

SPECTRAL KARYOTYPING (SKY) ESTABLISHES CHROMOSOMAL HOMOLOGIES BETWEEN A NEW WORLD MONKEY (PITHECIA PITHECIA) AND HUMANS (HOMO SAPIENS)

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ABSTRACT

Inspired by the already established chromosome homology between certain New World Monkeys and humans (Stanyon, et al., 2004), this cytogenetic study was designed to produce a homology map comparing the chromosomal arrangements of the *Pithecia pithecia* (White-faced Saki) and human karyotypes. After metaphase harvest and G-banding, a *P. pithecia* karyotype was constructed. Fluorescence in situ hybridization was then performed on a set of *P. pithecia* metaphase chromosomes using a Human Spectral Karyotype Reagent kit. Spectral hybridization allowed for the production of a *P. pithecia* karyotype labeled with a mosaic of colors representing the twenty-four human chromosomes. The extent of homology found is striking. Ten probes hybridized specifically with single sites on *P. pithecia* chromosomes, while the other fourteen human probes hybridized either with multiple *P. pithecia* chromosomes or in tandem arrangements along a single primate chromosome. The homology map illustrates the significant chromosomal conservation that links these two evolutionarily distant primates.

INTRODUCTION

The disruption of linkage groups by chromosomal rearrangements can present barriers to the interbreeding of two populations and facilitate subsequent speciation. These chromosomal changes can be demonstrated through cross-species painting (Ferguson-Smith et al., 2005). Homologies have been established between humans and many Old and New World monkeys and even some prosimians. Primate species that diverged approximately fifty million years ago show homologies of entire chromosomes.

The present investigation focuses on the *Pithecia pithecia* (White-faced Saki), a New World Monkey (infraorder Platyrrhini) of the family Cebidae and subfamily Pitheciinae. New World Monkeys are estimated to have diverged from the ancestral primate almost forty million years ago, and about thirty-five million years before the divergence of hominids.

Cross-species painting is most often accomplished by single chromosome hybridizations. However, some studies using spectral karyotyping with multiple probes produce results comparable to the more labor-intensive single chromosome paints (Best et al., 1998; Rens et al., 2001). The ASI Spectral View Imaging System produces human karyotypes in which each chromosome is painted a distinctive color. The process of spectral karyotyping (SKY) involves the hybridization of a set of whole chromosome painting probes, each labeled with a distinctive subset of one to four dyes from a set of five fluorochromes. This creates twenty-four distinct fluorochromosomes that serve as probes. Hybridization of each fluorochromosome is determined by the spectra detected at each pixel in an image field using an interferometer (Schrock et al., 1996). Spectral karyotypes of metaphase chromosomes from primates cross-painted with the ASI Human Spectral Karyotype Reagent Kit provide maps of homologies to human chromosomes in a single hybridization. A homology map, or color-coded diagram showing the chromosomal organization of the primate karyotype with respect to human chromosomes, is the major illustrative method of reporting cross-species homologies. Such homology maps have been published for representative species of the other Pitheciinae genera, but not *Pithecia* (Stanyon et al., 2004).

After successful spectral hybridization, the resulting homology map showed extensive chromosomal conservation between the *Pithecia pithecia* and human karyotype. Both conserved and derived chromosomes were traced back to their initial states in the karyotypes of common ancestors from the major primate groupings, in order to contrast the evolution of the *P. pithecia* and human karyotypes. The mapping results were then compared to a similar cross-species study by Stanyon, et al. (2004) in order to illustrate the common chromosomal links between the *Chiropotes* and *Pithecia* genera of Pitheciinae.

MATERIALS AND METHODS

Acquisition of cell line

A *Pithecia pithecia* fibroblast culture PR00239 was obtained from the Intergrated Primate Biomaterials and Informatics Resource (IPBIR).

Tissue culture

The culture was transformed, maintained, and subcultured by the fibroblast tissue culture laboratory staff at Coriell Institute for Medical Research.

