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Chromosome painting shows that skunks (Mephitidae, Carnivora) have highly rearranged karyotypes

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Abstract

The karyotypic relationships of skunks (Mephitidae) with other major clades of carnivores are not yet established. Here, multi-directional chromosome painting was used to reveal the karyological relationships among skunks and between Mephitidae (skunks) and Procyonidae (raccoons). Representative species from three genera of Mephitidae (*Mephitis mephitis*, 2n = 50; *Mephitis macroura*, 2n = 50; *Conepatus leuconotus*, 2n = 46; *Spilogale gracilis*, 2n = 60) and one species of Procyonidae (*Procyon lotor*, 2n = 38) were studied. Chromosomal homology was mapped by hybridization of five sets of whole-chromosome paints derived from stone marten (*Martes foina*, 2n = 38), cat, skunks (*M. mephitis*; *M. macroura*) and human. The karyotype of the raccoon is highly conserved and identical to the hypothetical ancestral musteloid karyotype, suggesting that procyonids have a particular importance for establishing the karyological evolution within the caniforms. Ten fission events and five fusion events are necessary to generate the ancestral skunk karyotype from the ancestral carnivore karyotype. Our results show that Mephitidae joins Canidae and Ursidae as the third family of carnivores that are characterized by a high rate of karyotype evolution. Shared derived chromosomal fusion of stone marten chromosomes 6 and 14 phylogenetically links the American hog-nosed skunk and eastern spotted skunk.

Abbreviations

Animals	
CLE	*Conepatus leuconotus*, American hog-nosed skunk
FCA	*Felis catus*, cat
HSA	*Homo sapiens*, human
MFO	*Martes foina*, stone marten
MMA	*Mephitis macroura*, hooded skunk
MME	*Mephitis mephitis*, striped skunk
PLO	*Procyon lotor*, northern raccoon
SGR	*Spilogale gracilis*, western spotted skunk
Other abbreviations	
ACK	ancestral carnivore karyotype
AMK	ancestral musteloid karyotype

BAC	bacterial artificial chromosome
C-bands	heterochromatic regions close to the centromeres and regions rich in satellite DNA
Cot-1	fraction of DNA consisting largely of highly repetitive sequences
DAPI	4′,6-diamidino-2-phenylindole
DOP–PCR	degenerate oligonucleotide primer PCR
FACS	fluorescence activated cell sorting
FISH	fluorescent *in-situ* hybridization
FITC	fluorescein isothiocyanate
G-banding	Giemsa banding
NOR	nucleolar organising region
PBS	phosphate buffered saline
PCR	polymerase chain reaction
SSC	standard saline citrate

Introduction

Skunks (Mephitidae) are part of Caniformia branch of the order Carnivora and comprise four genera (*Mephitis*, *Conepatus*, *Spilogale* and *Mydaus*) and 12 species. Early systematics usually included skunks as a subfamily, Mephitinae, within the weasel family Mustelidae (Simpson 1945, Wozencraft 1993). More recently, skunks were raised to family rank, the Mephitidae, based on evidence from morphological and molecular phylogenetic studies (Vrana *et al.* 1994, Ledje & Arnason 1996b, Dragoo & Honeycutt 1997, Flynn *et al.* 2000, 2005, Sato *et al.* 2004, Wozencraft 2005).

Surprisingly, the karyotypes of skunks are poorly known. In genus *Mephitis* only the karyotype of the striped skunk (*Mephitis mephitis*, 2n = 50) was studied, using conventional and differential staining procedures (Fredga 1966, Hsu & Benirschke 1967, Genest *et al.* 1986). For the genus *Conepatus* only the karyotype of *C. leuconotus* is known (2n = 46; Woodward 1994). The genus *Spilogale* has a wide range of diploid numbers (*S. pygmae* 2n = 48, *S. gracilis* 2n = 60) and *S. putorius* apparently shows a significant variation in chromosome number between different subspecies, 2n = 58–64 (Hsu & Benirschke 1967, Hsu & Mead 1969, Hsu & Arrighi 1971, Owen *et al.* 1996).

On the basis of conventional chromosome staining, Wurster and Benirshke noticed that ‘the skunks were remarkably different from other Mustelidae’ and that each species had ‘a highly individualistic karyotype’ (Wurster & Benirschke 1968). Graphodatsky *et al.* compared G-banded chromosomes of striped skunk presented by Genest *et al.* with banded chromosomes of other mustelids (Genest *et al.* 1986, Graphodatsky *et al.* 1989). Hardly a single chromosome of the skunk could be found without rearrangement in any other mustelid karyotype. Some authors have even hypothesized that multiple fissions of chromosomes probably occurred in the skunk lineage and that Mephitinae were phylogenetically distant from other mustelids (Graphodatsky *et al.* 1989).

In sharp contrast, karyotypes of procyonids were well described and are strikingly uniform (Wurster & Benirschke 1968; http://www.bionet.nsc.ru/chromosomes/). All known procyonid karyotypes have 38 apparently identical chromosomes (Wurster-Hill & Gray 1975). Recently, chromosome painting of the ringtail (*Bassariscus astutus*, 2n = 38) confirmed that procyonids have conserved the ancestral musteloid karyotype (Nash *et al.* 2008).

Chromosomal rearrangements are considered valuable characters for phylogenetic studies (Dobigny *et al.* 2004). Fortunately, Carnivora is one of the best-studied mammalian orders and there is chromosome painting on 35 species representing most major Carnivora families including Felidae, Vivveridae, Herpestidae, Hayenidae, Eupleridae, Canidae, Ursidae, Mustelidae, Ailuridae, and Pinnipedia (Frönicke *et al.* 1997, Hameister *et al.* 1997, Wienberg *et al.* 1997, Nash *et al.* 1998, 2001, 2008, Breen *et al.* 1999, Yang *et al.* 1999, 2000, Cavagna *et al.* 2000, Graphodatsky *et al.* 2000a,b, 2001, 2002, 2008, Nie *et al.* 2002, 2003, Tian *et al.* 2002, 2004, Yang & Graphodatsky 2004, Perelman *et al.* 2005). Global patterns of evolutionary genome changes were revealed and ancestral karyotypes were proposed for the whole order as well as for particular families (Nash *et al.* 1998, 2001, 2008, Graphodatsky *et al.* 2001, 2002, 2008, Murphy *et al.* 2001, Tian *et al.* 2004, Perelman *et al.* 2005).

In the Caniformia branch of Carnivora, two families stand out because of extremely high rate of chromosome evolution: Canidae and Ursidae. Karyotypes of extant species of these families diverged from a presumed ancestral Carnivora karyotype by multiple fusion–fission events (Nash *et al.* 1998, 2001, Breen *et al.* 1999, Yang *et al.* 1999, 2000, Graphodatsky *et al.* 2000a, 2001, 2008, Nie *et al.* 2003, Tian *et al.* 2004, Yang & Graphodatsky 2004). On the other hand, other families demonstrate

striking chromosomal conservatism: Mustelidae, Procyonidae, Pinnipedia, Lutrinae. In these families the karyotypes are distinguished by very few rearrangements from the various hypothesized ancestral Carnivora karyotypes (Arnason 1977, 1981, Frönicke *et al*. 1997, Hameister *et al*. 1997, Cavagna *et al*. 2000, Graphodatsky *et al*. 1989, 2000b, 2002, Nie *et al*. 2002, Tian *et al*. 2002, Nash *et al*. 2008).

However, Mephitidae were notably absent from these assessments. To remedy this lacuna we present here a comprehensive chromosome painting study of four skunk species, representing the three extant genera of Mephitidae, and of one species of Procyonidae—northern raccoon. We also discuss the karyotype evolution of Musteloidea and the phylogenetic implications of the chromosome rearrangements.

Materials and methods

All Mephitidae samples came from the USA. The samples included a male and two female striped skunks (*Mephitis mephitis*, MME, Maryland, road kills), a male hooded skunk (*Mephitis macroura*, MMA, Rodeo, New Mexico (NK 136991)) and a male American hog-nosed skunk (*Conepatus leuconotus*, CLE, Mason, Texas (NK136990)). A sample of female western spotted skunk (*Spilogale gracilis*, SGR) was collected by Rodney A. Mead (Department of Biological Sciences, University of Idaho, Moscow, Idaho) in Lane County, Oregon, and described as *Spilogale gracilis latifrons*. A male northern raccoon (*Procyon lotor*, PLO) was a road kill animal from Frederick, Maryland.

Metaphase preparations, G-banding and chromosome identification

Metaphase preparations were obtained from primary fibroblast or blood cell cultures that were established using conventional techniques (Stanyon & Galleni 1991, Nie *et al*. 2002). Conventional staining, G- and C-banding patterns were obtained following Seabright (1971) and Sumner (1972). Staining of nucleolus organizer regions (NOR-$AgNO_3$ staining) followed Howell & Black (1980). Nucleolus organizer region locations were also verified by fluorescence *in-situ* hybridization with a ribosomal DNA probe (Maden *et al*. 1987).

Flow sorting and generation of chromosome-specific paint probes

Human (*Homo sapiens*, HSA) and cat (*Felis catus*, FCA) painting probes were prepared as described previously (Carter *et al*. 1990, Yang *et al*. 1995, Wienberg *et al*. 1997). A set of painting probes of stone marten (*Martes foina*, MFO) prepared from flow-sorted chromosomes was also used in this study (Nie *et al*. 2002). Chromosomes were sorted from *Mephitis mephitis* and *M. macroura* on a dual laser cell sorter FACSDiVa (Becton Dickinson, NJ, VS) (Figure 1). Following the methods cited above, chromosome paints were generated from flow-sorted chromosomes using DOP-PCR with 6MW primer (5′-CCG ACT CGA GNN NNN NAT GTG G-3′) or G1-2 primers (first round of PCR primer G1 5′-GAG GAT GAG GTT GAG NNN NNH GTGG-3′; second round of PCR primer G2 5′-GTG AGT GAG AGG ATG AGG TTG AG-3′) (Telenius *et al*. 1992, Frönicke *et al*. 2003).

