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Chromosomal studies in *Callicebus donacophilus pallescens*, with classic and molecular cytogenetic approaches: Multicolour FISH using human and *Saguinus oedipus* painting probes

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Abstract

This paper presents the karyotype of *Callicebus donacophilus pallescens* for the first time. The analysis included G-, C-, NOR-banding techniques and FISH with chromosome painting probes from *Saguinus oedipus* and *Homo sapiens*. The results were compared with the karyotypes of *Callicebus moloch donacophilus* and *C. moloch* previously published. These three karyotypes display the same diploid number ($2n = 50$) but diverge about the number of biarmed and acrocentric chromosomes. The acrocentrics 14 and 15 from *C. m. donacophilus* and *C. moloch* have undergone an *in-tandem* fusion originating a large acrocentric (pair 10) in *C. d. pallescens*. The major submetacentric pair (pair 1) from *C. d. donacophilus* and *C. moloch* have undergone fission originating two acrocentric pairs in *C. d. pallescens* (pairs 15 and 22). Herein was evidence that, in spite of the high interspecific variation among *Callicebus*, most of the chromosomes remained conserved.

Introduction

The genus *Callicebus* is one of the most complex and diversified among the platyrrine Primates. These monkeys are medium size, have non-prehensile tails and are found mainly in the tropical forests of the Amazon and Orinoco basin, but also extend into the Atlantic forest region of Brazil and the *chaco* and dry forest of Paraguay (van Roosmalen *et al.* 2002).

According to Hershkovitz (1990), *Callicebus* encompasses 13 species and 16 subspecies. Kobayashi (1995) recognized five distinct clades of *Callicebus* species: *donacophilus*, *cupreus*, *moloch*, *personatus* and *torquatus*. Following this author, the diploid number is a good criterion for

Callicebus classification and suggests that each group may have an exclusive chromosome number. Recently, van Roosmalen *et al*. (2002) described two new species, *Callicebus stephennashi* and *C. bernardi* and recognized five species groups and a total of 28 species, elevating all the subspecies to full species status.

Cytogenetics studies have demonstrated a high chromosomal variability among karyotypes of *Callicebus* species (Table 1). Egozcue *et al*. (1969) and Benirschke & Bogart (1976) described the karyotype of *Callicebus torquatus* with $2n = 20$ chromosomes, the lowest diploid number found in Primates. Barros *et al*. (1999, 2000) described a new karyotype in *Callicebus torquatus* ssp. with $2n = 22$, and suggested the occurrence of *in-tandem* fusion events in the karyotipic evolution of *torquatus* group. The other species of *Callicebus* have diploid numbers ranging from $2n = 42$–50 chromosomes (Minezawa & Borda 1984, Pieczarka & Nagamachi 1988, Minezawa *et al*. 1989, Nagamachi *et al*. 1999, Rodrigues *et al*. 2001, Nagamachi *et al*. 2003). Stanyon *et al*. (2000) published a similar karyotype to that found by Minezawa & Borda (1984) but they identified the specimen as *C. moloch*. The karyotype of *C. donacophilus* was described by Minezawa & Borda (1984) in specimens from Bolivia, with 50 chromosomes. This karyotype was composed of 6 subtelocentric pairs, 5 metacentric or submetacentric pairs, 13 acrocentric pairs, a medium submetacentric X and a small metacentric Y.

In recent years, comparative genome maps between human and several Platyrrhini species have been established by cross-species chromosome painting (Sherlock *et al.* 1996, Richard *et al.* 1996, Consigliére *et al.* 1996, 1998, Morescalchi *et al.* 1997, Stanyon *et al.* 2000, 2001, Garcia *et al.* 2000, Müller *et al.* 2001, Neusser *et al.* 2001, de Oliveira *et al.* 2001). Stanyon *et al.* (2000), using the amount of data already published for different platyrrhini genera and including *Saimiri* and *Callicebus*, proposed a putative ancestral karyotype composed of 56 chromosomes. More recent papers, focusing on Callithrichidae (Neusser *et al.* 2001) and Atelidae (de Oliveira 2002) have agreed with this ancestral karyotype, with minor modifications. Hence, it seems that, in the near future, the chromosomal evolution that has taken place in the radiation of New World monkeys, originating diploid numbers from 20 to 62, will be resolved.