Metaphase cell harvest

When the cells appeared to be mitotic (round) under the inverted microscope, the culture was prepared for metaphase chromosome analysis by standard techniques. First, the culture was treated with colcemid to arrest cells in metaphase by disrupting spindle fiber assembly (mitotic arresting). The resulting monolayer of cells was detached from the flask by brief treatment with warm (37° C) Puck's Versene-trypsin (0.02% EDTA, 0.041% trypsin) and this suspension was centrifuged for 8 minutes, supernatant poured off, and the remaining pellet resuspended in the small amount of residual fluid. 10 ml of hypotonic solution (2:1 sodium chloride:sodium citrate) was added to increase cell volume and allow for expansion of the mitotic chromosomes, and this suspension incubated at 37° C for 15 minutes. Next, prefixation was achieved by adding a pipette of cold fixative (3:1 methanol:acetic acid) to the tube, mixing gently by inversion, and centrifuging for 8 minutes. The resulting pellet was resuspended in the residual fluid, and 10 ml of the cold fixative added slowly with continued agitation. Fixation was allowed to proceed at 4° C for 15 minutes. After the first fixation, the tube was centrifuged for 8 minutes, supernatant poured off, and the pellet resuspended in 10 ml of the cold fixative. This fixation process was repeated two more times to ensure that the metaphase chromosomes were completely preserved, membranes and chromatin hardened, and all cytoplasm removed by dehydration. Fixed pellets could be stored at 4° C or used immediately for chromosome analysis.

Slide preparation

Slides were made in a Thermotron set to approximately 43% humidity to prevent overspreading. A wet, sterile slide was held at a 45° angle and 3 drops of the fixed pellet dropped across the upper edge of the transparent region, allowing the cell dilution to spread uniformly down the slide. Once dry, slides were examined under a phase-contrast microscope setting to check for chromosome spreading and mitotic index (abundance of metaphase cells) to insure viability for staining.

G-banding

To prepare metaphase chromosomes for staining, the slide was first aged on a slide warmer at 55° C overnight, then heated in the oven at 90° C for 1 hour before G-banding. After the one-hour cooling period, standard G-banding procedures involving trypsin treatment followed by Wright's staining was performed. Following air-drying, slides were examined under the microscope to check for sharpness of banding.

Karyotyping

Using the microscope and computer imaging system, metaphase cell images with the best chromosome spreading, fewest overlaps, and sharpest banding were captured and analyzed. Homologous chromosome pairs from the metaphase image were arranged into a karyotype (by cutting and pasting) based on size, centromere position, and banding patterns.

Spectral Karyotyping (Fluorescence in situ hybridization)

In order to denature outer chromosomal proteins and expose DNA, the slide was treated with pepsin, covered with a glass coverslip, and observed under phase contrast microscopy to note any remaining cytoplasm, debris, etc. If sufficient, the slide was then washed, incubated in 1% formaldehyde, and dehydrated in 70%, 80%, then 100% ethanol at room temperature for 2 minutes each.

In order to denature the chromosomes before probe application, the slide was air-dried and then incubated in denaturation solution (35 ml formamide, 10 ml distilled H₂O, 5 ml 20x SSC, pH 7) at 70° C for about 1 minute. The slide was quickly removed dehydrated and air-dried.

Simultaneously, 10 ul of the Human Spectral Karyotyping probe was denatured by incubation in a water bath at 80° C for 7 minutes. It was imperative to protect the probe from direct light and store at -20° C until ready for use. Once the slide was dry, the denatured probe was applied within score marks on the slide, covered with a precleaned coverslip, and protected by sealing the edges with rubber cement. The slide was then transferred to a hybridization chamber and incubated at 37° C for 36 hours. Under these conditions, denatured (single-stranded) human DNA of the fluorescently-labeled probe could interact and anneal with complementary regions of the denatured primate chromosomal DNA fixed to the slide. These hybridizations could then be observed and interpreted through the ASI Spectral Imaging System.