However, the generation of skunk probes presented some particular difficulties. We had no success in hybridizing skunk probes back onto skunk metaphases. An extremely strong signal on the heterochromatin overshadowed euchromatic paint signals and did not allow us to determine which chromosome was in the sorted peak. Apparently the skunk genome has a significant amount of repetitive sequences that could not be blocked by conventional Cot-1 or genomic DNA pre-annealing. However, in distant hybridizations we did not have this problem (probably due to the divergence of repetitive sequences) and we were able to determine peak content by hybridizing onto cat chromosomes.

Fluorescent in-situ hybridization (FISH)

To facilitate chromosome identification, trypsin G-banding or pepsin slide pre-treatment was done prior to hybridization. Pepsin-treated slides were incubated with 0.01% pepsin in 10 mM HCl at 37°C for 5 min. G-banded metaphases were photographed, then the slides were destained in 100% methanol before hybridization. Slides were then fixed with 1% formaldehyde in PBS for 10 min at

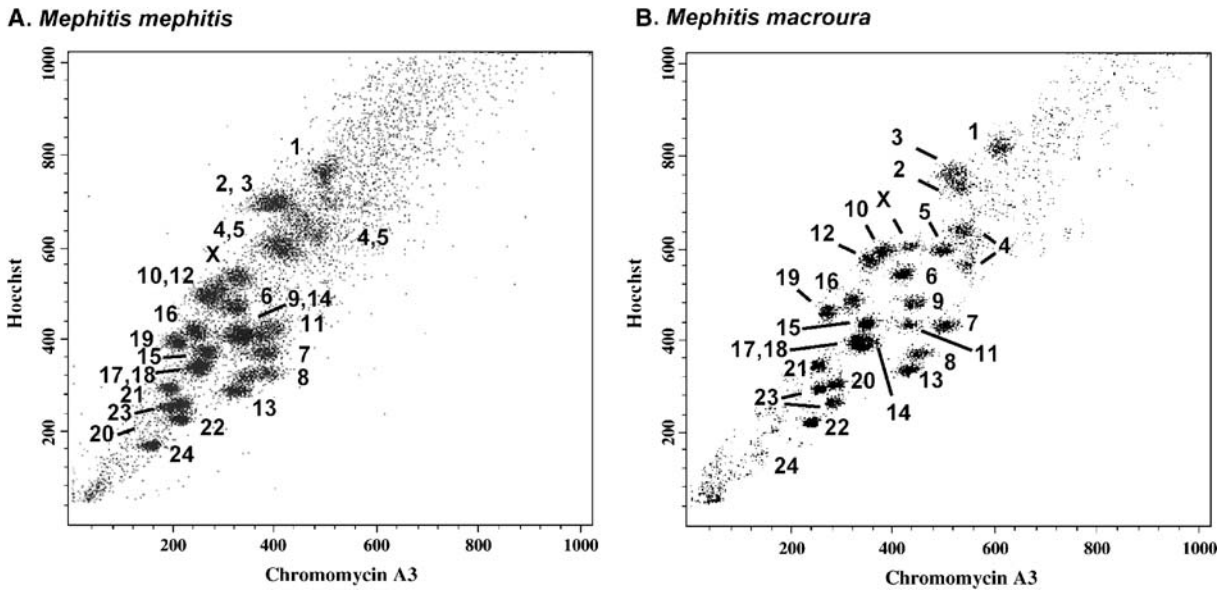


Figure 1. Bivariate flow karyotype of chromosomes from striped and hooded skunks (MME and MMA) with chromosome peak assignments.

room temperature and dehydrated through an ethanol series. For some hybridizations, slides were treated with 0.1 mg/ml RNAse A for 30 min. Chromosome painting was performed as previously described (Yang *et al.* 2000). FISH images were captured and processed following Graphodatsky *et al.* (2000a).

Briefly, biotin- or digoxigenin-labelled chromosome-specific probes (50–100 ng) were precipitated with 2 μg of Cot1 DNA of the same species and were made up to 15 μl with hybridization buffer (2× SSC, 50% deionized formamide, 10% dextran sulphate, 0.5 M phosphate buffer). The probes were denatured at 85°C for 10 min and pre-annealed by incubation at 37°C for 60 min. Slides were denatured by incubation in 70% formamide/2× SSC solution at 65°C for 1–2 min, quenched in ice-cold 70% ethanol, and dehydrated through an ethanol series. The pre-annealed paints were applied onto slides and allowed to hybridize overnight at 37–42°C depending on phylogenetic distance. Post-hybridization washes were two 5 min incubations in 50% formamide/2× SSC at 42–45°C, followed by 5 min incubations in 2× SSC, 1× SSC and 0.2× SSC at 42–45°C. Probes were visualized using FITC-avidin or anti-dig rhodamine. After detection, slides were stained with DAPI. For more details, see Rens *et al.* (2006).

Results

Karyotype description

Mephitis mephitis

The karyotype of the striped skunk has 50 chromosomes (Fredga 1966, 1967, Hsu & Arrighi 1966, Hsu & Benirschke 1967, Genest *et al.* 1986). Here we arranged chromosomes in order of decreasing chromosomal size, unlike Genest *et al.* who also presented a G-banded karyotype. There are 22 pairs of bi-armed and two pairs (20 and 23) of acrocentric autosomes (Figure 2a). C-bands were mainly localized at the centromeric regions, but chromosomes 5, 11 and 14 bear large heterochromatic arms, and chromosome 24 displayed interstitial C-bands. As with the majority of autosomes, the metacentric X-chromosome has a C-positive centromeric region. It is highly likely that there is a significant variation in size and location of heterochromatic blocks in *M. mephitis*, because all animals studied here had three chromosomes with huge blocks of heterochromatin. Genest *et al.* (1986) reported four chromosome pairs bearing entire heterochromatic arms in 6 animals from Canada. Chromosome 10 in one of our individuals had a translocation of chromosome 20 at the end of its long arm. None of the other

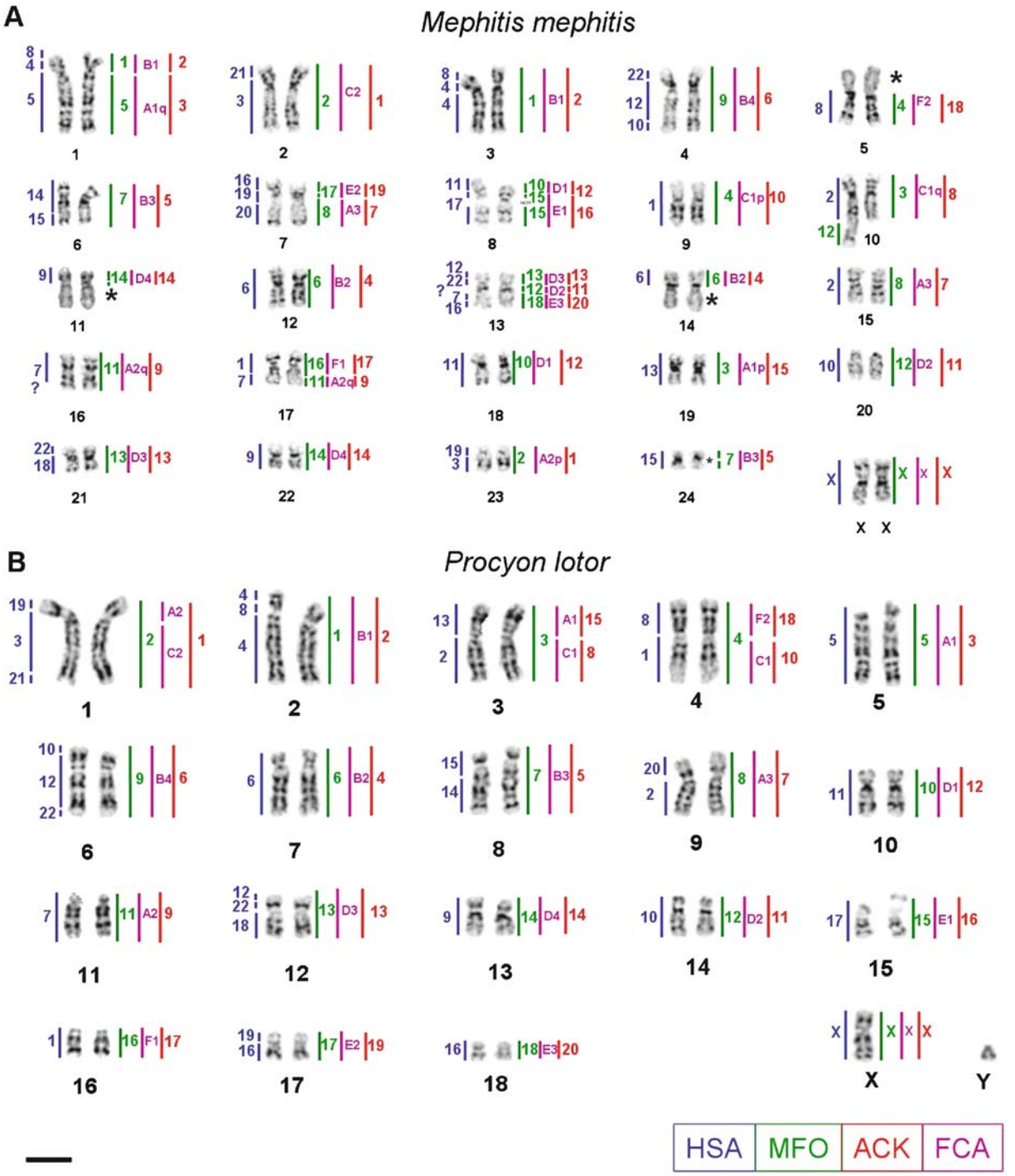


Figure 2. **(A)** G-banded karyotype of *Mephitis mephitis*. Asterisks mark regions of heterochromatin (confirmed by C-banding). **(B)** G-banded karyotype of *Procyon lotor*. On the right are shown homologies with stone marten (MFO, green) and corresponding chromosomes in cat (FCA, violet) and the ancestral carnivore karyotype (ACK, red, Murphy *et al*. 2001). On the left are results of localization of human painting probes (HSA, blue) onto skunk and raccoon chromosomes. Scale bar represents 10 μm.

individuals studied had this translocation. We think this translocation is actually present in the individual and is not a tissue culture artefact as we used low-passage cultures to make metaphase preparations. There is a large nucleolar organizing region (NOR) on chromosome 8q. In conventionally stained chromosomes it appears as a secondary constriction and is positive with $AgNO_3$ staining and by FISH with a ribosomal DNA probe (data not shown).