The aim of this paper is to describe for the first time the karyotype of *Callicebus donacophilus pallescens* through multidirectional chromosome painting, comparing it with those of *Callicebus moloch donacophilus* and *Callicebus moloch*, previously described in the literature. The data will contribute to a better understanding of the chromosomal diversity found in Platyrrhini species.

Table 1. Cytogenetic data in the genus *Callicebus*.

Species	2*n*	Bi	A	X	Y	G	C	NOR	FISH	References
C. moloch	46	20	24	SM	SM					4
C. moloch	50	24	24	SM	A	+			+	12
C. m. cupreus	46	20	24	SM	SM	+	+			1, 2, 3, 5, 6*
C. m. ornatus	46	20	24	SM	SM					5
C. m. moloch	48	20	26	SM	SM	+	+	+		7*
C. m. brunneus	48	20	26	SM	SM	+	+			9*
C. m. donacophilus	50	22	26	SM	SM	+	+			5, 8*
C. hoffmannsi	50	20	28	SM	A	+	+	+		11*
C. torquatus	20	8	10	SM	?					4
C. t. torquatus	20	8	10	SM	?	+	+			6*
C. torquatus ssp.	22	8	12	SM	?	+	+	+		10*
C. d. pallescens	50	18	30	SM	M	+	+	+		13
C. p. nigrifrons	42	14	6	SM	M	+	+	+		14, 15

Bi = bi-armed chromosomes; A = acrocentric chromosomes; SM = submetacentric; G = G-bands; C = C-bands; NOR = nucleolar organizer region staining.

References: [1]Bender & Mettler 1958; [2]Bender & Chu 1963; [3]Benirschke & Brownhill 1976; [4]Egozcue *et al.* 1969; [5]De Boer 1974; [6]Benirschke & Bogart 1976; [7]Pieczarka & Nagamachi 1988; [8]Minezawa & Borda 1984; [9]Minezawa *et al.* 1989; [10]Barros *et al.* 2000; [11]Rodrigues *et al.* 2001; [12]Stanyon *et al.* 2000; [13]Present study; [14]Nagamachi *et al.* 1999; [15]Nagamachi *et al.* 2003. *Studies with chromosome banding data.

Materials and methods

Cell cultures and banding techniques

We studied two specimens of *Callicebus donacophilus pallescens* (male and female) housed at the National Centre of Primates, FUNASA, Ananindeua, Pará State, Brazil. Metaphases were obtained following standard protocols for blood cell and fibroblast cultures.

Sequencial G-banding/FISH followed de Oliveira *et al.* (2002). The C-banding followed Sumner (1972). NOR labelling was made according to Howell & Black (1980).

In-situ *hybridization and probe detection*

Human and New World monkey chromosome-specific painting probes were the same as described before (Stanyon *et al.* 2000, Müller *et al.* 2001). Multicolor probe sets were labelled in Boolean combinations (Neusser *et al.* 2001). Probe labelling was performed by DOP-PCR (Telenius *et al.* 1992) using Biotin-dUTP, digoxigenin-dUTP (Roche) and TAMRA-dUTP (Applied Biosystems/PE). *In-situ* hybridization and probe detection were carried out as described by Neusser *et al.* (2001).

Miscroscopy and image analysis

Banded methaphases were photographed in a Zeiss III microscope, with Kodak Imagelink HQ 33-mm perforated film. Sequential G/FISH images were captured with a cooled CCD camera (Photometrics C250/A equipped with a KAF1400 chip, Kodak) coupled to a Zeiss Axiophot microscope. Microscope and camera control, digital image acquisiton, subsequent merging of metaphase images and false color assignment was performed by SmartCapture VP software (Digital Scientifics). Chromosome segments followed nomenclature proposed by by Neusser *et al.* (2001).