Spectral imaging

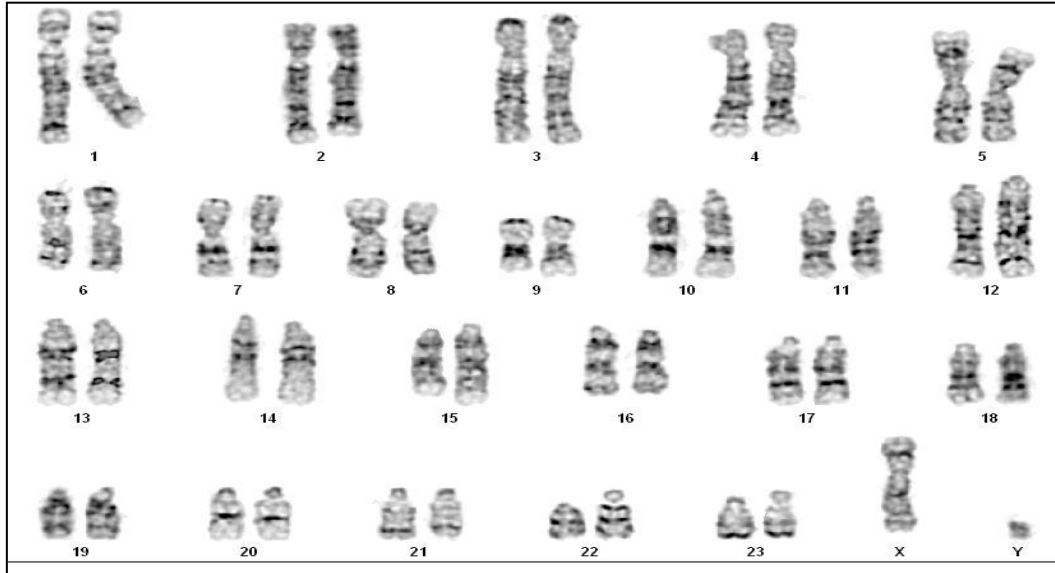
Each of the human chromosome probes of the Spectral Karyotyping Reagent was combinatorially labeled with a distinctive subset of one to four dyes from a set of five fluorochromes. Hybridization of each fluorochromosome was determined by the spectra detected at each pixel in an image field using an interferometer (Shrock, et al., 1996) in the ASI Spectral View Imaging System. This system allowed for the viewing and capture of metaphase chromosome hybridization images that were then arranged into a color-coded *P. pithecia* karyotype, or homology map, showing the organization of *P. pithecia* chromosomes with respect to human chromosomes.

RESULTS

Karyotype of *Pithecia pithecia*

A G-band karyotype of PR00239 was prepared (Figure 1) and compared to the karyotype for *P. pithecia* reported by Henderson et al. (1977), confirming that PR00239 was in fact a male *P. pithecia* (48, XY).

Figure 1. A male *Pithecia pithecia* G-band karyotype prepared by trypsinization and banding with Wright's stain.

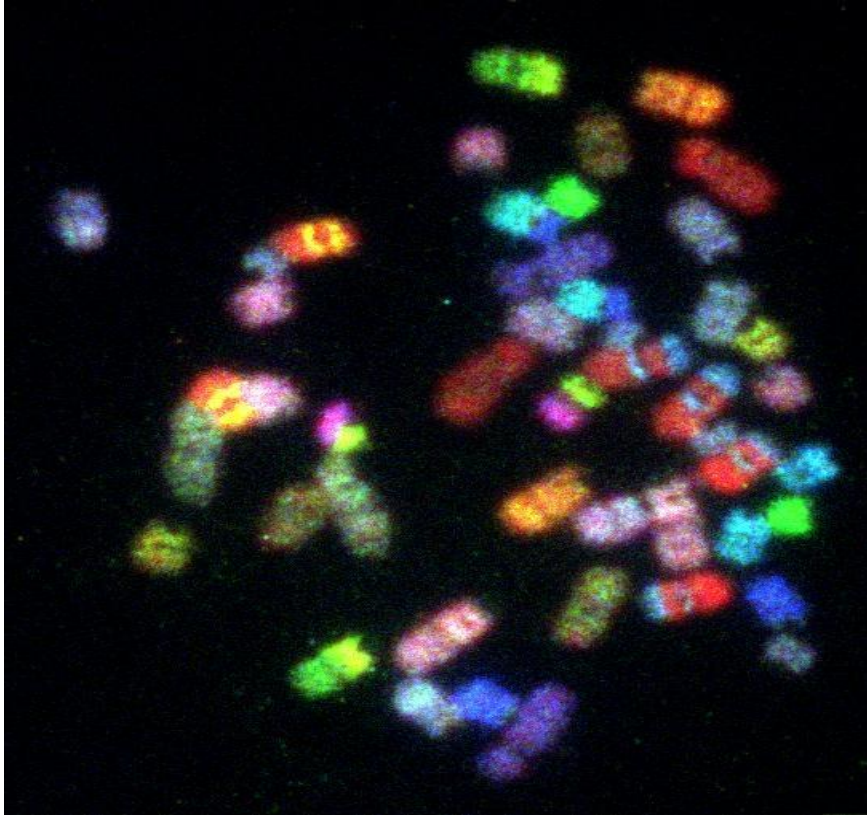


The diploid number ($2n$) is 48, with nine metacentric autosomal pairs and fourteen acrocentric autosomal pairs. The X is metacentric and similar in size and band pattern to most of the higher primates. The Y is acrocentric, quite small and non-descript, also comparable to other primates.

