ORIGINAL ARTICLE

Chromosomal rearrangements and karyotype evolution in carnivores revealed by chromosome painting

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Chromosomal evolution in carnivores has been revisited extensively using cross-species chromosome painting. Painting probes derived from flow-sorted chromosomes of the domestic dog, which has one of the most rearranged karyotypes in mammals and the highest diploid number ($2n=78$) in carnivores, are a powerful tool in detecting both evolutionary intra- and inter-chromosomal rearrangements. However, only a few comparative maps have been established between dog and other non-Canidae species. Here, we extended cross-species painting with dog probes to seven more species representing six carnivore families: Eurasian lynx (*Lynx lynx*), the stone marten (*Martes foina*), the small Indian civet (*Viverricula indica*), the Asian palm civet (*Paradoxurus hermaphrodites*), Javan mongoose (*Hepestes javanicas*), the raccoon (*Procyon lotor*) and the giant panda (*Ailuropoda melanoleuca*). The numbers and positions of intra-chromosomal rearrangements were found to differ among these carnivore species. A comparative map between human and stone marten, and a map among the Yangtze finless porpoise (*Neophocaena phocaenoides asiaeorientalis*), stone marten and human were also established to facilitate outgroup comparison and to integrate comparative maps between stone marten and other carnivores with such maps between human and other species. These comparative maps give further insight into genome evolution and karyotype phylogenetic relationships among carnivores, and will facilitate the transfer of gene mapping data from human, domestic dog and cat to other species.
Heredity (2012) **108**, 17–27; doi:10.1038/hdy.2011.107; published online 16 November 2011

INTRODUCTION

Carnivores have sharply contrasting genome organizations, and are among the best examples for studying the role of chromosomal rearrangements in speciation. Previous G-banding comparisons in Carnivora have demonstrated that the karyotypes of most carnivores are highly conserved with exceptions in Ursidae and Canidae (Wurster-Hill, 1973; Wurster-Hill and Gray, 1975; Wurster-Hill and Centerwall, 1982; Dutrillaux and Couturier, 1983). Although the phylogenetic relationships in Carnivora have undergone frequent revisions (see Eizirik *et al.*, 2010 and references therein), all available molecular evidence supports the monophyly of Carnivora, which consists of two monophyletic groups, Feliformia and Caniformia. The suborder Feliformia now includes seven families: Felidae, Hyaenidae, Viverridae, Herpestidae, Prionodontidae (Asian linsangs), Eupleridae (Malagasy carnivores) and the monotypic Nandiniidae (the African palm civet); the suborder Caniformia usually consists of Canidae, Ursidae, Procyonidae, Mustelidae, Ailuridae, Mephitidae (skunks), Otariidae, Odobenidae and Phocidae (Eizirik *et al.*, 2010).

During the last two decades, chromosome-specific painting probes have been made for nine carnivores: the domestic cat (*Felis catus*, FCA) (Wienberg *et al.*, 1997), the domestic dog (*Canis familiaris*, CFA) (Breen *et al.*, 1999a; Yang *et al.*, 1999; Graphodatsky *et al.*, 2000a), the red fox (*Vulpes vulpes*, VVU) (Yang *et al.*, 1999), the

Japanese raccoon dog (*Nyctereutes procyonoides*, NPR) (Nash *et al.*, 2001), the American mink (*Mustela vision*, MVI) (Graphodatsky *et al.*, 2002), the stone marten (*Martes foina*, MFO) (Nie *et al.*, 2002), the giant panda (*Ailuropoda melanoleuca*, AME) (Nash *et al.*, 1998), the striped skunk (*Mephitis mephitis*, MME) and the hooded skunk (*Mephitis macroura*, MMA) (Perelman *et al.*, 2008). A series of comparative chromosome maps have been established among carnivores, and karyotypic phylogenetic relationships in different carnivore groups have been revisited by chromosome painting (Nash *et al.*, 1998, 2001, 2008; Graphodatsky *et al.*, 2000a, 2001, 2002, 2008; Nie *et al.*, 2002; Tian *et al.*, 2004; Perelman *et al.*, 2005, 2008). Up to now, about 40 species representing most carnivore families have been studied by cross-species chromosome painting. Such studies allow a genome-wide view of inter-chromosomal rearrangements and the proposition of putative ancestral karyotypes for the entire order and some families (Nash *et al.*, 1998, 2001, 2008; Graphodatsky *et al.*, 2001, 2002, 2008; Murphy *et al.*, 2001a; Tian *et al.*, 2004; Perelman *et al.*, 2005, 2008). Nevertheless, comparative molecular cytogenetic studies in Carnivora so far have not taken account of both inter- and intra-chromosomal rearrangements in phylogenetic analyses.

Previous chromosome painting demonstrated that CFA, with the highest diploid number ($2n=78$) and the most rearranged karyotype in Carnivora, is an ideal reference species for high-resolution
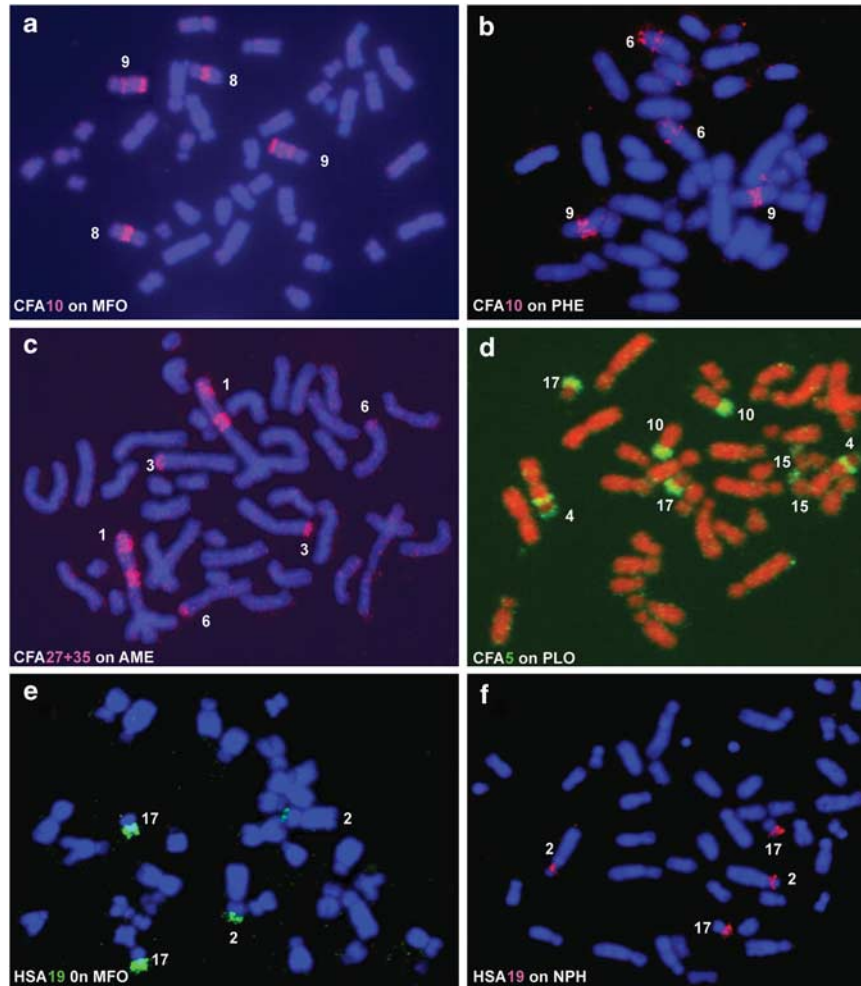
Figure 1 Examples of cross-species chromosome painting with human (HSA) and dog (CFA) chromosome-specific painting probes. (**a**) Hybridization of HSA 19 probe to metaphases of stone marten (MFO). (**b**) Hybridization of HSA 19 probe to metaphases of Yangtze finless porpoise (NPH). (**c**) Hybridization of CFA 10 probe to metaphases of stone marten (MFO). (**d**) Hybridization of CFA 10 probe to metaphases of Asian palm civet (PHE). (**e**) Hybridization of CFA 27+35 probes to metaphases of giant panda (AME). (**f**) Hybridization of CFA 5 probe to metaphases of raccoon (PLO).