Results

Chromosome banding experiments

Figure 1 shows the G-banded karyotypes of *Callicebus donacophilus pallescens*, together with the comparative genome map between this species and *Saguinus oedipus* and human painting probes. The diploid number of the specimens studied here is $2n = 50$. The autosomal complement is composed by 9 biarmed and 15 acrocentric pairs. The X chromosome is a medium submetacentric, while the Y is a small biarmed chromosome.

C-banding revealed the presence of constitutive heterocromatin in several parts of the karyotype: (1) in the centromeres of all the chromosomes; (2) in the whole short arm of the largest acrocentric (pair 10); (3) in the distal segments of the short arm of two submetacentric pairs (7 and 8); and (4) in the distal portion of the long arm of the X chromosome (Figure 2).

The nucleolar organising regions were observed on the distal region of the short arm of pair 10 and of the long arm of a small acrocentric (pair 20; Figure 3).

In-situ *hybridization experiments*

Figure 4 shows representative FISH experiments with human and *S. oedipus* probe sets to *C. d. pallescens*, while the comparative genome map is shown in Figure 1.

Human painting probes (Pools 1–4) produced 44 fluorescent signals in a haploid set of the male of *C. d. pallescens*. The Y probe did not hybridize. Chromosomes 3, 15, 19, 20 and X from *C. donacophilus pallescens* hybridized respectively to human painting probes 6, 13, 17, 20 and X. Pairs 1, 4, 5, 6, 7, 8, 10, 11, 12, 14 and 24 from *C. d. pallescens* constituted associations of human homologous segments: 15b/7b, 22/2a/22, 16b/2b/16b/2b, 16a/10a/16a/10a, 8a/18, 19/12, 9/7a/5a/7a/5a, 14/15a, 10b/11, 12/19, and 3a/21, respectively. Segments of human chromosome 1 hybridized to chromosomes 2, 9 and 23 of *C. d. pallescens*. Chromosome pairs 13 and 21 of *C. d. pallescens* hybridized to the segments of human chromosome 3 (3b and 3c), while pairs 17 and 18 hybridized to HSA 4. Chromosome pairs 16 and 22 hybridized to HSA 8b and HSA 5, respectively.

With *Saguinus oedipus* chromosome probes, we identified 33 hybridized homologous chromosome segments. The Y chromosome probe did not produce any hybridization signal. Chromosomes 2, 3, 5, 6, 7, 9, 11, 13, 16, 21, 23, 24 and X of

