Cytogenetic and Genome Research

Cytogenet Genome Res 2012;137:259–272
DOI: 10.1159/000339296

Published online: June 12, 2012

Chromosome Evolution in New World Monkeys (Platyrrhini)

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Key Words

Chromosome painting · Chromosome rearrangement · Karyotype evolution · New World monkey · Platyrrhini

Abstract

During the last decades, New World monkey (NWM, Platyrrhini, Anthropoideae) comparative cytogenetics has shed light on many fundamental aspects of genome organisation and evolution in this fascinating, but also highly endangered group of neotropical primates. In this review, we first provide an overview about the evolutionary origin of the inferred ancestral NWM karyotype of 2n = 54 chromosomes and about the lineage-specific chromosome rearrangements resulting in the highly divergent karyotypes of extant NWM species, ranging from 2n = 16 in a titi monkey to 2n = 62 in a woolly monkey. Next, we discuss the available data on the chromosome phylogeny of NWM in the context of recent molecular phylogenetic analyses. In the last part, we highlight some recent research on the molecular mechanisms responsible for the large-scale evolutionary genomic changes in platyrrhine monkeys.

New World monkeys (NWM) represent a monophyletic group of higher primates (infraorder Platyrrhini). The currently over 120 recognized species are assigned to at least 16 genera. Today these species are commonly classified in 3 families, the Cebidae, Atelidae and Pitheciidae [Opazo et al., 2006; Wildman et al., 2009; Perelman et al., 2011; Matsui and Hasegawa, 2012 for recent review]. Owl monkeys (genus *Aotus*) are usually included in the family Cebidae, while Groves [2001] classified *Aotus* as a fourth family. The Cebidae comprise the capuchins, squirrel monkeys, marmosets, and tamarins (and owl monkeys); the Atelidae include spider monkeys, woolly monkeys, howlers, and muriquis; and the Pitheciidae comprise sakis, uakaris and titis.

The majority of studies agreed on the classification of 11 genera in 3 monophyletic clades: (1) the large monkeys with prehensile tails from the family Atelidae with genera *Alouatta, Ateles, Lagothrix,* and *Brachyteles*; (2) the seed predator monkeys from the family Pitheciidae, with genera *Pithecia, Chiropotes* and *Cacajao*), and (3) the small clawed monkeys from the subfamily Callithrichinae (family Cebidae, genera *Saguinus, Leontopithecus*, *Calli-*

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thrix, and *Cebuella*) [Canavez et al., 1999; Schneider, 2000; Schneider et al., 2001; Singer et al., 2003; Steiper and Ruvolo, 2003; Ray and Batzer, 2005; Osterholz et al., 2009]. However, some disagreement remained with regard to the branching order between the clades and phylogenetic affiliations of the remaining genera *Cebus, Callimico, Saimiri*, *Callicebus*, and *Aotus*. Recent debate has been concerned with the interfamiliar relationship and the internal arrangements within Atelines and Callithrichines, but 2 recent comparative DNA sequence [Wildman et al., 2009; Perelman et al., 2011] and SINE integration [Osterholz et al., 2009] analyses, each including species from 15 or more genera, shed new light on some of these issues. Together with some earlier studies, these 3 phylogenetic reconstructions agreed on the branching sequence of the 3 families Pitheciidae{Atelidae-Cebidae}, on the linkage of *Callicebus* to the Pitheciidae and of *Aotus* to the Cebidae. Despite this, the position of *Aotus* is still not well resolved in these studies. Furthermore, the species affiliation has been continuously revised, for example the species record of *Callicebus* has been elevated from 13 [Hershkovitz, 1990] to 28 [van Roosmalen et al., 2002].

New World Monkey Comparative Molecular Cytogenetics

Because of the great karyological variability found in Platyrrhini with chromosome numbers ranging from 16 in a titi monkey to 62 in the woolly monkey, most species of this group have been subject of classical chromosome banding analyses since the 1970s. Due to their Mendelian pattern of inheritance, it is possible to use chromosome rearrangements as cladistic markers, to detect synapomorphies and to clarify sister-group relationships among taxa [Dobigny et al., 2004]. These characteristics, added to the fact that they are rare events, render chromosome rearrangements potentially powerful markers in phylogenetic investigations [Rokas and Holland, 2000].

The introduction of cross-species fluorescence in situ hybridization revolutionized the field of comparative cytogenetics and allowed to establish chromosome homology maps between human and other primate species including NWM at a resolution of 3–5 Mb [Müller, 2006; Wienberg and Stanyon, 1998 for review]. Since the mid-1990s, when the first NWM species where analysed by cross-species chromosome painting using human probes [Consigliére et al., 1996; Richard et al., 1996; Sherlock et al., 1996], comparative chromosome maps between humans and almost 40 species of Platyrrhini from all 16 genera have been published (table 1). In addition, chromosome-specific painting probes from several NWM species were established by fluorescence activated chromosome sorting and DOP-PCR amplification, namely from *Saguinus oedipus* [Müller et al., 2001], *Lagothrix lagotricha* [Stanyon et al., 2001], *Aotus trivirgatus* [Stanyon et al., 2004], *Callicebus pallescens* [Dumas et al., 2005], *Callithrix argentata*, *Cebuella pygmaea*, *Callimico goeldii*, and *Saimiri sciureus* [Dumas et al., 2007]. These NWM painting probes were used in reciprocal or multidirectional painting experiments and provided important additional information about sub-chromosome homologies between human and NWM as well as between different NWM.

Human-NWM chromosome homology maps established by chromosome painting served as a starting point for various downstream analyses, which will be reviewed in detail below: (a) the ancestral NWM karyotype could be reconstructed, (b) derived chromosome characters unique to individual NWM species or, more importantly, to a particular subgroup of NWM species were identified, (c) the succession of chromosome rearrangements including the various phylogenetic lineages was reconstructed, and landmark rearrangements were identified, (d) phylogenetic inferences could be made using chromosome data as cladistic markers, and (e) detailed analyses were carried out aiming to elucidate the molecular mechanisms of large-scale genome organisation and evolution in NWM.