Hybridization of human probes to *Pithecia pithecia* chromosomes

In Figure 2, the image of a full set of *P. pithecia* metaphase chromosomes hybridized with the twenty-four human chromosome probes of the Human Spectral Karyotyping Reagent Kit shows the spectral colors displayed.

Figure 2. Hybridized *P. pithecia* metaphase chromosomes in spectral colors.



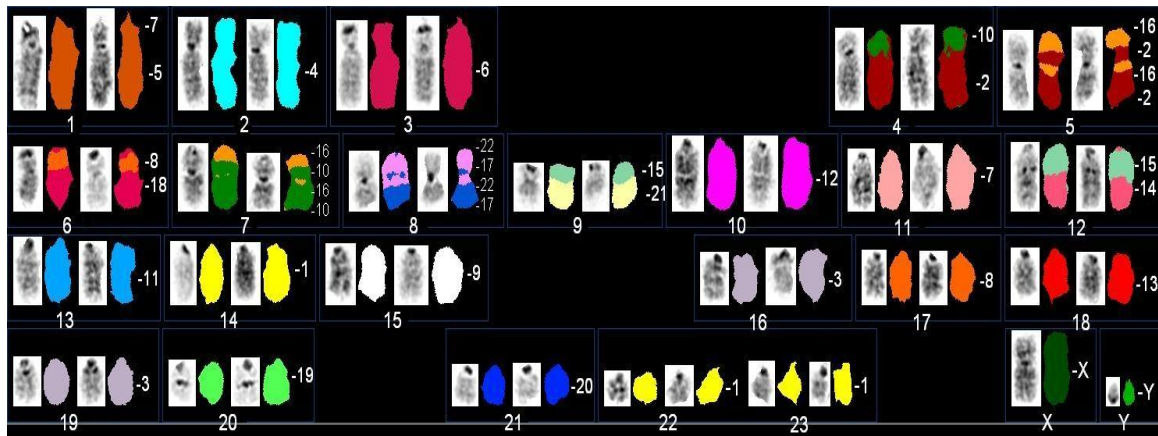
The primary value of this image is to estimate the quality of hybridization achieved. Although spectrally distinct, some of the dyes and dye combinations are not discernable microscopically. To enable viewing, each pixel in the image field is assigned a classified color based on the spectra detected. Figure 3 shows the combinatorial table used by the interferometer to convert spectral to classified colors.

Figure 3. Combinatorial table used to determine hybridization of each fluorochromosome and convert spectral to classified colors.

Ch	G	O	R	N	N	
1	x		x	x		Yellow
2					x	Red
3	x	x		x	x	Grey
4	x			x		Cyan
5	x	x	x		x	Red
6	x		x	x	x	Red
7			x	x		Pink
8	x					Yellow
9	x	x			x	White
1				x	x	Green
1	x	x		x		Blue
1			x		x	Magenta
1	x	x				Red
1			x			Pink
1		x	x	x		Cyan
1	x		x			Orange
1				x		Purple
1	x	x	x			Magenta
1		x		x		Green
2		x				Blue
2	x				x	Yellow
2		x	x	x	x	Pink
X		x			x	Green
Y	x			x	x	Green
	D	A	B	C	E	

The spectral karyotype (homology map) produced after conversion to classified colors is displayed in Figure 4. The black and white reverse-DAPI images, which are comparable to G-banded chromosomes, allow for arrangement into a karyotype.

Figure 4. Homology map showing the hybridization pattern of human chromosome probes within the *Pithecia pithecia* karyotype. Each color corresponds to a single human chromosome whose number is listed next to each color-coded segment. Black and white images show *P. pithecia* chromosomes in reverse-DAPI banding.



Probes for human autosomes 4, 6, 9, 11, 12, 13, 19 and 20 were found to hybridize each with a single *P. pithecia* chromosome pair (Table 1).

Table 1. Ten human chromosome probes hybridized completely and exclusively with a single *P. pithecia* metaphase chromosome.

Human chromosome probe	<i>P. pithecia</i> chromosome painted
4	2
6	3
9	15
11	13
12	10
13	18
19	20
20	21
X	X
Y	Y

By contrast, human chromosomes 1, 2, 3, 7, 8, 10, 15 and 16 were found to hybridize with multiple *P. pithecia* chromosomes (Table 2).

