Cytogenet Cell Genet 95:210–224 (2001)

Cytogenetics and Cell Genetics

The pattern of phylogenomic evolution of the Canidae

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Abstract. Canidae species fall into two categories with respect to their chromosome composition: those with high numbered largely acrocentric karyotypes and others with a low numbered principally metacentric karyotype. Those species with low numbered metacentric karyotypes are derived from multiple independent fusions of chromosome segments found as acrocentric chromosomes in the high numbered species. Extensive chromosome homology is apparent among acrocentric chromosome arms within Canidae species; however, little chromosome arm homology exists between Canidae species and those from other Carnivore families. Here we use Zoo-FISH (fluorescent in situ hybridization, also called chromosomal painting) probes from flow-sorted chromosomes of the Japanese raccoon dog *(Nyctereutes procyonoides)* to examine two phylogenetically divergent canids, the arctic fox *(Alopex lagopus)* and the crab-eating fox *(Cerdocyon thous)*. The results affirm intra-canid chromosome homologies, also implicated by G-banding. In addition, painting probes from domestic cat *(Felis catus)*, representative of the ancestral carnivore karyotype (ACK), and giant panda *(Ailuropoda melanoleuca)* were used to define primitive homologous segments apparent between canids and other carnivore families. Canid chromosomes seem unique among carnivores in that many canid chromosome arms are mosaics of two to four homology segments of the ACK chromosome arms. The mosaic pattern apparently preceded the divergence of modern canid species since conserved homology segments among different canid species are common, even though those segments are rearranged relative to the ancestral carnivore genome arrangement. The results indicate an ancestral episode of extensive centric fission leading to an ancestral canid genome organization that was subsequently reorganized by multiple chromosome fusion events in some but not all Canidae lineages.

Our view of chromosome evolution in the mammalian order Carnivora was sharpened considerably with the pioneering G-banding studies of Wurster-Hill and collaborators (Wurster-Hill and Gray, 1975; Wurster-Hill and Bush, 1980; Wurster-Hill and Centerwall, 1982). These authors demonstrated a remarkable degree of G-banded chromosome homology in species within and between Carnivore families, a result since confirmed by reciprocal chromosome painting or Zoo-Fish studies. An important derivative of their observations was the postulated chromosome organization of the common ancestor for all carnivore species (Wurster-Hill and Gray, 1975; Nash et al., 1987, 1998; Frönicke et al., 1997; Murphy et al., 2001). The ancestral carnivore karyotype (ACK) likely consisted of 2n = 42 chromosomes, most of which are conserved as full chromosomes or chromosome arms of the cat.

With the exception of two families, Ursidae and Canidae, carnivore families retain highly conserved karyotypes where the majority of the ACK G-banded chromosomes are found intact and only slightly rearranged. Reciprocal Zoo-FISH analysis has demonstrated that the reorganization of Ursidae species karyotypes involved a global centric fissioning of the ACK,

This publication has been funded in whole or in part with Federal funds from the National Cancer Institute, National Institutes of Health, under contract No. NO1-CO-56000. The content of this publication does not necessarily reflect the views or policies of the Department of Health and Human Services, nor does mention of trade names, commercial products, or organizations imply endorsement by the U.S. Government.

Received 13 September 2001; accepted 1 February 2002.

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which produced high numbered acrocentric karyotypes shared today by six species of ursine bears (Nash et al., 1987, 1998). Two subsequent, but independent centric fusions of these ancient acrocentric chromosome arms produced two Ursidae species with low numbered metacentric karyotypes, giant panda and spectacled bear. The chromosome arms that occur in these two species plus the acrocentric chromosomes of the ursine bears are with few exceptions homologous to the ACK chromosome arms as assessed by both Zoo-FISH and G-band comparisons (Nash et al., 1987; 1998).

The Canidae family appears to have taken a different track in their karyotype evolution. There is considerable segment and chromosome homology by Zoo-FISH among domestic dog *(Canis familiaris)*, red fox *(Vulpes vulpes)* and arctic fox *(Alopex lagopus)*; and it is possible to identify 42 conserved autosomal segments that are conserved across the three species. (Yang et al., 1999, 2000; Graphodatsky et al., 2000). Thirty-four of the conserved segments were equivalent to single domestic dog chromosomes. However, those segments are not identical to the primitive ACK segments (as they were in Ursidae) rather they involve multiple rearrangements relative to humans, cats, and ACK. Cat-dog comparisons revealed a minimum of 68 conserved sub-chromosomal segments and human/dog comparisons revealed 90 homology segments within the canid karyotype (Yang et al., 1999, 2000; Graphodatsky et al., 2000).

In this report, we examine the patterns of segment homology within Canidae and between other Carnivore families using painting probes derived from Japanese raccoon dog, domestic cat and giant panda. The results reveal a plausible evolutionary scenario for the chromosome exchanges that have occurred in the Canidae lineages based upon a parsimony approach to genome segment conservation and exchange. The analysis provides a detailed description of the events that characterized the extensive chromosome re-shuffling within canids and allows for an interpretation of these in the context of genome evolution in other families of Carnivore species.

Materials and methods

Flow-sorting procedures described previously for cat and giant panda chromosomes (Wienberg et al., 1997; Nash, 1998) were also performed for Japanese raccoon dog chromosomes (Fig. 1). Chromosome painting probes were prepared as described in Wienberg et al. (1997). Hybridization conditions used in this study differ from Wienberg et al. (1997) in that no low Cot-DNA was used for suppression hybridization.

Metaphase chromosome preparations for chromosome sorting were derived from primary skin fibroblast cultures from the domestic cat (*Felis catus*, cell line FCA-215), giant panda (*Ailuropoda melanoleuca* AME-13), and Japanese raccoon dog (*Nectereutes procyonoides*, NPR-2). Metaphase chromosome preparations for in situ hybridizations of cat (cell line FCA-215), giant panda (AME-13), Japanese raccoon dog (NPR-1 and NPR-2), arctic fox (*Alopex lagopus* ALA-2), and crab-eating fox (*Cerdocyon thous* CTH-1) derived from primary skin fibroblast cultures were made according to standard procedures (Modi et al., 1987).

To facilitate chromosome identification and specify the regions painted with different probes, chromosome preparations were G-banded prior to in situ hybridization. Chromosome nomenclature and numbering followed previous reports (Wurster-Hill et al., 1980, 1986; Nash and O'Brien, 1987; Wayne et al., 1987; Nash et al., 1998). When the chromosome order was changed, it was based on the size of the chromosomes, arranging them from largest to smallest.

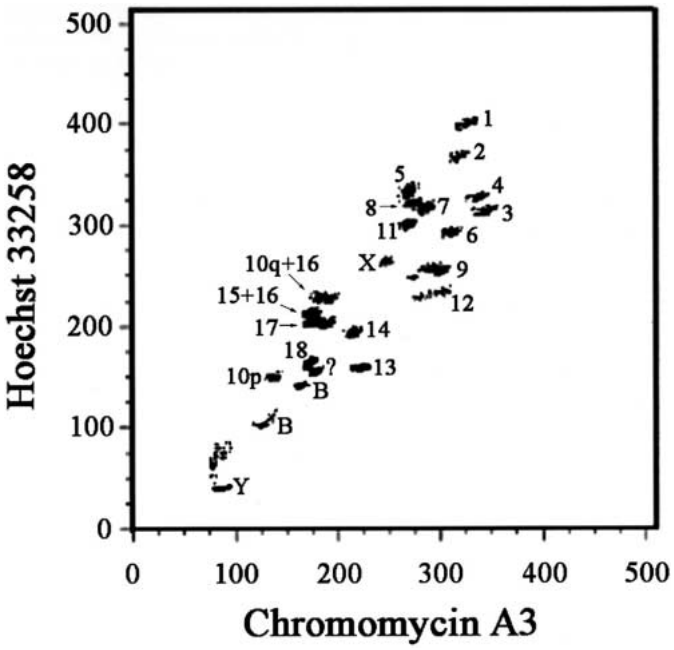


Fig. 1. Bivariate flow karyogram of Japanese raccoon dog chromosomes from a primary fibroblast culture. Painting probes obtained by DOP-PCR from flow-sorted chromosomes and in situ hybridization to raccoon dog metaphase chromosomes were numbered following the Japanese raccoon dog karyotype nomenclature from Wurster-Hill et al. (1986), and as modified in this article. The peak with the question mark was not recovered from the flow sort and may be the missing chromosome 10.

G-banded slides were kept in a 45°C oven for at least 1 week prior to hybridization. Prior to in situ hybridization the slides were de-stained for 1 min in two rinses of 3:1 methanol:acetic acid, rinsed twice (1 min each) with distilled water, then rinsed twice in 1× PBS for 1 min, and denatured in 70% formamide, 2× SSC (pH 7.0) in a 50-ml Coplin jar for 10 s at 55°C. This procedure produced both well-defined fluorescence signals and reverse DAPI (4′,6′-diamidino-2′-phenylindole) bands, observed by simply switching fluorescence filter sets on the same metaphase chromosome spreads.

Chromosomes of the raccoon dog, arctic fox, and crab-eating fox were hybridized with a commercially available telomeric probe (Chromophore 1 Pan Probe-Cytocell-Rainbow Scientific, Inc., Windsor, CT) according to their recommended protocol. Fluorescence signals were imaged separately with the appropriate filter set using a Zeiss Axioskop epi-fluorescence microscope equipped with a cooled CCD camera (Photometrics CH250). The digital 8-bit gray scale images were transferred to an Apple Macintosh computer for processing; the images were merged and pseudo-colored using Oncor Image software.

