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A standardized G-banded karyotype for the raccoon (*Procyon lotor*) compared with the domestic cat

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ABSTRACT

We propose a standardized karyotype for the raccoon (*Procyon lotor*; 2n = 38, FN 74) and compare it with that of the domestic cat (2n = 38, FN 72). Numerous chromosomes (12) have similar and sometimes identical G-banding and 14 chromosome pairs have remained intact. Other chromosomes apparently differ by Robertsonian translocations and inversions. The conservation of these karyotypes is remarkable considering that the divergence of procyonids and felids predates 50 million years B.P. However, the common diploid number of 38 is not a primitive retention, as sometimes hypothesized. Instead, cats and raccoons converged on this chromosome number by a different series of translocations. The unsolved problems of carnivore phylogeny could benefit from a combination of high resolution banding and molecular cytogenetic analyses.

KEY WORDS: Phylogeny - Cytogenetics - Caruivores - Procyonids.

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INTRODUCTION

Over the last two decades the chromosome banding patterns of numerous carnivore species have been reported (Wurster-Hill & Gray, 1973, 1975; Pathak & Wurster-Hill, 1977; Wurster-Hill & Bush, 1980; O'Brien & Nash, 1982; Wurster-Hill & Centerwall, 1982; Couturier & Dutrillaux, 1986). On the basis of banding, homologous chromosomes have been found between Procyonidae, Viverridae and even the Pinnipedia, but especially within the Felidae (Wurster-Hill & Gray, 1975). Some workers even report numerous homologous chromosomes between carnivores and other orders (Nash & O'Brien, 1982, Dutrillaux & Couturier, 1983).

Apparently, the carnivores are karyologically conservative (Sumner, 1990); however, the Ursidae and Canidae have experienced extensive karyological remodelling (Wurster-Hill & Bush, 1980; O'Brien *et al.*, 1985; Wayne *et al.*, 1987). The Canidae, for instance, have 2n numbers which range from 34 to 78 (among the highest diploid numbers in mammals).

Recently, in the ambit of an extensive monitoring of the ecology of urban wildlife we had an opportunity to karyotype a number of raccoons, *Procyon lotor*. In spite of the wide distribution of this species in North America (and secondarily in Europe), information on the chromosomes of raccoons are scarce. The only banded karyotype available from the literature is based on a single female specimen in which the G-banding resolution is barely adequate for recognizing homologs and the identification of the X-chromosome is not certain (Wurster-Hill & Gray, 1975). The C-banded karyotype of the raccoon was reported in Pathak & Wurster-Hill (1977). All chromosomes had C-bands, but as is typical for carnivores the amount of C-band material was limited.

On the basis of banded karyotypes from three other procyonid species, and comparisons with numerous viverrids and felids, Wurster-Hill & Gray (1975) proposed that the raccoon had a nearly complete felid karyotype.

Here we report on the karyotypes of raccoons of both sexes and propose a karyotype standardization based on medium to high resolution G-banding. Finally, we have compared the chromosomes of the raccoon to those of the domestic cat. This comparison allows the raccoon karyotype to be more securely related to the chromosomes of the most intensively studied carnivore species from both a karyological and a gene mapping perspective.

MATERIALS AND METHODS

Heparinized whole blood samples were taken from a total of 16 raccoons (8 males, 8 females), *Procyon lotor lotor* (PLO), live-trapped in Rock Creek Park, Washington, D.C. (USA). About 1 ml of blood was then transferred to 12 ml test tubes filled with nutrient medium (RPMI 1640, 5% FCS and antibiotics). The remaining part of the blood was left in the syringes. The samples were then shipped by express courier to Italy or Germany where they were cultured.

The tissue culture consisted of 0.3 ml of whole blood inoculated into 5 ml of tissue culture medium (RPMI 1640, 15% FCS, 50 µg/ml Concavalin-A type III [SIGMA]). After 90 hours of culture at 38.5° C colcemid (0.05 µg/ml) was added for 90 minutes. The cultures were then harvested according to standard methods (hypotonic treatment with 0.075 M KCl for 15 min).

Domestic cat (*Felis catus*, but more properly considered *Felis silvestris* according to opin. 465 ICZN, 1957) fibroblast culture was established according to the method of Stanyon & Galleni (1991).

Chromosome spread were banded as follows: G-banding with trypsin according to Small *et al.* (1985), C-banding according to Sumner (1972), AgNOR-banding according to Howell & Black (1980).

The idiogram was constructed from 15 karyotypes measured using the CAMO interactive computer program as described by Wienberg *et al.*, (1988). The banding pattern of the idiogram was based on medium to high resolution karyotypes pooled from all animals studied.

