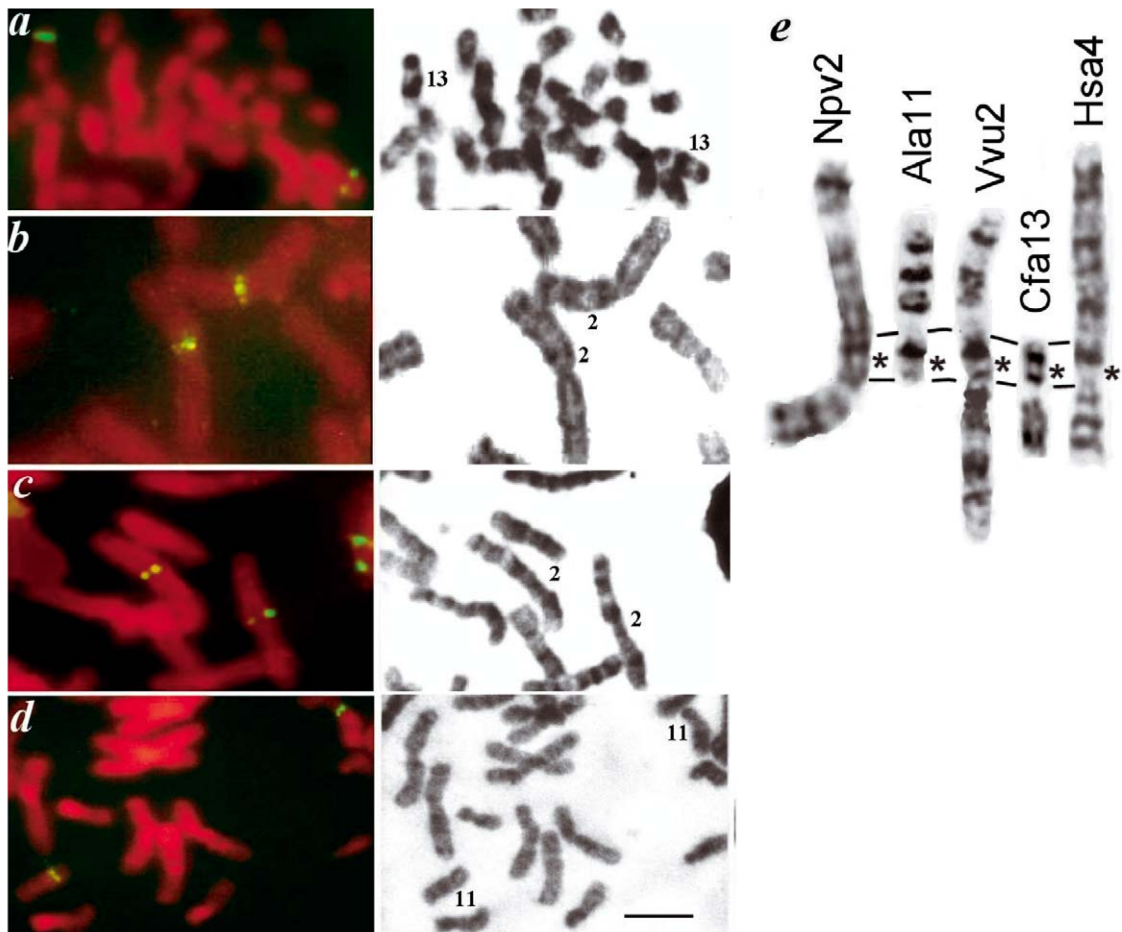


*Figure 1.* Localization of C-KIT probe (left) onto G-banded (right) A-chromosomes of *Canidae* species: dog (***a***), red fox (***b***), Japanese raccoon dog (***c***), Arctic fox (***d***). (***e***) G-banding comparison of chromosomal segments of conserved synteny between the human chromosome 4 (*Hsa 4*), dog chromosome 13 (*Cfa13*), fox chromosome 2 (*Vvu2*), Arctic fox chromosome 11 (*Ala11*) and raccoon dog chromosome 2 (*Npv2*) defined by chromosome painting. *denotes the sites of C-KIT probe localization. Scale bar = 10 μm.

C-KIT BAC probe in an attempt to identify Bs without C-KIT. The ten red foxes studied herein have 2–7 B-chromosomes, the two Japanese raccoon dogs have 3–5 B-chromosomes and two Chinese raccoon dogs have 2 and 3 B-chromosomes. In all cells so far examined, FISH has revealed the C-KIT gene on all B-chromosomes (Figure 2).