Mephitis macroura

The karyotype of the hooded skunk has 50 chromosomes and is almost identical to that of the striped skunk (Figure 3a). There are 23 pairs of bi-armed and one pair (23) of acrocentric autosomes. The G- and C-banded chromosomes of the hooded skunk are shown in Figure 3. C-bands were mainly localized at the centromeric regions, but chromosomes 5, 11 and 14 bear large heterochromatic arms, and chromosome 24 displayed interstitial C-bands. However, the size of heterochromatic arms in our hooded skunk was smaller than in our striped skunk sample. Note that the two homologues of chromosome 11 have differently sized heterochromatic regions. As with the majority of autosomes, the metacentric X-chromosome has a C-positive centromeric region and the small acrocentric Y-chromosome has a small terminal C-positive band. As in the striped skunk, chromosome 8 also bears a large NOR on the q arm.

Conepatus leuconotus

The karyotype of the American hog-nosed skunk possesses 46 chromosomes (Figure 3b). There are 19 pairs of bi-armed and three pairs (16, 21 and 22) of acrocentric autosomes. Faint C-bands were mainly localized at the centromeric regions, but chromosomes 16 and 19, as well as the Y-chromosome, displayed large pericentromeric C-bands (not shown). The metacentric X-chromosome has a C-positive centromeric region and the Y-chromosome is small and subtelocentric.

Spilogale gracilis

The karyotype of the western spotted skunk has 60 chromosomes (Hsu & Arrighi 1966, Hsu & Benirschke 1967, Lee & Modi 1983). There are six pairs (1–6) of bi-armed and 23 pairs of acrocentric autosomes (Figure 3c). Hsu & Mead (1969) describe the karyotype of *Spilogale gracilis latifrons* (2n = 60, 6 pairs of bi-armed chromosomes) that matches the karyotype of our specimen. Faint C-bands were mainly localized at the centromeric regions of metacentric chromosomes, while all acrocentrics displayed large centromeric C-bands. The metacentric X-chromosome also has a C-positive centromeric region (not shown). Recent consensus suggests *Spilogale putorius* (eastern spotted skunk) and *Spilogale gracilis* (western spotted skunk) represent separate species (Verts *et al.* 2001). In earlier taxonomies (Wozencraft 1993) *S. gracilis* was considered a synonym of *S. putorius*. However, Wozencraft (2005) considered the two taxa to be valid species. The specimen we studied here belongs to the western spotted skunk, subspecies *S. g. latifrons*.

Procyon lotor

The karyotype of the northern raccoon possesses 38 chromosomes (Figure 2b). It is in agreement with previously published descriptions (Benirschke & Low 1966, Hsu & Benirschke 1967, Wurster-Hill & Gray 1975, Pathak & Wurster-Hill 1977) and arranged according to Stanyon *et al.* 1993. All autosomes except for chromosomes 11 (acrocentric) and 16 (telocentric) are meta- or submetacentrics. C-bands were mainly localized at the centromeric regions, but chromosomes 4 and 5 possess large heterochromatic regions on the p-arm (not shown). Both the metacentric X-chromosome and the small acrocentric Y have large C-positive centromeric regions. Chromosome 15 is NOR bearing.

Chromosome painting

In our study we used five sets of painting probes (human, cat, stone marten, striped skunk and hooded skunk) to determine chromosome homology regions in skunks and the raccoon. It was shown that the stone marten *Martes foina* has a karyotype identical to the ancestral Mustelidae karyotype (Nie *et al.* 2002). This set of probes is unique as each chromosome of the stone marten completely corresponds to an ancestral mustelid chromosome. To facilitate comparisons we also used two sets of traditional reference species: cat and human. Since skunks have not been studied by chromosome painting before, we also made chromosome paints

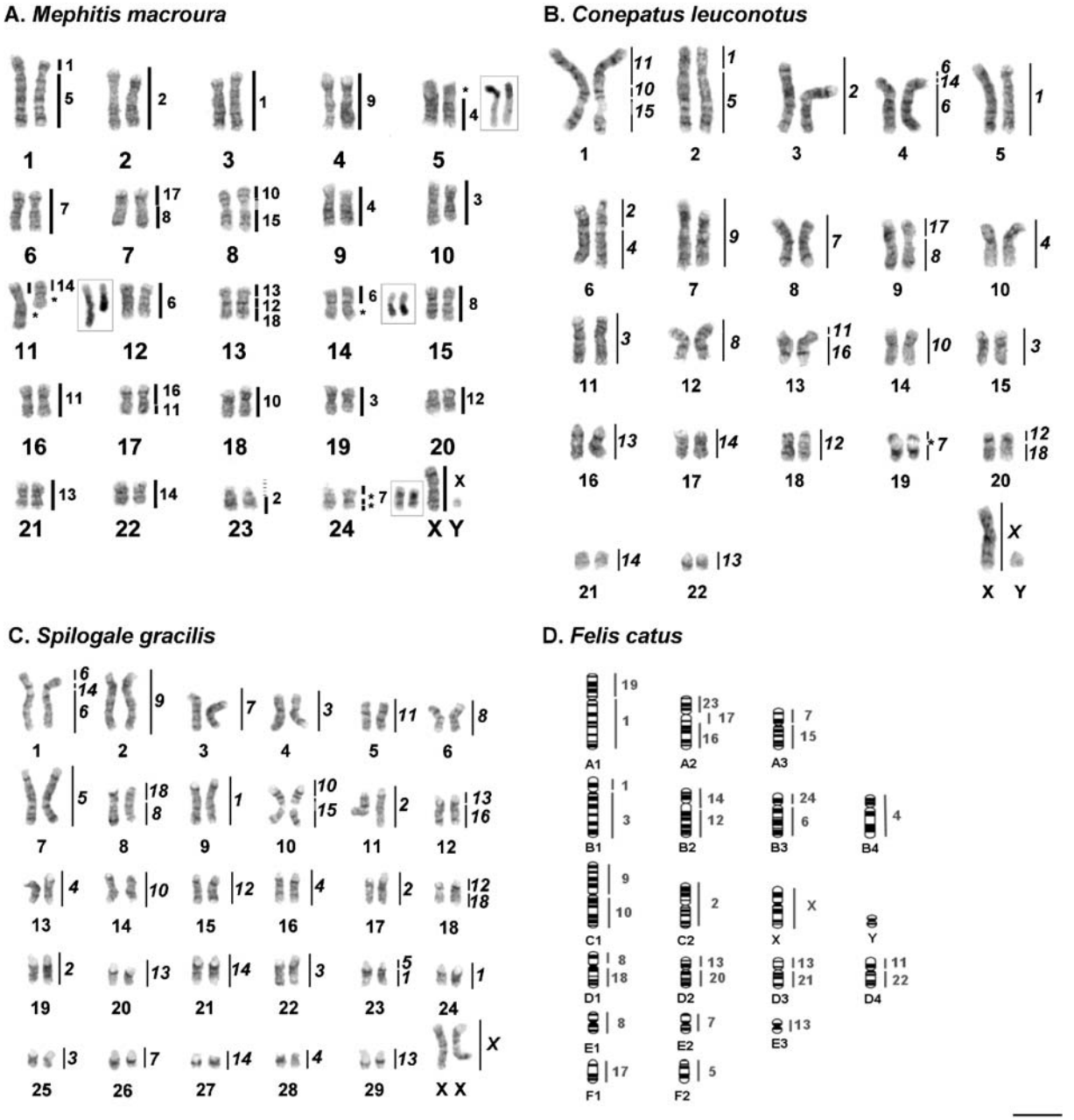


Figure 3. **(A, B, C)** G-banded karyotypes of three skunk species (*Mephitis macroura*, *Conepatus leuconotus*, *Spilogale gracilis*) with localization of stone marten painting probes shown on the right. Asterisks mark regions of heterochromatin (confirmed by C-banding). For *Mephitis macroura* C-banded chromosomes bearing large heterochromatin blocks are shown, framed, on the right. **(D)** Ideogram of cat (*Felis catus*) chromosomes with results of localization of striped and hooded skunk painting probes shown on the right. Scale bar represents 10 μm.

from the two skunks in order to perform 'reverse' painting to cat chromosomes (Figure 3d).