Results

The male raccoon dog cell line (NPR-2) used for chromosome sorting was heteromorphic for chromosome 10. The normal chromosome 10 is biarmed. The variant chromosome 10 is composed of two acrocentrics that correspond to the short and long arms of the normal 10. Of the 22 painting probes generated, 18 were specific for individual raccoon dog chromosomes, including two different B chromosomes and the Y. Raccoon dog chromosomes 15 and 16 were sorted together (Fig. 1). The normal chromosome 10 was not identified in any peak, but

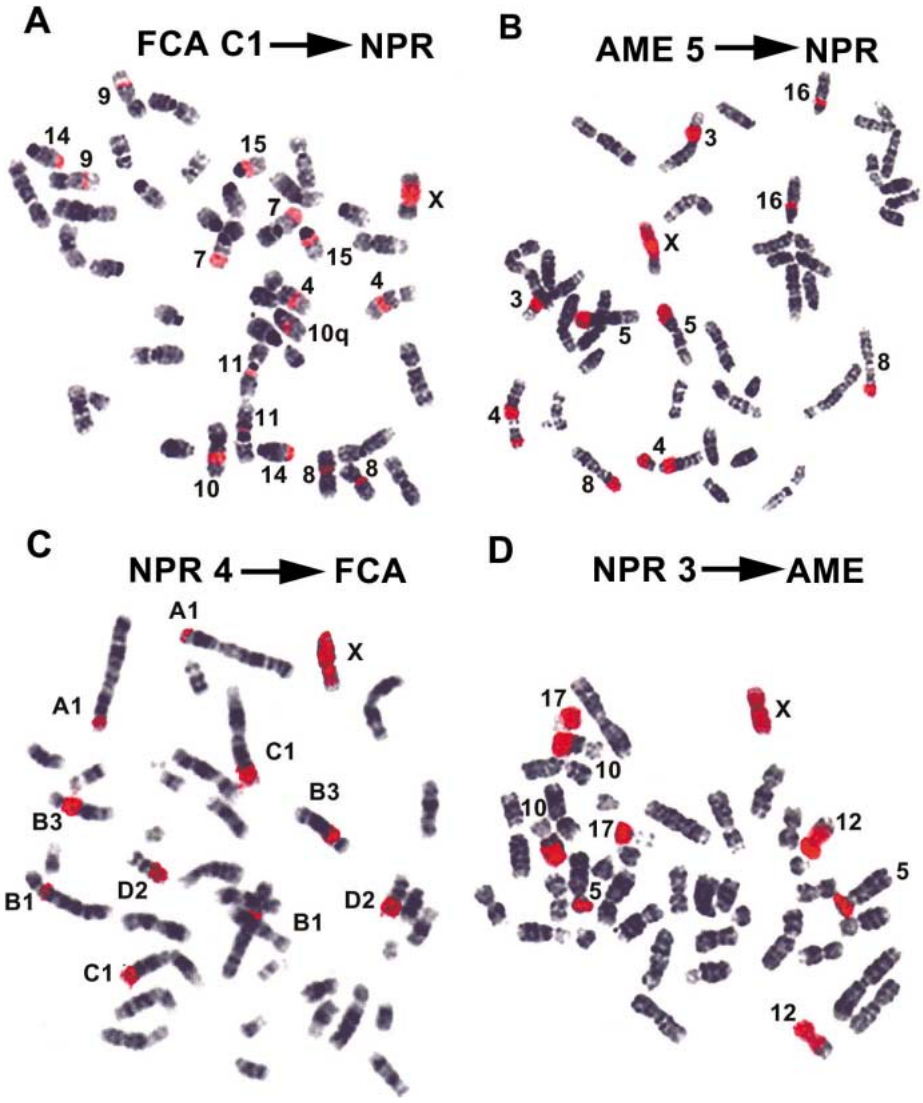


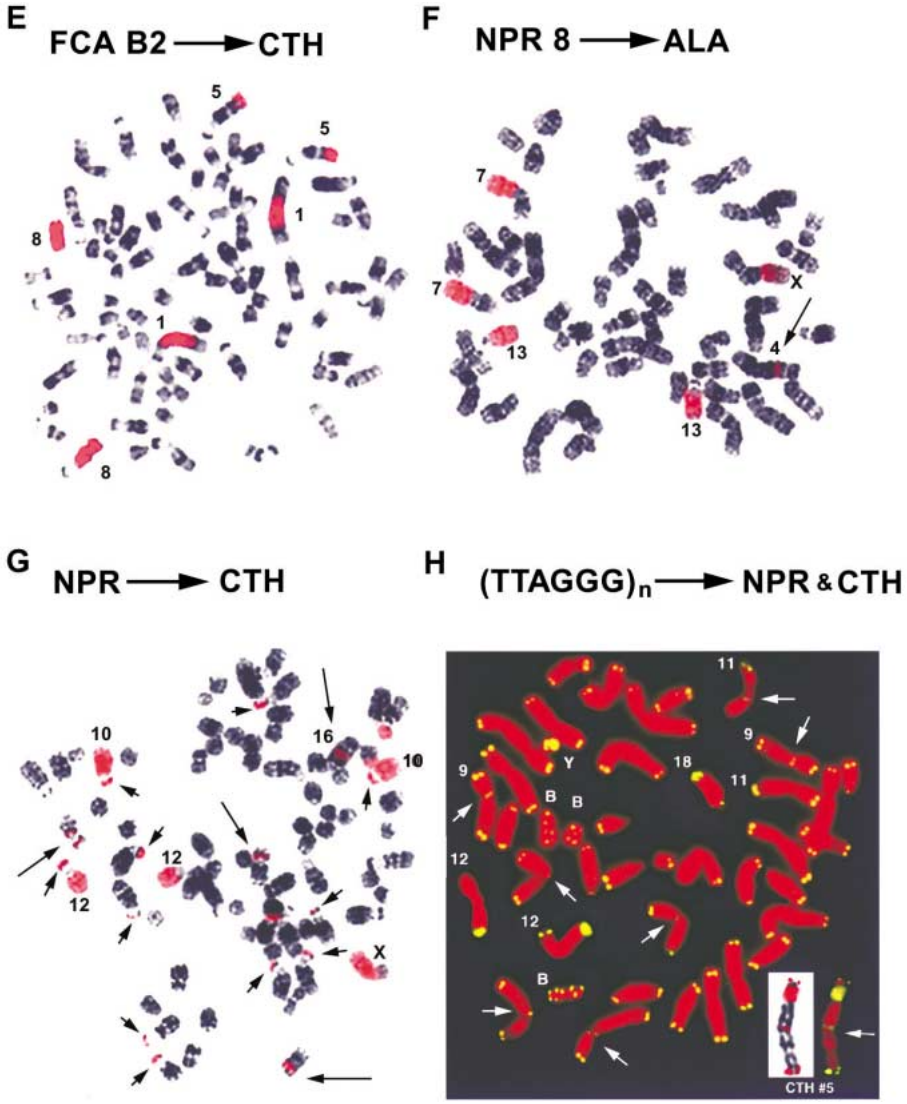
Fig. 2. (A–G) Examples of in situ hybridization of DOP-PCR generated cat, giant panda chromosomes and Japanese raccoon dog painting probes. Metaphase spreads were G-banded prior to hybridization and this figure shows the hybridization signal (red) superimposed on reverse DAPI-banded chromosomes. Chromosomes with a hybridization signal are identified with a species-specific chromosome number. The non-specific X-chromosome paint generated by all autosomal and Y painting probes is shown. (A) Cat chromosome C1 painting probe on raccoon dog metaphase spread; (B) giant panda chromosome 5 painting probe on raccoon dog; (C) raccoon dog chromosome 4 painting probe on cat; (D) raccoon dog chromosome 3 painting probe on giant panda; (E) cat chromosome B2 painting probe on crab-eating fox (F) raccoon dog chromosome 8 painting probe on arctic fox (G) and raccoon dog chromosome 8 painting probe on crab-eating fox metaphase spread. Arrow in (F) indicates non-specific hybridization signals on chromosome 4 (often signals are found on both homologues). The short arrows in (G) show non-specific signals on the G-dark caps of heterochromatic short arms, the long arrows indicate non-specific signals routinely found at other chromosome sites.

may be the peak identified by the question mark in Fig. 1. The variant acrocentric chromosome arm 10p sorted independently and the variant acrocentric chromosome 10q arm sorted with chromosome 16. As a result, using the complete set of raccoon dog probes, we could independently resolve the painting pattern of each chromosome.

Chromosome painting of cat and giant panda probes onto raccoon dog chromosomes

G-banding studies have shown that canid chromosomes are very distinct from other carnivore species (Wurster-Hill and Centerwall 1982; Wayne et al., 1987a, 1987b). To determine the nature of this difference, domestic cat chromosomes were painted onto raccoon dog chromosomes (Fig. 2A). Cat chromosomes were used because they can be directly compared to the ACK (Fig. 3), and therefore provide a reference point for dog chromosome divergence. Raccoon dog metaphase spreads were also painted with giant panda chromosome probes (Fig. 2B) to confirm the cat assignments and to further dissect the chromosome homologies. For example, are the ancestral breakpoints between bears and dogs the same or different? Examples of fluorescent in situ hybridization (FISH) with cat and giant panda probes painting raccoon dog chromosomes are shown in Figs. 2A, B, reciprocal raccoon dog chromosome paints in Figs. 2C, D, and full genome results are summarized in Fig. 4.

The 18 cat painting probes hybridized to all raccoon dog chromosomes, except for the B and Y-chromosomes (Fig. 4). A small interstitial region of chromosome 12 and the heterochromatic short arms of chromosomes 14–18 were also not painted. Of the 33 chromosome arms of the raccoon dog (excluding the Y), only two correspond to complete cat chromosome arms (domestic cat A2p and A3p paint raccoon dog chromosome arms 6p and 12p, respectively and have the same G-banding pattern). In addition to raccoon dog chromosome arms 6p and 12p, eleven additional complete raccoon dog chromosome arms (1p, 2p, 5p, 7p, 9p, 10p, 13p, 16q, 17q, Xp, and Xq) are



The long arrow pointing to chromosome 16 is a site homologous to arctic fox chromosome 4 (F). The significance of the non-specific chromosome painting presented in the results will be discussed in a future publication. (H) Telomere DNA sequence probe $(TTAGGG)_n$ hybridized to raccoon dog metaphase spread and crab-eating fox chromosome 5 (inset). In addition to signals at all raccoon dog chromosome telomeres, centromere located signals are also present (arrows). The interstitial telomere band (ITB) of chromosome 9 is quite strong, whereas ITB's found at other biarmed chromosome centromeres are very weak. Multiple ITB's are seen on the B chromosomes. The NOR regions of chromosomes 11, 12 and 18 and the Y chromosome show highly amplified telomere signals. Inset shows chromosome 5 of the crab-eating fox. In addition to the signals at the telomeres an ITB (arrow) is found at the site corresponding to the tandem fusion of two previously acrocentric chromosomes (see text). Species abbreviations: FCA – *Felis catus*, domestic cat; AME – *Ailuropoda melanoleuca*, giant panda; NPR – *Nyctereutes procyonoides*, Japanese raccoon dog; CTH –*Cerdocyon thous*, crab-eating fox, ALA – *Alopex lagopus*, arctic fox.

painted by individual cat chromosome arms. When the reciprocal (NPR on FCA) paints were performed (Fig. 5) it became clear that most of these raccoon dog arms are homologous to only portions of cat chromosome arms. The remaining 20 raccoon dog chromosome arms are mosaics of 2–4 cat chromosome arms (Fig. 4). The cat chromosomes shown in Fig. 4 are color coded with reference to their ACK homologues (Fig. 3) and one can see what is true for the cat chromosome arms can also be applied by logical inference to the ACK chromosome arms. Relative to the raccoon dog chromosomes, the cat karyotype is broken into 67 homology segments.