comparative genomic analysis of carnivores (Yang et al., 1999, 2000), and that CFA painting probes could reveal cryptic inversions that would have escaped detection using painting probes from species with synteny-conserved genomes such as human (Homo sapiens, HSA) and FCA (Yang et al., 2000). CFA painting probes have been widely used to establish comparative chromosomal maps between CFA and other canids (Yang et al., 1999; Graphodatsky et al., 2000a, 2001, 2008; Nie et al., 2003). Nevertheless, only eight species from other families were studied using CFA painting probes, including FCA (2n=38, Yang et al., 2000), African lion (Panthera leo, PLE, 2n=38) and the clouded leopard (Neofelis nebulosa, NNE, 2n=38) (Tian et al., 2004) in Felidae; the spotted hyena (Crocuta crocuta, CCR, 2n=40) in Hyaenidae (Perelman et al., 2005); the masked palm civet (Paguma larvata, PLA, 2n=44) in Viverridae (Perelman et al., 2005); Malayan sun bear (Helartos malayanus, HMA, 2n=74) (Tian et al., 2004) and the spectacled bear (Tremarctos ornatus, TOR, 2n=50) (Yang and Graphodatsky, 2004) in Ursidae; red panda (Ailurus fulgens, AFU, 2n=36) in Ailuridae (Tian et al., 2002) and MVI (2n=30) in Mustelidae (Graphodatsky et al., 2000b). In addition, Nash et al. (2008) also established comparative chromosome maps between NPR, another species from the Canidae, and three carnivores (ringtails,

Bassariscus astutus, BAS, 2n=38; Dwarf mongoose, Helogale parvula, HPA, 2n=36; Malagasy civet, Fossa fossa, FFO, 2n=42). More carnivores representing different families remain to be studied by chromosome painting using CFA probes or probes from other carnivore species with highly rearranged karyotypes.

Molecular phylogenetic studies placed Carnivora, together with Eulipotyphla, Pholidota, Chiroptera, Perissodactyla and Cetartiodactyla, in one superordinal clade called Laurasiatheria (Murphy et al., 2001b). Within the Laurasiatheria, Pholidota, Perissodactyla and Cetartiodactyla were considered to be sister clades of Carnivora, and Pholidota was the closest living relatives of carnivores (Murphy et al., 2001b). In some molecular phylogenetic studies of Carnivora, the pangolins, whales and moles were selected as outgroups (Yu et al., 2004; Eizirik et al., 2010). Comparative chromosome maps between HSA and 10 carnivores, including FCA (Rettenberger et al., 1995; Wienberg et al., 1997; Yang et al., 2000), CFA (Breen et al., 1999b; Yang et al., 1999; Sargan et al., 2000), MVI (Hameister et al., 1997), the harbor seal (Phoca vitulina, PVI, Frönicke et al., 1997), AME (Nash et al., 1998), the domestic ferret (Mustela putorus furo, MPU, Cavagna et al., 2000), CCR (Perelman et al., 2005), PLA (Perelman et al., 2005), MME (Perelman et al., 2008) and the northern raccoon (Procyon lotor,
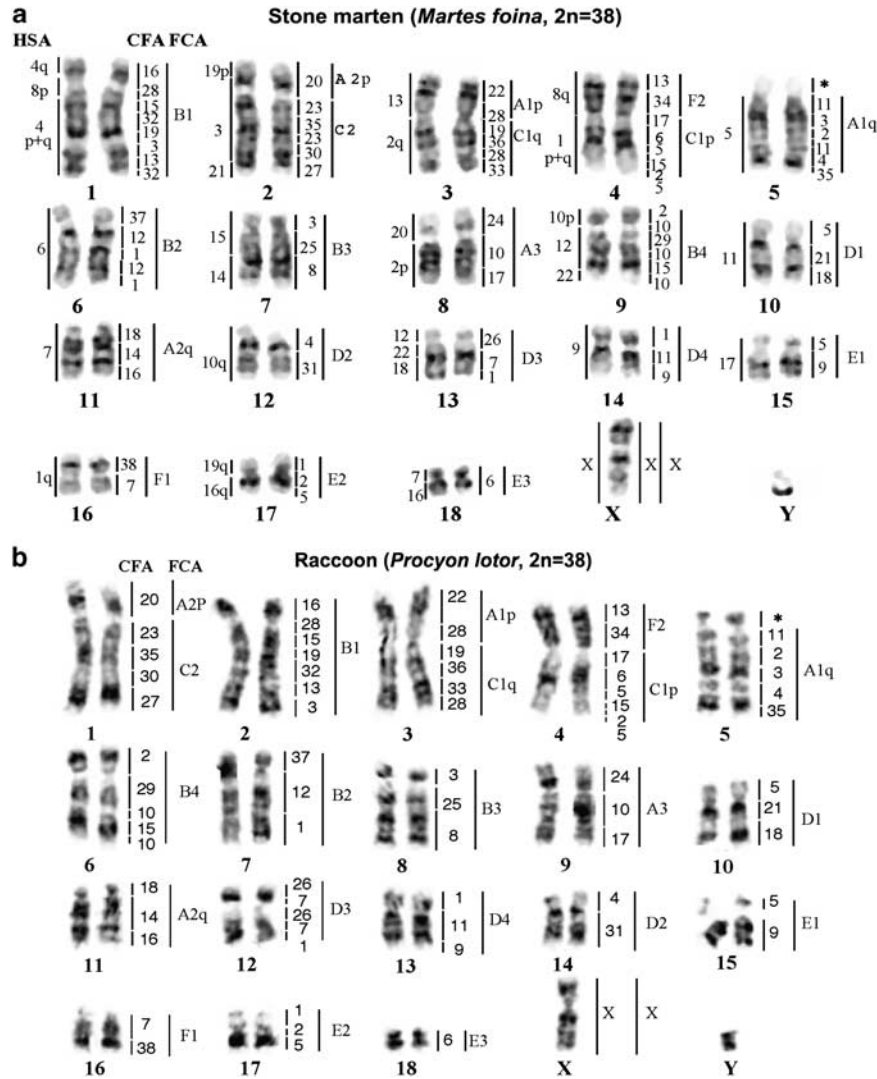
Figure 2 (**a**) G-banded karyotype of stone marten (MFO) with the assignment of homologies to human (HSA), dog (CFA) and cat (FCA) chromosomes. *The segment is not painted by any dog probes. (**b**) G-banded karyotype of raccoon (PLO) with the assignment of homologies to dog (CFA) and cat (FCA) chromosomes. *The segment is not painted by any dog probes.