RESULTS

Of the 16 raccoon blood samples, 10 provided metaphases suitable for karyotyping. Six whole blood cultures were either contaminated or too old to give good results. Samples arrived in the laboratory from four to ten days after being taken, however, cultures made within seven days of blood draws provided good *in vitro* growth. All raccoons analyzed had 2n = 38 chromosomes, with a fundamental number of 74 and apparently identical G-banded karyotypes (Fig. 1). The X-chromosome is a medium sized submetacentric and the Y-chromosome is a small acrocentric. Table I reports the chromosome measurements and Figure 2 presents a standardized idiogram at about the 400 band level of resolution.

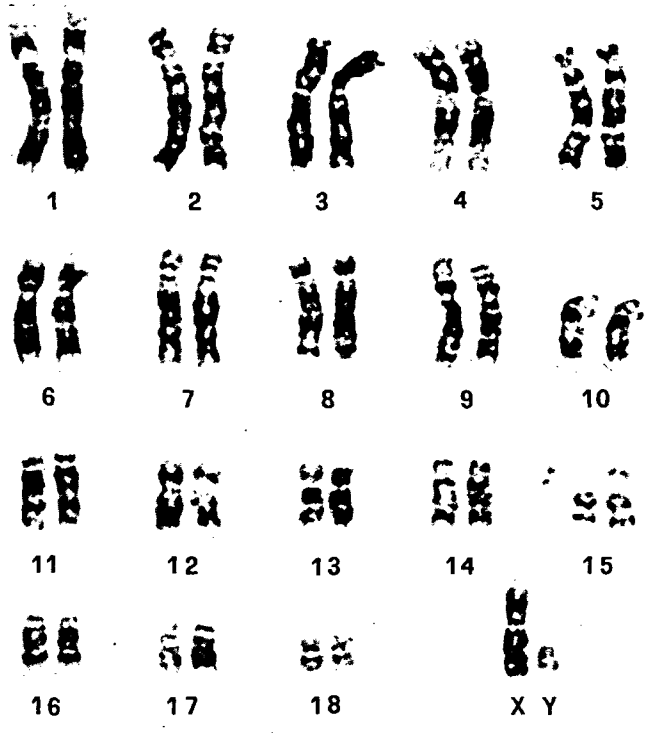

Fig. 1 - G-banded karyotype of *Procyon lotor* (raccoon).

TABLE I - *Measurements for chromosomes of* Procyon lotor.

Chromosome	RHCL*	SD	S/L
1	8.8	0.54	26.2
2	8.0	0.61	20.0
3	7.8	0.35	41.9
4	7.7	0.45	42.7
5	7.0	0.51	15.3
6	6.2	0.34	29.0
7	6.1	0.31	19.4
8	5.9	0.47	18.6
9	5.6	0.32	33.7
10	4.5	0.34	28.4
11	4.2	0.26	15.0
12	3.9	0.25	39.1
13	3.5	0.25	25.3
14	3.5	0.30	34.0
15	3.3	0.47	42.7
16	2.8	0.32	17.3
17	2.4	0.26	43.5
18	1.8	0.22	42.5
X	5.2	0.29	36.8
Y	1.5	0.34	6.0

* RHCL = Relative Haploid Chromosome Length

A single pair, n. 15, was positively stained after silver staining for NORs. After C-banding, only chromosomes 5 (entire short arm), 15 (entire short arm proximal to the NOR), and the Y (almost all) had notable C-bands; centromeric C-bands on other chromosomes were barely visible.

A comparison of PLO with the G-banded chromosomes of the domestic cat (FCA) showed that the two species have similar karyotypes. Both species have 38 chromosomes, but differ in FN: PLO = 74, FCA = 72. Among the autosomals: eight chromosome pairs are very similar or identical (PLO/FCA: 7/B2, 8/B3, 9/A3, 10/D1, 13/D3, 14/D4, 17/E2, 18/E3); two chromosome pairs differ by single pericentric or paracentric inversions (6/B4, 16/F1). PLO chromosome 5 (with the exception of the short arm which is entirely heterochromatic) is homologous to cat Alq. Chromosome 15 differs from cat E1 only by the presence of a heterochromatin segment on the short arm proximal to the NOR. Chromosome 2 appears to differ from cat B1 by multiple, intrachromosomal rearrangements, probably both pericentric and paracentric inversions. Inversions could also help explain minor differences in chromosome banding in PLO 7, 8, 10, 12, 13.