Each of the 21 giant panda chromosome paint probes is specific for a single giant panda chromosome with the exception of chromosomes 10 and 11 which sorted together (Nash et. al., 1998). The giant panda Y chromosome did not paint the raccoon dog Y chromosome. The remaining 20 giant panda probes hybridized to all other raccoon dog chromosomes except for the B chromosomes (Fig. 4). As with the cat painting probes, a small interstitial region of raccoon dog chromosome 12 and the heterochromatic short arms of chromosomes 14–18 were not painted. The pattern of hybridizations of cat painting probes on the raccoon dog is in agreement in every case with the hybridizations of homologous giant panda painting probes as predicted by previously identified homology segments between cat and giant panda (Nash et al., 1998). For example, raccoon dog chromosome 1 is painted by segments of cat chromosomes A2q, F2, A1q, and C2, which are homologous to segments of giant panda chromosomes 1q, 9q, 3q prox, and 1p respectively (Nash et al., 1998). The parallel color-coding of giant panda and cat chromosomes (based on ACK homology from Fig. 3 but shown in Fig. 4) reflects cat-giant panda homologies plus their relationship to the ACK. This chromosome homology information was used to resolve individual chromosome painting patterns of the aggregate cat chromosome D3+D4 painting probe, and giant panda 10+11 chromosome painting probe shown in Fig. 4. For example, cat D3p (NPR3) and D3q (NPR10) match

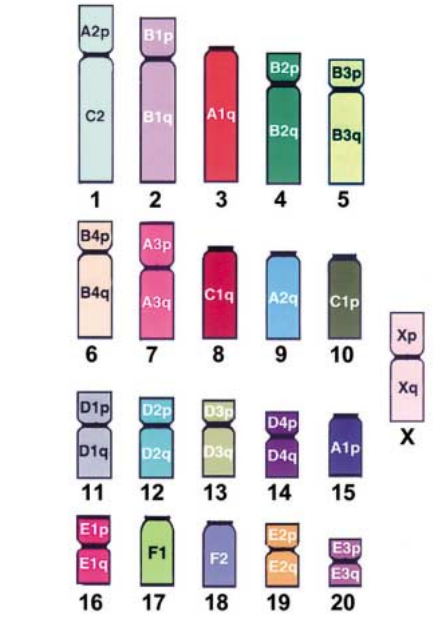


Fig. 3. Proposed ancestral carnivore karyotype (ACK) shows the relative length and centromere positions for the 20 autosomal chromosomes (Wurster-Hill and Gray 1975; Frönicke et al., 1997; Nash et al., 1998; Murphy et al., 2001). All the chromosome arms of the ACK have been identified with respect to their cat chromosome arm homologues. The ancestral carnivore X chromosome is like that found in most other mammals. The relative length and centromere position of the ancestral carnivore Y chromosome is unknown and not included. Chromosomes were judged to be ancestral, primarily as a result of being commonly found in species of several families from both the canoid and feloid branch of the Carnivora order (Murphy et al., 2001). Two modifications of the previous ACK (Murphy et al., 2001) are proposed: 1) ACK chromosome 11 (equals FCAD1) and 12 (FCAD2) are reversed to indicate that ACK11 (FCAD1) is slightly larger than ACK12 (FCAD2); 2) ACK 17 (FCAF1) is an acrocentric chromosome.

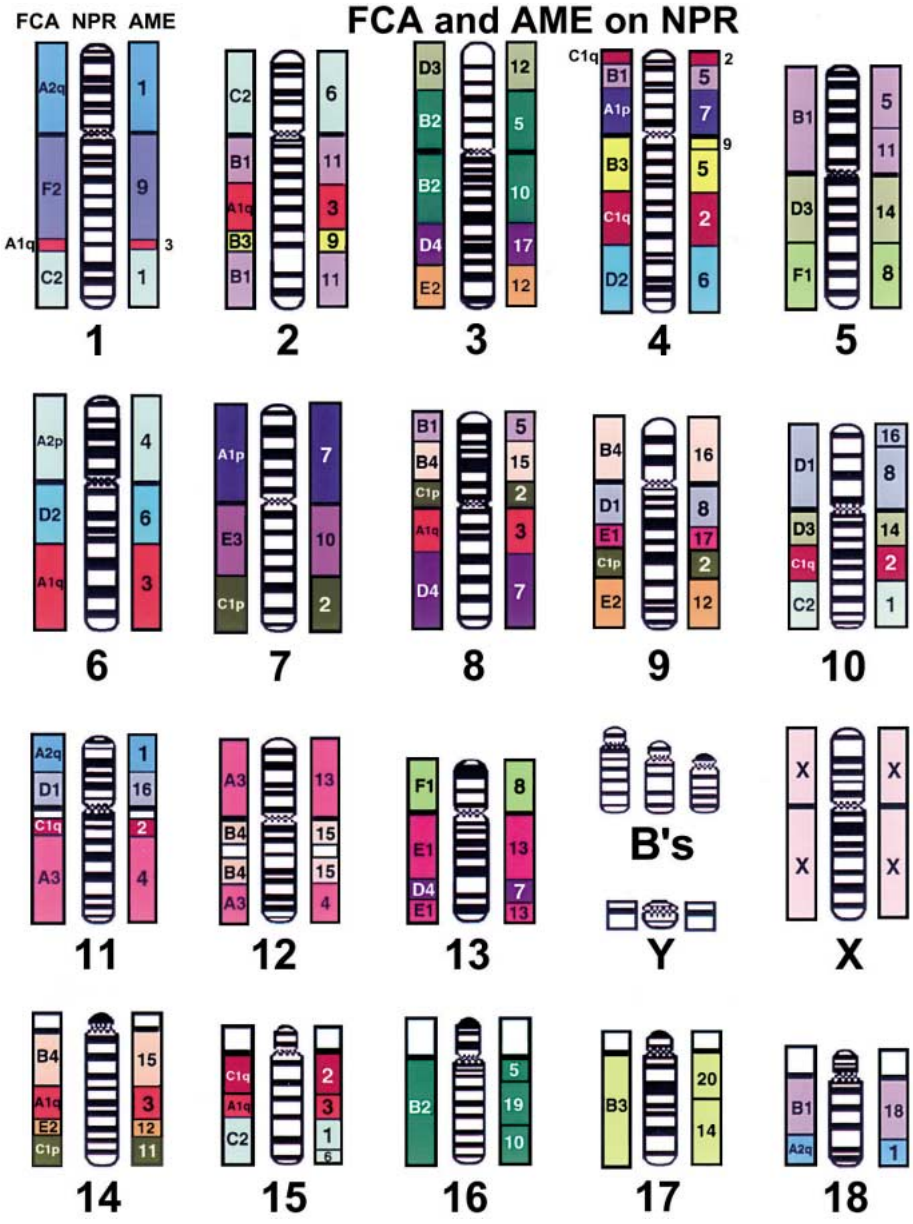


Fig. 4. Hybridization results of cat (FCA) and giant panda (AME) chromosome-specific paints on raccoon dog (NPR) are summarized adjacent to the raccoon dog ideogram (2n = 38+B). The hybridization patterns of cat chromosome painting probes on raccoon dog are shown to the left of each chromosome, giant panda is shown to the right. Colors correspond to ancestral homologies of the 21 autosomes and X chromosome of the ACK of Fig. 3. Note the parallel color (ACK homologies) indicated by independent FCA versus AME painting results. White indicates no hybridization signal. Many of the raccoon dog chromosome arms are mosaics of 2–4 chromosome arm fragments of the ACK. The giant panda painting probes were used to verify homology regions implicated with the cat painting probes. Every homologous chromosome region between cat and giant panda (same color), formerly established by reciprocal paints between cat and giant panda (Nash et al., 1998), hybridized to the same regions on the raccoon dog chromosomes. The breakpoint junctions in ACK chromosomes that gave rise to the bear (giant panda) and to the canid chromosomes are different. For example, raccoon dog chromosome 16 (which is also an intact chromosome in the arctic fox, crab-eating fox, and domestic dog) is a single chromosome fragment of the ACK. This same fragment is part of three different giant panda chromosomes (5, 19, and 10).

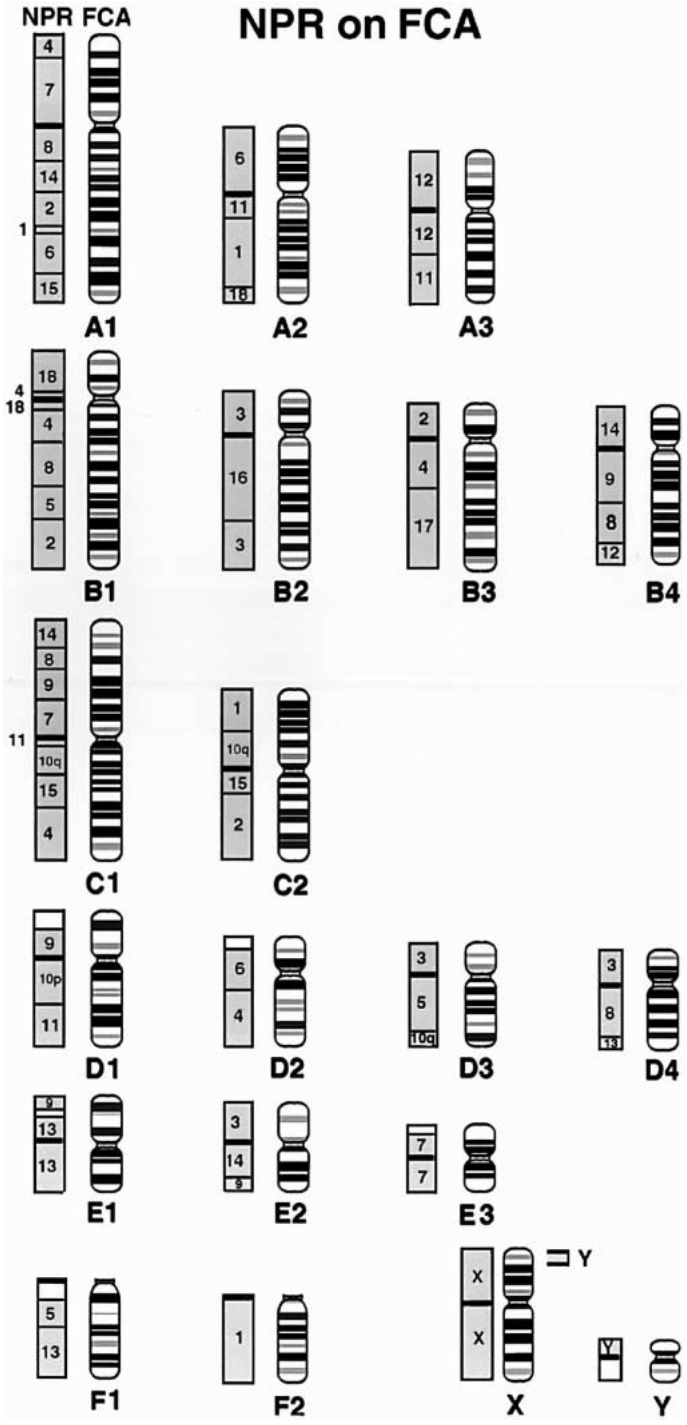


Fig. 5. Results of raccoon dog (NPR) chromosome-specific paints hybridized to cat chromosomes (FCA) (2n = 38). The hybridization patterns of raccoon dog painting probes on cat chromosomes are shown to the left of the cat ideogram. Gray shaded boxes indicate hybridization with raccoon dog probes. White boxes indicate no hybridization. The bracket to the right of Xpter shows where the raccoon dog Y chromosome painting probe hybridizes to the cat X chromosomes.

their homologous giant panda chromosomes 12p and 14q respectively.