PLO, Perelman et al., 2008), and between HSA and at least one representative of all other orders in Laurasiatheria (see Yang et al., 2006 and references therein) have been established; within Laurasiatheria only two non-carnivore species, that is, the domestic pig (Sus scrofa, 2n=38) and Javan pangolin (Manis javanica, 2n=38) have been studied with probes from carnivores (CFA and MFO) (Biltueva et al., 2004; Yang et al., 2006). Although the Canifornia and Feliformia could each act as an outgroup for the other branch owing to Carnivora splitting into these two branches very early in the Carnivore radiation (Nash et al., 2008), comparison of chromosomal rearrangements between carnivores and outgroup species from other Laurasiatheria orders will be helpful in determining the ancestral state of chromosome rearrangements within Carnivora.

Here, we established comparative chromosome maps between CFA and seven species representing six families of Carnivora: MFO (2n=38), PLO (2n=38), AME (2n=44), Eurasian lynx (Lynx lynx, LLY, 2n=38), Javan mongoose (Herpestes javanicus, HJA, 2n=36), the small Indian civet (Viverricula indica, VIN, 2n=36) and the Asian palm civet (Paradoxurus hermaphroditus, PHE, 2n=42). We also present here the results of chromosome painting studies between

HSA and MFO, and among HSA, MFO and the Yangtze finless porpoise (Neophocaena phocaenoides asiaeorientalis, NPH, 2n=44), a freshwater Cetacea species from Cetartiodactyla. Combined with previously published chromosomal painting data, our data provide further evidence for inter- and intra-chromosomal rearrangements in the genomes of different carnivores and insights into the phylogenetic relationships of carnivores.

MATERIALS AND METHODS
Cell culture, chromosomal preparation and G-banding
Fibroblast cell lines derived from LLY (KCB 200020), MFO (KCB 92037), PLO (KCB 200224), NPH (KCB 200820), PHE (KCB 200632), VIN (KCB 85022), HJA (KCB 83003) and AME (KCB200405) were provided by Kunming Cell Bank of the Chinese Academy of Sciences, Kunming, Yunnan, China. The cells culture, chromosomal preparation and G-banding followed the methods as described previously (Nie et al., 2002).

Fluorescence in situ hybridization
Chromosome-specific painting probes for CFA, MFO and HSA were prepared by degenerate oligonucleotide-primed PCR (Telenius et al., 1992) amplification of flow-sorted chromosomes as previously described (Yang et al., 1999;
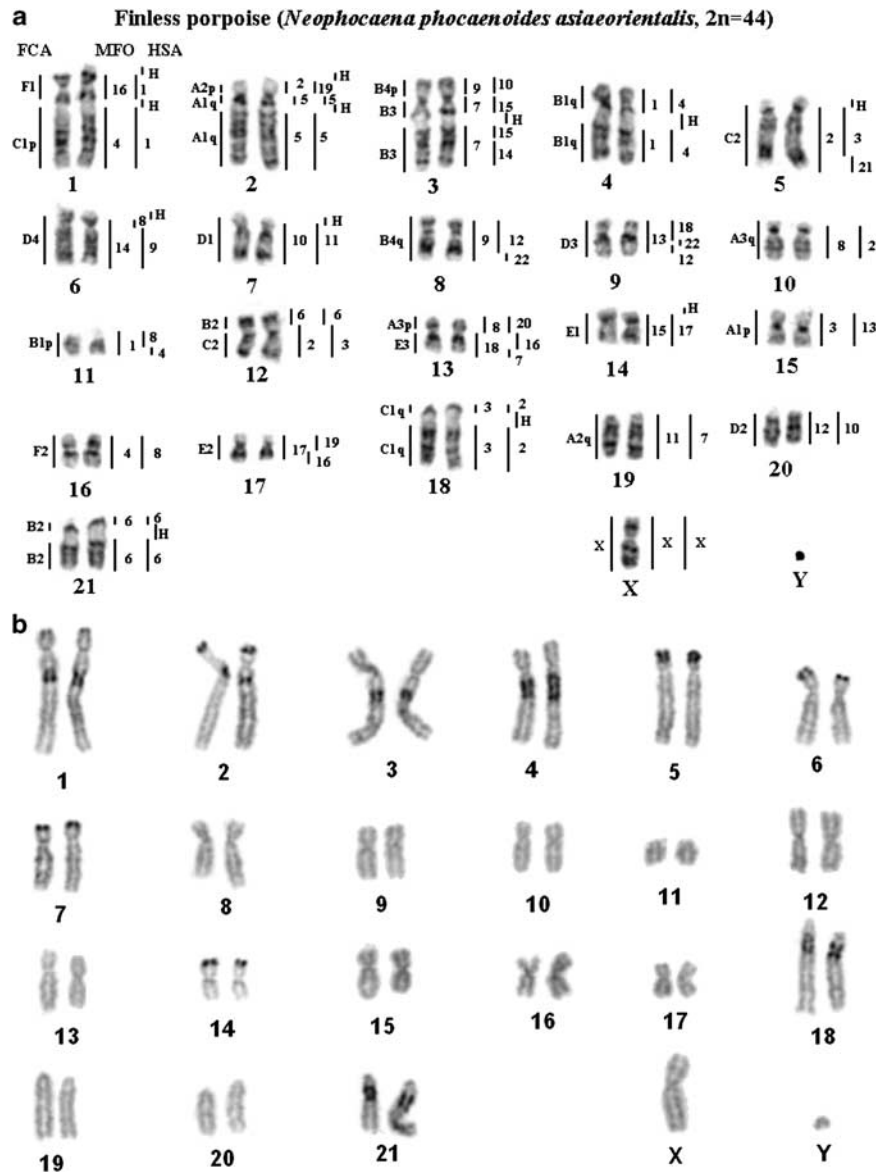
Figure 3 (a) G-banded karyotype of Yangtze finless porpoise (NPH) with the assignment of homologies to human (HSA), cat (FCA) and stone marten (MFO) chromosomes. (b) C-banded karyotype of Yangtze finless porpoise (NPH).

Nie *et al.*, 2002). Fluorescence *in situ* hybridization, detection, image capture and processing were carried out following Yang *et al.* (2000) and Nie *et al.* (2002).

Chromosome nomenclature

CFA chromosome nomenclature followed Yang *et al.* (1999), its correspondence with Breen *et al.* (1999a) nomenclature has been reported by Sargan *et al.* (2000). Chromosomes of PHE, AME and PLO were numbered according to the G-banding karyotypes reported previously (Wurster-Hill and Gray, 1975; Nash and O'Brien, 1987; Stanyon *et al.*, 1993). The arrangement of LLY chromosomes referred to the widely accepted FCA chromosomal nomenclature (Wurster-Hill and Centerwall, 1982). To facilitate the integration of cytogenetic maps, the chromosomes of NPH were arranged according to the nomenclature of the Atlantic bottlenose dolphin (*Tursiops truncatus*) (Bielec *et al.*, 1998) and the long-finned pilot whale (*Globicephala melas*) (Kulemzina *et al.*, 2009). The chromosomes of other carnivores were arranged based on their relative length, from the longest to the shortest.

RESULTS

Hybridizing HSA probes onto MFO chromosomes

Chromosomal homologies between MFO and several Mustelidae species have been established using MFO chromosome-specific probes (Nie *et al.*, 2002). To extend the homology link with HSA to more carnivore species, the metaphase spreads of MFO were also painted with HSA chromosome paints. Hybridization example of HSA probes is shown in Figure 1a. Homologous HSA chromosomal segments are summarized to the left of each MFO chromosome (Figure 2a). The 22 HSA autosomal probes detected 32 homologous chromosomal segments in the genome of MFO. Besides the conserved syntenic segment associations characteristic for eutherian mammals (that is, HSA 3/21, 4/8, 7/16, 10/12/22, 12/22, 14/15 and 16/19; Murphy *et al.*, 2001a; Yang *et al.*, 2003), five more syntenic segment associations (HSA 1/8, 2/13, 2/20, 3/19 and 18/22/12) were found in the genome of MFO.