### *Localization of C-KIT neighbouring genes*

The presence of autosomal genes on the B-chromosomes would favour the theory of an autosomal origin of additional elements. If B-chromosomes bearing C-KIT originated from a large autosomal segment, they might contain some other genes near to the C-KIT sequences. C-KIT is adjacent on autosomes to the genes KDR (kinase insert domain receptor) and PDGFRA (platelet-derived growth factor receptor) in all mammals so far investigated (http://www.ncbi.nlm.nih.gov). We localized KDR and PDGFRA using canine BAC clones as probes on the fox autosomes (revealing, as predicted, co-localization with C-KIT) but detected no signals on B-chromosomes. PCR screening of flow-sorted fox B-chromosome-specific library (Sargan *et al.* 2000) revealed the presence of C-KIT but failed to detect KDR and PDGFRA genes on fox B-chromosomes (Figure 3).

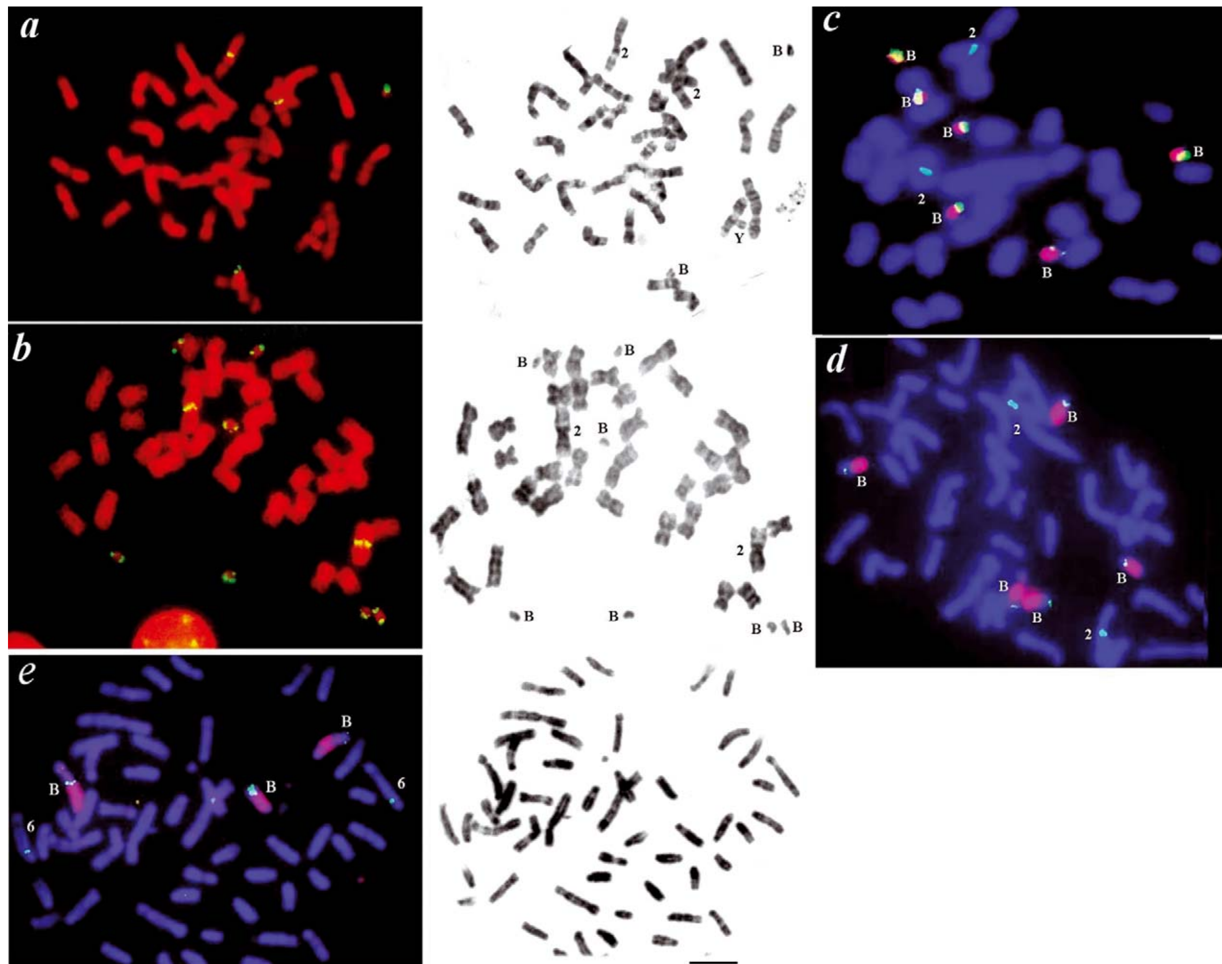


*Figure 2.* Examples of FISH with the C-KIT probe. (***a***) FISH of the C-KIT probe (left) onto G-banded (right) chromosomes of red fox male specimen with 2 Bs. (***b***) FISH of the C-KIT probe (left) onto G-banded (right) chromosomes of red fox female specimen with 7 Bs. (***c***) FISH of the C-KIT probe (green) and B-chromosome painting probe (red) onto chromosomes of red fox female specimen with 6 Bs. (***d***) FISH of the C-KIT probe (green) and B-chromosome painting probe (red) onto chromosomes