One additional point of interest can be inferred from Fig. 4. The chromosomes B1 through B4 of the cat (respectively chromosomes 2, 4, 5, and 6 of the ACK) are highly fragmented in the chromosomes of both ursids (13 breakpoints) and canids (15 breakpoints). Except for the breakpoints at the centromeres, the remaining breakpoints are independent in the two families. This is most easily seen by looking at the raccoon dog chromosomes 16 and 17 which represent single homology segments of cat chromosome arms B2q and B3q, but three and two different homology segments respectively, in the giant panda.

Chromosome painting of raccoon dog probes onto cat and giant panda

The results of the Zoo-FISH using 22 raccoon dog painting probes on cat and giant panda metaphase spreads are illustrated in Figs. 2C, D, 5 and 6. The raccoon dog painting probes painted the entire cat karyotype, with the exception of the pter regions of cat chromosomes D1, D2, and E3, the pinter region of E1, and the centromere proximal region of F1. Interestingly, the raccoon dog Y chromosome painting probe hybridized to the short arm of the cat Y chromosome and also to the pter region of the X chromosome (Fig. 5). Relative to the cat, the raccoon dog chromosomes are broken into 65 homology segments compared to 67 homology segments in the reciprocal Zoo-FISH (Fig. 4). The discrepancy arises from cases like cat chromosome B1, which hybridizes to two interrupted sites on raccoon dog chromosome 2 (Fig. 4), whereas the homologous region of NPR-chromosome 2 of the raccoon dog hybridizes to one site on cat B1 (Fig. 5).

The raccoon dog probes painted the entire giant panda karyotype with the exception of the pter regions of giant panda chromosomes 4, 11, and the qter region 16, and the centromere proximal regions of 8p and 13q (Fig. 6). In addition, the heterochromatic short arms of 18, 19 and 20, and the NOR region of chromosome 17 were not painted (Fig. 6). The raccoon dog Y chromosome painting probe hybridized to the qter region of the Y and the pter region of the X chromosome (Fig. 6). Relative to the raccoon dog chromosomes, the giant panda chromosomes are broken into 74 fragments (Fig. 6). In the reciprocal hybridization the giant panda chromosomes are broken into 70 fragments (Fig. 4). The discrepancy arises from interrupted homology segments as described above for the raccoon dog/cat hybridizations.

Chromosome painting of cat probes onto crab-eating fox

The 18 cat painting probes (Wienberg et al., 1997) hybridized to all the euchromatic arms of the crab-eating fox (Fig. 2E and Fig. 7, FCA). Only the p proximal region of chromosome 15 was not painted. Cat chromosome arms A2p, A3p and D3 correspond to complete crab-eating fox chromosomes (chromosomes 23, 27 and 30, respectively). Eighteen additional crab-eating fox chromosome arms were painted by portions of a single cat chromosome arm (Fig. 7, FCA). The remaining seventeen of the 36 crab-eating fox euchromatin autosomal arms are mosaics of 2–4 cat and ACK chromosome arms. The crab-

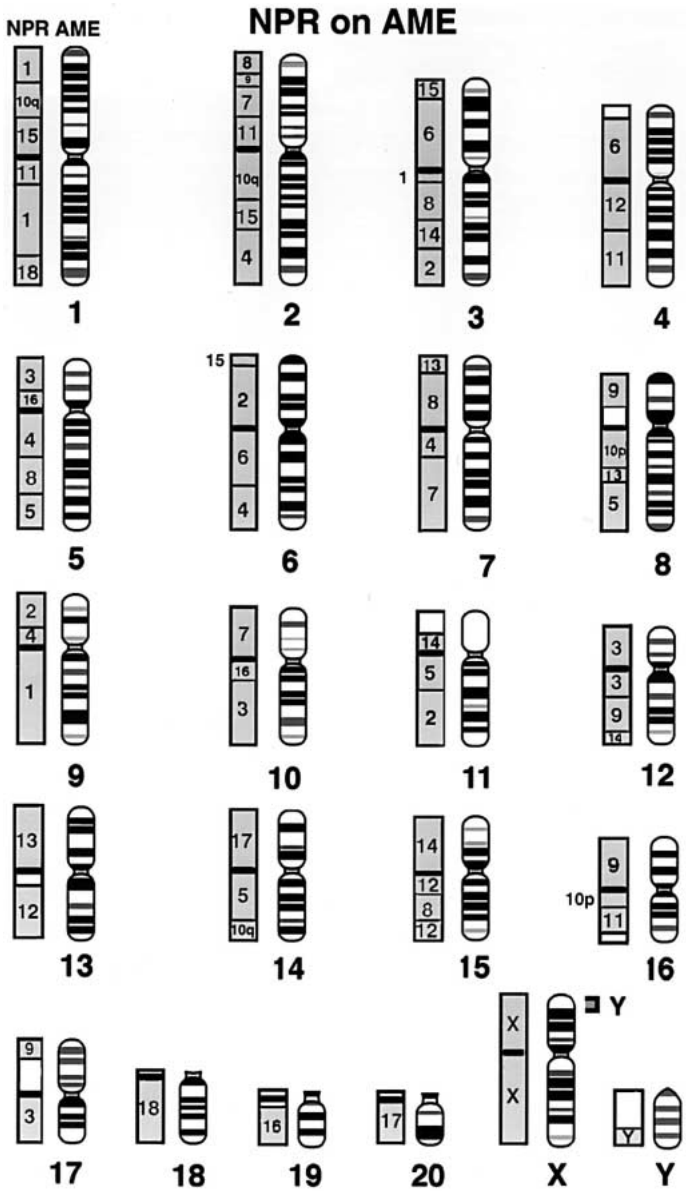


Fig. 6. Hybridization results of raccoon dog (NPR) painting probes on giant panda (AME) (2n = 42) chromosomes are shown to the left of the giant panda ideogram. The format of this figure is identical to Fig. 5.

eating fox karyotype consists of 65 homology segments relative to domestic cat and ACK.

Chromosome painting of raccoon dog probes onto crab-eating fox

The results of Zoo-FISH using raccoon dog painting probes on crab-eating fox metaphase spreads are illustrated in Fig. 2G and summarized in Fig. 7 (NPR). All crab-eating fox chromosomes were hybridized by the raccoon dog painting probes with the exceptions of the heterochromatic short arms of chromosomes 1–14. G banding reveals these arms to contain a proximal non-staining stalk, topped with a dark staining cap (Figs. 2E, G). The Giemsa-dark caps of these heterochromatic p arms hybridized randomly and non-specifically with all 22 raccoon dog probes (Fig. 2G). A non-specific hybridization signal is defined as a signal produced on the metaphase chromosome spreads of one species by several or all the chromosome painting probes of a second species. Five other regions (on chromosomes 3, 15, 21, X, Y) hybridized non-specifically with most if not all raccoon dog probes, indicated by brackets in Fig. 7. The raccoon dog Y-chromosome gives an intense hybridization signal on the crab-eating fox Y-chromosome as well as Xpter. The raccoon dog Y probe also uniquely hybridizes weakly to all short arm stalk regions. Except for chromosomes 1, 5, 18, and 21, all euchromatic chromosome arms (N = 37) of the crab-eating fox were hybridized by a single raccoon dog painting probe (Fig. 7). This is in contrast to the situation with the cat and giant panda where multiple raccoon dog probes were required to paint a single chromosome arm (Figs. 5, 6).

Chromosome painting of raccoon dog probes onto arctic fox

The results of Zoo-FISH using raccoon dog painting probes on the arctic fox are illustrated in Fig. 2F and summarized in Fig. 8. All of the arctic fox chromosomes were hybridized with the raccoon dog painting probes with the following exceptions; the heterochromatic p arms of chromosomes 12 and 14–24 failed to hybridize with any of the raccoon dog painting probes. As also seen with crab-eating fox-raccoon dog comparisons (Fig. 7), 34 of 36 arctic fox euchromatic chromosome arms are painted by a single raccoon dog painting probe. An interstitial region of arctic fox chromosome 4q showed a non-specific signal with all 22 raccoon dog painting probes. The raccoon dog Y-chromosome probe painted the arctic fox Y-chromosome intensely and also painted a portion of Xpter (Fig. 8, brackets indicate non-specific hybridization sites).

G-banded comparisons of canid karyotypes

Figure 9A–C shows the G-banded karyotypes of the crab-eating fox (2n = 74), the raccoon dog (2n = 38+B), and arctic fox (2n = 50) at the same level of extension (~500 bands). Each species represents a different phylogenetic lineage of the Canidae radiation (Wayne, 1993). Figure 9D presents a G-banded karyotype comparison plus identified homology segments for the crab-eating fox (CTH), the raccoon dog (NPR) and the arctic fox (ALA) based on chromosome painting. Extensive G-banded homology between the three species is apparent even though chromosome numbers of the three species differ widely. Twenty of the 36 crab-eating fox autosomal chromosomes for example, show convincing G-banded homology to whole chromosome or chromosome arms of both the arctic fox and raccoon dog. Fifteen of the remaining 17 smaller crab-eating fox chromosomes are found intact but fused together in the larger chromosome arms of the low chromosome number karyotypes of the arctic fox and raccoon dog.