Giant panda (*Ailuropoda melanoleuca*, 2n=44)
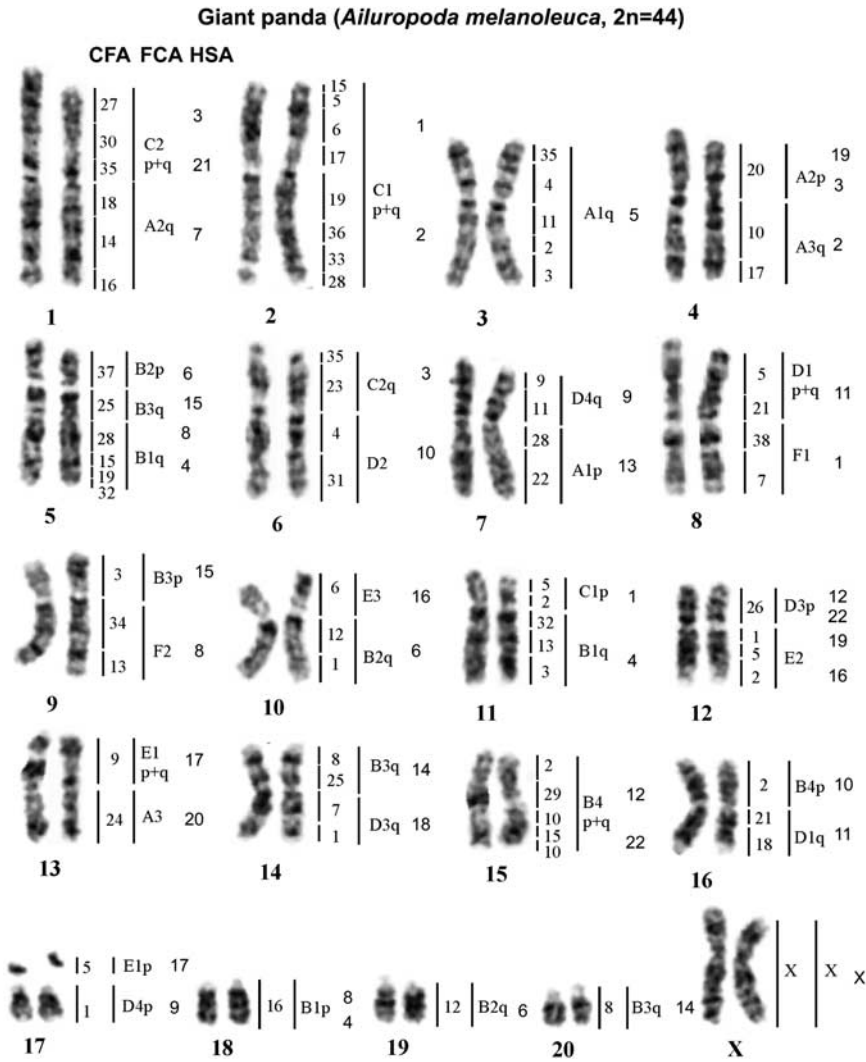
Figure 4 G-banded karyotype of giant panda (AME) with the assignment of homologies to dog (CFA), cat (FCA) and human (HSA) chromosomes. HSA painting data are cited from Nash *et al*. (1998).

Karyotype description of the Yangtze finless porpoise and cross-species painting with HSA and MFO probes

The Yangtze finless porpoise (NPH) is the sole freshwater subspecies of the finless porpoise. It has a 2n=44 karyotype, the same as most Cetacea species (Arnason, 2006). There are 17 pairs of bi-armed and four pairs of acrocentric autosomes. The X chromosome is metacentric; the acrocentric Y is the smallest chromosome (Figure 3a). C-bands, generated by over-denaturing metaphases with 70% formamide/30% 2× SSC (standard saline citrate) solution, were mainly localized at the telomeric regions of chromosomes 1, 2, 5–7 and 14, while chromosomes 1–4, 18 and 21 displayed interstitial C-bands (Figure 3b).

CFA paint probes failed to work on the chromosomes of NPH after several attempts. Thus, only probes from HSA and MFO were utilized to paint its chromosomes. An example of hybridization of HSA probes is shown in Figure 1b. Hybridization patterns of HSA painting probes onto NPH chromosomes are consistent with previous data on the long-finned pilot whale (2n=44, Kulemzina *et al*., 2009), except that some homologous chromosome segments were disrupted by heterochromatic blocks (Figure 3a). The 22 human autosomal probes detected 40 homologous chromosomal segments in the NPH genome.

Six additional syntenic segment associations (that is, HSA 3/6, 5/19, 8/9, 10/15, 16/20 and 18/22/12) were present in the NPH genome besides those conserved syntenic segment associations ancestral for eutherian mammals. The 18 MFO autosomal probes detected 31 homologous chromosomal segments in the NPH genome (Figure 3a). Painting probes from 10 MFO chromosomes (#5, 10–18) each painted one segment or one entire NPH chromosome, while the other MFO chromosome probes each gave two or three pairs of signals on NPH chromosomes. To facilitate comparison of homologous chromosomal segments among species, the homologous chromosomal segments of FCA were also indicated to the left of NPH chromosomes based on a published comparative chromosome map between MFO and FCA (Nie *et al*., 2002).

Hybridizing CFA probes onto chromosomes of seven carnivore species

Chromosome painting probes from CFA were hybridized to metaphase spreads of MFO, PLO, AME, LLY, HJA, VIN and PHE. Each CFA probe yielded 1 to 4 pairs of hybridization signals on the metaphase spreads of these seven species. Hybridization examples
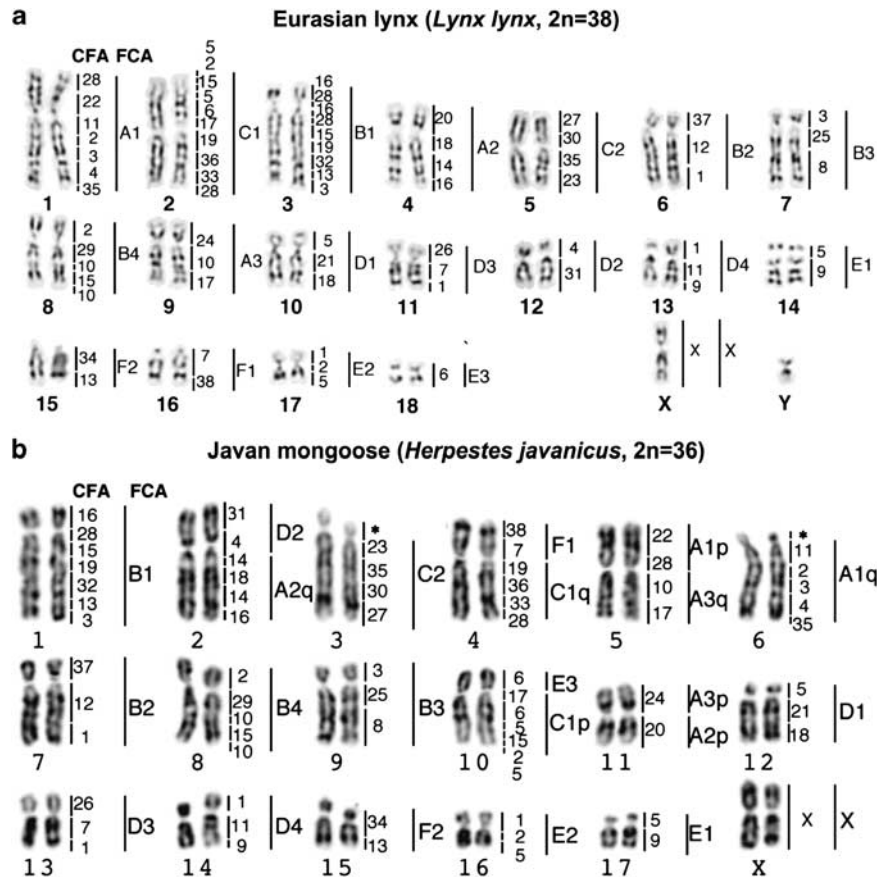
Figure 5 (**a**) G-banded karyotype of Eurasian lynx (*Lynx lynx*) with the assignment of homologies with dog (CFA) and cat (FCA) chromosomes. (**b**) G-banded karyotype of Javan mongoose (HJA) with the assignment of homologies with dog (CFA) and cat (FCA) chromosomes. *The segment is not painted by any dog probes.