The Ancestral NWM Karyotype

When the cross-species chromosome painting data on the various NWM (table 1) are integrated, the ancestral NWM karyotype can be inferred. It comprises chromosome forms which were observed throughout NWM. According to several studies [Neusser et al., 2001; Stanyon et al., 2001; and for recent reviews Müller, 2006; Stanyon et al., 2008], the ancestral NWM karyotype had 2n = 54 chromosomes (fig. 1). Human chromosome 4, 6, 9, 11, 12, 13, 17, 19, 20, 22, X, and Y homologs are found entirely conserved as separate chromosomes. Chromosome 5, 14, 18, and 21 homologs show conserved synteny; however, they are in syntenic association (5/7, 14/15, 8/18, and 3/21). The remaining human homologs are fragmented: chromosome 1 (3 fragments), chromosome 2 (2 fragments), chromosome 3 (3 fragments), chromosome 7 (2 fragments), chromosome 8 (2 fragments), chromosome 10 (2 fragments), chromosome 15 (2 fragments), and chromosome 16 (2 fragments).

Table 1. Summary of all NWM species analysed by chromosome painting to date

Family	Genus	Species	Reference
Atelidae	*Alouatta*	*A. belzebul*	Consigliére et al., 1998
		A. sara	Consigliére et al., 1996
		A. seniculus arctoidea	Consigliére et al., 1996
		A. s. macconnelli	de Oliveira et al., 2002
		A. caraya	de Oliveira et al., 2002
		A. fusca	de Oliveira et al., 2002
		A. guariba guariba	Stanyon et al., 2011
	Ateles	*A. geoffroyi*	Morescalchi et al., 1997
		A. belzebuth hybridus	Garcia et al., 2002
		A. b.h. marginatus	de Oliveira et al., 2005
		A. paniscus	de Oliveira et al., 2005
	Brachyteles	*B. arachnoides*	de Oliveira et al., 2005
	Lagothrix	*L. lagothricha*	de Oliveira et al., 2005
Pitheciinae	*Callicebus*	*C. moloch*	Stanyon et al., 2000
		C. donacophilus	Barros et al., 2003
		C. lugens	Stanyon et al., 2003
		C. cupreus	Dumas et al., 2005
		C. pallescens	Dumas et al., 2005
		C. personatus	Rodrigues et al., 2011
	Chiropotes	*C. utahicki*	Stanyon et al., 2004
		C. israelita	Stanyon et al., 2004
	Pithecia	*P. irrorata*	Finotelo et al., 2010
	Cacajao	*C. calvus rubicundus*	Finotelo et al., 2010
Cebidae	*Callithrix*	*C. jacchus*	Neusser et al., 2001; Sherlock et al., 1996
		C. argentata	Neusser et al., 2001
	Cebuella	*C. pygmaea*	Neusser et al., 2001
	Callimico	*C. goeldii*	Neusser et al., 2001
	Saguinus	*S. oedipus*	Müller et al., 2001
	Leontopithecus	*L. chrysomelas*	Gerbault-Serreau et al., 2004
	Samiri	*S. sciureus*	Stanyon et al., 2000
	Cebus	*C. capucinus*	Richard et al., 1996
		C. apella	Garcia et al., 2000
		C. nigrivitatus	Garcia et al., 2002
		C. olivaceus	Amaral et al., 2008
		C. albifrons	Amaral et al., 2008
	Aotus	*A. nancymae*	Stanyon et al., 2004
		A. sp.	Ruiz-Herrera et al., 2005
		A. lemurinus griseimembra	Stanyon et al., 2011

NWM species classification follows Perelman et al. [2011].

By comparison of the NWM data set with the chromosome painting results available on other primate and non-primate mammals, the evolutionary origin of all chromosome forms present in the ancestral NWM karyotype can be readily reconstructed [Müller, 2006; Stanyon et al., 2008 for review]. NWM conserved human chromosome 4, 6, 9, 11, 13, 17, 20, X, and Y homologs were already found as separate entities in the primate ancestral karyotype as well as 2 chromosome 2 homologous segments and the larger of the 2 chromosome 7 segments. In addition, primate ancestral syntenic associations 14/15 and 3/21 were conserved, although the NWM homologs were further rearranged (see below). The human chromosome 10 and 16 homologs were still found conserved as each 2 independent units in the NWM ancestor, but the primate ancestral syntenic association 7/16 was lost, and both chromosome 16 units and the larger chromosome 10 unit were involved in further rearrangements (see below).