Localization of telomere DNA sequences in the raccoon dog, crab-eating fox and arctic fox

FISH hybridization signals using the telomere DNA sequence probe $(TTAGGG)_n$ revealed telomeres at the ends of all chromosomes of the raccoon dog, arctic fox and crab-eating fox

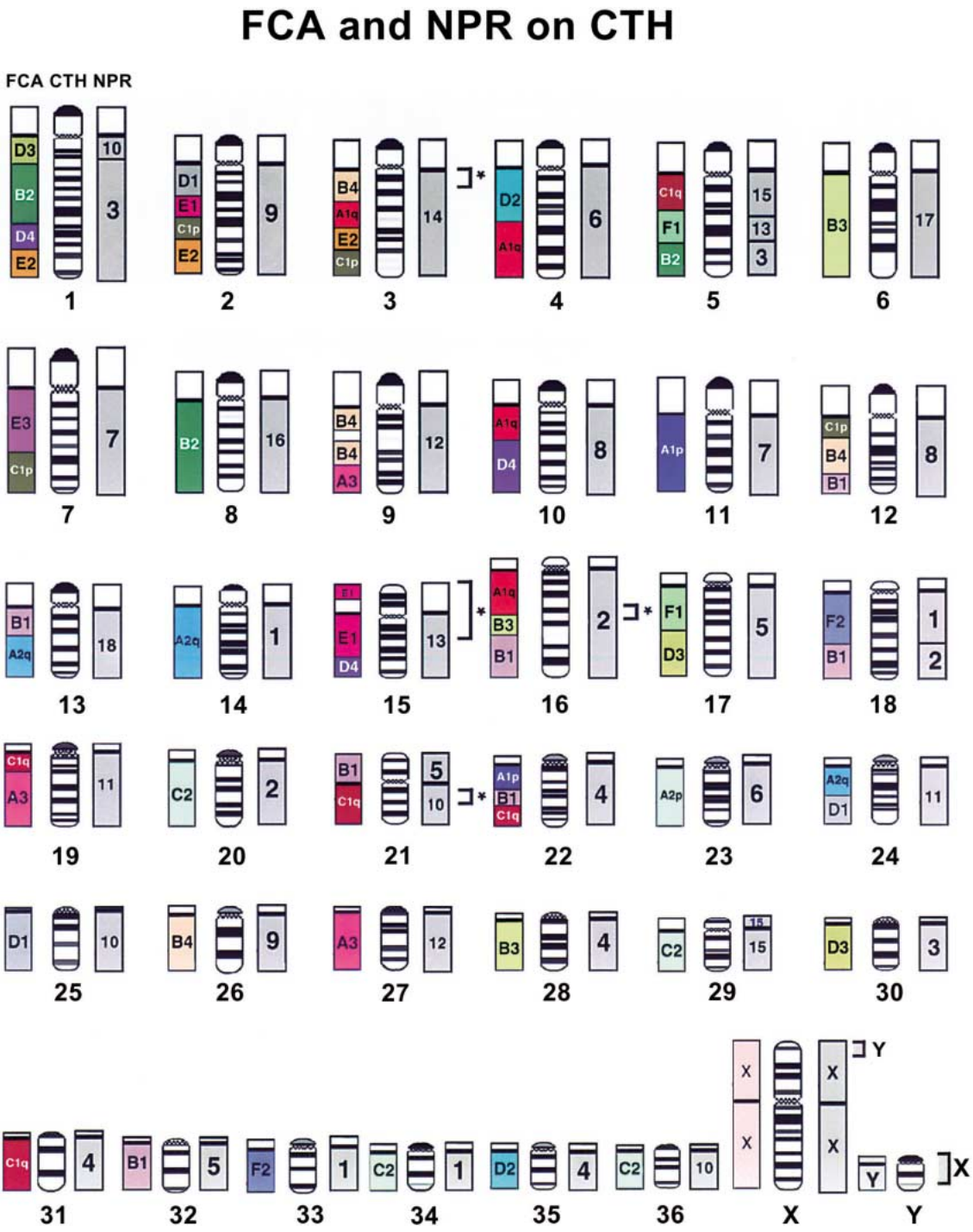


Fig. 7. The hybridization results of cat (FCA) chromosome-specific painting probes on crab-eating fox (CTH) chromosomes (2n = 74). The hybridization pattern of cat chromosomes is summarized to the left of the crab-eating fox ideogram. The color-coding is the same as Fig. 4, and therefore shows the hybridization pattern relative to the cat and ACK. As was the case with the chromosome arms of raccoon dog, most of the larger crab-eating fox chromosomes are mosaics of two to four chromosome arm fragments of the ACK. Most of the patterns of mosaicism between the crab-eating fox and raccoon dog chromosome arms relative to the ACK are identical. Relative to the cat, the crab-eating fox chromosomes are broken into 67 homology segments. The hybridization pattern of raccoon dog (NPR) painting probes on crab-eating fox (CTH) is shown to the right of the crab-eating fox ideogram. The format of this portion of the figure is identical to that described in Fig. 5. The brackets with asterisks to the right of crab-eating fox chromosomes indicate regions that were non-specifically hybridized with all raccoon dog painting probes. The raccoon dog Y painting probe hybridized to the crab-eating fox Y chromosome and to the Xpter region (bracket to right of X chromosome). The raccoon dog X painting probe hybridized to the crab-eating fox X and often gave a weak signal on the Y chromosome (bracket to right of Y chromosome). Thirty-two of the 36 autosomal crab-eating fox chromosomes were painted by a single raccoon dog chromosome probe. The juxtaposition of the hybridization patterns of the cat and raccoon dog next to the crab-eating fox ideogram demonstrates two important features of canid chromosomes. Relative to each other they are highly conserved (crab-eating fox 1qter, 2q, 3q, 4q = raccoon dog 3q, 9q, 14, 6q, respectively). Relative to the domestic cat/ACK, they are extensively rearranged (the color coding to the left of crab-eating fox ideogram can be transferred to the boxes to the right, one for one).

(Fig. 2H). A strong interstitial telomere band (ITB) (Meyne et al., 1990) was present within the pericentromeric region of the raccoon dog chromosome 9. Much weaker ITB's were found at the centromeres of other biarmed chromosomes, phylogenetic footprints of their evolutionary history of chromosome fusions.

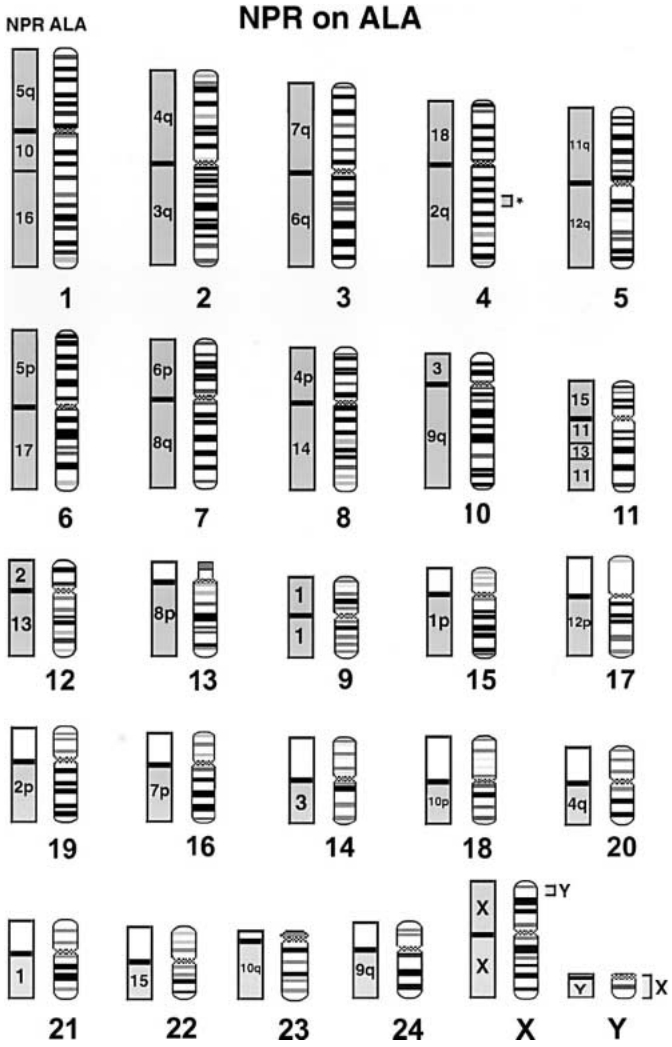


Fig. 8. The chromosome numbering system for the arctic fox ideogram and karyotype throughout this publication conforms as closely as possible to the standard karyotype of Mäkinen (1985). Due to the low resolution of that karyotype however, matching chromosomes 14–20 to more recent high quality karyotypes was problematic. While retaining the Mäkinen numbering system, we used a more compact 5 × 5 format which allows presentation of chromosomes at the maximum size. The hybridization pattern of raccoon dog (NPR) painting probes on arctic fox (ALA) is shown to the left of the arctic fox ideogram (2n = 50). The format of this figure is identical to that described in Fig. 5. The bracket next to chromosome 4, marked by an asterisk, shows a region that was non-specifically hybridized with all raccoon dog painting probes. The raccoon dog Y painting probe hybridized to the arctic fox Y chromosome and to the Xpter region (bracket to right of X). The raccoon dog X painting probe hybridized to the arctic fox X chromosome and often gave a weak signal on the Y (bracket to right of Y). Thirty-three of the 36 euchromatic chromosome arms of the arctic fox were painted by a single raccoon dog chromosome probe.

B chromosomes had multiple ITB's. The whole Y chromosome was intensely painted along with the NOR regions of raccoon dog chromosomes 11, 12 and 18 (Fig. 2H). ITB's were observed within the pericentromeric regions of most of the arctic fox biarmed chromosomes. Telomere DNA sequences were amplified throughout all the heterochromatic chromosome short arms (14–22+24) of the arctic fox (data not shown). All the heterochromatic G-light stalk regions of the crab-eating fox chromosomes had amplified telomere DNA sequences. Interestingly, an ITB was observed within the euchromatic arm of crab-eating fox chromosome 5 (Fig. 2H inset).

Discussion

The karyotypes of species from all carnivore families except for the Ursidae and the Canidae are highly conserved relative to the ancestral carnivore karyotype (ACK). Thus, many G-banded chromosomes that are specific to carnivores are found in all families except the bears and dogs (Wurster-Hill and Centerwall, 1952; Arnason, 1972, 1974; Wurster-Hill and Gray, 1975; Nash et al., 1987, 1998; Wayne et al., 1987a, b; Modi and O'Brien, 1988). A recent Zoo-FISH and G-banding study has shown that the ursid karyotypes, while rearranged relative to ACK, have three full-length ACK chromosomes and 12 conserved ACK chromosome arms (Nash et al., 1998). The present study was initiated to more fully characterize the divergence of the canid karyotype from the ACK. Definition of the ACK from which the canid chromosomes evolved is extremely useful since it provides a primitive ancestral organization baseline which was modified directionally during Canidae evolution (Wurster-Hill and Gray, 1975; Dutrillaux and Couturier, 1983; Nash et al., 1987, 1998; Frönicke et al., 1997; Murphy et al., 2001).

Fig. 9. (A) Karyotype of the crab-eating fox (*Cerdocyon thous*-CTH); (B) karyotype of the Japanese raccoon dog (*Nyctereutes procyonoides*-NPR); (C) karyotype of the arctic fox (*Alopex lagopus*-ALA); (D) G-banded karyotypic comparison of the arctic fox and crab-eating fox to the raccoon dog based on Zoo-FISH hybridizations. The heterochromatic arms of the crab-eating fox and arctic fox have no homology in the raccoon dog and are therefore not shown in this comparison. The intact raccoon dog chromosomes are in the middle and numbered below. Crab-eating fox chromosomes and chromosome regions homologous to the raccoon dog are shown to the left and arctic fox to the right. Lines perpendicular to the chromosomes delineate the separate chromosome or chromosome regions. When biarmed chromosomes of the low-chromosome number arctic fox and raccoon dog are both compared to the high numbered crab-eating fox (for example, raccoon dog chromosome comparisons 6, 7, 8, and 9) the chromosome arm associations of the raccoon dog and arctic fox are different for each chromosome. Therefore, with reference to the high-chromosome number crab-eating fox, the raccoon dog and arctic fox biarmed chromosomes likely represent independent fusions of ancestral crab-eating fox acrocentric chromosomes (heterochromatic short arms represent a more recent addition to the ancestral euchromatic acrocentrics).