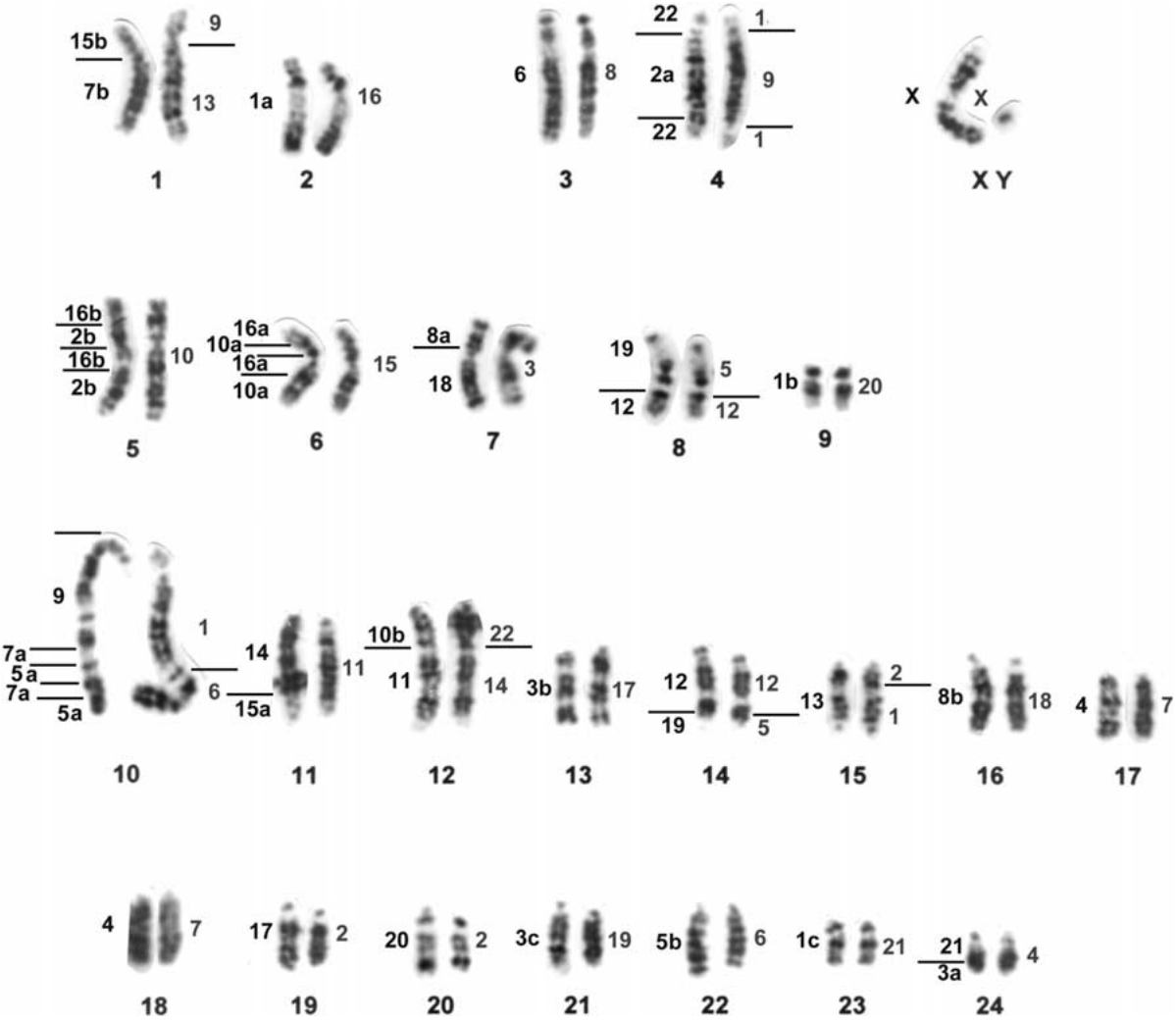

Figure 1. Karyotype G-banded from *Callicebus donacophilus pallescens* showing the comparative genome map with human and *Saguinus oedipus* genomes. Numbers on the left of the chromosomes represent the human homologous segments, while the numbers on the right represent homologies with *Saguinus oedipus*.

C. d. pallescens are syntenic groups conserved between this species and *S. oedipus*, and hybridized respectively to SOE painting probes: 16, 8, 10, 15, 3, 20, 11, 17, 18, 19, 21, 4 and X. Pairs 1, 4, 8, 10, 12, 14 and 15 of *C. d. pallescens* correspond to rearranged associations of *S. oedipus* chromosomes: 9/13, 1/9/1, 5/12, 1/6, 22/14, 12/5, 2/1, respectively. SOE painting probes 7 and 2 hybridized to two pairs of *C. d. pallescens* each: 17 and 18, and 19 and 20, respectively. Chromosome pair 22 of *C. d. pallescens* hybridized to a segment of chromosome 6 of SOE.

Discussion

The results of hybridization experiments showed that *C. d. pallescens* has mantained fourteen autosomic syntenic groups found in the putative ancestral karyotype of Platyrrihi (Stanyon *et al.* 2000, Neusser *et al.* 2001): 6, 14/15a, 8a/18, 8b, 11, 13, 1a, 3b, 17, 3c, 1b, 20, 1c, 3a/21. Two more ancestral associations were maintened, although they have suffered inversions: 2b/16b and 10a/16a. The other sysntenic groups have been rearranged. Derived association of human chromosomes 7b/