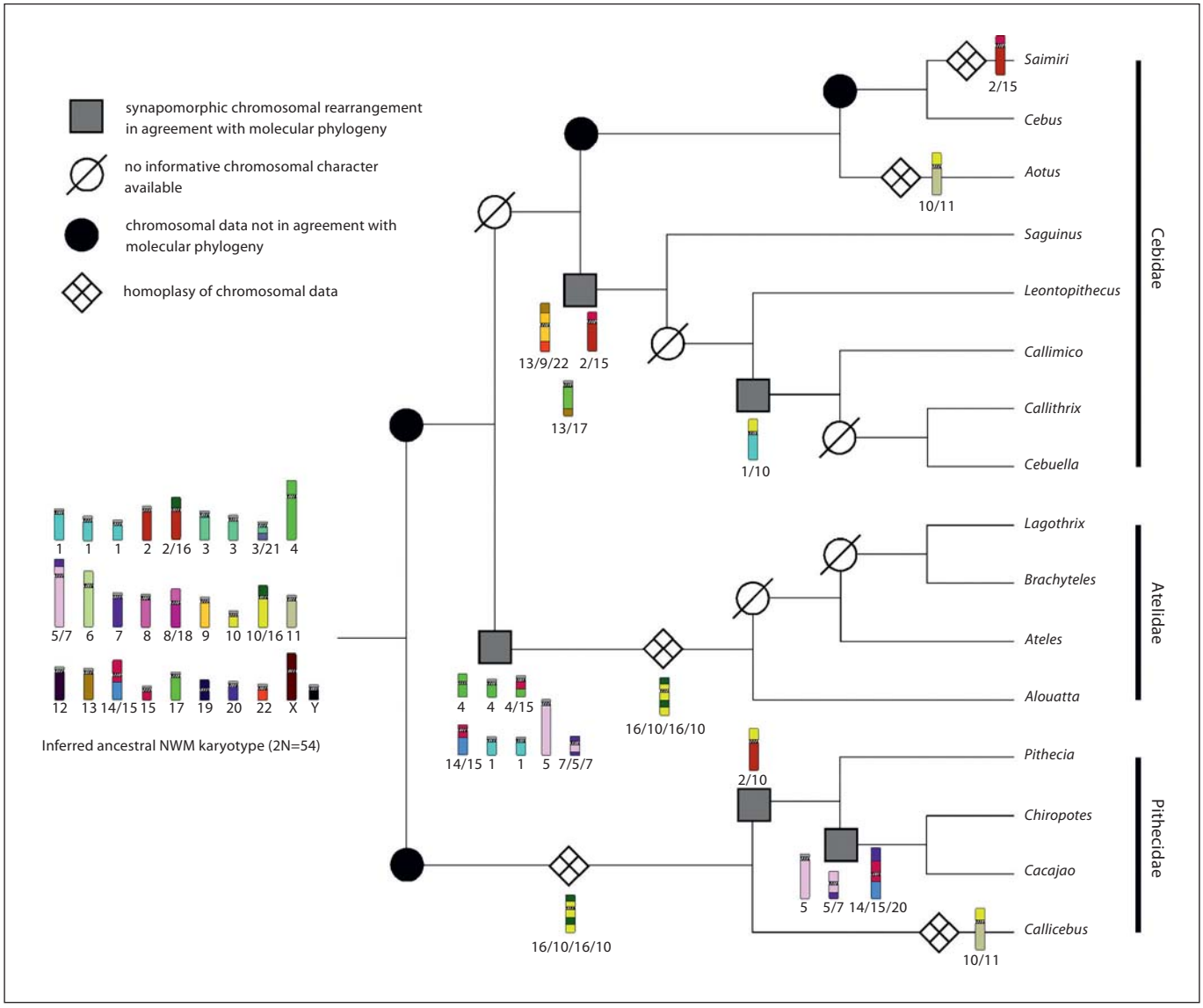

Fig. 1. The NWM ancestral karyotype and evolutionary landmark rearrangements superimposed on the molecular phylogenetic tree of Platyrrhini presented by Perelman et al. [2011] and Wildman et al. [2009]. Chromosomes are colour coded and numbered according to their homology with human chromosomes.

NWM conserved chromosome 12, 19 and 22 homologs are of more recent evolutionary origin and can be assigned to the simian ancestral karyotype. The remaining NWM ancestral chromosome forms represent exclusive NWM synapomorphies: fragmentation of the primate ancestral human chromosome 1 homolog by 2 fissions and of the 3/21 homolog by 2 fissions with breakpoints in the human chromosome 3 homolog, fission of the chromosome 8 homolog and subsequent fusion of the 8p segment with chromosome 18, fusion of the 2 chromosome 16 segments to form 2/16 and 10/16 homologs, respectively, fusion of the smaller chromosome 7 segment resulting in the 5/7 homolog, and fission of a small chromosome 15 homologous segment from the 14/15 homolog.

It is of note that the syntenic associations 5/7, 2/16, 10/16, and 8/18 were observed in most, but not in all NWM species. As far as the chromosome 5/7 is concerned, it is likely that for technical reasons the small chromosome 7 segment was missed in some evaluations in species from different families. In contrast, technical

reasons are unlikely the cause for the absence of the association 2/16 in all howler and owl monkeys and associations 10/16 and 8/18 in owl monkeys. It could be speculated that these characters mark a polyphyly of NWM; however, the monophyly of NWM was never questioned in previous studies using molecular, morphological or ecological data sets [Groves, 2001; Matsui and Hasegawa, 2012 for review]. Association 2/16 and 8/18 may rather have been lost secondarily, and the association 10/16 was probably obscured by a derived rearrangement resulting in 10/22/16 (see below for details).

Atelidae

The inclusion of *Alouatta, Ateles, Brachyteles*, and *Lagothrix* in a monophyletic clade is widely accepted in most phylogenetic proposals, and the chromosome data unequivocally support this classification. Atelidae exclusively share common derived fission of a chromosome 1 homologue, fission of the 5/7 homolog with a break in the chromosome 5 segment and followed by an inversion 7/5/7, an additional fission of the NWM ancestral 14/15 homolog with break in the 15 homologous segment and subsequent fusion 4/15, and fission of the chromosome 4 homolog. Further, a derived inversion of the chromosome form 10/16, resulting in 16/10/16/10 was observed in *Alouatta, Brachyteles* and *Lagothrix*, while in *Ateles* the presumably related chromosome form 16/10/16 was present. The ancestral Atelidae karyotype would therefore be comprised of 2n = 62 chromosomes, similar to the chromosome complement found conserved in extant *Lagothrix lagotricha* and *Brachyteles arachnoides* [Stanyon et al., 2001; de Oliveira et al., 2005] (table 2).

In *Ateles*, 17 tandem or Robertsonian type fusions and 3 fissions resulted in a dramatic reduction of the diploid chromosome number to 34 as found conserved in *Ateles b. marginatus* [Morescalchi et al., 1997; Garcia et al., 2002; de Oliveira et al., 2005]. A shared derived inversion 1/6/1 phylogenetically linked *A. p. paniscus, A. geoffroyi* and *A. b. hybridus*, while another synapomorphic inversion 16/2/16/2/1 defined *A. geoffroyi* and *A. b. hybridus* as sister taxa (table 2). This chromosome phylogeny would, however, require the reclassification of *A. b. marginatus* and is in disagreement with a previous molecular phylogeny based on mitochondrial sequence comparison by Collins and Dubach [2001].

In *Alouatta*, in addition to many autapomorphies found in different species because of extensive chromosome reshuffling, the molecular cytogenetic analyses could identify the fission of 2/16, association 3/15 and possibly a Y-autosomal translocation as chromosome signatures, confirming the monophyly of this clade [de Oliveira et al., 2002] (table 2). Associations 2/20, 5/7/5/7 and 4/16 define *A. belzebul* and *A. caraya* as sister clades, while *A. seniculus*, *A. sara*, *A. macconelli*, and *A. guariba* share chromosome form 2/4 [Consigliére et al., 1996, 1998; Stanyon et al., 2011]. Further, only *A. seniculus*, *A. macconelli* and *A. sara* showed synapomorphic chromosome forms 1/20 and 8/7/5/7, and another 6 derived chromosome rearrangements occurred in the last common ancestor of *A. seniculus* and *A. sara*.