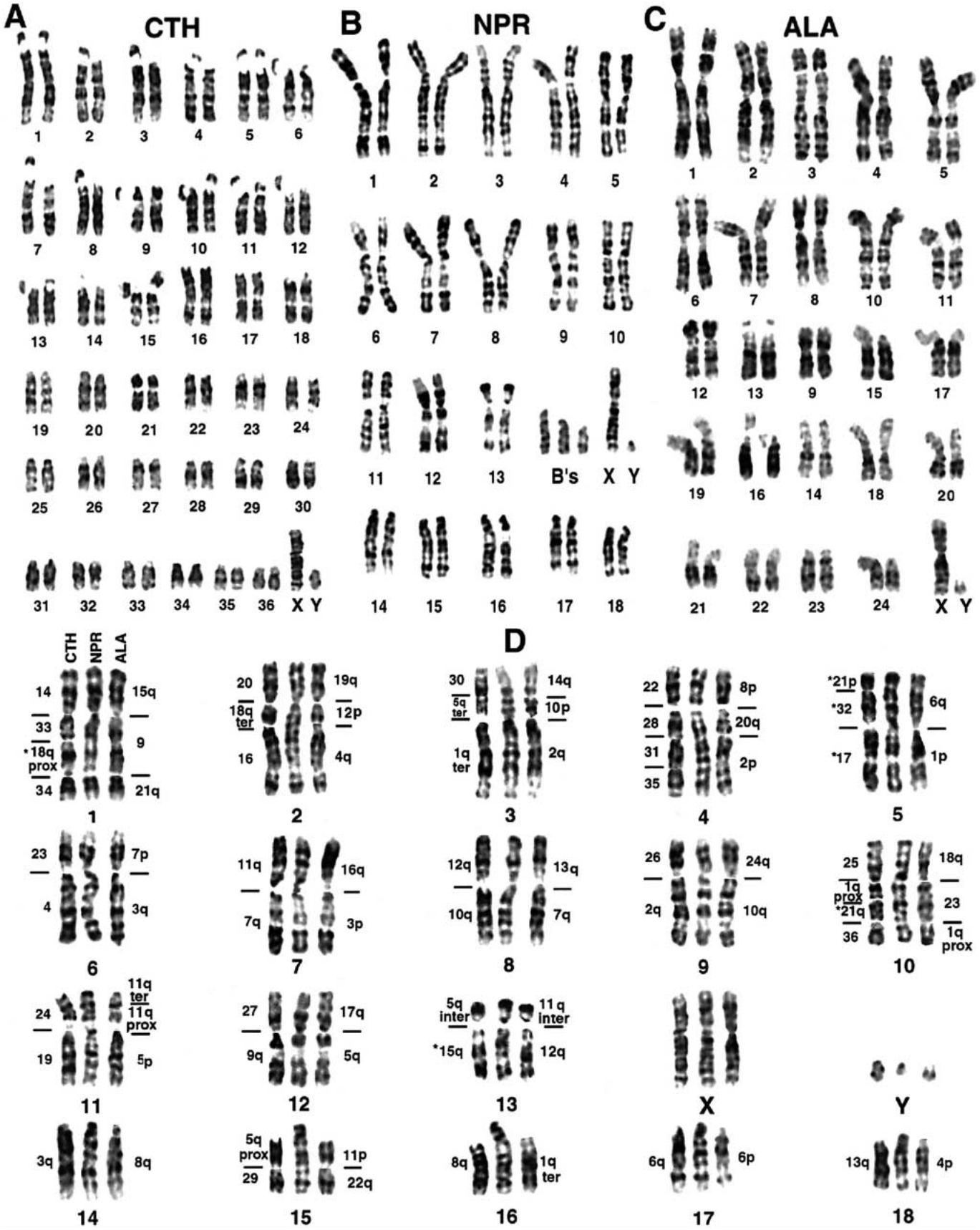

A
CTH
1 2 3 4 5 6
7 8 9 10 11 12
13 14 15 16 17 18
19 20 21 22 23 24
25 26 27 28 29 30
31 32 33 34 35 36 X Y
B
NPR
1 2 3 4 5
6 7 8 9 10
11 12 13 B's X Y
14 15 16 17 18
C
ALA
1 2 3 4 5
6 7 8 10 11
12 13 9 15 17
19 16 14 18 20
21 22 23 24 X Y
D
CTH NPR ALA
14 33 *18q prox 34 15q 9 21q
1
20 18q ter 16 19q 12p 4q
2
30 5q ter 1q ter 14q 10p 2q
3
22 28 31 35 8p 20q 2p
4
*21p *32 *17 6q 1p
5
23 4 7p 3q
6
11q 7q 16q 3p
7
12q 10q 13q 7q
8
26 2q 24q 10q
9
25 1q prox *21q 36 18q 23 1q prox
10
24 19 11q ter 11q prox 5p
11
27 9q 17q 5q
12
5q inter *15q 11q inter 12q
13
X
Y
3q 8q
14
5q prox 29 11p 22q
15
8q 1q ter
16
6q 6p
17
13q 4p
18

Table 1. A comparison of homologous chromosome segments in five Canid species as revealed by Zoo-FISH[a]

Domestic dog[b]	Crab-eating fox[c]	Arctic fox	Raccoon dog	Red fox[b]
1	1q	2q + 23qprox[d]	3q + 10qprox[d]	1p + 5pprox[d]
2	3q	8q	14	2q
3	16	4q	2qter	14q
4	4q	3q	6q	4q
5	2q	10q	9q	12qter
6	10q	7q	8q	3q
7	17	1p	5q	13qter
8	6q	6p	17	6q
9	15q	12q	13q	2pter
10	9q	5q	12q	16q
11	7q	3p	7q	12p
12	8q	1qter	16	1qter
13	18	12p + 9q	1qinter+2qprox	2pprox + 13p
14	14	15q	1p	7q
15	11q	16q	7p	10q
16	13q	4p	18	7p
17	19	5p	11q	8q
18	24	11qprox+11qter	11p	5qprox+5qter
19	21	6pter+23qter	5pter+10qinter	4pter + 5pter
20	23	7p	6p	9q
21	25	18q	10p	11p
22	12q	13q	8p	6p
23	20	19q	2p	11q
24	27	17q	12p	14p
25	28	20q	4qprox	15qprox
26	30	14q	3pter	10p
27	34	21q	1qter	15p
28	22	8p	4p	9p
29	26	24q	9p	8p
30	36	1qprox	10qter	1qprox
31	35	2pter	4qter	15qter
32	32	6qprox	5pprox	4pprox
33	31	2pprox	4qinter	16p
34	33	9p	1qprox	13qprox
35	29	22q	15qter	3pprox
36	5qprox	11p	15prox	3pter
37	5qter	10p	3pprox	12qprox
38	5qinter	11qinter	13p	5qinter

[a] prox = proximal; inter = internal; ter = terminal.
[b] Data modified from AS. Graphodatsky et al. (2000). Raccoon dog chromosome numbers were modified in our report as described in the Methods. The 35 non-shaded rows show the currently defined conserved chromosomes of the AKEC.
[c] The crab-eating fox karyotype differs from the domestic dog karyotype by just two fusions and one inversion. Domestic dog chromosomes 36, 37 and 38 are fused together in chromosome 5 of the crab-eating fox. Domestic dog chromosome 19 (acrocentric) is homologous to bi-armed chromosome 21 of the crab-eating fox.
[d] Conserved regions of chromosome 1.

Reciprocal chromosome painting between the raccoon dog and the cat, and confirmed with the giant panda, shows that only two small chromosome arms of the ACK (cat A2p and A3p) are intact in the raccoon dog (Figs. 4, 5). This is consistent with the Zoo-FISH results of Graphodatsky et al. (2000) and Yang et al. (2000) which show the domestic dog, red fox, and arctic fox to be highly fragmented with respect to the cat. In fact, a comparison of the data of Graphodatsky et al. (2000) and Yang et al. (2000) with our results for the raccoon dog, arctic fox, and crab-eating fox, demonstrates that all these Canidae species retain these two small intact ACK chromosome arms (Fig. 3). The domestic dog karyotype retains two additional small ACK chromosome arms (FCA B2 and D3). The extensive chromosome rearrangements is the reason why earlier G-banding studies (Wayne et al. 1987a, b) failed to observe any significant homology between canids and other carnivores except for the raccoon dog, which was reported to show some similarity between the cat, but which was later shown to be incorrect (Yang et al., 2000; Nash et al., present study). Unlike other carnivores, canid karyotypes are composed of chromosome arms that are mosaics of two-four fragments of ACK chromosome arms (Figs. 4–7).

Zoo-FISH (Figs. 7, 8) and G-band comparisons (Fig. 9) of three canid species with varying chromosome number revealed a high degree of chromosome arm homology between all three species. Our results combined with those of Yang et al. (2000) and Graphodatsky et al. (2000) allowed us to define explicit chromosome arm homologies for the domestic dog, crab-eating fox, arctic fox, raccoon dog, and red fox (Table 1). Thirty-five domestic dog chromosomes (excluding chromosomes 13, 18, and 19) are homologous to single chromosomes or chromosome regions in three to four of the other canid species, and can be considered as ancestral to extant canids. (A small proximal portion of dog chromosome 1 is not homologous to low chromosome number canids. See Table 1.) For other canid species, G-banding studies have shown that the kit fox karyotype is identical to that of the arctic fox (Creel and Thornton, 1974), in that the wolf *(Canis lupus)*, coyote *(Canis lustrous)*, maned wolf *(Chrysocyon brachyurus)*, and bush dog *(Speothus venaticus)* are very similar to the domestic dog (Mäkinen and Gustevsson, 1982; Wurster-Hill et al., 1982; Yoshida et al., 1983; and Wayne et al., 1987a, b).

Most of the chromosome arms of low numbered canid species show G-banding homology to acrocentric chromosomes of species with the high number karyotypes. However, when the biarmed chromosomes of low number species are compared to each other, the acrocentric fragments (arms) are joined in different combinations in different species (Fig. 9D). These observations suggest that the low numbered karyotype canids evolved from a common high number ancestral karyotype, mainly through a series of independent centric fusions, as previously has been suggested by Yoshida et al. (1983).