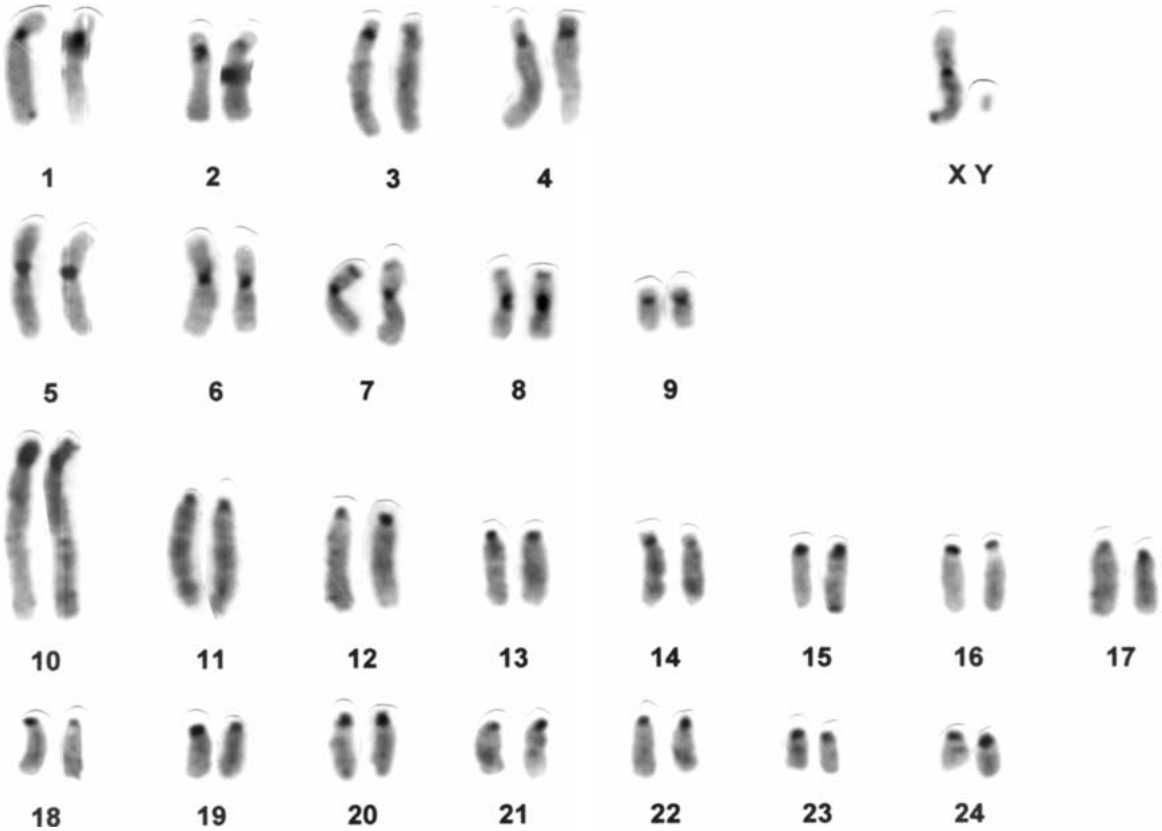

Figure 2. C-banded karyotype from *C. d. pallescens*.

15b, 22/2a/22, 19/12, 9/7a/5a/7a/5a and 10b/11 homologues were exclusively identified in *Callicebus*. Of these, associations 9/7a/5a/7a/5a and the fragmentation of association 19/12 in two different chromosomes were not described in *C. moloch* by Stanyon *et al.* (2001). Moreover, these authors have not detected associations 5a/7a, which has been detected in all genera of Platyrrhini analysed by FISH so far, as well as the inversion in 16a/10a observed in the karyotype of *C. d. pallencens*. Inversions in associations 5a/7a and 16a/10a have also been detected in Atelinae (de Oliveira 2001, de Oliveira *et al.* 2002) and *Chiropotes satanas* (Neusser 1999). Therefore, they seem to be homoplastic conditions of *C. d. pallescens* instead of sinapomorphies shared by *Callicebus* and these groups because they were not detected in *C. moloch* by Stanyon *et al.* (2000). However, only the analysis of other taxa of *Callicebus* will be able to clarify this point.