of Japanese raccoon dog specimen with 5 Bs. (***e***) FISH of the C-KIT probe (green) and B-chromosome painting probe (red) onto chromosomes of Chinese raccoon dog specimen with 3 Bs (left). DAPI-inverted image (right). Scale bar = 10 μm.

### *Exon–intron boundaries of C-KIT on fox B-chromosomes*

One might propose that B-chromosomes are derived from retroviral elements (homology with Hardy-Zuckerman 4 feline sarcoma virus strongly suggests this proposition), in which case B-chromosomes should lack all introns. To test this hypothesis, we undertook PCR amplification of C-KIT exons from the fox B-chromosome library using canine intron-specific primers (Table 1B). Ten C-KIT exons (4, 5, 6, 7, 8, 11, 12, 17, 18, 21) were sequenced and the exon–intron boundaries were observed as was expected. In addition, intron 3 was located on fox Bs by FISH with a labelled PCR-fragment. The presence of C-KIT introns in B-chromosomes indicates that the copy of C-KIT on additional chromosomes is not Hardy-Zukerman 4 feline sarcoma virus derived.

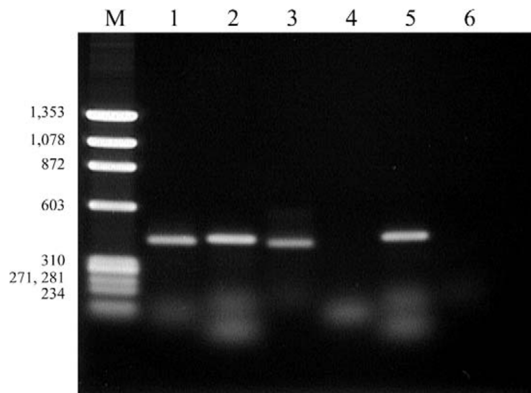


*Figure 3.* PCR amplification of PDGFRA, C-KIT and KDR genes from fox genomic DNA and fox B-chromosome library. Lines 1–3 represent PCR on fox genomic DNA; Lines 4–6 represent PCR on DNA from B-chromosome library. Lines 1, 4 show the PCR results with PDGFRA primers, lines 2, 5 – with C-KIT primers, and lines 3, 6 – with KDR primers.