Chromosome painting of human chromosomes onto striped skunk chromosomes was consistent with previous data on carnivores (Figure 2a). Ancestral placental mammalian (HSA 3/21, 4/8, 10/12/22, 14/15, 16/19, 12/22, 7/16) and carnivore-specific associations (HSA 3/19, 18/22) of human homologous segments were present in the skunk karyotype. We could not detect all homologous segments from

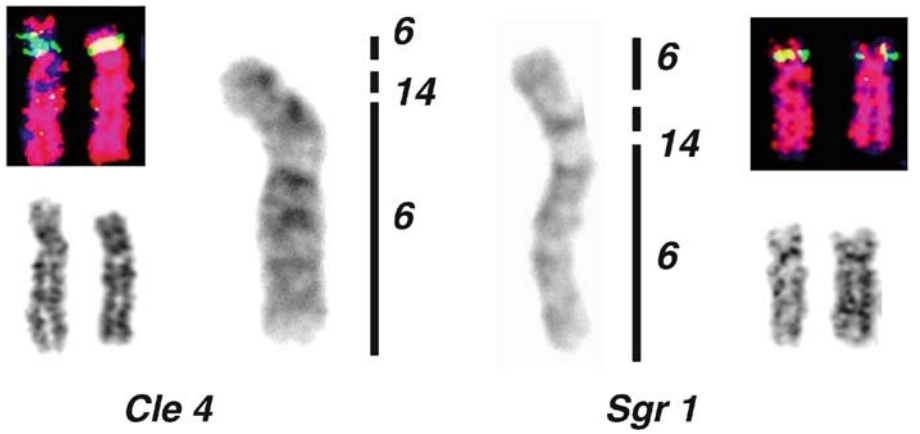


Figure 4. Common rearrangement of ACK 4 and 14 in *Conepatus leuconotus* and *Spilogale gracilis* (*Martes foina* chromosomes 6 and 14, *Felis catus* chromosomes B2 and D4 respectively). G-banding of *Conepatus leuconotus* chromosome 4 and *Spilogale gracilis* chromosome 1 and bicolour hybridization of *Martes foina* chromosome paints (6 in red and 14 in green) onto CLE4 and SGR1.

HSA10q owing to their weak hybridization signals (MME13, HSA 10?/7/16). There was also a segment of MME16q that was not labelled with any of the human probes. Human chromosome associations HSA 16/19/20, 11/17, 1/7, 10?/7/16 were present in all four skunk species and could be considered characteristic for this family.

Striped and hooded skunk painting probes were hybridized to cat chromosomes (Figure 3d) and revealed 30 conserved segments in the cat karyotype. As we had no individual probe for skunk chromosome 3 (it was present as a very weak fraction in the same peak as chromosome 2), the signal on the q-arm of the B1 chromosome was very weak. The pattern of signals on the cat chromosomes was identical for the two skunks.

The full set of chromosome-specific painting probes of stone marten (*Martes foina*, 2n = 38) was mapped onto representatives of three genera of the skunk family Mephitidae. Eighteen autosomal stone marten painting probes revealed 30 conserved segments in striped skunk (*M. mephitis*, 2n = 50) and in hooded skunk (*M. macroura*, 2n = 50), 31 segments in the karyotype of the American hog-nosed skunk (*Conepatus leuconotus*, 2n = 46), and 36 in western spotted skunk (*Spilogale gracilis*, 2n = 60) (Figure 3). Chromosomal fusion of stone marten chromosomes 6 and 14 was revealed for the American hog-nosed skunk and eastern spotted skunk, joining them in one lineage (Figure 4).

Karyotypes of the northern raccoon and the stone marten appeared identical according to our painting results. Eighteen autosomal probes of stone marten revealed 18 conserved regions in northern raccoon (*Procyon lotor*, 2n = 38). However, comparison of banding patterns of marten and raccoon suggested possible inversions. This result needs to be further checked by using higher-resolution chromosome paints such as those from the dog, or by BAC-FISH. Human and cat painting probes revealed 31 and 21 conserved autosomal segments, respectively, with all expected signature mammalian and carnivore ancestral chromosomal associations being present (Figure 2).

Discussion

In this study we characterized, by multi-directional chromosome painting, the karyotypes of four skunk species and one raccoon. Until this study, despite more than a decade of painting studies of carnivores, there were no molecular cytogenetic reports on the family Mephitidae and only one study on Procyonidae. We created chromosome paint probe sets from two species of skunks, which were assigned by painting to cat chromosomes. We also mapped probes of stone marten (*Martes foina*) onto four skunk species and the raccoon. The karyotype of the stone marten was shown to be identical to the ancestral musteloid karyotype (Nie *et al.* 2002), so it is a good tool for investigating musteloid karyotypes. Finally, human and cat probes were also

hybridized to the striped skunk and raccoon chromosomes to facilitate comparisons with other mammals.

Chromosome relationships within Mephitidae

Currently, four extant skunk genera are recognized: *Mephitis* (hooded and striped skunks, 2 species), *Spilogale* (spotted skunks, 4 species), *Conepatus* (hog-nosed skunks, 4 species), and *Mydaus* (stink badgers, 2 species) (Wozencraft 2005). Even though the monophyly of the skunk family was confirmed by morphological and molecular data (Wozencraft 1989, 1993, Dragoo *et al*. 1993) there is no agreement about phylogenetic relations between skunk genera. For instance, sister taxa relationships are not clear and three hypotheses were recently discussed in the literature: *Spilogale*/*Mephitis*, *Spilogale*/*Conepatus*, or *Mephitis*/*Conepatus* (Dragoo *et al*. 1993, 2004, Dragoo & Honeycutt 1997, Flynn *et al*. 2005, Wang *et al*. 2005) with *Mydaus* always as the basal lineage. Many subspecies were described in New World skunk genera and species and subspecies status continues to be revised (Dragoo *et al*. 2003). Current taxonomies can hardly be considered final and further investigation of South American skunks is badly needed.

Our painting data show that all three skunk genera diverged from the ancestral carnivore karyotype (ACK; here and further in the text all numbers of ACK chromosomes refer to Murphy *et al*. 2001) by 10 fission and 5 fusion events (Figure 5). Fission of chromosome ACK14 and fusion with ACK4 and possibly inversion led to the branch uniting *Conepatus* and *Spilogale*. Five further fissions gave origin to the 2n = 60 karyotype of *Spilogale gracilis*. Two

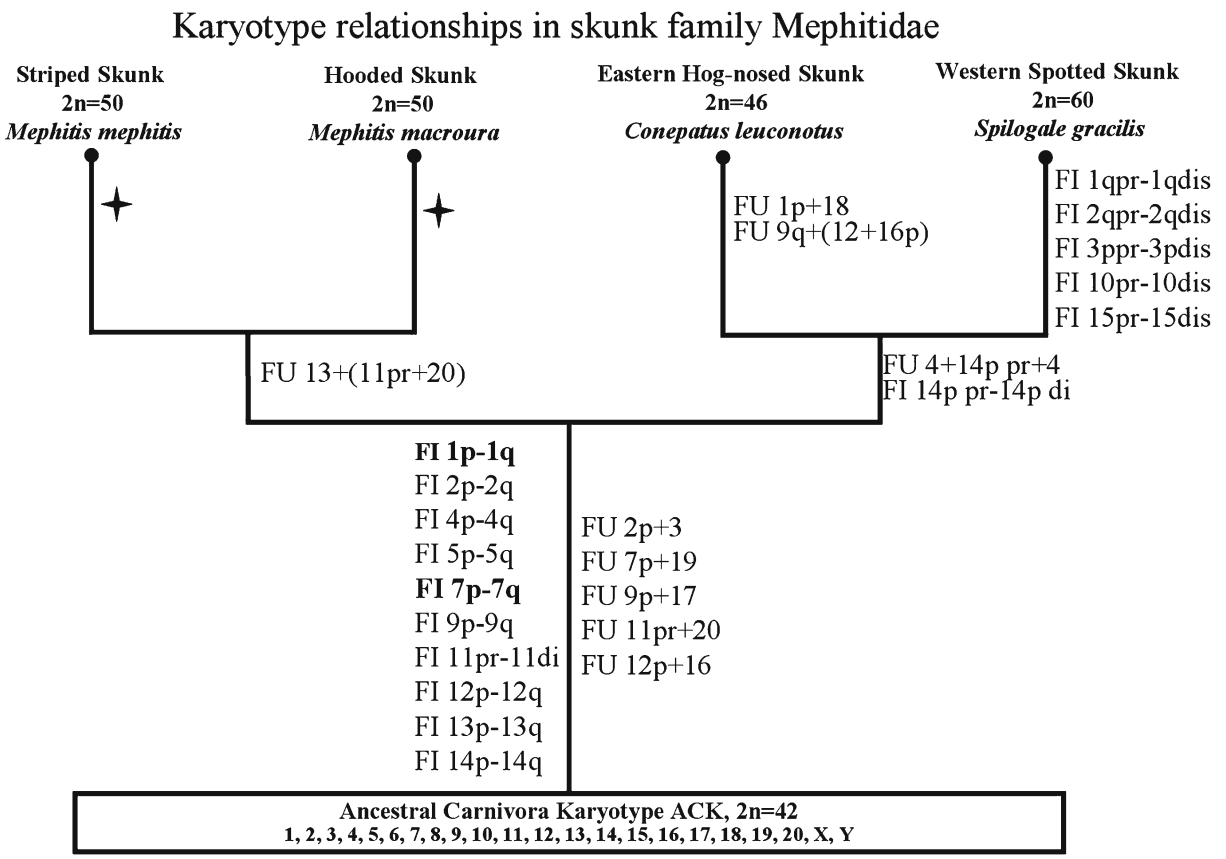
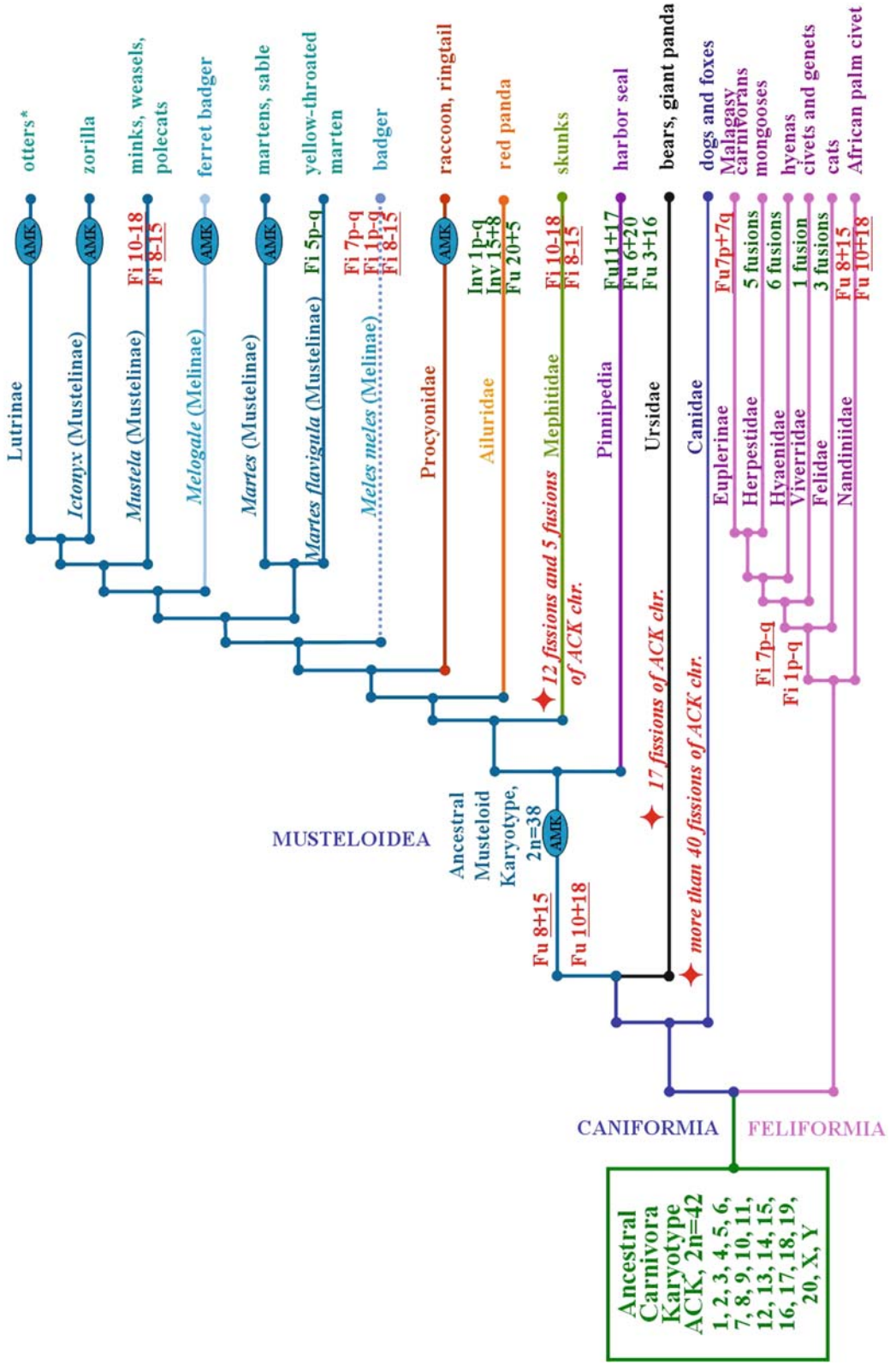