The homology of other chromosomes is more difficult to establish, but some hypotheses can be made. Chromosome 1 appears to be the result of a translocation between FCA A2p + C2. Two chromosomes appear to be the result of Robertsonian translocations between various cat chromosome arms: PLO 3 = FCA F2 + C1q, PLO4 = A1p + C1p.

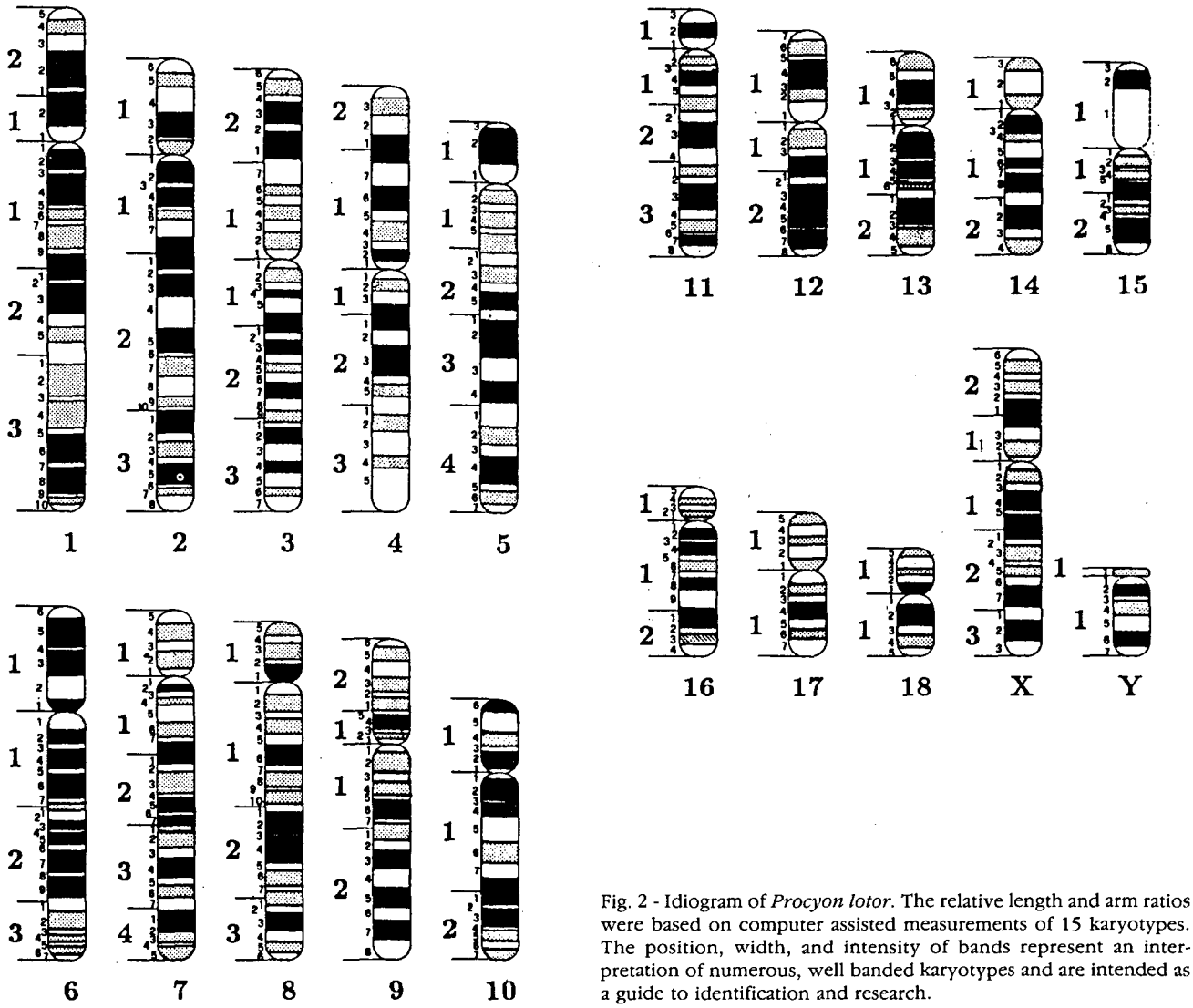

Fig. 2 - Idiogram of *Procyon lotor*. The relative length and arm ratios were based on computer assisted measurements of 15 karyotypes. The position, width, and intensity of bands represent an interpretation of numerous, well banded karyotypes and are intended as a guide to identification and research.

Finally, the X-chromosomes are identical, but the acrocentric raccoon Y-chromosome is about 40% smaller than the metacentric Y-chromosome of the cat.