are shown in Figures 1c–f. The hybridization patterns of 38 CFA autosomal probes and the X probe were summarized onto G-banded karyotypes of these seven species. Chromosomal correspondence with FCA as inferred from the CFA–FCA comparative chromosomal map (Yang *et al*., 2000) was also indicated beside the CFA segments on G-banded karyotypes. In total, 38 CFA autosomal probes revealed 72, 69, 74, 69, 68, 68 and 67 homologous segments in the genomes of MFO (Figure 2a), PLO (Figure 2b), AME (Figure 4), LLY (Figure 5a), HJA (Figure 5b), VIN (Figure 6a) and PHE (Figure 6b), respectively.

DISCUSSION

Implications for the signature rearrangements of Carnivora and for the putative ancestral carnivore karyotype

The establishment of comparative maps between CFA and the pig (Biltueva *et al*., 2004), between MFO and Javan pangolin (Yang *et al*., 2006), and between MFO and NPH (this study) provides a chance to compare directly chromosome homology between species in Carnivora and species in other orders from the superordinal clade Laurasiatheria, especially species used as outgroups in carnivore molecular phylogenetic studies. Of 18 MFO autosome paints, nine (MFO 10–18) and six (MFO 11, 12, 14, 15, 17 and 18) paints each hybridized to one segment or one chromosome in NPH (Figure 3a) and Javan pangolin (Yang *et al*., 2006), respectively. One association (MFO 6+2) seems to be common to NPH and Javan pangolin, but the results of chromosome painting with HSA probes confirmed that segments homologous to MFO 2 in this association were of different origins

in NPH (homologous to HSA3) and Javan pangolin (homologous to HSA19p).

In Carnivora, together with MFO, chromosome homologies between human and 11 species have been established (Rettenberger *et al*., 1995; Frönicke *et al*., 1997; Hameister *et al*., 1997; Wienberg *et al*., 1997; Nash *et al*., 1998; Yang *et al*., 1999, 2000; Cavagna *et al*., 2000; Sargan *et al*., 2000; Perelman *et al*., 2005, 2008; this study). Previous comparison of maps between HSA and these carnivores suggested that the HSA 2p/20, 18/22/12 and 19/3 associations could be specific signatures for Carnivora (Murphy *et al*., 2001a; Perelman *et al*., 2008). Except for the conserved syntenic segment associations characteristic for eutherian mammals, we found no common associations between carnivores and Javan pangolin, but one common association (HSA 18/22/12) between carnivores and NPH. The HSA 18/22/12 association was also detected in the Atlantic bottlenose dolphin (*T. truncatus*, Bielec *et al*., 1998) and the long-finned pilot whale (*G. melas*, Kulemzina *et al*., 2009). Therefore, the HSA 18/22/12 association could not be considered as a character specific for Carnivora; instead it could be a cytogenetic character linking Carnivora and Cetartiodactyla.

Reconstruction of ancestral karyotypes of different mammalian taxa will be helpful in determining the mode and tempo of evolutionary changes that have occurred in mammalian phylogenetic lineages (Murphy *et al*., 2001a). Two types of ancestral carnivore karyotypes (ACKs) with different diploid numbers (2*n*=42 and 2*n*=38) have been proposed based on comparisons of R-banded karyotypes and fluorescence in situ hybridization data (Dutrillaux and Couturier,
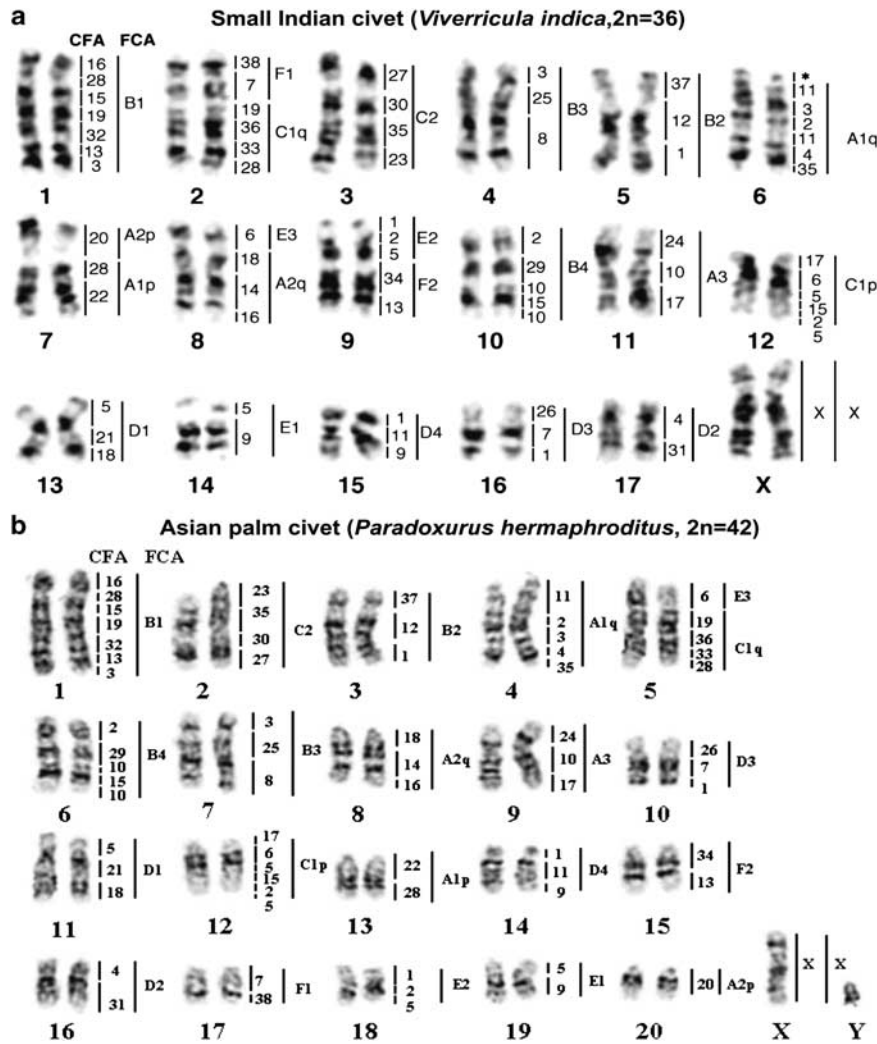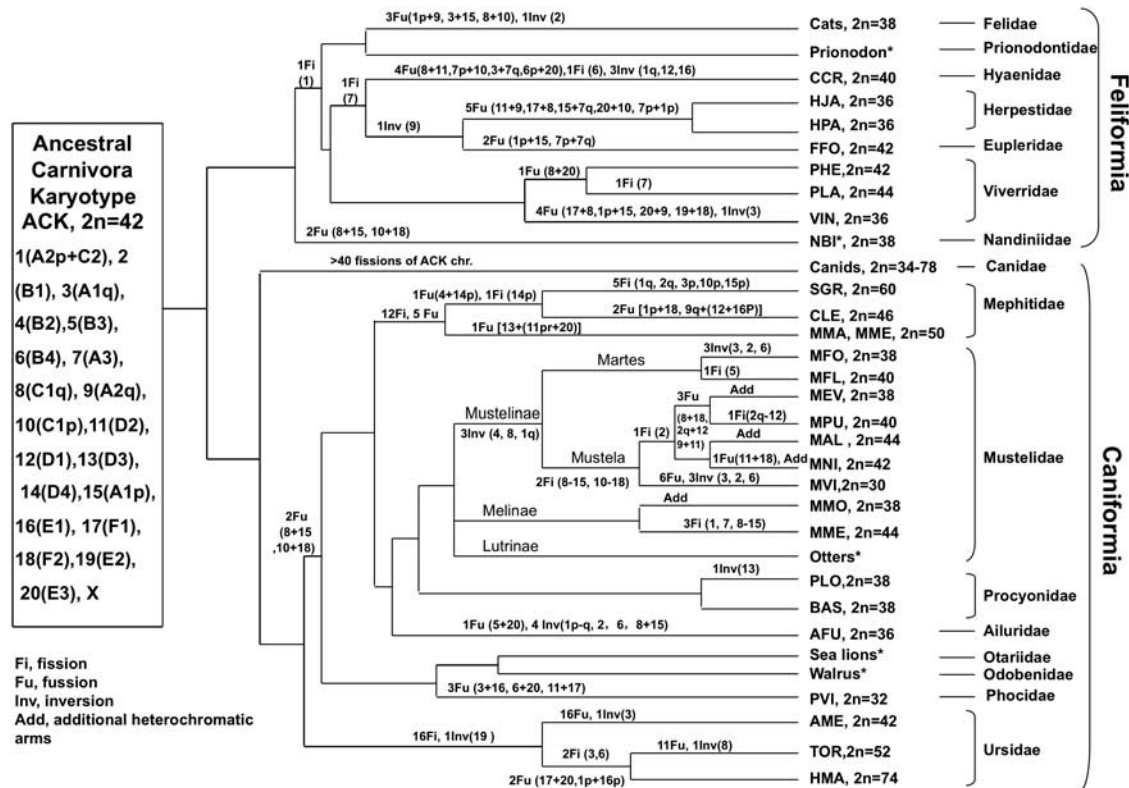
Figure 6 (a) G-banded karyotype of small Indian civet (VIN) with the assignment of homologies to dog (CFA) and cat (FCA) chromosomes. *The segment is not painted by any dog probes. (b) G-banded karyotype of Asian palm civet (PHE) with the assignment of homologies to dog (CFA) and cat (FCA) chromosomes.