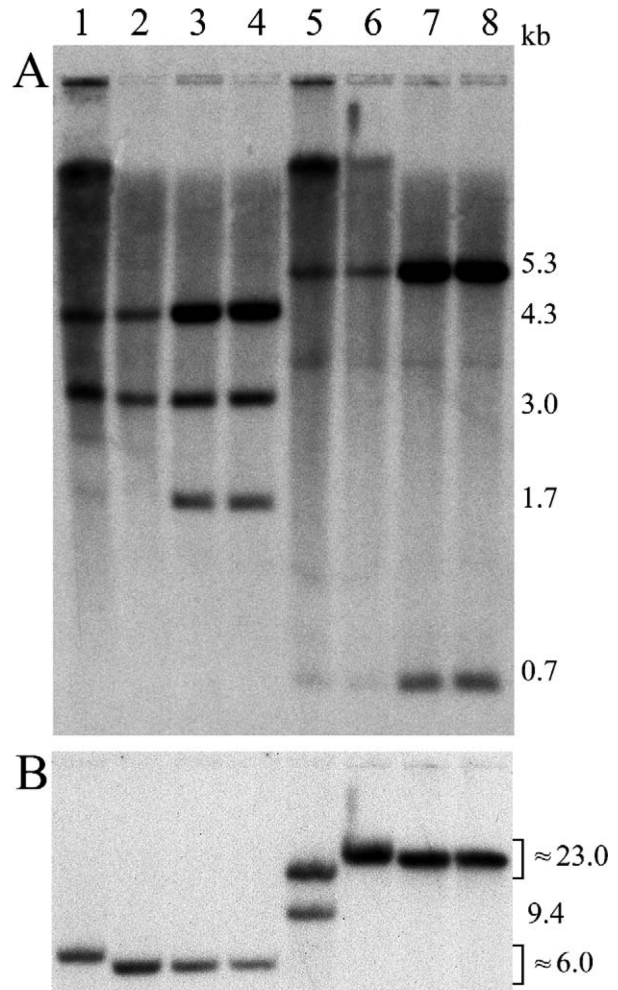


*Figure 4.* Southern blot of dog and fox genomic DNA hybridized with (***a***) C-KIT probe. Lines 1 and 5 contain dog gDNA; lines 2 and 6 contain gDNA from mosaic fox with 0–1 B-chromosomes; lines 3 and 7 contain gDNA from fox with 4 B-chromosomes; lines 4 and 8 contain gDNA from a mosaic fox with 3–7 B-chromosomes. DNA samples were digested: lines 1–4 with EcoR1, lines 5–8 with HindIII. (***b***) Same DNA blot rehybridized with the β-globin probe.

### *Correlation of C-KIT copy number and a number of B-chromosomes in fox*

To estimate the correlation of B-chromosomes and C-KIT copy number, we performed Southern and dot hybridization with dog, fox, and raccoon dog genomic DNA, and semi-quantitative PCR. (Figures 4 & 5). Southern blot hybridization with C-KIT intron 20 (KITF2/KITR2) and β-globin probes was performed on DNA from three foxes with different numbers of B-chromosomes and a dog (Figure 4 A, B). In the control experiment with β-globin probe, the signal intensity was measured on the lines 5, 6, 7, 8. The variation of the signal intensity among lines did not exceed 16% (on line 5 (dog DNA), both β-globin bands were analysed together). The difference in intensity of C-KIT signals between line 5 (dog) and line 6 (fox with 0–1 Bs) was 20%. The intensity of C-KIT signal on line 7 (fox with 4 Bs) was 2.6 times higher than the intensity of the C-KIT signal on line 6 (fox with 0–1 Bs). The C-KIT signal on line 8 (fox with 3–7 Bs) was 2.9 times stronger than on line 6 (fox with 0–1 Bs). The positive correlation between C-KIT copy number and the number of Bs was also observed in dot hybridization and semiquantitative PCR experiments (Figure 5).

### *Origin and evolution of* Canidae *Bs*

The molecular cytogenetic characterization of fox and raccoon dog B-chromosomes has been reported previously (Wurster-Hill *et al.* 1988, Yang *et al.* 1999, Trifonov *et al.* 2002). B-chromosomes of the red fox were isolated by flow sorting to generate a B-specific chromosomal paint probe that revealed heterochromatin homology with autosomal centromeric regions and

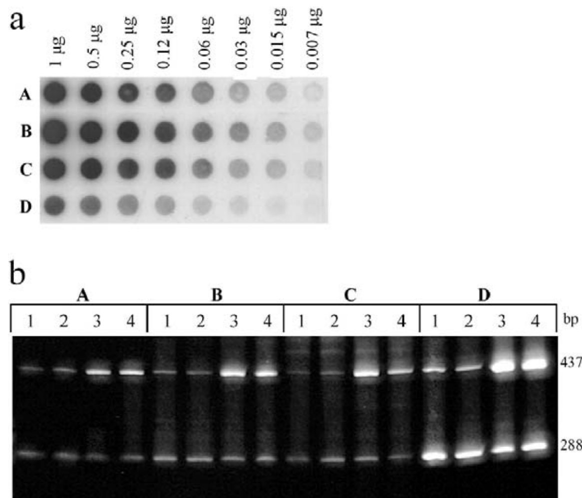


*Figure 5.* (**a**) Dot hybridization of C-KIT intron 3 with different gDNA quantities of Chinese raccoon dog with 3 Bs (***A***), fox with 7 Bs (***B***), fox with 1 B (***C***), dog (***D***). (**b**) Semiquantitative PCR on dog and fox genomic DNA with C-KIT and gamma tubulin primers. The aliquots (1–4) were collected after the 20th (***A***), 25 (***B***), 30 (***C***) and 35 (***D***) cycles. Line 1 contains PCR product amplified on dog genomic DNA; line 2 contains PCR product amplified on gDNA from fox with 0–1 B-chromosomes; line 3 contains PCR product amplified on gDNA from fox with 4 B-chromosomes; line 4 contains PCR product amplified on gDNA from fox with 3–7 B-chromosomes. The 437-bp band corresponds to C-KIT product and 288-bp band corresponds to gamma tubulin product.

interstitial heterochromatin blocks (Yang *et al.* 1999). The whole chromosomal probe derived from a single B-chromosome painted all other Bs suggesting a similar composition of fox additional elements. A centromere-specific repeat was isolated from fox genomic DNA (Potapov *et al.* 1987) and its presence on fox Bs was detected by FISH (unpublished data).