DISCUSSION

In agreement with Wurster-Hill & Gray (1975) we found that the raccoon, *Procyon lotor*, has a diploid number of 38; however they did not report the fundamental number (74), could not securely identify the X-chromosome and did not describe the G-banding pattern of the Y-chromosome, because they studied only one female animal. Our results present G-banding at a higher level of resolution and show that the X-chromosome is a medium sized submetacentric (about 5% of the total haploid karyotype length) and has a G-banding pattern typical of mammalian X-chromosomes. The Y-chromosome is the smallest chromosome (about 1.5% and is an acrocentric as reported by Pathak & Wurster-Hill (1977). There are no previous reports on NOR staining in the raccoon. Only one pair of NORs was detected by silver staining and as expected, this was located on the «marker chromosomes» n. 15.

Our results also support previous observations that the procyonids have many chromosomes in common with the felids (Wurster-Hill & Gray, 1975; Couturier & Dutrillaux, 1986). The difference in FN (PLO = 74, FCA = 72) is probably due to the short arm of chromosome 5 which is composed entirely of heterochromatin.

Wurster-Hill & Gray (1975) were the only authors to directly compare *Procyon lotor* with felids. With respect to the karyotype of FCA, they found 13 autosomal homologs of which most are in agreement with our results. They did not assign homologs for five cat chromosomes (A2, C2, D3, F1, F2) for which we were able to propose homologs. For C1 we agree with Wurster-Hill & Gray that C1p is homologous to PLO4, but instead propose that C1q is homologous to PLO3q.

Fig. 3 - A comparison of the proposed chromosomal homologies between raccoon (*Procyon lotor*) and domestic cat chromosomes. Raccoon chromosomes are numbered below. Cat chromosomes are placed to the right of raccoon chromosome and identified laterally. When two cat chromosomes or cat chromosome arms are homologous to a raccoon chromosome, the raccoon chromosome is placed between the proposed cat homologs (cat chromosome numbering follows Jones, 1965). Cat chromosome C2 has been inverted, while cat chromosomes A1, A2, C1 were cut at the centromere and the p and q arms were separately placed in the figure to better show the banding similarities. The homologies proposed for chromosomes 5-18 appear certain; those for chromosomes 1-4 must be considered as only working hypotheses. Chromosomes from both the raccoon and cat were selected from various metaphases.

We also propose homologies for chromosomes 1, 3 and 11 which they did not assign to any felid. These chromosome would derive from three different translocations. They also assigned two raccoon chromosomes to felid chromosomes which are supposedly not present in FCA; we assigned these chromosomes directly to FCA chromosomes D3, and F1. They also proposed a pericentric inversion in PLO 15, but our C-banding results show that the origin of this short arm is probably only due to the addition of heterochromatin.

Our results agree better with those of Couturier & Dutrillaux (1986) even if they did not directly compare PLO and FCA. From their reconstruction of the ancestral karyotype they propose that procyonids and felids differ by six Robertsonian translocations and seven inversions. On the basis of our limited results comparing only two species we can agree with their proposal that the common diploid number (2n = 38) of proyconids and felids is the result of convergence and that the ancestral karyotype probably had 42-44 chromosomes.

It is clear, however, that establishing chromosomal homology solely on the basis of chromosome banding can only be considered a first step in cytogenetic comparison and evolutionary reconstruction. To more

TABLE II - *Proposed raccoon/cat chromosome homologs.*

Raccoon chromosome	Proposed domestic cat homolog
1p	A2p
1q	C2
2	B1
3p	F2
3q	C1q
4p	A1p
4q	C1p
5	A1q
6	B4
7	B2
8	B3
9	A3
10	D1
11	A2q
12	D2
13	D3
14	D4
15	E1
16	F1
17	E2
18	E3

securely establish homology and the validity of chromosome phylogenies, it is necessary to combine banding results with gene mapping data. Gene mapping data is quite plentiful in the cat, and more limited results have been reported for three other carnivores: mink, fox and dog (O'Brien & Marshall Graves, 1991). Recently, we have introduced a new strategy for establishing chromosomal homology based on *in situ* suppression hybridization (CISS hybridization or chromosome painting) (Wienberg *et al.*, 1991, 1992; Stanyon *et al.*, 1992). The results we obtained in comparing the raccoon and cat chromosomes suggest that carnivore cytogenetics could be profitably re-examined using a combination of high resolution banding and molecular cytogenetic methods. Such a research programme could make valuable contributions to resolving problems in carnivore phylogeny for which there is not yet any clear consensus (Novacek, 1992).

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