Figure 5. Karyotype relationships in the family Mephitidae as revealed by chromosome painting. FI, fission; FU, fusion; asterisk, heterochromatic blocks; pr, proximal part of p-arm; di, distal part of p-arm. Chromosome numbers correspond to chromosomes of the ancestral carnivore karyotype (ACK) (Murphy *et al*. 2001).


otters*
zorilla
minks, weasels, polecats
ferret badger
martens, sable
yellow-throated marten
badger
raccoon, ringtail
red panda
skunks
harbor seal
bears, giant panda
dogs and foxes
Malagasy carnivorans
mongooses
hyenas
civets and genets
cats
African palm civet
AMK
Lutrinae
Ictonyx (Mustelinae)
Mustela (Mustelinae)
Fi 10-18
Fi 8-15
Melogale (Melinae)
Martes (Mustelinae)
Martes flavigula (Mustelinae)
Fi 5p-q
Meles meles (Melinae)
Fi 7p-q
Fi 1p-q
Fi 8-15
Procyonidae
Ailuridae
Inv 1p-q
Inv 15+8
Fu 20+5
Mephitidae
Fi 10-18
Fi 8-15
12 fissions and 5 fusions of ACK chr.
Pinnipedia
Fu11+17
Fu 6+20
Fu 3+16
Ursidae
17 fissions of ACK chr.
Canidae
more than 40 fissions of ACK chr.
Euplerinae
Fu7p+7q
Herpestidae
5 fusions
Hyaenidae
6 fusions
Viverridae
1 fusion
Felidae
3 fusions
Nandiniidae
Fu 8+15
Fu 10+18
Fi 7p-q
Fi 1p-q
MUSTELOIDEA
Ancestral Musteloid Karyotype, 2n=38
Fu 8+15
Fu 10+18
CANIFORMIA
FELIFORMIA
Ancestral Carnivora Karyotype ACK, 2n=42
1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, X, Y

additional fusions formed the karyotype of *Conepatus leuconotus*. Another fusion led to the *Mephitis* branch. Karyotypes of both striped and hooded skunks are almost identical. These karyotypes were both characterized by large heterochromatin blocks on the same three pairs. The only difference found was a slight size variation of those blocks between species, but this variability was frequently seen within species. Only one molecular phylogeny has suggested a *Conepatus*/*Spilogale* grouping (Dragoo *et al*. 2003). However, our results clearly indicated a link between these two genera. Fusion MFO6–MFO14–MFO6 (FCA B2–D4–B2) was characteristic for these two genera and absent in *Mephitis* or any other carnivore studied so far (Figure 4). It might be worth testing the *Conepatus*/*Spilogale* grouping using more extensive molecular phylogenetic techniques. Also, study of karyotypes for additional *Spilogale* including *S. pygmae*, *S. putorius*, *S. angustifrons* and *S. gracilis*, will be required as the chromosomal numbers within this genus are variable. Additionally, other species within the genus *Conepatus* might shed more light on the validity of this peculiar phylogenetic rearrangement.

Chromosome relationships within Musteloidea

Two branches are generally recognized in the Carnivora tree: Feliformia (Nandiniidae, Felidae, Viverridae, Hayenidae, Herpestidae, Euplerinae) and Caniformia (Canidae, Ursidae, Pinnipedia, Musteloidea (Mephitidae, Ailuridae, Procyonidae, Mustelidae) (cf. Ledje & Arnason 1996b, Sato *et al*. 2006, Arnason *et al*. 2007) (Figure 6). However, the position of Mephitidae among other arctoids remains controversial and the relationships within superfamily Musteloidea are not well resolved. For instance, grouping of sister species within this superfamily is highly debatable and many hypotheses exist in the literature (e.g. Bryant *et al*. 1993, Vrana *et al*. 1994, Flynn *et al*. 2000, 2005, Sato *et al*. 2006). Various authors have suggested contrasting sister groupings including Mephitidae/Procyonidae, Mephitidae/Ailuridae, Mephitidae/Melinae, Mephitidae/Lutrinae (cf. Dragoo & Honeycutt 1997, Flynn & Nedbal 1998, Flynn *et al*. 2000, 2005, Sato *et al*. 2004, Fulton & Strobeck 2006).

Members of the family Procyonidae were shown to have strikingly uniform karyotypes that strongly resemble karyotypes of mustelids, pinnipeds and felids (Hsu & Benirshke 1967, Wurster-Hill & Gray 1975, Arnason 1977). Here we confirmed that the raccoon karyotype is highly conserved and identical to the ancestral mustelid karyotype (Graphodatsky *et al*. 2002). The karyotype of another Procyonidae species studied recently by chromosome painting—the ringtail (*Bassariscus astutus*)—is identical to the *Procyon lotor* karyotype, at least at the level of resolution of cat paints (Nash *et al*. 2008). Many other taxa among Musteloidea have preserved the ancestral musteloid karyotype in their chromosomal complement (Lutrinae, *Ictonyx, Melogale*, *Martes*) (Figure 6). Because raccoons share the ancestral musteloid karyotype with so many other species, it is very difficult to place Procyonidae on the Caniformia tree using only chromosome data.

However, extending chromosome painting studies to skunks allows us to examine many controversial issues of Caniformia phylogeny. Unexpectedly, fission and fusion events that occurred in course of the formation and evolution of the skunk family genome were unique and are not shared with any other carnivore species studied so far by chromosome painting. Clearly, our painting data do not provide any support for a close relationship between skunks and raccoons. There is also no support for grouping skunks with Ursidae or Canidae. None of these taxa have any common derived rearrangement

Figure 6. Karyotype relationships in the order Carnivora as revealed by chromosome painting studies. Tree topology for the Caniformia branch is from Fulton & Strobeck (2006) and for the Feliformia branch from Flynn *et al*. 2005. FI, fission; FU, fusion; AMK, ancestral musteloid karyotype (Graphodatsky *et al*. 2002); ACK, ancestral carnivore karyotype (Murphy *et al*. 2001). Fusion and fission events underlined in red are reverse rearrangements. Chromosome numbers correspond to chromosomes of the ancestral carnivore karyotype (ACK) (Murphy *et al*. 2001). Published data on chromosome painting of Canidae (Breen *et al*. 1999, Yang *et al*. 1999, 2000, Graphodatsky *et al*. 2000a, 2001, 2008, Nash *et al*. 2001, Nie *et al*. 2003), ferret (Cavagna *et al*. 2000), harbour seal (Frönicke *et al*. 1997), Felidae (Wienberg *et al*. 1997, Yang *et al*. 2000, Tian *et al*. 2004), mustelids (Hameister *et al*. 1997, Graphodatsky *et al*. 2000b, 2002, Nie *et al*. 2002), bears (Nash *et al*. 1998, 2001, Tian *et al*. 2004, Yang & Graphodatsky 2004), viverrids and hyena (Perelman *et al*. 2005), Malagasy civet, mongoose and ringtail (Nash *et al*. 2008), red panda (Tian *et al*. 2002, Nie *et al*. 2002), and Lutrinae (Stanyon, unpublished) were used for this figure.

with any of the Mustelidae. Thus karyological data support the separate family status of skunks.