1983; Frönicke et al., 1997; Murphy et al., 2001a; Nash et al., 2008). The majority of the ancestral chromosomes are identical in these two types of ACKs. The difference between 2n=42 and 2n=38 putative ancestral karyotypes concerns two chromosomes, homologous to FCA A1p+C1q and C1p+F2. In the 2n=42 ACK, these two chromosomes were supposed to be four single chromosomes homologous to cat A1p, C1q, C1p and F2 (Dutrillaux and Couturier, 1983; Murphy et al., 2001a), while they were retained as two whole chromosomes in the 2n=38 ACK (Frönicke et al., 1997; Nash et al., 2008). Recently, Perelman et al. (2008) also questioned the ancestral state of four ACK chromosomes, homologous to FCA A2p+C2, A3p+A3q, A1p+C1q and C1p+F2, but it proved difficult to determine whether fissions or fusions of these four chromosomes represented the ancestral state when analyzing the distribution of these four chromosomes on different branches of the Carnivora tree.

Comparative maps between HSA and representatives of all the orders in Laurasiatheria have been established (see Yang et al., 2006 and references therein). Comparing these maps with those between carnivores, we found that no association was common to species in Laurasiatheria if we excluded the putative ancestral syntenic associations for eutherian mammals. Nevertheless, one fission event

occurring on the chromosome homologous to FCA A3p+A3q is noteworthy. The FCA A3p+A3q homologues were present as two chromosomes or chromosomal segments (that is, A3p and A3q) in species from other orders such as the pig (Cetartiodactyla, Biltueva et al., 2004), Javan pangolin (Pholidota, Yang et al., 2006) and NPH (Cetartiodactyla, Figure 3a). Furthermore, FCA A3p and A3q were also found to be homologous to two discrete chromosomal segments in HSA (that is, a representative species of Euarchontoglires, Yang et al., 1999, 2000). However, both fusion and fission states of FCA A3 were found in different carnivore families and even in species with different diploid numbers in the same family (Figure 7). These data taken together appear to support the idea that the fission state (rather than the fusion state) of FCA A3p+A3q should be regarded as the ancestral condition. In other words, chromosomal segments homologous to FCA A3p and A3q could represent two separate chromosomes in the ACK.

Cryptic inversions in carnivores revealed by CFA painting probes
Using the CFA–FCA comparative chromosomal map (Yang et al., 2000) as the common reference and comparing the hybridization patterns of CFA painting probes on the large blocks of synteny-

Ancestral Carnivora Karyotype ACK, 2n=42

1(A2p+C2), 2 (B1), 3(A1q), 4(B2),5(B3), 6(B4), 7(A3), 8(C1q), 9(A2q), 10(C1p),11(D2), 12(D1),13(D3), 14(D4),15(A1p), 16(E1), 17(F1), 18(F2),19(E2), 20(E3), X

Fi, fission
Fu, fusion
Inv, inversion
Add, additional heterochromatic arms

3Fu(1p+9, 3+15, 8+10), 1Inv (2) — Cats, 2n=38 — Felidae
Prionodon* — Prionodontidae
1Fi (1)
4Fu(8+11,7p+10,3+7q,6p+20),1Fi (6), 3Inv (1q,12,16) — CCR, 2n=40 — Hyaenidae
1Fi (7)
5Fu (11+9,17+8,15+7q,20+10, 7p+1p) — HJA, 2n=36 / HPA, 2n=36 — Herpestidae
1Inv (9)
2Fu (1p+15, 7p+7q) — FFO, 2n=42 — Eupleridae
1Fu (8+20) — PHE,2n=42
1Fi (7)
PLA, 2n=44 — Viverridae
4Fu (17+8,1p+15, 20+9, 19+18), 1Inv(3) — VIN, 2n=36
2Fu (8+15, 10+18) — NBI*, 2n=38 — Nandiniidae