The chromosome painting approach demonstrated that the karyotypes of *C. d. pallescens* and *C. moloch* diverged mainly by two rearrangements: an *in-tandem* fusion between pairs 14 and 15 of *C. moloch* (association 9/7a/5a/7a/5a of CDP) and the fragmentation of chromosome 1 of *C. moloch*, homologous to pairs 17 and 18 from *C. d. pallescens*, homologous to human chromosome 4 (Table 2).

The comparison of the karyotypes of *Callicebus donacophilus pallescens, C. moloch* (Stanyon *et al.* 2000), both analysed through FISH experiments, and *C. moloch donacophilus* (Minezawa & Borda 1984), with classical banding techniques, showed that, although the three taxa have the same diploid number ($2n = 50$), they differ in the number of biarmed chromosomes (11 pairs in *C. moloch donacophilus* and *C. moloch*, and 9 pairs in *C. d. pallescens*) and number of acrocentrics (13 pairs in *C. m. donacophilus* and *C. moloch*, and 15 pairs in

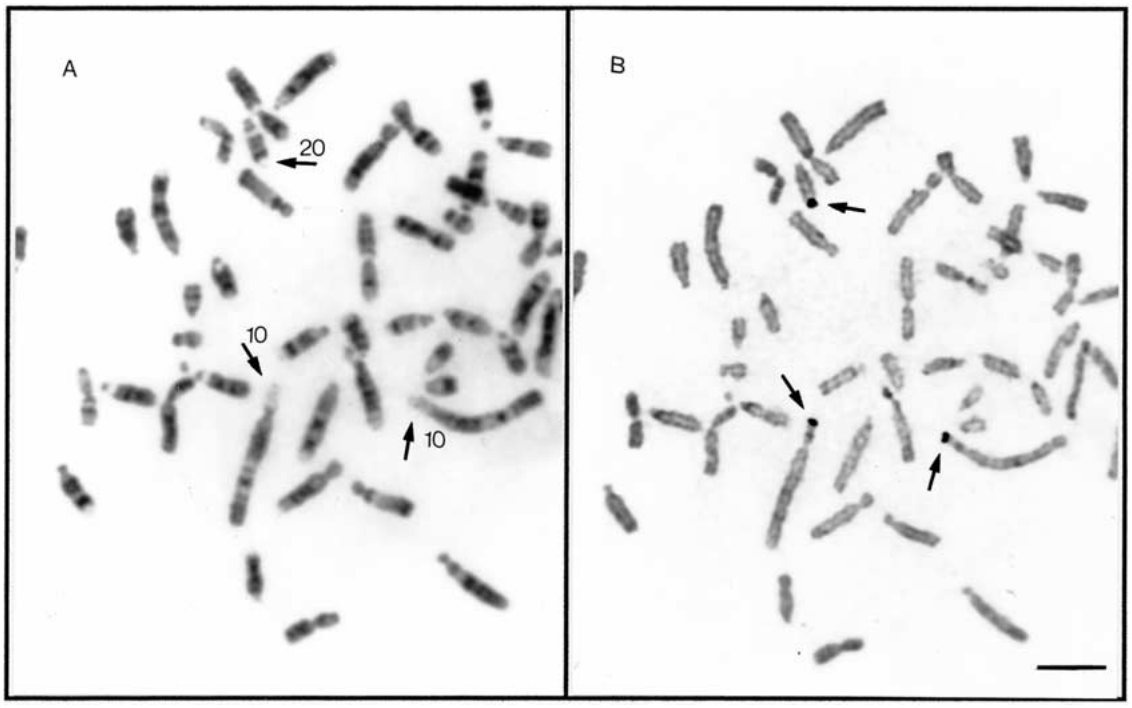

Figure 3. Sequentially G-banded (**A**) and NOR-staining (**B**) metaphase, showing NORs in the chromosome pairs 10 and 20. Bar = 5 μm.