With the painting data on skunks, we can examine some additional controversial taxonomic hypotheses related to this group. The African zorilla or striped polecat (*Ictonyx striatus*) is sometimes described as a skunk because of its similar striped coat (Nowak 1991). However, morphological and molecular phylogenies indicate that *Ictonyx* does not group with skunks (Bryant *et al.* 1993, Dragoo & Honeycutt 1997). The karyotype of *Ictonyx* is identical to the ancestral mustelid karyotype; the only difference being giant blocks of heterochromatin found on five autosomes (Graphodatsky *et al.* 2002). Skunks and *Ictonyx,* therefore, do not share any common derived chromosomal rearrangements and our painting data do not suggest any special phylogenetic relationship between skunks and the striped polecat.

Some phylogenetic studies hypothesize grouping skunks with Lutrinae (Hunt 1974, Wozencraft 1989, Wyss & Flynn 1993). According to G-banding and unpublished painting data, otter karyotypes are highly conserved and identical to the AMK (Graphodatsky *et al.* 1989, 2002, Stanyon, unpublished). Therefore, the chromosome data do not indicate any close phylogenetic relationship between Lutrinae and skunks.

It will certainly be informative to hybridize higher-resolution probes such as dog paints and cloned DNA probes such as BACs and fosmids to both skunks and raccoons. These probes could reveal intrachromosomal rearrangements and provide cytogenetic markers to resolve outstanding questions of family relations in Musteloidea.

Ancestral carnivora karyotype

There have been several attempts to reconstruct the ancestral Carnivora karyotype (Frönicke *et al.* 1997 (2n = 38), Murphy *et al.* 2001 (2n = 42), Nash *et al.* 2008 (2n = 38)). Most agree on the majority of ancestral chromosomes, but four ancestral chromosomes remain unclear. Certainly an ever-increasing and richer array of carnivore species provides a database for improvement of ancestral genome reconstructions and will eventually resolve questions about the exact content of the carnivore ancestral genome. Here we refer to the ACK chromosome numbers and nomenclature in reference to Murphy *et al.* (2001). A number of chromosomes deserve attention, in particular ACK1p + 1q (= FCA A2p + C2), ACK7 (FCA A3p + A3q), ACK 8 + 15 (FCA C1q + A1p) and ACK 10 + 18 (FCA C1p + F2) (Table 1).

Two fusions of ancestral chromosomes, ACK8 + ACK15 and ACK10 + ACK18, are present on the Caniformia branch in all Musteloidea with the exception of skunks and the genus *Mustela.* Fusion ACK8 + 15 is absent in the badger. Neither of these fusions is present in Canidae and Ursidae. On the Feliformia branch these combinations are present only in Nandiniidae (based on G-banding studies by Wurster-Hill & Gray 1975, Nash *et al.* 2008) and absent in all other families (Felidae, Viverridae, Hayenidae, Herpestidae and Euplerinae) (Figure 6). However, the presence of ACK8 + ACK15 and ACK10 + ACK18 in the *Nandinia binotata* karyotype still needs to be confirmed with molecular methods such as chromosome painting. We need to question the ancestral state of these four chromosomal elements. Nash and co-authors (2008) conclude that as musteloids and Nandiniidae, on different branches of the carnivore tree (Caniformia and Feliformia), both have these two fusions (ACK8 + ACK15 and ACK10 + ACK18), this represents the ancestral state. The facts that Nandiniidae is the basal branch on the Feliformia and that karyotypes of musteloids are characterized by high levels of chromosome conservatism favour this hypothesis. However, if we accept the hypotheses of ACK8 + ACK15 and ACK10 + ACK18 being fused in the ancestral karyotype, we have to consider that it has undergone fissions in canids, bears, skunks and *Mustela*, and one of them (ACK8, ACK15) in the Eurasian badger (*Meles meles*), as well as independently fissioned in all Feliformia higher on the tree of Nandiniidae.

If we stand by the alternative hypothesis of the separated state of these four elements, we have to assume a reversal in fission in *Mustela*, Eurasian badger and skunks (Figure 6). The second hypothesis (of ACK8, ACK15, ACK10 and ACK18 being present in the ancestral karyotype as separate chromosomes) involves fewer events (three evolutionary fission events versus six) and thus can be considered more parsimonious. It should be remembered that Robertsonian fusion–fission events such as these are often considered more subject to convergence than any other type of chromosome rearrangements. Thus, Robertsonian fission–fusions are of

Table 1. The presence of eight ancestral chromosomal elements in different branches of the Carnivora tree.[a] These ancestral chromosomal elements were involved in the same Robertsonian fusion-fission events throughout the evolution of the order Carnivora. Robertsonian rearrangements are prone to convergence, making it difficult to determine the ancestral condition

ACK, Murphy *et al.* (2001)	ACK1p + 1q	ACK7	ACK 8 + 15	ACK 10 + 18
ACK, Nash *et al.* (2008)	ACK 1	ACK 9	ACK 3	ACK 4
FCA	FCA A2p + C2	FCA A3p + A3q	FCA C1q + A1p	FCA C1p + F2
Feliformia				
Nandiniidae	X	X	X	X
Felidae	X	X	0	0
Viverridae	0	0	0	0
Hyaenidae	0	0	0	0
Herpestidae	0	0	0	0
Euplerinae	0	X	0	0
Caniformia				
Canidae[b]	0	0	0	0
Ursidae[b]	0	0	0	0
Pinnipedia	X	X	X	X
Mephitidae[b]	0	0	0	0
Ailuridae	X	X	X	X
Procyonidae	X	X	X	X
Meles meles	0	0	0	X
Mustelinae	X	X	X	X
Mustela	X	X	0	0

ACK, ancestral Carnivora karyotype: Murphy *et al.* (2001), Nash *et al.* (2008).
[a]X, ancestral chromosome is present in the karyotypes of the taxon; 0, ancestral chromosome is absent from the karyotypes of the taxon.
[b]Families characterized by particularly high level of chromosomal rearrangements.

lower phylogenetic weight and their ancestral state can be difficult to determine (see Rens *et al.* 2003, Mao *et al.* 2008).

Two other ancestral chromosomes ACK1 (FCA A2p + C2) and ACK7 (FCA A3p + A3q) are also questionable. ACK1 (FCA A2p + C2) is present in musteloids (Ailuridae, Mustelidae, Procyonidae), Pinnipedia, Felidae and Nandiniidae but has undergone fission in all other families of the Feliformia branch (Viverridae, Hayenidae, Herpestidae and Euplerinae) and in Canidae, Ursidae, Mephitidae and *Meles meles* on the Caniformia branch. ACK7 (FCA A3) is present in Nandiniidae, Felidae, Euplerinae and musteloids (Ailuridae, Pinnipedia, Mustelidae, Procyonidae) and fissioned in Viverridae, Hayenidae, Herpestidae, Canidae, Ursidae, Mephitidae and *Meles meles*. If ACK1 and ACK7 were each present in the ancestral karyotype as an entire chromosome, then independent fissions must have occurred in the Feliformia branch (Viverridae, Hayenidae and Herpestidae) and in Canidae, Ursidae, Mephitidae and the Eurasian badger on the Caniformia branch, and reverse fusion happened in Euplerinae. If ACK1 and ACK7 were each present in the ancestor as two chromosomes, then independent fusion would have happened in musteloids and Nandiniidae, followed by reverse fissions in Eurasian badger, skunks, Felidae, Hayenidae, Herpestidae, Viverridae and reverse fusion of ACK7 in Euplerinae. Therefore, it is hardly possible to decide whether or not these two chromosomes underwent fission in the ancestral karyotype, although the first scenario (ACK1 and ACK7 each present in the ancestral karyotype as an entire chromosome) seems more parsimonious.

Convergence of two identical chromosome rearrangements in separate phylogenetic lines is usually considered unlikely. However, a current subject in comparative cytogenetics is how to weight various types of chromosome rearrangements. Indeed, Robertsonian fission/fusion events are the least convincing phylogenetic markers as they are most prone to convergence. These eight chromosomal elements might represent hotspots of evolutionary rearrangements that for some reason underwent repeated and independent fission/fusion events over 55 million years of the evolution of Carnivora. Thus, these ancestral chromosomes seem to be not very informative for

the carnivore tree and do not help to resolve complicated family-level relationships. On the basis of the available data it is very difficult to decide on their ancestral state. More detailed mapping of carnivore genomes from more representative species might help to clarify this problem. Certainly, additional difficulties are posed by lineages characterized by high evolutionary rates of chromosome rearrangements such as Canidae, Ursidae and Mephitidae, since the problems of distinguishing homology from homoplasy are increased. Otherwise, if we do consider ancestral rearrangements discussed above as phylogenetic characters, then carnivore phylogeny needs reassessment to clarify the position of skunks among caniformia and their relationships to badgers as well as some other issues such as the position of Euplerinae.

