Spectral Karyotyping (SKY) Establishes Chromosomal Homologies Between a New World Monkey (*Pithecia pithecia*) and Humans (*Homo sapiens*)

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The disruptions of linkage groups by chromosomal rearrangements can present barriers to the interbreeding of two populations and lead to speciation. These chromosomal changes can be demonstrated through cross-species painting (Ferguson-Smith et al., 2005). Homologies have been established between human and each of the other hominids as well as many old and new world monkeys and even some prosimians. The extent of homology is striking. Primate species that diverged 10 to 40 million years ago show homology of entire chromosomes.

Cross-species painting is most often accomplished by single chromosome painting. However, some studies using Spectral Karyotyping produced results comparable to the more labor intensive single chromosome paints (Best et al., 1998, Rens et al., 2001). The Spectral View Imaging System from Applied Spectral Imaging produces human karyotypes in which each chromosome is painted a distinctive color. The process of Spectral Karyotyping or SKY involves the hybridization of a set of whole chromosome painting probes each labeled combinatorially with a distinctive subset of one to four dyes from a set of five fluorochromes. Hybridization of each fluorochromosome is determined by the spectra detected at each pixel in an image field using an interferometer (Shrock et al., 1996). Spectral karyotypes of metaphases from lower primates cross-painted with the ASI Human Spectral Karyotype Reagent Kit provide maps of homologies to human chromosomes in a single hybridization.

Chromosomal homologies have been found between some species of the New World Monkey infraorder (Platyrrhini) and humans through fluorescence in situ hybridization (FISH). The present investigation focuses on the *Pithecia pithecia* (White-faced Saki), a New World Monkey of the family Cebidae and subfamily Pitheciinae, the least studied subfamily of the six Cebidae groupings. *Pithecia* is the most basal of three Pitheciinae genera: *Pithecia, Chiropotes* and *Cacajao* based on morphologic and molecular analyses (Bonvicino et al. 2003). New World Monkeys are estimated to have diverged from the ancestral primate approximately forty million years ago, and about thirty-five million years before the divergence of hominids.
A karyotype 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*.

*A Pithecia pithecia* fibroblast culture PR00239 was obtained from the Integrated Primate Biomaterials and Informatics Resource (IPBIR). The culture was maintained, subcultured, harvested and prepared for metaphase chromosome analysis by standard techniques. The G-band karyotype seen in Figure 1 was based on the karyotype for *P. pithecia* reported by Henderson et al., 1977). It confirms that PR00239 is *P. pithecia*. The diploid number is 2n = 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 and quite small and non-descript.

*In situ* hybridization to *P. pithecia* metaphases using a Human Spectral Karyotype Reagent Kit was performed according to the manufacturer’s instructions, except for a reduction of the stringency of the wash buffers. In Figure 2, an image of a metaphase image shows the spectral colors seen through the microscope. The primary value of this view is for estimation of hybridization quality. Although spectrally distinct, some of the dyes and dye combination are not discernable by eye. To enable viewing, each pixel is assigned a classified color based on the spectra detected. The Spectral Karyotype after conversion to classified colors is displayed in Figure 3.

Probes for human autosomes 4, 6, 9, 11, 12, 13, 19 and 20 were found to hybridize each with a single *Pithecia* chromosome pair. Furthermore, probes for the sex chromosomes X and Y, which have been shown to remain completely conserved throughout primate karyotype evolution, hybridized completely and exclusively with their *Pithecia* chromosome counterparts.

In contrast, human chromosomes 1, 2, 3, 5, 7, 8, 10, 14, 15, 16, 17, 18, 21 and 22 were found to hybridize either with multiple *Pithecia* chromosomes or in tandem associations along one chromosome. For example, human chromosome 1 (HSA 1) hybridized with *Pithecia* 14, 22, and 23, while two fragments of HSA 3 hybridized with *Pithecia* 16 and 19. Furthermore, three particular *Pithecia* chromosomes were hybridized in four-segment alternating syntenic associations by human chromosome probes: *Pithecia* 5 (HSA 16 and 2), *Pithecia* 7 (HSA 16 and 10), and *Pithecia* 8 (HSA 22 and 17). Other *Pithecia* chromosomes hybridized by human probes in two-segment tandems included *Pithecia* 1 (HSA 7/5), *Pithecia* 4 (HSA 10/2), *Pithecia* 6 (HSA 8/18), *Pithecia* 9 (HSA 15/21), and *Pithecia* 12 (HSA 15/14).

These chromosome homologies between human and Saki are strikingly similar to the homologies found between human and *Chiropotes utahicki*, a member of the *Chiropotes* genera within the Pitheciinae subfamily (Stanyon et al. 2004). This previous study by Stanyon demonstrated nineteen chromosomal hybridizations of human probes (not including the conserved sex chromosomes) that were also found in the *Pithecia* karyotype. Of these nineteen common homologies, thirteen involved whole chromosome hybridizations, while the other six involved associations of multiple probes on a single primate chromosome. Chromosomal rearrangements resulted in 7 remaining *Chiropotes* autosomes with hybridization patterns varying
from those of the *Pithecia* and the greater diploid number in the *Chiroptes* (2n = 54), and effectively, the divergence of these two genera.

However, some of the homologies shared by these two representative species of the Cebidae subfamily Pitheciinae can also be found in the Platyrrhini ancestral karyotype (Stanyon et al., 2004). These homologies include the conservation of single syntenies homologous to HSA 1 (three chromosomes), 3 (two chromosomes), 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 *Chiroptes* 1).

This high level of conservation between the ancestral karyotype and these two genera suggests that Pitheciinae is of the more basal subfamilies of the Cebidae family and Platyrrhini infraorder. However, the chromosomal conservation between this evolutionarily distant primate family and humans is remarkable, to say the least, especially when considering the 30-40 million years separating their divergences.

References


Figure 1 is a G-band karyotype of a male *Pithecia pithecia*, from the IBPIR culture PR00239.

Figure 2
Figure 2 is an image of a spectral image of a Pithecia metaphase hybridized with the ASI Human Spectral Karyotype Paint Probe. This metaphase is arranged in karyotype form in Figure 3.
Figure 3 displays a Spectral Karyotype of Pithecia in two ways. The upper panel shows the spectral colors, while the lower panel shows the same chromosomes with classified colors.