Feliformia

>40 fissions of ACK chr. — Canids, 2n=34-78 — Canidae
1Fu(4+14p), 1Fi (14p)
5Fi (1q, 2q, 3p,10p,15p) — SGR, 2n=60
12Fi, 5 Fu
2Fu [1p+18, 9q+(12+16P)] — CLE, 2n=46
1Fu [13+(11pr+20)] — MMA, MME, 2n=50 — Mephitidae
Martes
3Inv(3, 2, 6) — MFO, 2n=38
1Fi (5) — MFL, 2n=40
3Fu — Add — MEV, 2n=38
Mustelinae (8+18, 1Fi(2q-12) — MPU, 2n=40
3Inv (4, 8, 1q) 2q+12 Add — MAL , 2n=44
1Fi (2) 9+11) 1Fu(11+18), Add — MNI, 2n=42
Mustela — MVI,2n=30
2Fi (8-15, 10-18) 6Fu, 3Inv (3, 2, 6)
Add — MMO, 2n=38
Melinae 3Fi (1, 7, 8-15) — MME, 2n=44 — Mustelidae
Lutrinae — Otters*
1Inv(13) — PLO,2n=38
BAS, 2n=38 — Procyonidae
1Fu (5+20), 4 Inv(1p-q, 2, 6, 8+15) — AFU, 2n=36 — Ailuridae
Sea lions* — Otariidae
Walrus* — Odobenidae
3Fu (3+16, 6+20, 11+17) — PVI, 2n=32 — Phocidae
2Fu (8+15 ,10+18)
16Fu, 1Inv(3) — AME, 2n=42
16Fi, 1Inv(19)
2Fi (3,6) 11Fu, 1Inv(8) — TOR,2n=52 — Ursidae
2Fu (17+20,1p+16p) — HMA, 2n=74

Caniformia

Figure 7 Karyotype relationships of the order Carnivora. Tree topology for Carnivora is modified from Eizirik et al. (2010). Published chromosome painting data for Felidae (Wienberg et al., 1997; Yang et al., 2000; Tian et al., 2004), Hyaenidae and Viverridae (Perelman et al., 2005), Herpestidae and Eupleridae (Nash et al., 2008), Canidae (Breen et al., 1999b; Yang et al., 1999, 2000; Graphodatsky et al., 2000a, 2001, 2008; Nash et al., 2001; Nie et al., 2003), Mephitidae (Perelman et al., 2008), Mustelidae (Hameister et al., 1997; Cavagna et al., 2000; Graphodatsky et al., 2000b, 2002; Nie et al., 2002), Procyonidae (Nash et al., 2008; Perelman et al., 2008), Ailuridae (Nie et al., 2002; Tian et al., 2002), Phocidae (Frönicke et al., 1997), Ursidae (Nash et al., 1998, 2001; Tian et al., 2004; Yang and Graphodatsky, 2004) and the data in this study were used for this figure. The ACK was from Murphy et al. (2001a). Chromosome numbers in brackets on the tree correspond to ACK chromosomes. *Species in the branches have no chromosome painting data or not confirmed by chromosome painting. CLE, Conepatus leuconotus; MAL, Mustela altaica; MFL, Martes flavigula; MME, Meles meles; MMO, Melogale moschata; MNI, Mustela nivalis; MLU, Mustela lutreola; SGR, Spilogale gracilis.

conserved chromosomes (homologous FCA chromosomes or chromosomal segments), we detected cryptic inversions, with varying numbers and positions, in the genomes of MFO, PLO, AME, LLY, HJA and VIN. However, no inversions were detected in the genome of PHE. Combined analysis of the current data with the previously published data allows an overview of the inversions that occurred in carnivores belonging to different families (Table 1).

In Felidae, paints from CFA chromosomes 16 and 28 on chromosomes homologous to FCA B1 displayed the same painting pattern of CFA 16/28/16/28 in LLY (Figure 5a), FCA (Yang et al., 2000), NNE and PLE (Tian et al., 2004), but the arrangement of CFA chromosomes 16 and 28 homologous segments in other carnivores was CFA 16/28, suggesting a common inversion occurred in felids.

In Herpestidae, only HJA (this study) and Dwarf mongoose (HPA) (Nash et al., 2008) have chromosome painting data. These two species have the same diploid number (2n=36) and an identical chromosomal homology pattern with FCA. An inversion was detected by CFA and NPR painting probes respectively in chromosomal segments homologous to FCA A2q in HJA (CFA 14/18/14/16, Figure 5b) and HPA (NPR 1/11/1/18, Nash et al., 2008). Chromosome painting results showed that CFA chromosomes 14, 18 and 16 corresponded to NPR chromosomes 1p, 11p and 18, respectively (Graphodatsky et al., 2001). The inversion detected by the CFA and NPR painting probes in HJA and HPA appears to be the same. The same inversion

was also detected in the same homology segment in Malagasy civet (FFO) by NPR painting probes (NPR 1/11/1/18, Nash et al., 2008). Previous molecular phylogenetic study indicated that Malagasy carnivores actually formed a separate feliform family Eupleridae, not included in the Herpestidae and Viverridae (Yoder et al., 2003). Therefore, the inversion detected in HJA, HPA and FFO could be a common cytogenetic signature for the Herpestidae and Eupleridae.

In Viverridae, only one inversion (CFA 11/3/2/11/4/35) in VIN chromosome 6 (equivalent to FCA A1q, Figure 6a) was detected by CFA painting probes.

In Hyaenidae, three inversions (CFA 27/30/23/35, CFA 21/5/21/5/18 and CFA 5/9/5) were found in chromosomes homologous to FCA C2, D1, E1 in CCR (2n=40) (Perelman et al., 2005).

In Ursidae, the pattern of CFA paints 1, 2 and 5 on the homologues to FCA E2 was the same (CFA 1/5/2) in AME (Figure 4), TOR (Yang and Graphodatsky, 2004) and HMA (Tian et al., 2004), while the painting pattern of CFA paints 1, 2 and 5 on the equivalents of FCA E2 in other carnivores was CFA 1/2/5. Although chromosome equivalents of FCA E2 in TOR and HMA had a different centromere position, this inversion has been proposed as one common character for Ursidae (Tian et al., 2004). Another two inversions (CFA 35/4/11/2/3 and CFA 19/33/36/28) were found in the genomes of AME (chromosome 3, equivalent to FCA A1q, Figure 4) and TOR (chromosome 6, equivalent to FCA C1q, Yang and Graphodatsky, 2004).

Table 1 Inversions revealed by dog paint probes in large chromosome segments of conserved synteny in different carnivores

Species (2n)		Homologous cat chromosomes or chromosome segments										References
		A1q	A2q	B1	B2	B4	C1q	C2	D1	D3	E1	E2	
Feliformia	**Felidae**												
	FCA (38)			○									Yang et al. (2000)
	PLE (38)			○									Tian et al. (2004)
	NNE (38)			○									Tian et al. (2004)
	LLY (38)			○									This study
	Herpestidae												
	HJA (36)		●										This study
	Viverridae												
	VIN (36)	■											This study
	Hyaenidae												
	CCR (40)							▽	✱		⟦		Perelman et al. (2005)
Caniformia	**Ursidae**												
	TOR (52)						☆					◆	Yang and Graphodatsky (2004)
	HMA (74)											◆	Tian et al. (2004)
	AME (44)	◇										◆	This study
	Ailuridae												
	AFU* (36)			∫		∮	#	※					Tian et al. (2002)
	Procyonidae									〖			This study
	PLO (38)												
	Mustelidae												
	MVI (30)	□		◎	§	△	★	▼					Graphodatsky et al. (2000b)
	MFO (38)	■		⊙	§	▲	★	▼					This study