Pitheciidae

From the clade of the seed predator monkeys *Pithecia, Chiropotes* and *Cacajao*, members from all 3 genera share a derived fusion resulting in chromosome form 2/10 [Stanyon et al., 2004; Finotelo et al., 2010]. In addition, FISH analyses favour the proximity of *Cacajao* and *Chiropotes* by fission of the chromosome 5 homologous segment of the NWM ancestral 5/7 homolog with a breakpoint distinctly different from that observed in Atelidae and by a fusion 20/15/14 (table 3).

In *Callicebus*, molecular cytogenetic analyses [Stanyon et al., 2000, 2003; Barros et al., 2003; Dumas et al., 2005; Rodrigues et al., 2011] confirmed the extensive intrageneric karyotype variability observed in earlier chromosome banding studies. Moreover, despite being the species with the lowest chromosome number among Platyrhhini, *Callicebus lugens* (2n = 16) showed the evolutionary conservation of 11 human chromosomes [Stanyon et al., 2003]. Syntenic associations 7/15, 16/2/16/2, 10/11, and 22/2/22 were found in all 6 species analysed so far and thus, represent ancestral *Callicebus* chromosome forms (table 3). Interestingly, syntenic associations 13/17 and 17/20 were present in 4 species of *Callicebus* as well as in Callithrichinae (see below). However, a more detailed analysis of the translocated segments in *Callicebus donacophilus*, using *S. oedipus* paints as subregional probes, indicates the involvement of different chromosome 13 homologous segments in the rearrangement in the 2 NWM species groups and hence, an independent evolutionary origin of the syntenic associations 13/9 and 13/17/20 in Callithrichinae compared to chromosome forms 13/17 and 17/20 in *Callicebus* [M. Neusser, unpublished results].

Table 2. Atelidae-derived chromosome forms observed in at least 2 species, excluding chromosome forms present in the ancestral NWM karyotype

Chromosome trait	*Ateles*				*Lag.*	*Bra.*	*Alouatta*					
	AHB	ABM	APP	AGE	LLA	BAR	ABE	ACA	AGG	ASA	ASE	AMA
1 fission	+	+	+	+	+	+	+	+	+	+	+	+
1/2/16	+	+	+	+								
1/2/16/2/16	+			+								
1/6/1	+	+	+	+								
1/6/1/6/1	+		+	+								
1/7/3	+	+	+	+								
1/20										+	+	+
2/3/15/22	+	+	+	+								
2/4									+	+	+	+
2/10	+	+	+	+								
2/16 fission							+	+	+	+	+	+
2/20							+	+				
3/15								+	+	+	+	+
6/3/21	+	+	+	+								
4 fission	+	+	+	+	+	+	+	+	+	+	+	+
4/15	+	+	+	+	+	+	+	+	+	+	+	+
4/16							+	+				
5 fission*	+	+	+	+	+	+	+	+	+	+	+	+
4/7/5/7	+	+	+	+			+	+				
5/7/5/7							+	+				
5/8	+	+	+	+						+	+	
5/11							+			+	+	
7/5/7	+	+	+	+	+	+	+	+	+	+	+	+
8/7/5/7										+	+	+
9/18/8	+	+	+	+								
10/19										+	+	
12/15/14/1/4/15	+	+	+	+								
15/16										+	+	
16/10/16	+	+	+	+								
16/10/16/10					+	+	+	+	+	+	+	+
19/20	+	+	+	+								
20/22										+	+	

* Fission in 5 with a breakpoint distinctly different compared to Pitheciidae, as revealed by *L. lagotricha* painting probes. ABH = *Ateles belzebuth hybridus*; ABM = *A. b. marginatus*; APP = *A. paniscus paniscus*; AGE = *A. geoffroyi*; LLA = *Lagothrix lagotricha*; BAR = *Brachyteles arachnoids*; ABE = *Alouatta belzebul*; ACA = *A. caraya*; AGG = *A. guariba guariba* (formerly also classified as *A. fusca*); ASA = *A. sara*; ASE = *A. seniculus arctoidea*; AMA = *A. macconelli*.

For references see table 1.

Cebidae

The putative ancestral NWM karyotype of 2n = 54 was found conserved in *Cebus capucinus* [Richard et al., 1996], from which the *Cebus apella* karyotype can be derived by an inversion 14/15/14 [García et al., 2000] (table 4). Each one species-specific fusion explains the reduction of diploid number to 52 in *C. albifrons* (12/15) and *C. olivaceus* (8/15/8), respectively [Amaral et al., 2008].

The chromosome phylogeny of the smallest NWM, the callithrichines, is resolved reasonably well at the genus level. All Callithrichinae studied so far share synapomorphic associations 2/15, 13/9, 13/22, and 13/17 [Sherlock et al., 1996; Stanyon et al., 2000; Müller et al., 2001; Neusser et al., 2001; Gerbault-Serreau et al., 2004] (table 4). Fusion

Table 3. Pitheciidae-derived chromosome forms observed in at least 2 species, excluding chromosome forms present in the ancestral NWM karyotype

Chromosome trait	*Chir.*	*Cacajao*		*Pith.*	*Callicebus*					
	CUT	CIS	CCA	PIR	CDO	CLU	CCU	CPA	CMO	CPE
2/10	+	+	+	+						
4 fission					+		+			
5 fission*	+	+	+							
7/5/7/5					+		+	+		
7/15					+	+	+	+	+	+
9/7/5/7/5					+		+			
10/11					+	+	+	+	+	+
12/19					+		+	+	+	+
13/17					+**		+	+		
16/2/16/2					+	+	+	+	+	+
16/10/16/10	+	+	+	+	+	+	+	+		+
17/20					+**	+	+	+		
20/15/14	+	+	+							
22/2/22					+	+	+	+	+	+

* Fission in 5 with a breakpoint distinctly different compared to Atelidae, as revealed by *L. lagotricha* painting probes. ** Association not observed by Barros et al. [2003], but clearly present in *C. donacophilus* [M. Neusser, unpublished results]. CUT = *Chiropotes utahicki*; CIS = *Cacajao israelita*; CCA = *C. calvus rubicundus*; PIR = *Pithecia irrorata*; CDO = *Callicebus donacophilus*; CLU = *C. lugens*; CCU = *C. cupreus*; CPA = *C. pallescens*; CMO = *C. moloch*; CPE = *C. personatus*.