Interstitial telomere DNA sequences present at the centromeres of most biarmed arctic fox and raccoon dog chromosomes (Fig. 2H) lend support to their derivation from centric fusions of ancestral acrocentric chromosomes (Meyne et al., 1990). A postulated high chromosome number mostly acrocentric Ancestral Karyotype of Extant Canids (AKEC), containing the 35 currently defined conserved autosomal chromosomes is shown in Table 1 (the 35 conserved chromosomes of the domestic dog were found as single contiguous pieces in the other four canid species). A small amount of chromosomal material is still unaccounted for and may consist of one to three additional AKEC chromosomes. Because of this uncertainty we show the AKEC as having a range from 74–78 (2n) chromosomes (Fig. 10). The AKEC, which resembles the karyotype of the domestic dog and other wolf-like canids, is composed of a large number of chromosomes that are mosaics of two to four arm fragments of the ACK. Thus, most of the chromosome changes that make the canid karyotype so distinctive relative to the ACK, and other carnivores, probably occurred during the

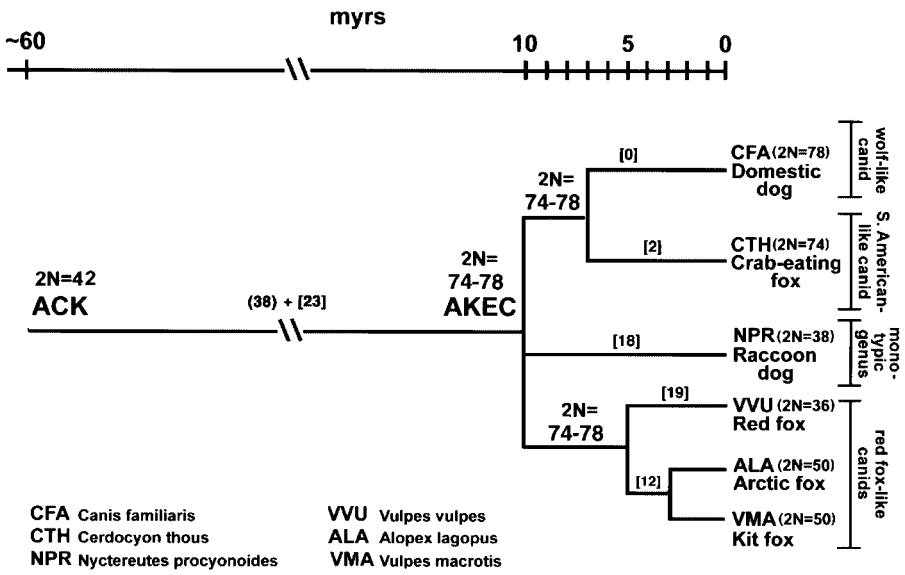


Fig. 10. Patterns of chromosome evolution in the family Canidae. The time-line and phylogenetic relationship of canids is modified from Wayne (1993). Most of the chromosome arm reshuffling observed in the evolution of canids occurred in the descent from the low numbered ancestral carnivore karotype (ACK) to the emergence of the ancestral karyotype of extant canids (AKEC). The wolf-like, and South American-like canids, which are represented here by the domestic dog and crab-eating fox, respectively, comprise most present day species and have 78 all or mostly acrocentric (crab-eating fox heterochromatic arms excluded) autosomes. The number in brackets indicates the number of chromosomal fusions that occurred on each lineage. The number in parenthesis indicates chromosome fissions that occurred between the ACK and AKEC. The red fox (Yang et al., 1999), arctic fox/kit fox, and raccoon dog were derived primarily from the accumulation of independent centric fusions of the AKEC. Thirty-five of the 37 acrocentric autosomal chromosomes required to generate the independent fusions in these species have been defined by Zoo-FISH. The centric fusions that differentiate the arctic fox from the AKEC occurred in a time period of no more than two million years. Kit fox karyotype is identical to arctic fox. For raccoon dog only "A" chromosomes were counted.

origin of the AKEC and before the divergence of extant canid species.

In Figs. 10 and 11, we propose a scenario based upon the principle of maximum parsimony to explain the development of canid species' chromosome mosaicism. Modern canids can be separated roughly into four phylogenetic groups (Fig. 10; Wayne, 1993): (1) the wolf-like canids; (2) South American canids; (3) monotypic genera canids; and (4) red fox-like canids. The wolf-like and South American groups have high chromosome number karyotypes (2n = 74–78), while the latter two groups contain the low chromosome number karyotypes (2n = 36–50).

The initial transition from ACK (2n = 42) to AKEC (2n = 74–78) involved centric fission since modern dog karyotypes still record the presence of most ACK centromere breakpoints. Additional fissions require the generation of new centromeres likely through acrocentric-metacentric (AM) inversions (Imai, 1993) whereby an acrocentric chromosome internalizes the centromere (Fig. 11A). For example, domestic dog acrocentric chromosome 19 underwent an AM inversion to produce biarmed chromosome 21 in the crab-eating fox. A centric fission produced the two small acrocentric chromosomes, which eventually became the short arms of 4p and 5p in the red fox. It is important to note that AM inversions lead to fragments of ancestral chromosome arms and later, independent fusions resulted in the mosaic chromosome arms found in the AKEC and all modern canids.

Chromosome number reductions in modern canids occurred primarily by Robertsonian centric fusions. Reduction in chromosome number beyond acrocentric chromosome fusions requires the formation of new acrocentrics. This can occur by metacentric-acrocentric (MA) inversion whereby a metacentric chromosome undergoes a "centromere-telomere" pericentric inversion to produce an acrocentric chromosome (Fig. 11B). "Centromere-telomere inversions" are common in canid chromosomes (for example, all the chromosomes within Fig. 9D that have asterisks; see also Graphodatsky et al., 2000). MA inversions produce karyotypes whose chromosome arms are mosaics of ancestral arms. Tandem fusion between two acrocentric chromosomes where the internal centromere is eliminated by inactivation is a second mechanism that reduces chromosome number and results in mosaic chromosome arms. Relic telomere sequences at the fusion site as seen in crab-eating fox chromosome 5 (Fig. 2H, inset) indicate a tandem fusion. This chromosome, which includes telomeric DNA sequence footprints, derives from the fusion product of three ancestral chromosomes (AKEC 33, 34 and 35 = domestic dog 36, 37 and 38, Table 1).

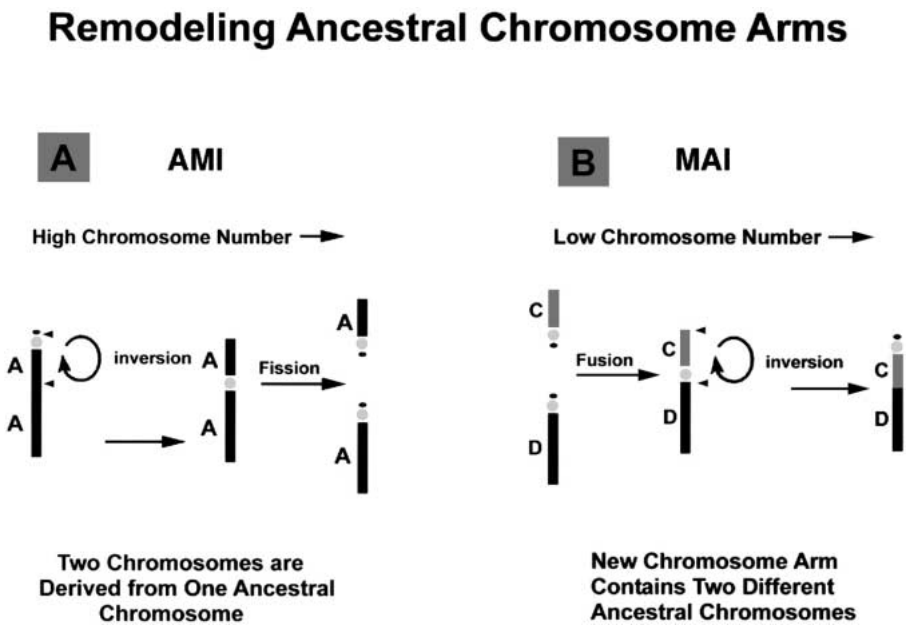


Fig. 11. Two types of chromosome rearrangements that altered chromosome numbers during Canidae evolution: (A) The centromere of an ancestral acrocentric chromosome is internalized by a pericentric inversion. A subsequent centric fission breaks an ancestral chromosome arm into two fragments, increasing chromosome numbers. (B) Chromosomes C and D depicted in panel (B) can be either whole chromosome arms or fragments of chromosome arms relative to the ancestral chromosome condition. Centric fusion followed by a "centromere-telomere inversion" reduces the chromosome number and reshuffles ancestral chromosome arms. AMI = acrocentric metacentric inversion; MAI= metacentric acrocentric inversion.

The AKEC which preceded the divergence of modern canids remained virtually unchanged in the high numbered wolf-like and South American canid species. The divergence of three lineages leading to the raccoon dog, red fox, and arctic fox/kit fox karyotypes are accompanied by at least three independent series of global centric fusion events that reorganized the AKEC. These fusions were extensive and occurred abruptly, within 2–5 million years, emphasizing the punctuated or episodic tempo of evolutionary global chromosome exchange in this group.

If we compare the karyotypes of low chromosome number canids to the AKEC, we can determine if chromosome arm mosaicism has occurred in the last ten Myrs. If we consider only the 35 conserved chromosomes of the AKEC inferred from the data in Table 1, we find that chromosome arms 1q and 2p of the arctic fox are mosaics of two AKEC chromosomes. In the raccoon dog, chromosome 15 and chromosome arms 1q, and 3p are mosaics of two AKEC chromosomes whereas 4q consists of three AKEC chromosomes. In the red fox chromosome arms 1q, 3q, 12q, 13q and 15q are mosaics of the two AKEC chromosomes. Most of the chromosome arm mosaicism found in modern canids relative to the ACK however, occurred before the emergence of the AKEC (see Fig. 12). The combined data of all Zoo-FISH hybridizations with canids shows that 14 of the 35 conserved chromosomes of the AKEC are mosaics of from two to four chromosome arm fragments of the ACK. Twenty-one chromosomes of the AKEC are composed of single chromosome arms (2) or arm fragments (19) of the ACK. Eight are composed of 2, three are composed of 3, and three are composed of 4 chromosome arms or arm fragments of the ACK. The 50 Myrs between the ACK and AKEC provided ample time for the formation of the 14 (8 + 3 + 3) mosaic chromosomes that separates these two karyotypes. The absence of telomere signals at internal arm fusion sites in the raccoon dog and arctic fox, and only one signal in the crab-eating fox, suggests MA inversions are more common than tandem fusions in the production of canid arm mosaicism. We also rule out a significant contribution from reciprocal translocations, since Zoo-FISH studies to date have not revealed any verifiable examples in canids and other carnivores.