Mephitidae and Melinae chromosomal relationships

The relationships of skunks and the badger subfamily Melinae (genera *Arctonyx*, *Melogale*, *Meles*) are a fascinating topic. If we consider the rearrangements discussed above, then several interpretations are possible. For instance, the Eurasian badger (*Meles meles*) has fissions of ancestral chromosomes (ACK1, ACK7) and a fission of musteloid ancestral association ACK8-ACK15. The phylogenetic implication of these fissions would place *Meles meles* as a sister taxon of skunks. Thus the fission ACK8-15 will place the Eurasian badger outside of Mustelidae and close to skunks, which also have fissions of ACK1 and ACK7. This hypothesis would confirm the paraphyly of Melinae. It was shown previously that the *Melogale* karyotype is identical to the ancestral musteloid karyotype and phylogenetic studies also confirmed that this genus is deeply nested within Mustelidae and isolated from other badgers (*Arctonyx* and *Meles*) which occupy a more basal position among musteloids (Sato *et al*. 2004, Fulton & Strobeck 2006, Koepfli *et al*. 2008).

The inclusion of oriental stink badgers *Mydaus* within skunks is supported by many molecular and morphological studies (Pocock 1921, Simpson 1945, Petter 1971, Radinsky 1973, Bryant *et al*. 1993, Dragoo & Honeycutt 1997, Wang *et al*. 2005). Traditionally, *Mydaus* was grouped with badgers in the *Melinae* (Simpson 1945). It would certainly be interesting to study karyotypes of *Mydaus* and *Taxidea taxus* (the American badger that is recognized as the subfamily Taxidiinae and whose chromosomes look quite unusual for mustelids (Wurster-Hill 1973)) to better determine the relationships between the families of skunks and badgers and to know whether there is chromosomal support for the placement of *Mydaus* within the Mephitidae.

Increased rate of karyotype evolution in skunks

The Order Carnivora is a good example of chromosome evolution with variable rates. On the one hand, felids, pinnipeds, Ailuridae and most of the mustelids have strikingly conserved karyotypes (Arnason 1977, 1981, Frönicke *et al*. 1997, Hameister *et al*. 1997, Wienberg *et al*. 1997, Cavagna *et al*. 2000, Yang *et al*. 2000, Graphodatsky *et al*. 2002, Nie *et al*. 2002, Tian *et al*. 2002). On the other hand, chromosome painting showed that Canidae have extremely rearranged genomes. More than 40 fissions of ancestral chromosomes separate the ACK from the ancestral karyotype of all canids (Graphodatsky *et al*. 2000a, 2008). It was shown that Ursidae also have a high rate of chromosome evolution (Nash *et al*. 1998, Tian *et al*. 2004, Yang & Graphodatsky 2004). The ursid ancestral karyotype can be derived from the carnivore ancestor by 17 fissions.

Now we have a third taxon in the order Carnivora with highly derived karyotypes—Mephitidae. Ten fissions and five fusions are necessary to form the ancestral skunk karyotypes from the ACK. The high evolutionary rate of rearrangements strikingly distinguishes them from other musteloids, which generally have highly conserved karyotypes, and would also support raising skunks to the family level–Mephitidae—as was proposed by earlier studies (Ledje & Arnason 1996a,b, Dragoo & Honeycutt 1997). Thus, our results show that independent, massive reshuffling of the ancestral genome occurred at least three times in Carnivora, because dogs, bears and skunks have few common fissions of ancestral chromosomes.

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References

Arnason U (1977) The relationship between the four principal pinniped karyotypes. *Hereditas* **87**(2): 227–242.

Arnason U (1981) Localization of nucleolar organizing regions in pinniped karyotypes. *Hereditas* **94**(1): 29–34.

Arnason U, Gullberg A, Janke A, Kullberg M (2007) Mitogenomic analyses of caniform relationships. *Mol Phylogenet Evol* **45**(3): 863–874.

Benirschke K, Low RJ (1966) Chromosome studies on four carnivores. *Mammal Chrom Newsl* **21**: 148.

Breen M, Thomas R, Binns MM, Carter NP, Langford CF (1999) Reciprocal chromosome painting reveals detailed regions of conserved synteny between the karyotypes of the domestic dog (*Canis familiaris*) and human. *Genomics* **61**: 145–155.

Bryant HN, Russel AP, Fitch WD (1993) Phylogenetic relationships within the extant Mustelidae (Carnivora): appraisal of the cladistic status of the Simpsonian subfamilies. *Zool J Linn Soc* **108**: 301–334.

Carter NP, Ferguson-Smith ME, Affara NA, Briggs H, Ferguson-Smith MA (1990) Study of X chromosome abnormality in XX males using bivariate flow karyotype analysis and flow sorted dot blots. *Cytometry* **11**(1): 202–207.

Cavagna P, Menotti A, Stanyon R (2000) Genomic homology of the domestic ferret with cats and humans. *Mamm Genome* **11**: 866–870.

Dobigny G, Ducroz JF, Robinson TJ, Volobuev V (2004) Cytogenetics and Cladistics. *Syst Biol* **53**(3): 470–484.

Dragoo JW, Honeycutt RL (1997) Systematics of mustelid-like carnivores. *J Mammal* **78**(2): 426–443.

Dragoo JW, Bradley RD, Honeycutt RL, Templeton JW (1993) Phylogenetic relationships among the skunks: a molecular perspective. *J Mamm Evol* **1**: 255–267.

Dragoo JW, Honeycutt RL, Schmidly DJ (2003) Taxonomic status of white-backed hog-nosed skunks, genus *Conepatus* (Carnivora: Mephitidae). *J Mammal* **84**: 159–176.

Dragoo JW, Matthes D, Aragon A, Hass CC, Yates TL (2004) Identification of skunk species submitted for rabies testing in the desert southwest. *J Wildlife Dis* **40**: 371–376.

Flynn JJ, Nedbal MA (1998) Phylogeny of the Carnivora (Mammalia): congruence vs incompatibility among multiple data sets. *Mol Phylogenet Evol* **9**(3): 414–426.

Flynn JJ, Nedbal MA, Dragoo JW, Honneycutt RL (2000) Whence the red panda? *Mol Phylogenet Evol* **17**(2): 190–199.

Flynn JJ, Finarelli JA, Zehr S, Hsu J, Nedbal MA (2005) Molecular phylogeny of the Carnivora (Mammalia): assessing the impact of increased sampling on resolving enigmatic relationships. *Syst Biol* **54**(2): 317–337.

Fredga K (1966) Chromosome studies on six species of Mustelidae and one of Procyonidae. *Mamm Chromosomes Newsl* **21**: 145.

Fredga K (1967) Comparative chromosome studies of the family Mustilidae (Carnivora, Mammalia). *Hereditas* **57**: 295.

Frönicke L, Müller-Navia J, Romanakis K, Scherthan H (1997) Zoo-FISH maps of the harbor seal (*Phoca vitulina*) and the putative ancestral carnivore karyotype. *Chromosoma* **106**: 108–113.

Frönicke L, Wienberg J, Stone G, Adams L, Stanyon R (2003) Towards the delineation of the ancestral eutherian genome organization: comparative genome maps of human and the African elephant (*Loxodonta africana*) generated by chromosome painting. *Proc R Soc Lond B* **270**: 1331–1340.

Fulton TL, Strobeck C (2006) Molecular phylogeny of the Arctoidea (Carnivora): effect of missing data on supertree and supermatrix analyses of multiple gene data sets. *Mol Phylogenet Evol* **41**: 165–181.

Genest FB, Morisset P, Patenaud RP (1986) Caryotype de la mouffette rayee, *Mephitis mephitis*. *Genet Sel Evol* **18**(2): 111–112.

Graphodatsky AS, Perelman PL, Sokolovskaya NV, Beklemisheva VR, Serdukova NA, Dobigny G, O'Brien SJ, Ferguson-Smith MA, Yang F (2008) Phylogenomics of the dog and fox family (Canidae, Carnivora) revealed by chromosome painting. *Chromosome Res* **16**(1): 129–143.

Graphodatsky A, Sharshov A, Ternovsky D, Ternovskaya Yu (1989) Comparative cytogenetics of Mustelidae (Carnivora). *Zool Zh* **68**(12): 96–106.

Graphodatsky AS, Yang F, O'Brien PC *et al*. (2000a) A comparative chromosome map of the Arctic fox, red fox and dog defined by chromosome painting and high resolution G-banding. *Chromosome Res* **8**: 253–263.

Graphodatsky AS, Yang F, Serdukova N, Perelman P, Zhdanova N, Ferguson-Smith MA (2000b) Dog chromosome-specific paints reveal evolutionary inter- and intrachromosomal rearrangements in the American mink and human. *Cytogenet Cell Genet* **90**: 275–278.

Graphodatsky AS, Yang F, O'Brien PCM *et al*. (2001) Phylogenetic implications of the 38 putative ancestral chromosome segments for four canid species. *Cytogenet Cell Genet* **92**: 243–247.

Graphodatsky AS, Yang F, Perelman P *et al*. (2002) Comparative molecular cytogenetic studies in the order Carnivora: mapping chromosomal rearrangements onto the phylogenetic tree. *Cytogenet Genome Res* **96**: 137–145.

Hameister H, Klett C, Bruch J, Dixkens C, Vogel W, Christensen K (1997) Zoo-FISH analysis: the American mink (Mustela vison) closely resembles the cat karyotype. *Chromosome Res* **5**: 5–11.

Howell WM, Black DA (1980) Controlled silver-staining of nucleolus organizer regions with a protective colloidal developer: a 1-step method. *Experientia* **36**: 1014–1015.

Hsu TC, Arrighi FE (1966) *Mammal Chromosomes Newsl* **21**: 155–159.

Hsu TC, Arrighi FE (1971) Distribution of constitutive heterochromatin in mammalian chromosomes. *Chromosoma* **34**: 243–253.

Hsu TC, Benirschke K (1967) *Atlas of Mammalian Chromosomes*, vol 1. Berlin, Heidelberg, New York: Springer Verlag.

Hsu TC, Mead RA (1969) Mechanisms of chromosomal changes in mammalian speciation. In: Benirschke K, ed. *Comparative Mammalian Cytogenetics* New-York: Springer-Verlag, pp. 8–17. http://www.bionet.nsc.ru/chromosomes/.