For references see table 1.

1/10 is further shared by *Cebuella pygmaea*, *Callithrix argentata*, *C. jacchus*, and *Callimico goeldii* to the exclusion of *Leontopithecus chrysomelas* and *Saguinus oedipus*, placing *C. goeldii* next to *Callithrix* and *Cebuella*. This observation provides further evidence for the taxonomic and phylogenetic integration of *Callimico* within Callithrichinae [Neusser et al., 2001].

Importantly, the syntenic association 2/15 present in *Saimiri sciureus* demonstrated that *Saimiri* shares a synapomorphy otherwise found only in Callithrichinae, thus arguing for a closer relationship of *Saimiri* to this clade than to any other group of Platyrrhini [Stanyon et al. 2000; Neusser et al., 2001] (table 4).

Aotus, which shows a high karyological diversity at the species level, was found not to have retained the 2 NWM ancestral chromosomal associations 2/16 and 10/16. Both associations were absent in *A. lemurinus griseimembra, A. nancymae* and *Aotus* sp. [Stanyon et al., 2004, 2011; Ruiz-Herrera et al., 2005]. A closer look at the syntenic group 10/22/16 in *A. nancymae* observed by multidirectional chromosome painting, using human and *Lagothrix lagotricha* probes, indicates that the chromosome association may be the result of an insertion or of a fusion of the NWM ancestral chromosome 10/16 with 22 followed by an inversion. Concerning association 2/16, a fission followed by a fusion with a chromosome 1 homologous segment would explain the absence of this ancestral association in *Aotus*. When considering chromosome data to try to clarify the position of *Aotus* with respect to other Platyrrhini, all but one synapomorphy for *Aotus* found so far, namely the mentioned disruptions of 2/16 and 16/10, but also association 1/3, 1/16, 2/20, 4/15, 7/11, 16/22 and inversion 15/14/15/14 are autapomorphic for this genus (table 4). Importantly, as an exception to this rule, all *Aotus* and *Callicebus* species analysed until now exclusively share the derived association 10/11, which would also indicate a phylogenetic link between these 2 clades, but is in contrast to the recent trend to classify *Aotus* with the Cebidae (fig. 2).

NWM Phylogenetic Reconstructions and Chromosome Evolution

During the last decades, numerous studies have attempted phylogenetic reconstructions in NWM using a broad spectrum of different approaches, including mor-

Table 4. Cebidae-derived chromosome forms observed in at least 2 species, excluding chromosome forms present in the ancestral NWM karyotype

Chromosome trait	*Cebus*			*Callithrix*		*Callim.*	*Ceb.*	*Leon.*	*Sag.*	*Saim.*	*Aotus*		
	CAP	CCA	CNI	CJA	CAR	CGO	CPY	LCH	SOE	SSC	ALG	ANA	Asp.
1/3											+	+	+
1/16											+	+	+
1/10				+	+	+	+						
2/12												+	+
2/15				+	+	+	+	+	+	+			
2/16 fission											+	+	+
2/20											+	+	+
3/14												+	
4/15											+	+	+
5/15												+	+
7/11											+	+	+
9/15												+	+
9/22				+	+	+	+	+	+				
10/11											+	+	+
10/16 fission											+	+	+
10/22												+	+
13/9				+	+	+	+	+	+				
13/9/22				+	+		+	+	+				
13/17				+	+	+	+	+	+				
13/17/20				+	+		+	+	+				
14/15/14	+	+	+										
16/22											+	+	+

CAP = *Cebus apella*; CCA = *C. capucinus*; CNI = *C. nigrivitatus*; CJA = *Callithrix jacchus*; CAR = *C. argentata*; CGO = *Callimico goeldii*; CPY = *Cebuella pygmaea*; LCH = *Leontopithecus chrysomelas*; SOE = *Saguinus oedipus*; SSC = *Saimiri sciureus*; ALG = *Aotus lemurinus griseimembra*; ANA = *A. nancymae*; Asp = *Aotus* sp.

For references see table 1.

phology, biogeography, behaviour, molecular genetics, and cytogenetics. It is beyond the scope of this review to discuss the strengths or limitations of approaches other than comparative cytogenetics. Instead, we will first attempt to integrate the molecular cytogenetic data summarized above in the context of recent trends towards a 'consolidated branching order' of NWM [Osterholz et al., 2009] based on molecular genetic data sets. Next, we will propose an alternative NWM phylogenetic tree using chromosome data alone, and finally, we make an attempt to reconcile both approaches. In doing so, we will try to weigh the value of the NWM chromosome landmark rearrangements described above as markers for phylogenetic reconstructions.

Most recent molecular phylogenetic reconstructions agree on the classification of NWM in 3 families and also show a clear tendency towards a consensus branching sequence at the level of individual genera [Matsui and Hasegawa, 2012 for recent review]. We will first superimpose the chromosome data onto the tree proposed by Perelman et al. [2011] and Wildman et al. [2009] based on the comparative analysis of nuclear sequence data (fig. 1).