Table 2. Eight human chromosome probes hybridized with multiple *P. pithecia* chromosomes.

Human chromosome probe	<i>P. pithecia</i> chromosome painted
1	14, 22, 23
2	4, 5
3	16, 19
7	1, 11
8	6, 17
10	4, 7
15	9, 12
16	5, 7

Furthermore, three particular *P. pithecia* chromosomes were hybridized by human probes in four-segment alternating syntenic associations : PPI 5, PPI 7, PPI 8. Five other *P. pithecia* chromosomes were hybridized by two human probes. but in two-segment tandem associations: PPI 1, PPI 4, PPI 6, PPI 9, and PPI 12 (Table 3).

Table 3. Eight *P. pithecia* chromosomes were hybridized in two- or four-segment combinations of two human chromosome probes.

Human chromosome probe(s)	<i>P. pithecia</i> chromosome painted
7/5	1
10/2	4
16/2/16/2	5
8/18	6
16/10/16/10	7
22/17/22/17	8
15/21	9
15/14	12

DISCUSSION

Comparative genetic studies between humans and non-human primates began in the early 1960s, even before banding techniques were developed (Ferguson-Smith et al., 2005). Initially, visual comparisons were performed using the unbanded karyotypes of humans and the great apes, but when banding techniques became available, these more advanced studies generated even more promising results. G-banding not only allowed for the accurate identification and karyotyping of chromosomes, but also provided the potential for detecting intra- and interchromosomal rearrangements, or aberrations. The discovery of chromosomal aberrations is made possible by the recognition of banding patterns which vary from the established banding pattern for a species. However, the technique of G-banding becomes somewhat inaccurate in precisely locating the gene regions involved in rearrangements. This requires the use of a molecular technique that allows the interaction of multiple sets of chromosome DNA, even those from two or more different species.

The development of individual chromosome-specific painting probes by flow cytometry and polymerase chain reaction in the 1990s provided the necessary tools for fluorescence *in situ* hybridization (FISH) of metaphase chromosomes and the DNA-based detection of interspecies chromosome homology. FISH, unlike G-banding, can identify the specific chromosome aberrations which result from illegitimate meiotic recombination. These fusions, fissions, translocations and inversions lead to differences in the organization of genes within a genome, and essentially, the evolutionary changes which may lead to the divergence of species.

FISH has allowed for great progress in the study of karyotype evolution, and has contributed much to the construction of the phylogenetic history of primates. Since its introduction into the field of cytogenetics, FISH has allowed for the cross-species painting of all three infraorders of primates (Catarrhini, Platyrrhini and Prosimii) by both unidirectional and reciprocal hybridization. These studies provide the data necessary to map the landmark chromosomal rearrangements responsible for karyotype evolution beginning with the ancestral primate in the Eocene period more than fifty-five million years ago and ending with the divergence of hominids a mere five million years ago (Holden 2005).

Hybridization using FISH probes specific for whole chromosomes or specific gene regions has been used extensively as a screening method for identifying and detecting chromosomal aberrations

typical of clinical genetic disorders. Initially, only one to three probes could be used at a time because of limited availability of fluorochromes. In 1996, Schrock et al. reported the development of a FISH technique called spectral karyotyping, or SKY, which utilized spectrally overlapping probes and therefore allowed the simultaneous hybridization and detection of probes for all twenty-four human chromosomes. This breakthrough system utilized Fourier spectroscopy, charge-coupled device (CCD) imaging, and optical microscopy, which combined to detect the subtle differences in the emission spectra of each pixel as the fluorophores from each hybridized probe pass through an interferometer. The sensitivity of spectral karyotyping allows for the discrimination of each of the twenty-four fluorophore combinations of Cy2, Spectrum Green, Cy3, Texas Red and Cy5, and therefore the identification of each human chromosome in a metaphase hybridization. SKY soon became a major technique used in tumor cytogenetics, because of its ability to identify the chromosomal material which comprised the notorious "marker" chromosomes of tumor cell lines.