Hunt RM Jr (1974) The auditory bulla in Carnivora: an anatomical basis for reappraisal of carnivore evolution. *J Morphol* **143**: 21–76.

Koepfli KP, Deere KA, Slater GJ *et al.* (2008) Multigene phylogeny of the Mustelidae: resolving relationships, tempo and biogeographic history of a mammalian adaptive radiation. *BMC Biol* **6**: 10.

Ledje C, Arnason U (1996a) Phylogenetic analyses of complete cytochrome *b* genes of the order Carnivora with particular emphasis on the *Caniformia*. *J Mol Evol* **42**: 135–144.

Ledje C, Arnason U (1996b) Phylogenetic relationships within caniform carnivores based on analyses of the mitochondrial 12S rRNA gene. *J Mol Evol* **43**: 641–649.

Lee MR, Modi WS (1983) Chromosomes of *Spilogale pigmae* and *S. putorius leucoparia*. *J Mammal* **64**(3): 493–495.

Maden BE, Dent CL, Farrell TE, Garde J, McCallum FS, Wakeman JA (1987) Clones of human ribosomal DNA containing the complete 18 S-rRNA and 28 S-rRNA genes. Characterization, a detailed map of the human ribosomal transcription unit and diversity among clones. *Biochem J* **246**(2): 519–527.

Mao X, Nie W, Wang J, Su W, Feng Q, Wang Y, Dobigny G, Yang F (2008) Comparative cytogenetics of bats (Chiroptera): the prevalence of Robertsonian translocations limits the power of chromosomal characters in resolving interfamily phylogenetic relationships. *Chromosome Res* **16**(1): 155–170.

Murphy WJ, Stanyon R, O'Brien SJ (2001) Evolution of mammalian genome organization inferred from comparative gene mapping. *Genome Biol* **2**(6): 1–8.

Nash WG, Wienberg J, Ferguson-Smith MA, Menninger JC, O'Brien SJ (1998) Comparative genomics: tracking chromosome evolution in the family Ursidae using reciprocal chromosome painting. *Cytogenet Cell Genet* **83**: 182–192.

Nash WG, Menninger JC, Wienberg J, Padilla-Nash HM, O'Brien SJ (2001) The pattern of phylogenomic evolution of the *Canidae*. *Cytogenet Cell Genet* **95**: 210–224.

Nash WG, Menninger JC, Padilla-Nash HM, Stone G, Perelman PL, O'Brien SJ (2008) The ancestral carnivore karyotype (2n = 38) lives today in ringtails. *J Hered* **99**(3): 241–253.

Nie W, Wang J, O'Brien PCM *et al.* (2002) The genome phylogeny of domestic cat, red panda and five mustelid species revealed by comparative chromosome painting and G-banding. *Chromosome Res* **10**: 209–222.

Nie W, Wang J, Perelman P, Graphodatsky AS, Yang F (2003) Comparative chromosome painting defines the karyotypic relationships among the domestic dog, Chinese raccoon dog and Japanese raccoon dog. *Chromosome Res* **11**(8): 735–740.

Nowak RM (1991) *Walker's Mammals of the World*, 5th edn, vol. 2. Baltimore and London: The Johns Hopkins University Press.

Owen JG, Baker RJ, Williams SL (1996) Karyotypic variation in spotted skunks (Carnivore: Mustelidae: *Spilogale*) from Texas, Mexico and El Salvador. *Texas J Sci* **48**(2): 119–122.

Pathak S, Wurster-Hill DH (1977) The distribution of constitutive heterochromatin in carnivores. *Cytogenet Cell Genet* **18**: 245–254.

Perelman PL, Graphodatsky AS, Serdukova NA *et al.* (2005) Karyotypic conservatism in the suborder Feliformia (Order Carnivora). *Cytogenet Genome Res* **108**(4): 348–354.

Petter G (1971) Origine, phylogenie et systematique des blaireaux. *Mammalia* **35**: 567–597.

Pocock RI (1921) On the external characters and classification of the Mustelidae: *Proc Zool Soc Lond* 803–837.

Radinsky L (1973) Are stink badgers skunks? Implications of neuroanatomy for mustelid phylogeny. *J Mammal* **54**: 585–593.

Rens W, O'Brien PC, Fairclough H, Harman L, Graves JA, Ferguson-Smith MA (2003) Reversal and convergence in marsupial chromosome evolution. *Cytogenet Genome Res* **102**(1–4): 282–290.

Rens W, Fu B, O'Brien PC, Ferguson-Smith M (2006) Cross-species chromosome painting. *Nat Protoc* **1**(2): 783–790.

Sato JJ, Hosoda T, Wolsan M, Suzuki H (2004) Molecular phylogeny of arctoids (Mammalia: Carnivora) with emphasis on phylogenetic and taxonomic positions of the ferret-badgers and skunks. *Zool Sci* **21**: 111–118.

Sato JJ, Wolsan M, Suzuki H *et al.* (2006) Evidence from nuclear DNA sequences sheds light on the phylogenetic relationships of pinnipedia: single origin with affinity to musteloidea. *Zool Sci* **23**(2): 125–146.

Seabright M (1971) A rapid banding technique for human chromosomes. *Lancet* **30**;2(7731): 971–972.

Simpson GG (1945) The principles of classification and classification of mammals. *Bull Am Museum Nat Hist* **85**: 1–350.

Stanyon R, Galleni L (1991) A rapid fibroblast culture method for mammalian chromosome. *Boll Zool* **58:** 81–83.

Stanyon R, Bigoni F, Wienberg J, Hadidian J (1993) A standardized G-banded karyotype for the raccoon (*Procyon lotor*) compared with the domestic cat. *Boll Zool* **60**: 41–45.

Sumner AT (1972) A simple technique for demonstrating centromeric heterochromatin. *Exp Cell Res* **75**: 304–306.

Telenius H, Pelmear AH, Tunnacliffe A *et al.* (1992) Cytogenetic analysis by chromosome painting using DOP-PCR amplified flow-sorted chromosomes. *Genes Chromosomes Cancer* **4**: 226–257.

Tian Y, Nie WH, Wang JH, Yang YF, Yang FT (2002) Comparative chromosome painting shows the red panda (*Ailurus fulgens*) has a highly conserved karyotype. *Yi Chuan Xue Bao* **29**(2): 124–127.

Tian Y, Nie W, Wang J, Ferguson-Smith MA, Yang F (2004) Chromosome evolution in bears: reconstructing phylogenetic relationships by cross-species chromosome painting. *Chromosome Res* **12**(1): 55–63.

Verts BJ, Carraway LN, Kinlaw A (2001) Spilogale gracilis. *Mammalian Species* **674**: 1–10. Retrieved from http://www.bioone.org/perlserv/?request=get-archive&issn=1545-1410 11/10/2008.

Vrana PB, Milinkovitch MC, Powell JR, Wheeler WC (1994) Higher level relationships of the arctoid Carnivora based on sequence data and 'total evidence'. *Mol Phylogenet Evol* **3**: 47–58.

Wang X, Whistler DP, Takeuchi GT (2005) A new basal skunk Martinogale (Carnivora, Mephitinae) from late Miocene dove spring formation, California and origin of new world Mephitines. *J Vertebrate Paleontol* **25**(4): 936–949.

Wienberg J, Stanyon R, Nash WG *et al.* (1997) Conservation of human vs. feline genome organization revealed by

reciprocal chromosome painting. *Cytogenet Cell Genet* **77**(3–4): 211–217.

Woodward RL (1994) *The generic relationships of skunks* (*Mustelidae, Mephitinae*) based on chromosome morphology and banding patterns. MS thesis. Angelo State University, San Angelo, Texas.

Wozencraft WC (1989) The phylogeny of the recent Carnivora. In: Wilson DE, Reeder DM, eds. *Carnivore Behaviour, Ecology, and Evolution*. Washington, DC: Smithsonian Institution Press, pp. 279–348.

Wozencraft WC (1993) Order Carnivora. In: Wilson DE, Reeder DM, eds. *Mammal Species of the World: a Taxonomic and Geographic Reference*. Washington, DC: Smithsonian Institution Press, pp. 495–535.

Wozencraft WC (2005) Order Carnivora. In: Wilson DE, Reeder DM, eds. *Mammal Species of the World*. Baltimore and London: Johns Hopkins University Press, pp. 532–628.

Wurster-Hill DH (1973) Chromosomes of eight species from five families of Carnivora. *J Mammal* **54**(3): 753–760.

Wurster DH, Benirschke K (1968) Comparative cytogenetic studies if the order Carnivora. *Chromosoma* **24**: 336–382.

Wurster-Hill DH, Gray CW (1975) The interrelationship of chromosome banding patterns in procyonids, viverrids, and felids. *Cytogenet Cell Genet* **15**: 306–331.

Wyss AR, Flynn JJ (1993) A phylogenetic analysis and definition of the Carnivora. In: Szalay F, Novacek M, McKenna M eds., *Mammal Phylogeny: Placentals*. New York: Springer-Verlag, pp. 32–52.

Yang F, Graphodatsky AS (2004) Integrated comparative genome maps and their implications for karyotype evolution of carnivores. In: Schmid M, Nanda I, eds. *Chromosomes Today*, pp. 215–224.

Yang F, Carter NP, Shi L, Ferguson-Smith MA (1995) A comparative study of karyotypes of muntjacs by chromosome painting. *Chromosoma* **103**(9): 642–652.

Yang F, O'Brien PC, Milne BS *et al*. (1999) A complete comparative chromosome map for the dog, red fox, and human and its integration with canine genetic maps. *Genomics* **62**: 189–202.

Yang F, Graphodatsky AS, O'Brien PC *et al*. (2000) Reciprocal chromosome painting illuminates the history of genome evolution of the domestic cat, dog and human. *Chromosome Res* **8**: 392–404.