Both the molecular and the cross-species chromosome painting data with 7 derived chromosome forms unequivocally agree on the monophyly of Atelidae. At present, no molecular cytogenetic evidence is available to support the internal Atelidae branching sequence (fig. 2) because *Brachyteles* and *Lagothrix* have conserved the ancestral Atelidae karyotype. This polytomy could be resolved when also considering comparative G-banding data, which would suggest a common derived inversion of the human chromosome 8 homolog in all Atelidae, except for *Alouatta*, and inversion of the chromosome 13 homolog in support of a *Lagothrix*/*Ateles* clade [de Oliveira et al., 2005]. Here, high-resolution analysis of the marker order on Atelidae chromosome 8 and 13 homologs by FISH, using locus-specific probes (e.g. BAC probes) would be required to verify the G-banding data. Even then the resulting branching sequence {*Brachyteles*{*Lagothrix-Ateles*} would not be in agreement with the molecular phylogeny presented in figure 1. In these molecular phylogenies,

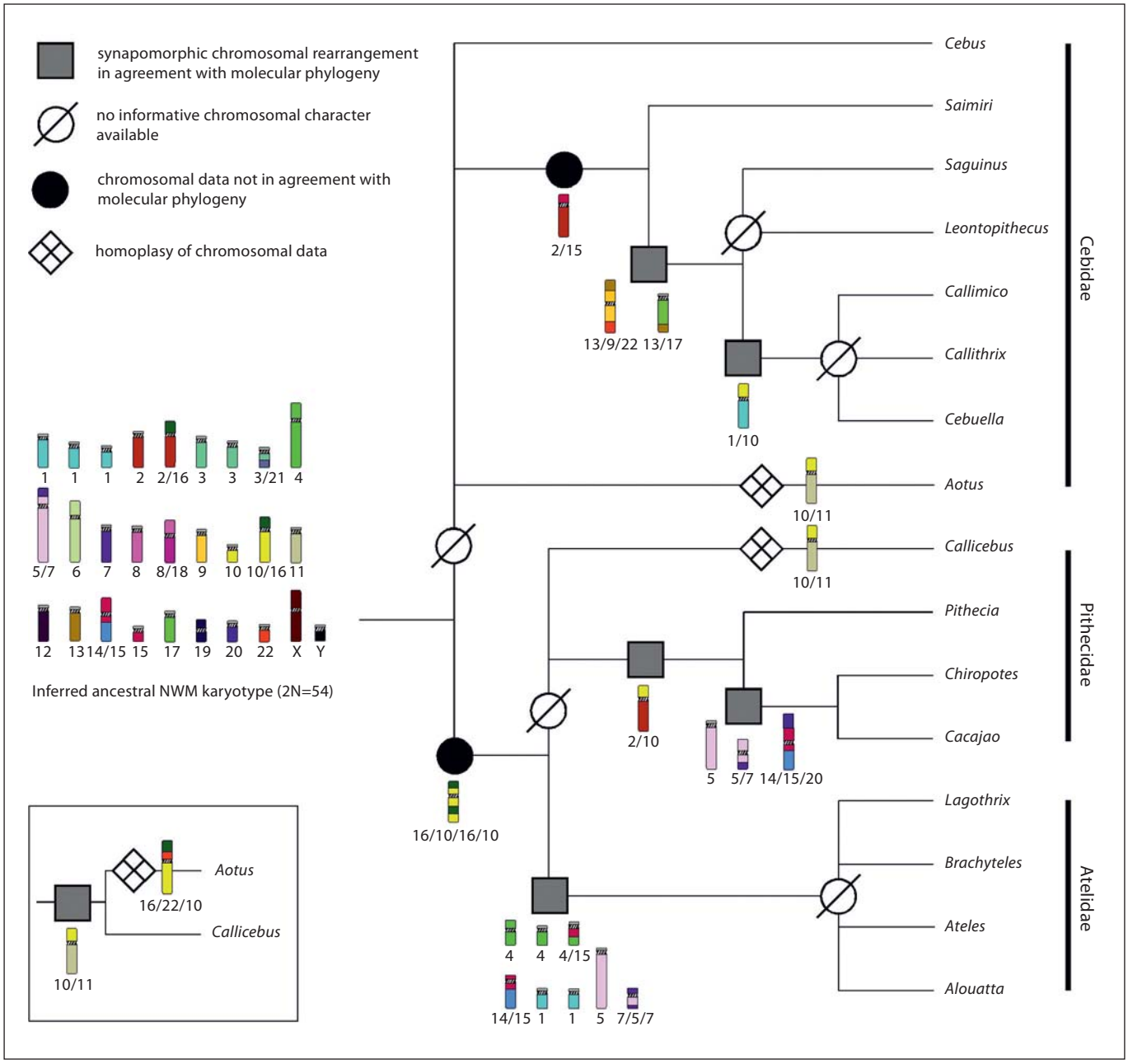

Fig. 2. Chromosomal phylogeny of NWM and evolutionary landmark rearrangements (inset: alternative phylogeny assuming that syntenic association 10/11 represents a phylogenetic link between *Aotus* and *Callicebus*, see text for details). Chromosomes are colour coded and numbered according to their homology with human chromosomes.

however, low bootstrap values also provided no unequivocal support for a *Lagothrix-Ateles* clade [Wildman et al., 2009; Perelman et al., 2011].

In Pithecidae, the DNA sequence and the molecular cytogenetic trees are in agreement when only considering the genera *Pithecia, Chiropotes* and *Cacajao*. Both trees agree on their monophyly, and also each one chromosome landmark rearrangement supports the branching sequence {*Pithecia*{*Chiropotes*/*Cacajao*} [Finotelo et al., 2010]. In contrast, no molecular cytogenetic evidence is

presently available in support of *Callicebus* as the basal Pitheciidae clade (fig. 1). None of the 4 shared derived chromosome forms allocated to the inferred ancestral *Callicebus* karyotype, nor any other derived chromosome rearrangement observed in one of the 6 *Callicebus* species so far was found to be shared with other Pitheciidae [Dumas et al., 2005; Finotelo et al., 2010]. Furthermore, the presence of a unique shared derived chromosome association 10/11 found in all *Callicebus* and *Aotus* species so far would argue for a different tree topology with *Callicebus* and *Aotus* as sister clades without any chromosome affinity to other NWM species (fig. 2). Assuming that the DNA sequence tree reflects the correct phylogeny – which may well be the case since the placement of *Callicebus* as a basal Pitheciidae is well supported in most molecular analyses [Matsui and Hasegawa, 2012], and only few studies using morphological characters favoured a *Callicebus*/*Aotus* clade [Rosenberger, 1981; Ford, 1986] – chromosome form 10/11 would be the product of a convergent fusion (fig. 1). In any case, a detailed FISH analysis of the associated segments 10/11 using locus-specific probes would be required to determine whether the associated syntenic segments in *Callicebus* and *Aotus* are truly homologous or not (preliminary, unpublished results indicate they are indeed homologous, M. Neusser).