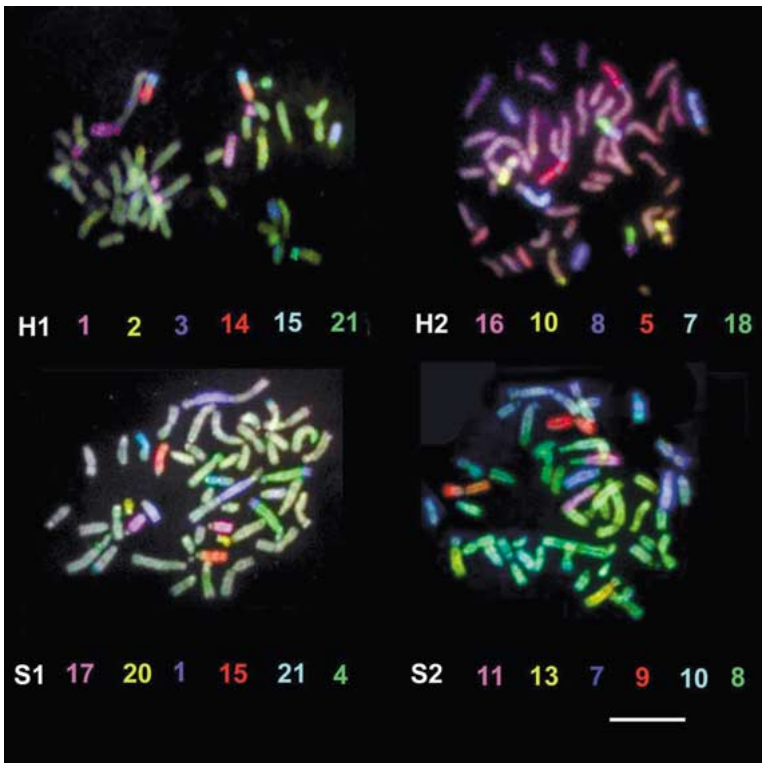

Figure 4. Representative FISH experiments with human and *S. oedipus* multicolour probe sets to *C. d. pallescens*. Respective probe composition and false colour assignment is given below each metaphase (H = human, S = *S. oedipus*). Bar = 10 μm.

Table 2. Chromosomal relationships based on comparison of FISH informative data between *C. d. pallescens* (CDP), *Homo sapiens* (HSA), *Saguinus oedipus* (SOE), *C. moloch donacophilus* (CMD) and *C. moloch* (CM).

CDP	HSA	SOE	CMD/CM*
1	15b/7b	9/13	5
2	1a	16	8(inv)
3	6	8	4
4	22/2a/22	1/9/1	2
5	16b/2b/16b/2b	10	6(inv)
6	16a/10a/10a/10a	15	7
7	8a/18	3	9
8	19/12	5/12	11
9	1b	20	12
10	9/7a/5/7a/5	1/6	14/15
11	14/15a	11	13
12	10b/11	22/14	3q(inv)
13	3b	17	16
14	12/19	12/5	10
15	13	2/1	18
16	8b	18	17
17	4	7	1a
18	4	7	1b
19	17	2	20
20	20	2	21
21	3c	19	22
22	5	6	19
23	1c	21	23
24	3a/21	4	24
X	X	X	X

*From Minezawa & Borda 1984; Stanyon *et al.* 2000.

C. d. pallescens). Our results showed that chromosome pairs 14 and 15 (acrocentrics) of *C. moloch* (and probably *C. m. donacophilus*) had fusioned *in tandem*, originating a large acrocentric (pair 10) in the karyotype of *C. d. pallescens*. The opposite hypothesis also can be considered but there is already evidence that *in-tandem* fusion is not an uncommon event in *Callicebus* chromosomal evolution, as demonstrated by Barros *et al.* (2000), in the evolution of the *torquatus* group.

Considering the high chromosomal variation found in this genus, avaliable data is still too incomplete to allow conclusions about the direction of chromosomal changes or to propose an ancestral karyotype to this group. Nevertheless, it seems that ancestral Platyrrhini chromosome forms homologous to human chromosomes 6, 14/15a, 8a/18, 8b, 11, 13, 1a, 3b, 17, 3c, 1b, 20, 1c, 3a/21, 10a/16a and probably 5a/7a would be found in the ancestral karyotype of *Callicebus*.

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