Abbreviations: AFU, *Ailurus fulgens*; AME, *Ailuropoda melanoleuca*; CCR, *Crocuta crocuta*; FCA, *Felis catus*; HJA, *Herpestes javanicu*; HMA, *Helartos malayanus*; LLY, *Lynx lynx*; MFO, *Martes foina*; MVI, *Mustela vision*; NNE, *Neofelis nebulosa*; PLE, *Panthera leo*; PLO, *Procyon lotor*; TOR, *Tremarctos ornatus*; VIN, *Viverricula indica*.
AFU*, inversions each was detected to occur between two chromosome segments (homologous to FCAA1p+C1q and FCAA2p+C2) in red panda. Different symbols indicated different inversions. Identical inversion in different species was indicated by the same symbol. ■, CFA 11/3/2/11/4/35; ◇, CFA 35/4/11/2/3; □, CFA 3/2/11/4/35; ●, CFA 14/18/14/16; ○, CFA 16/28/16/28/15/19/32/13/3; ∫, CFA 16/28/16/15/19/32/13/3; ◎, CFA 16/15/28/32/19/13/3; ⊙, CFA 16/28/15/32/19/3/13/32; §, CFA 37/12/1/12/1; ∮, CFA 29/2/29/10/15/10; △, CFA 2/10/15/29/10/15/10; ▲, CFA 2/10/29/10/15/10; ☆, CFA 19/33/36/28; #, CFA 22/19/28/36/33/28; ★, CFA 19/36/28/33; ▽, CFA 27/30/23/35; ※, CFA 20/23/20/23/35/30/27; ▼, CFA 23/35/23/30/27; ✱, CFA 21/5/21/5/18; 〖, CFA 26/7/26/7/1; ⟦, CFA 5/9/5; ◆, CFA 1/5/2.

In Ailuridae, four inversions (CFA 20/23/20/23/35/30/27, CFA 22/19/28/19/36/33/28, CFA 16/28/16/15/19/32/13/3 and CFA 29/2/29/10/15/10) were detected in chromosomes homologous to FCA A2p+C2, A1p+C1q, B1 and B4 in AFU (2n=36) by CFA paints (Tian et al., 2002).

In Procyonidae, the painting pattern generated by CFA chromosomes 1, 7 and 26 paints on PLO chromosome 12 (equivalent to FCA D3) was CFA 26/7/26/7/1 (Figure 2b), which was different from the pattern CFA 26/7/1 on the equivalent chromosomes of other carnivores, suggesting that an inversion occurred in PLO chromosome 12.

In Mustelidae, inversions were detected in six homologous chromosomes or chromosomal segments (equivalents to FCA A1q, B1, B2, B4, C1q and C2) in MFO (2n=38, Figure 2a) and MVI (2n=30, Graphodatsky et al., 2000b) by CFA painting probes. Three common inversions (CFA 37/12/1/12/1, CFA 19/36/28/33 and CFA 23/35/23/30/27) were recognized in chromosomes of MFO and MVI homologous to FCA B2, C1q and C2. These inversions could be common cytogenetic signatures for the Mustelidae. In addition, the probe from CFA chromosome 10 gave three signals on chromosomes homologous to FCA B4 in MFO (Figure 1c) and MVI (Graphodatsky et al., 2000b). This inversion seems to be a common character for MFO and MVI. But another inversion revealed by the probe of CFA chromosome 15 was also found on the same chromosome in MVI, resulting in the different hybridization patterns on the homologues to FCA B4 in MFO and MVI.

After comparing inversions occurring in species from different families, we found an obviously identical inversion in a Feliformia (VIN) and a Caniformia species (MFO) (Table 1). A probe from CFA chromosome 11 painted two segments on VIN 6 and MFO 5 (equivalents to FCA A1q), resulting in the change of the CFA probes hybridization pattern on chromosomes homologous to FCA A1q from CFA 11/2/3/4/35 in other carnivores to CFA 11/3/2/11/4/35 in VIN and MFO, and MFO 5 and VIN 6 showed similar G-bands (Figures 2a and 6a). Nevertheless, it is difficult to say if this inversion is a common character for Feliformia and Caniformia as it could have evolved independently in these two different clades.

To sum up, inversions have been revealed in most carnivores studied by CFA paints, and some could represent cytogenetic signatures for a given carnivore species group, while others were specific for a given species. For instance, one inversion on the homologues to FCA A2q appears to be common to all species so far studied in the Herpestidae and Eupleridae; one inversion on the homologues to FCA B1 seems to be specific for all studied Felidae species, one inversion on the homologues to FCA E2 seems to be common to all species studied in Ursidae, and three common inversions on the homologues to FCA B2, C1q and C2 appear to be shared by two Mustelidae species. Our findings suggest that inversions have had an important role in the karyotype divergence of carnivores and, in particular, in species with synteny-conserved karyotypes such as the mustelids.

Mapping chromosome rearrangements onto the phylogenetic tree of Carnivora

On the basis of the multiple nuclear gene sequences, Eizirik *et al.* (2010) proposed a complete molecular phylogeny for 50 different genera representing all carnivoran families and constructed a molecular timescale for the evolution of Carnivora. Many species with painting data were also included in this study. Mapping the chromosome rearrangements identified by chromosomal painting onto the relevant lineages of the phylogenetic tree proposed by Eizirik *et al.* (2010) enabled us to trace the characteristic chromosomal rearrangements and karyotypic evolution relationships in the major phylogenetic lineages in the order Carnivora (Figure 7).

Here, we used the $2n=42$ ACK as the starting point to map the chromosome rearrangements that have occurred during the divergence of Carnivora. In Feliformia, except for the African palm civet (*Nandinia binotata*, NBI, Nandiniidae), the fission of ACK 1 (FCA A2p+C2) was the common character for all the species studied. Two fusions (ACK8+15 and 10+18) differentiated the karyotype of NBI from that of other species in Feliformia. Three fusions (ACK 1p+9, 3+15 and 8+10) and one inversion (ACK2) characterized the Felidae branch. One fission (ACK 7) supported the clade of Hyaenidae+Herpestidae+Eupleridae. A further inversion (ACK9) linked Herpestidae and Eupleridae. In Viverridae, different chromosome rearrangements were found in species with different diploid numbers.

In Caniformia, >40 fissions differentiated the karyotypes of species in Canidae from the ACK. Sixteen fissions and one inversion were common to all species studied in Ursidae. A further 16 fusions and 1 inversion occurred in AME. Two fusions and two fissions were common to TOR and bears with $2n=74$. A further 11 fusions and 1 inversion differentiated the karyotype of TOR from that of other bears. Two common fusions (ACK8+15 and 10+18) were found in species in Mustelidae, Procyonidae, Ailuridae and Phocidae. They could be considered as common characters for species in the Arctoidea excluding Ursidae. Mephitidae (skunks) are the third family of Carnivora that was found to have highly rearranged karyotypes, besides Canidae and Ursidae (Perelman *et al.*, 2008). More than 10 rearrangements differentiated the karyotypes of species in Mephitidae from the ACK. Species in Procyonidae have the same diploid number and similar painting patterns with some species in Mustelidae, supporting the idea that these two families have close relationships. The karyotypic evolutionary relationships among species in Mustelidae have been detailed by Graphodatsky *et al.* (2002). Furthermore, three common inversions were revealed in two species from the Mustelidae in our study. Some specific chromosomal rearrangements were found in species from the Phocidae and Ailuridae (Frönicke *et al.*, 1997; Nie *et al.*, 2002; Tian *et al.*, 2002). The role of chromosome rearrangements in speciation remains controversial, but some landmark chromosomal rearrangements have been found at the major nodes of the Carnivora phylogenetic tree.

DATA ARCHIVING

This article does not report new empirical data or software.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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