The phylogenetic relationships among the 3 Cebidae subgroups Aotinae, Cebinae and Callithrichinae are much less well understood compared to Pithecidae. Although the placement of *Aotus* with Cebidae has been confirmed in most recent molecular studies, its position with respect to the other 2 subfamilies received no or only particularly low support in recent SINE insertion and nuclear sequence analyses, respectively [Osterholz et al., 2009; Wildman et al., 2009; Perelman et al., 2011]. Molecular cytogenetic data offer no deeper insight; since so far, *Aotus* was not found to share any synapomorphic traits with other Cebidae, and the paucity of evolutionary chromosome changes in genus *Cebus* hampered its phylogenetic classification with respect to other Cebidae. For the same reason, the classification of *Cebus* and *Saimiri* as sister clades could not be confirmed (fig. 1). Instead, the shared derived association 2/15 placed *Saimiri* in a basal position of a clade together with marmosets and tamarins [Stanyon et al., 2000; Neusser et al., 2001]. Hence, this chromosome trait is representing another discrepancy with generally accepted molecular phylogenies where *Cebus* and *Saimiri* are sharing a last common ancestor (fig. 1). Again, under the assumption that the present NWM molecular phylogenies are correct, 2 alternative scenarios may explain the cytogenetic findings: association 2/15 is an ancestral Cebidae chromosome form which was lost again in *Cebus* (and possibly also in *Aotus* when accepting the phylogeny presented in fig. 1) or is a convergent fusion which occurred independently in *Saimiri* and in the *Callithrichinae* ancestor. Since this association is a potentially important cladistic marker, high-resolution FISH analysis with fusion point flanking BAC probes in *Cebus* and *Aotus* may be indicated to clarify if a cryptic association 2/15 below the resolution of chromosome painting was retained in these 2 species.

At least 3 derived chromosome rearrangements highlight the monophyly of *Callithrichinae*. One additional derived chromosomal fusion places *Callimico* next to *Callithrix* and *Cebuella* [Neusser et al., 2001], in agreement with recent molecular phylogenies (fig. 1, 2).

Finally, when considering the relationship between the 3 NWM families, molecular approaches favour the branching sequence Pitheciidae{Atelidae-Cebidae} with low–moderate support by sequence analysis [Wildman et al., 2009; Perelman et al., 2011] and by a substantially high number of SINE integrations [Ray and Batzer, 2005; Osterholz et al., 2009], whereas solid molecular cytogenetic data are not available to date. All but one shared derived chromosome rearrangements detected in NWM so far can be either assigned to the NWM common ancestor or are at least confined to the ancestor of 1 of the 3 families. The sole exception is a derived inversion of the chromosome form 10/16, resulting in 16/10/16/10, which was found in all Pitheciidae including 5/6 species of *Callicebus* and in all Atelidae, except for genus *Ateles*. It can be speculated that the chromosome form 16/10/16 present in all *Ateles* species analysed until now is in fact a derivative of the ancestral segment association 16/10/16/10 by translocation of the terminal chromosome 10 homologous segment. Under these presumptions, chromosome form 16/10/16/10 would represent a phylogenetic link between Atelidae and Pitheciidae, leading to a NWM branching sequence Cebidae{Atelidae-Pitheciidae}, as proposed in the molecular phylogeny by Schneider [2000] and Schneider et al. [2001]. From the present cross-species chromosome painting and from G-banding data it is, however, difficult to determine if the inversion breakpoints in all clades are identical and, by consequence, whether chromosome form 16/10/16/10 is truly homologous. In order to substantiate this finding, detailed comparative chromosome maps established by BAC-FISH would be required. If confirmed, this landmark rearrangement would contradict the presumed synapomorphic association 10/11 shared by *Aotus* and *Callicebus* and consequently the phylogenetic link between the 2 species [Dumas et al., 2005] (fig. 2).

The Subregional Organisation of NWM Chromosomes

Although cross-species chromosome painting using human probes to delineate interspecies chromosomal homologies provides a comprehensive overview of syntenic segments, the subregional organisation of these segments and also the precise localisation of breakpoints cannot be resolved, and most intrachromosomal rearrangements escape detection.

Reverse painting of NWM chromosome-specific probes to human chromosomes was used to map the breakpoints involved in NWM specific chromosome rearrangements with reference to human chromosomes. According to these studies, the ancestral NWM karyotype of 2n = 54 chromosomes would comprise of the 28 syntenic units 1p, 1q32–qter, 1q21–q31, 2q13–qter, 2pter–q13/16q, 3p12–q12/21, 3p24–pter/3p12–p14/3q12–q21/3q27–qter, 3p21–p24/3q21–q26, 4, 5/7p22/7q11.2/7q22, 6, 7p11–21/7q11.2–q21/7q22–qter, 8p/18, 8q, 9, 10q/16p, 10p, 11, 12, 13, 14/15q14–24, 15q11–q13/15q25–qter, 17, 19, 20, 22, X and Y [Neusser et al., 2001; Stanyon et al., 2001; Dumas et al., 2007]. Of note, individual NWM ancestral chromosomes showing multiple syntenic segments for chromosomes 3, 7 or 15 homologs in (e.g. 3p21–p24/3q21–q26) resulted at least in part from derived intrachromosomal rearrangements which occurred in the higher Old World primate/human lineage [Müller et al., 2004; Ventura et al., 2004; Stanyon et al., 2008, see below for details]. The ancestral Atelidae karyotype of 2n = 62 chromosomes resulted by additional synteny breaks in human homologous regions 1p36, 4q24, 4q31, 5q31, and 15q21 [Stanyon et al., 2001].