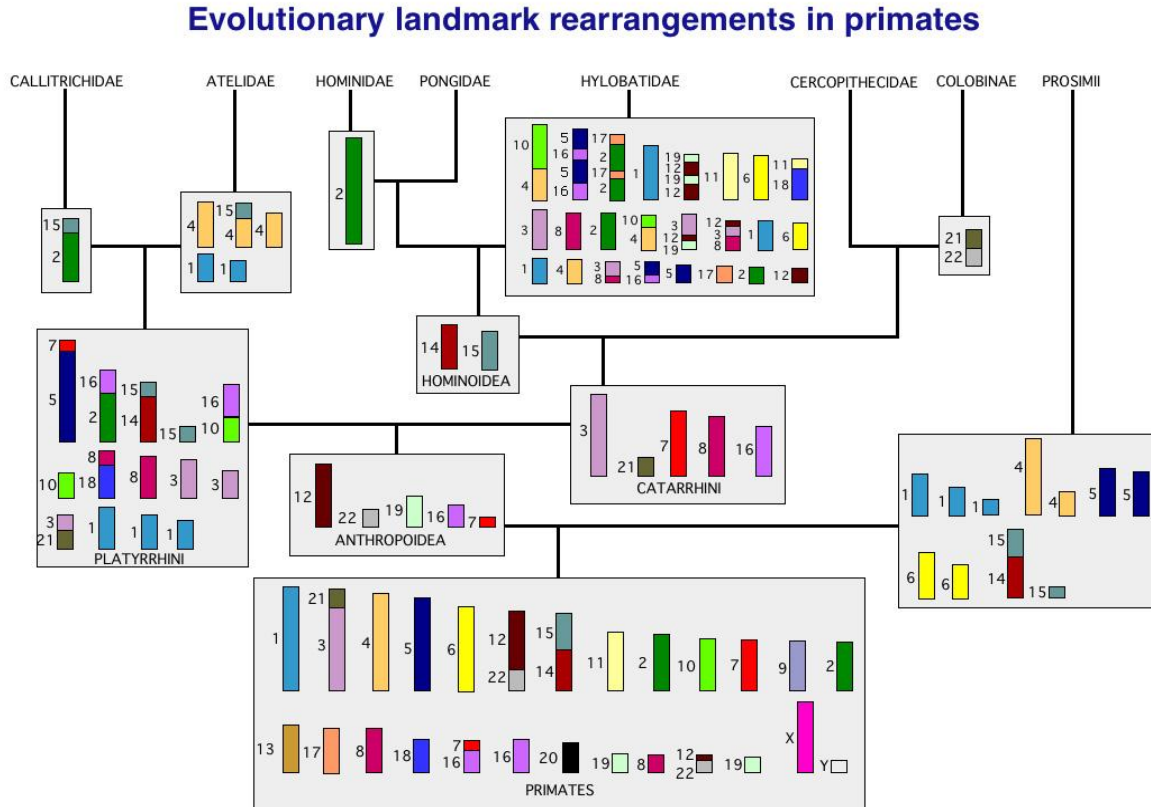
The efficiency of SKY makes it an excellent method for a single cross-species painting experiment, as opposed to the more time-consuming and tedious method of performing twenty-four separate hybridizations for each individual human probe. In the case of this study, SKY has been used to create a karyotypic homology map for the *Pithecia pithecia* which identifies the chromosomal rearrangements that have occurred between the divergence of this New World Monkey and that of our own species, *Homo sapiens*.

The *Pithecia pithecia*, or White-faced Saki, is a New World Monkey, also known as a neotropical primate, and is indigenous to Venezuela, the Guyanas, and northeastern Brazil. This seed-eating species is known for its excellent leaping skills and passive behavior (Rowe, 1996). The male *Pithecia*, with the fringe of white fur surrounding its face, is responsible for the common name of this species. According to Bonvicino et al. (2003), *Pithecia pithecia* resides in the most basal of the three Pitheciinae genera (*Chiropotes*, *Cacajao* and *Pithecia*). Also, X-linked DNA sequence studies by Steiper and Ruvolo in 2003 locate the Pitheciinae branch at or very near the base of the Platyrrhini divergence (Stanyon et al., 2004), suggesting an even larger period of evolution separates *Homo sapiens* and *Pithecia pithecia*.

The organization of human chromosomal material within the *P. pithecia* karyotype as illustrated in the homology map can be examined to show how the karyotypes of both the hominid line (*Homo sapiens*) and the New World Monkey line (*P. pithecia*) have been derived from the chromosomes of the first primate. It is possible to follow how chromosomal rearrangements of the ancestral primate karyotype (APK) caused the branching of the suborder Anthropeidea, the common ancestor of both New World and Old World Monkeys, and thus, both *Pithecia pithecia* and humans. From the ancestral Anthropeidea karyotype (AAK), chromosomal rearrangements led to the split of the infraorders Platyrrhini (New World Monkeys) and Catarrhini (Old World Monkeys, apes and humans). By comparing the hybridization pattern of human probes in this study to those found previously to be typical of the ancestral New World Monkey karyotype (ANWK), the ancestral Catarrhini karyotype (ACK), the AAK and the APK, it is possible to highlight the landmark chromosome rearrangements which not only allowed for the divergences of these larger branches, but also represent the cytogenetic links between *Pithecia pithecia* and *Homo sapiens* (Figure 5).