B-chromosomes of the raccoon dog have been studied using GTG-, C-banding, Ag-staining and FISH with a telomere repeat probe. C-banding revealed individual patterns that varied between animals but were consistent within an animal. Clusters of telomeric sequences were revealed along the length of B-chromosomes (Wurster-Hill *et al.* 1988). We generated Chinese raccoon dog and Japanese raccoon dog B-chromosome specific libraries using microdissection. These probes were localized on B-chromosomes of both subspecies but gave very faint signals on autosomes and sex chromosomes (Trifonov *et al.* 2002, our data). The large size of raccoon dog Bs has allowed us to microdissect the specific segments of B-chromosomes (in both subspecies) and we detected that raccoon dog Bs consist of at least two types of B-specific heterochromatin, localized in the same order (Trifonov *et al.* 2002, our data). Polymorphism in block size among the chromosomes was evident. We suggest that all raccoon dog Bs could have originated from one initial B-chromosome with subsequent uneven amplification of different DNA sequences in different Bs (Trifonov *et al.* 2002). Inactive NOR-like sequences were revealed within the proximal half and at the telomeres of raccoon dog B-chromosomes (Szczerbal & Switonski 2003). Fox centromere-specific probe gave very faint signals in raccoon dog Bs.

Fox B-specific probes did not detect any signals on either A- or B-chromosomes of the raccoon dog, and the raccoon dog B-specific paint gave no signal on fox Bs either. This indicates that fox and raccoon dog Bs have different heterochromatin compositions. The C-KIT localization in Bs of both species could suggest either the common origin of these elements with subsequent heterochromatin divergence or C-KIT sequences were integrated into additional chromosomes of both species independently following the gene amplification event.

Inactive ribosomal genes are found on Bs of other mammalian species (Stitou *et al.* 2000), in invertebrates (Green 1990), and in plants (Puertas 2002). Mouse ribosomal intergenic spacer contains two short amplification-promoting sequences (APS1 and APS2), which stimulate amplification of cis-linked plasmid DNA in cell culture (Wegner *et al.* 1989). The presence of inactive NOR-like sequences and their colocalization with C-KIT on the distal part of raccoon dog B-chromosomes favours the gene amplification hypothesis. C-KIT gene amplification has been reported previously in the minute marker chromosomes observed in the human leukaemia cell line Kasumi-1 (Beghini *et al.* 2002) and C-KIT duplication was found in a pig (Johanson Moller *et al.* 1996).

If the B-chromosomes of the raccoon dog and fox have originated from the karyotype of a putative common ancestor, they have become lost in all other extant 25 *Canidae* species lacking Bs. Since the 12.5 MYA when the fox and raccoon

dog first diverged, their karyotypes have become highly rearranged. In this situation, one would expect that the supernumerary chromosomes usually degenerate via the mechanism of Muller's Ratchet (Green 1990), and that the C-KIT sequences would not have been conserved for such a long time. However, comparison of fox C-KIT exons amplified from B-chromosome library and canine C-KIT sequence revealed very little variation. Three synonymous and one non-synonymous mutation were observed between ten C-KIT exons of the two species (T/C in position 68758 bp; G/A in position 73083 bp; G/A in position 82570 bp; A/G in position 84460 bp; reference sequence is AC142093, canine nucleotides are sited first). Two of these synonymous mutations were present in fox B-chromosome library as single nucleotide polymorphisms (SNPs) and, in both cases, canine alleles were also observed (G/A in position 73083 bp; G/A in position 82570 bp; reference sequence is AC142093). The homology of C-KIT coding regions argues in support of common ancestry of canine and fox B-chromosome C-KIT copies.

Active cytochrome P450 gene was found in supernumerary chromosome of plant pathogenic fungus (*Nectria haematococca*) (Han *et al.* 2001). This gene confers vitally important resistance to antibiotic produced by plants. Our observation that C-KIT genes are located on these canid B-chromosomes raises questions about their functional significance. If the presence of the C-KIT gene on B-chromosome is not beneficial for the host cell, we would expect an accumulation of multiple mutations in its coding region. So far the sequence data has failed to confirm it. Further experiments should show if B-chromosomal and autosomal copies are identical, if B-chromosomal C-KIT genes are expressed, and if C-KIT genes are present in other mammalian B-chromosomes. It can be concluded, however, that the detection of the same gene on the Bs of two different species argues against a current view that B-chromosomes represent parasitic elements of certain genomes.

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