High-resolution comparative maps between human and NWM chromosomes could be established by cross-species FISH using BAC probes which were sequenced during the course of the human genome project and are anchored in the human reference sequence [Stanyon et al., 2008 for review]. These studies delineated the marker order of human homologs in index species from all major primate clades, including at least 1 species from NWM, in some cases even species from each of the tree NWM families (see table 1 in Stanyon et al. [2008] for a comprehensive reference list). For example, the order of 23 BAC-FISH markers along chromosome 1 homologs was found conserved between human and the inferred ancestral primate, from which the 4 *Lagothrix lagotricha* homologs originated by 3 fissions with breakpoints at approximately 84–97 Mb, 1 centromere and 186–195 Mb of the human reference sequence (UCSC build May 2004), respectively. Strikingly, when comparing the marker order present on chromosomes in the inferred ancestral primate and in the ancestral NWM karyotype, almost all human homologs show completely conserved subregional organisation at a resolution defined by the genomic distance each BAC pair used in the respective studies [Stanyon et al., 2008 and references therein]. Only the human chromosome 3 and 9 homologs showed a large NWM-specific inversion and 2 nested inversions, respectively [Montefalcone et al., 1999; Ventura et al., 2004]. From these studies, it can be concluded that the vast majority of NWM shared derived chromosome forms are the product of the above-mentioned interchromosomal rearrangements and that inversions were rather rare.

Patterns of Large-Scale Genome Rearrangement in NWM

In the 38 NWM species analysed by cross-species chromosome painting to date (table 1), a total of 182 chromosome rearrangements were recorded after correction for changes that occurred in the inferred ancestral NWM or in the inferred ancestor of the various NWM phylogenetic lineages. Of these, 129 changes account for fusions (71%), 39 for fissions (21%) and 13 for inversions (7%). Hence, Robertsonian type fusions, centromere-telomere and tandem fusions are the predominant mechanism of evolutionary change in NWM. Only 2 reciprocal translocations were observed (1%), indicating that reciprocal exchanges occur at exceptionally low rates in Platyrrhini or, more likely, that reciprocal translocations have a very low chance to be fixed in any population of NWM.

The number of rearrangements between the NWM ancestor and extant species, however, varies greatly between zero in *Cebus capucinus* [Richard et al., 1996] and over 20 in *Callicebus lugens* [Stanyon et al., 2003]. Equally, the direction of changes does not appear to follow a continuous trend in any of the NWM clades. For example, in the ancestor of Atelidae, a series of fissions occurred, increasing the chromosome number from 54 to 62. Since then, the karyotypes in *Brachyteles* and in *Lagothrix* remained essentially conserved, whereas in *Ateles* a dramatic reduction to 2n = 32 chromosomes took place.

From the genomics perspective, but also to determine the robustness of chromosome rearrangements as phylogenomic markers, it is of interest to estimate the probability that identical chromosome rearrangements are recurrent. Taking into account the evolutionary changes resulting in the 13 derived chromosome forms present in

the NWM ancestor and in the 57 different chromosome forms summarized in tables 2, 3 and 4, and assuming further that the molecular phylogeny illustrated in figure 1 is correct, over 90% of these chromosome changes were unique evolutionary events. Only a maximum of 6/57 rearrangements (9%) may represent recurrent and therefore convergent gains: associations 5/8 in *Alouatta/Ateles*, 10/11 in *Aotus/Callicebus*, 10/16/10/16 in Atelidae/Pitheciidae, 2/20 in *Aotus/Alouatta*, 2/15 in *Saimiri/Callithrix*, and 2/10 in *Ateles/Chiropotes*. Not surprisingly, the break or fusion points in 5 of these rearrangements are located in centromeric regions.

Strikingly, besides the conventional structural changes mentioned above, the detailed FISH analysis of the marker order in NWM revealed the emergence of evolutionary neocentromeres (ENC) in 20 NWM cases so far [Rocchi et al., 2012 for recent review]. The majority of ENC appeared during the process of non-centromeric fission, while some represent 'classical' ENC, including the X chromosome centromere of squirrel monkeys *(Saimiri sciureus)* [Rocchi et al., 2012]. The latter ENC emerged by a yet poorly understood process of inactivation and degeneration of the ancestral centromere and simultaneous epigenetic seeding of the new centromere in a different location of the same chromosome. In addition, several instances of telomere conversion into centromeres were recorded, for example, on a chromosome 3 homolog in *Callicebus moloch* [Ventura et al., 2004] and on a chromosome 1 homolog in *Callithrix jacchus* [Neusser et al., 2001]. Further, amplification and rapid sequence divergence of pericentromeric, interstitial and subtelomeric heterochromatin appears to be commonplace also in New World primates. As determined by interspecies comparative genomic hybridization and FISH using a microdissected DNA probe, even in the close related marmosets *Callithrix argentata* and *Cebuella pygmaea*, rapid and species-specific amplification of repetitive sequences could be observed when compared to the *Callithrix jacchus* genome [Neusser et al., 2005]. The same observation was made when comparing the genomes of *Cebus libidinosus* with other species of NWM [Fantini et al., 2011; Nieves et al., 2011], where the amplification of *Cebus*-specific repeat sequences in *C. libidinosus* accounted for an increase in genome size of around 10%.

Conclusions and Perspectives

During the last decades, NWM comparative classical and, in particular, molecular cytogenetics have highlighted many fundamental aspects of genome organisation and evolution in this fascinating, but also highly endangered group of primates. In summary, NWM; most probably represent the mammalian clade with the most comprehensive molecular cytogenetic data set available at present. These studies have impact in various fields including comparative genomics, taxonomy and phylogeography but also in species conservation and management. This work aspired to gain fundamental insight into the evolutionary principles, which shaped the genomes of extant primates and to solve the jigsaw puzzle of Platyrrhini chromosome reshuffling, but also by the expectation that sequencing the entire genomes of all 120 and more NWM species would not be feasible in the foreseeable future. However, in recent years, with novel time- and cost-effective parallel sequencing techniques emerging, the situation has changed dramatically. It can be envisioned that at least low-coverage whole genome sequence of 1 species per NWM genus will become publicly available within the next few years. This data set will help to resolve the remaining open questions concerning NWM phylogeny and will also complement or even supersede the cytogenetic data available today because it will resolve syntenic segments and structural rearrangement breakpoints at the ultimate level of resolution.

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