The ten human chromosome probes that hybridized each with a single *P. pithecia* metaphase can be found in various conditions among the ancestral karyotypes of the diagram (below). For example, the sex chromosomes X and Y have been completely conserved throughout primate evolution from the ancestral primate karyotype (APK) all the way through both the *P. pithecia* and *Homo sapiens* karyotypes. *P. pithecia* chromosome 2 (PPI 2), which is homologous to human chromosome 4 (HSA 4); PPI 3, the homolog to HSA 6; PPI 13, the homolog to HSA 11; PPI 18, the homolog to HSA 13; PPI 15, the homolog to HSA 9; and PPI 21, the homolog to HSA 20, have all been conserved from the APK. These eight homologies represent that chromosomal material which has undergone little or no change since its initial appearance in the karyotype of the first primate. The other two completely conserved homologies between *P. pithecia* and *Homo sapiens* are those between PPI 10 and HSA 12 and PPI 20 and HSA 19. Unlike the first eight homologies mentioned, these common chromosomes first appeared in the ancestral Anthropeidea karyotype (AAK), a less distant, but still common relative of the two distant species.

Figure 5. Evolutionary landmark rearrangements in primates. A diagrammatic representation of ancestral karyotypes from major branches of the primate phylogenetic tree. Numbers and colors show typical hybridization patterns of human chromosome probes. Each box contains the new chromosomal rearrangements which may have contributed to the divergences of the primate grouping. Figure taken from Primate Cytogenetics website of Mariano Rocchi.



There are also *P. pithecia* chromosomes that have been conserved from the APK, but are only homologous to fragments of human chromosomes, such as PPI 11, which corresponds to part of HSA 7. Likewise, PPI 12, which corresponds to HSA 15/14, is a chromosome that has been conserved from the APK through the ANWK and *P. pithecia*, while the fission of HSA 15/14 into two separate chromosomes first appears in the ancestral hominoidea karyotype. This fission is a landmark rearrangement in the divergence of hominids from other Catarrhines. Conversely, PPI 14, 21 and 22, which are all homologous to fragments of HSA 1, are three chromosomes which are derived from the fissions of a single large chromosome seen in the APK. These three *P. pithecia* chromosomes, which first appear in the ANWK, represent another landmark rearrangement that separates Platyrrhines from Catarrhines, as the large HSA 1 has remained conserved from the APK through the hominid karyotype. The last *P. pithecia* chromosome completely conserved from the APK is PPI 17, which is homologous to a fragment of HSA 8. In the derivation of the ACK and later human karyotype, this HSA 8 fragment fused with the other smaller fragment of HSA 8 that appears in the APK.

There are four *P. pithecia* chromosomes which are conserved through humans from the less distant ANWK, including PPI 1, 6, 16 and 19. PPI 1 is homologous to the fusion of HSA 7/5, which occurred during the divergences of Platyrrhines and Catarrhines. Similarly, the small fragment of HSA 7 (seen in the AAK) involved in this association fused with the larger fragment of HSA 7 (homologous to PPI 11) to form the complete HSA 7 which first appears in the ACK and remains conserved in the hominids. PPI 6 is homologous to the association of HSA 8/18, seen only in Platyrrhines, in contrast to the fusion of the two HSA 8 fragments to form the complete HSA 8, which first appears in the ACK. PPI

16 and 19 are both homologous to fragments of HSA 3, but by only using spectral karyotyping, it is impossible to determine which corresponds to the whole fragment of HSA 3 seen in the APK, and which corresponds to that fragment which resulted from the fission of the ANWK association of HSA 3/21 and subsequent fusion with the smaller of the two free HSA 3 fragments also seen in the ANWK.