Just after our manuscript was sent in for review, we became aware of a paper by Graphodatsky et al. (2001) who proposed an alternative model to ours based on similar independently derived data. They suggest that the ancestral karyotype of modern canids was low numbered (2n = 38) and this would eliminate the need for "extensive" independent centric fusions required by a model like ours, for example to account for the low numbered chromosome canids with independent chromosome arm associations. When starting with an ancestral low chromosome numbered karyotype it is difficult to derive multiple low numbered karyotypes whose arms are independently associated without extensive simultaneous centric fissions and fusions. For example, if the raccoon dog karyotype (2n = 38) is taken as ancestral, then derivation of modern red fox or arctic fox genome arrangement requires that every raccoon dog biarmed autosome be separated at their centromeres to allow for the independent associations of chromosome arms seen in their derived species.

Graphodatsky et al. (2001) suggest that "extensive" reciprocal translocations are primarily responsible for the chromosome changes that make the canid karyotype so distinctive from other carnivores. The chromosomes of extant canids are highly conserved and when compared to each other do not differ by obvious reciprocal translocations. We have shown that most of the chromosome reshuffling that led to the mosaic chromosome arms of canids occurred during the transition from the ACK to AKEC. If extensive reciprocal translocations mediated this transition, then reciprocal pairs of ancestral chro-

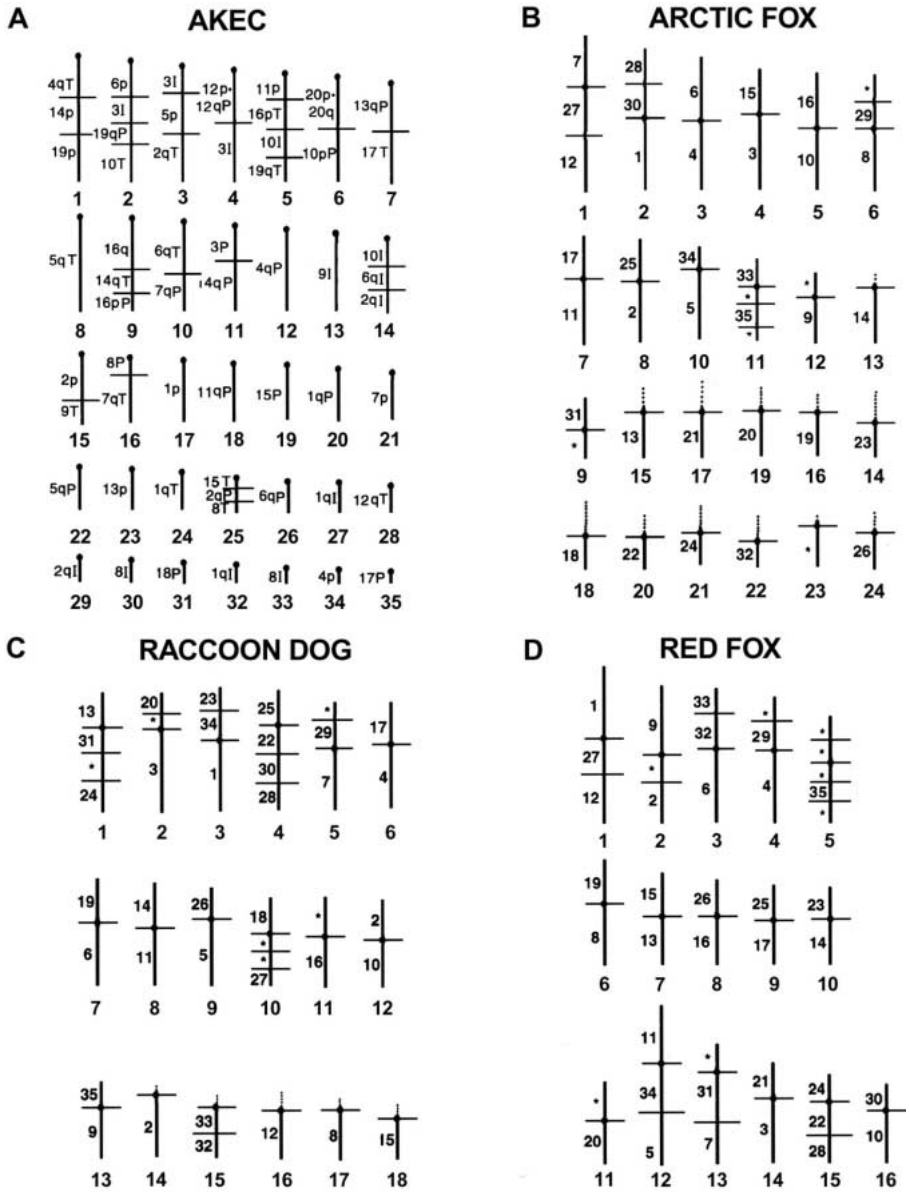


Fig. 12. (A) AKEC karyotype with ACK chromosome segments at left. Most small AKEC chromosomes are single ACK fragments while larger AKEC chromosomes are mosaics of ACK segments. Both fusions and fissions were involved in the construction of these chromosomes. (B) Arctic fox, (C) raccoon dog, and (D) red fox karyotypes with homologous AKEC chromosomes shown to the left. Canids with low chromosome number karyotypes differ from the AKEC primarily by extensive independent centric fusions. In the very low chromosome number karyotypes (C and D), chromosomes resulting from multiple fusions become more common. Horizontal lines indicate breakpoints. Vertical hatch marks in (B) and (C) indicate heterochromatin. p = short arm; q =long arm; P = proximal; I = interstitial; T = terminal; • = centromere; * = chromosome regions homologous to as yet undefined AKEC chromosomes.

mosome fragments should be common in canid chromosomes. Empirically, ancestral chromosomes are only very rarely observed. One such pair, C1p-E2 on chromosome 9 and E2-C1p on chromosome 14 of the raccoon dog (Fig. 4) could be a reciprocal pair, but even in this exceptional case the two fragments of C1p are derived from non-contiguous regions of the ancestral C1p chromosome arm.

Acknowledgments

The authors are grateful to Professor Malcolm Ferguson-Smith and Patricia O'Brien for providing flow-sorted chromosomes. In addition, the authors wish to thank Dr. Roscoe Stanyon and Dr. Lutz Frönicke for critical reading of the manuscript. Animal care was provided in accordance with the procedures outlined in the 'Guide for the Care and Use of Laboratory Animals' (NIH Publication No. 86-23, 1985).

References

Arnason U: Comparative chromosome studies in Pinnipedia. Hereditas 76:179 (1974).

Arnason U: The relationship between the four principal pinniped karyotypes. Hereditas 87:227 (1977).

Creel GC, Thornton WA: Comparative study of a *Volpes fulva-Vulpes macrotis* hybrid fox karyotype. Southwestern Naturalist 18:465 (1984).

Dutrillaux B, Couturier J: The ancestral karyotype of Carnivora: comparison with that of platyrrhine monkeys. Cytogenet Cell Genet 35:200 (1983).

Frönicke L, et al: Chromosomal homeologies between human, harbor seal (*Phoca vitulina*) and the putative ancestral carnivore karyotype revealed by Zoo-FISH. Chromosoma 106:108 (1997).

Graphodatsky AS, et al: A comparative chromosome map of the Arctic fox, red fox and dog defined by chromosome painting and high resolution G-banding. Chrom Res 8:253 (2000).

Graphodatsky AS, et al: Phylogenetic implications of the 38 putative ancestral chromosome segments for four canid species. Cytogenet Cell Genet 92:243 (2001).

Imai HT: A theoretical approach to chromosome banding analysis. Jpn J Genet 68:97 (1993).

Mäkinen A, Gustavsson I: A comparative chromosome banding study in the silver fox, the blue fox, and their hybrids. Hereditas 97:289 (1982).

Mäkinen A: The standard karyotype of the blue fox (*Alopex lagopus* L.). Hereditas 103:33 (1985).

Meyne J, et al: Distribution of non-telomeric sites of the $(TTAGGG)_n$ telomeric sequence in vertebrate chromosomes. Chromosoma 99:3 (1990).

Modi WS, et al: Cytogenetic methodologies for gene mapping and comparative analyses in mammalian cell culture systems. Gene Anal Tech 4:75 (1987).

Modi WS, O'Brien SJ: Quantitative cladistic analyses of chromosomal banding data among species in three orders of mammals: Hominoid primates, felids and arvicolid rodents, in Gustafson JP, Appels R (eds): Chromosome Structure and Function, pp 215–242 (Plenum, New York 1988).

Murphy WJ, et al: Evolution of mammalian genome organization inferred from comparative gene mapping. Genome Biol 2:REVIEWS0005 (2001).

Nash WG, O'Brien SJ: A comparative chromosome banding analysis of the Ursidae and their relationship to other carnivores. Cytogenet Cell Genet 45:206–212 (1987).

Nash WG, et al: Comparative genomics: tracking chromosome evolution in the family Ursidae using reciprocal chromosome painting. Cytogenet Cell Genet 83:182 (1998).

Wayne RK, et al: Chromosomal evolution of the Canidae. I. Species with high diploid numbers. Cytogenet Cell Genet 44:286 (1987a).

Wayne RK, et al: Chromosomal evolution of the Canidae. II. Divergence from the primitive carnivore karyotype. Cytogenet Cell Genet 44:134 (1987b).

Wayne RK: Molecular evolution of the dog family. Trends Genet 9:218 (1993).

Wienberg J, et al: Conservation of human vs. feline genome organization revealed by reciprocal chromosome painting. Cytogenet Cell Genet 77:211 (1997).

Wurster-Hill DH, Bush M: The interrelationship of chromosome banding patterns in the giant panda (*Ailuropoda melanoleuca*), hybrid bear (*Ursus middendorfi* × *Thalarctos maritimus*), and other carnivores. Cytogenet Cell Genet 27:147 (1980).

Wurster-Hill DH, Centerwall WR: The interrelationships of chromosome banding patterns in canids, mustelids, hyena, and felids. Cytogenet Cell Genet 34:178 (1982).

Wurster-Hill DH, Gray CW: The interrelationships of chromosome banding patterns in procyonids, viverrids, and felids. Cytogenet Cell Genet 15:306 (1975).

Wurster-Hill DH, et al: Banded chromosome studies and B chromosomes in wild-caught raccoon dogs, *Nystereutes procyonoides viveirinus*. Cytogenet Cell Genet 42:85 (1996).

Yang F, et al: Reciprocal chromosome painting illuminates the history of genome evolution of the domestic cat, dog and human. Chrom Res 8:393 (2000).

Yang F, et al: A complete comparative chromosome map for the dog, red fox, and human and its integration with Canine genetic maps. Genomics 62:189 (1999).

Yoshida MA, et al: Karyotypic kinship between the blue fox (*Alopex lagopus* Linn.) and the silver fox (*Vulpes fulva* Desm.). Cytogenet Cell Genet 35:190 (1983).