Finally, there are five *P. pithecia* chromosomes which are unique to the species, and therefore derived from the ANWK, including PPI 4, 5, 7, 8 and 9. The p arm of PPI 4 is homologous to a small fragment of HSA 10, while its q arm is homologous to a large fragment of HSA 2. This fusion is unique to the *P. pithecia*, while the fusion of the two HSA 2 fragments (seen in the APK) to form the large human chromosome 2 is the one which sets humans apart from other hominids. PPI 5, which is composed of a four-segment, alternating association of HSA 16/2/16/2, was mostly likely formed from a pericentric inversion of the ANWK chromosome corresponding to the fusion of HSA 16 and 2. In the ACK, the fusion of the two fragments of HSA 16 originally seen separated in the AAK is one unique to the Catarrhini line. Similarly, in PPI 7, a pericentric inversion of the ANWK chromosome corresponding to HSA 16/10 is probably responsible for the four-segment alternating association of HSA 16/10/16/10 in the *P. pithecia*. By contrast, HSA 10 has remained completely conserved as a single chromosome between the APK and hominid karyotype. Furthermore, the fusion of HSA 22 and HSA 17 (with probable deactivation of one of the two centromeres), followed by a pericentric inversion, led to the derivation of PPI 8, which shows homology to HSA 22/17/22/17. HSA 17 is conserved from the APK through the human karyotype, and HSA 22 is similarly conserved from the AAK stage. Finally, PPI 9, which is homologous to HSA 15/21, is derived from the ANWK, where the HSA 3/21 chromosome underwent a fission, followed by the fusion of the free HSA 21 fragment and HSA 15 fragment (not involved in HSA 15/14 fusion).

The complicated rearrangements seen in these derived chromosomes illuminate the cytogenetic differences between the *P. pithecia* and *Homo sapiens*. However, by tracing the less rearranged chromosomes back to the common ancestors of these two species, it is possible to see the significant amount of homology that has survived the millions of years of evolution separating their divergences.

The chromosomal homologies found between humans and *P. pithecia* can be related phylogenetically to the results of a similar comparative cytogenetic investigation involving *Chiropotes utahicki*, a member of the *Chiropotes* genera within the Pitheciinae subfamily (Stanyon et al., 2004). This previous study by Stanyon produced nineteen hybridizations of human autosomal probes that were also found in the *P. pithecia* homology map. Of these nineteen homologies, thirteen involved whole chromosome hybridizations, while the other six involved associations of multiple human probes on a single primate chromosome. These shared conservations and rearrangements illustrate the cytogenetic connection between these two closely related genera, *Pithecia* and *Chiropotes*. The seven remaining *C. utahicki* autosomes with spectral hybridization patterns varying from those of the *P. pithecia*, as well as the greater diploid number ($2n = 54$), represent the cytogenetic barriers which contributed to the divergences of these two genera.

Many of the homologies shared by these two representative species of the Pitheciinae subfamily are typical of the ancestral Platyrrhini karyotype (Stanyon et al., 2004). These homologies include the conservation of single syntenies homologous to HSA 1 (three chromosomes); HSA 3 (two chromosomes); HSA 4, 6, 7, 8, 9, 11, 12, 13, 19 and 20 (*Pithecia* only); as well as the associations of HSA 7/5, HSA 16/10, HSA 8/18, HSA 10/2, HSA 16/2, and HSA 15/14 (fused with HSA 20 to form *Chiropotes* 1). This high level of conservation between the ancestral karyotype and these two genera confirms that Pitheciinae is of the more basal subfamilies of the Cebid family and Platyrrhini infraorder overall, as previously suggested by Bonvicino et al. (2003). This information is especially striking when considering that the *Pithecia pithecia* karyotype represents that of an early Platyrrhine, but still shows so much homology to the late evolving hominid karyotype.

Despite the significant results found in this study, future work is needed more clearly to define the chromosomal homology between humans and *P. pithecia*. According to Rens et al. (2001), the resolution of chromosome painting is not sensitive enough to detect homologous regions which are less than three megabases wide. Therefore, spectral karyotyping is a good method for detecting homology

visually, but only a rudimentary step in the complete comparison of genomes, as many intrachromosomal rearrangements may be missed. A more complete method for genome comparison would be a combination of cytogenetic and molecular techniques, including multidirectional chromosome painting as a visual tool, and comparative genome hybridization (CGH) microarray technology as a more accurate detector of the content and breakpoints of rearranged chromosomes.

However, as illustrated through SKY, the chromosomal conservation that is visible between the evolutionarily distant primate, *Pithecia pithecia*, and humans is remarkable, especially when considering the thirty to forty millions years separating their divergences.

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