Evolutionary stasis of the pseudoautosomal boundary in strepsirrhine primates

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ABSTRACT:

Sex chromosomes are typically structured in a non-recombining region and a recombining pseudoautosomal region. The relative size of these regions is critical for sex chromosome biology both at the functional level (i.e. number of sex-linked genes) and evolutionary one (i.e. extent of Y degeneration and X-Y heteromorphy). The evolution of the pseudoautosomal boundary (PAB) - the limit between the recombining and the non-recombining regions of the sex chromosomes - is well documented in haplorrhines (apes and monkeys) but not in strepsirrhines (lemurs and lorises), which represent almost 30% of all primates. Here we studied the PAB of seven species representing the main strepsirrhine lineages by sequencing a male and a female genome in each species and using a male:female coverage ratio approach to identify the PAB. We found that during primate evolution, the PAB has remained unchanged in strepsirrhines whereas several recombination suppression events moved the PAB in haplorrhines. Strepsirrhines are well known to have much lower sexual dimorphism than haplorrhines. We suggest that mutations with antagonistic effects between males and females have driven recombination suppression and PAB evolution in haplorrhines. Our work supports the view that sexually antagonistic mutations have influenced the evolution of sex chromosomes in primates.

INTRODUCTION

The human sex chromosomes are strongly heteromorphic as they exhibit extensive differences in size, gene number, DNA repeat abundance and heterochromatin composition (Skaletsky et al. 2003; Ross et al. 2005). The X chromosome comprises a large X-specific region recombining only in females whereas the Y comprises a male-specific region that does not recombine at all. Both sex chromosomes share two regions called pseudoautosomal regions (PAR1 and 2) that recombine in both males and females. Strikingly, our strongly heteromorphic sex chromosomes originated from a pair of nearly identical autosomes ~150 millions years ago (Lahn and Page 1999; Skaletsky et al. 2003; Hughes and Rozen 2012). It all started with the emergence of *Sry* – the master male-determining gene in mammals - from *Sox3* just prior to the divergence of placentals and marsupials (Lahn and Page 1999; Skaletsky et al. 2003; Hughes and Rozen 2012). Since then, at several moments throughout evolutionary history, vast regions of the Y chromosome have at once stopped recombining with the X, likely through inversions on the Y, forming the so-called evolutionary strata (Lahn and Page 1999; Van Laere et al. 2008; Lemaitre et al. 2009; Pandey et al. 2013). The most recent strata (4 and 5) have originated in the history of Catarrhini (Old World monkeys and apes) respectively, ~40 and ~25 Mya, and now only a very small PAR continues to recombine between X and Y in those primates (Hughes et al. 2012). In humans, PAR1 is the consequence of that process, while PAR2 is a recent addition (Skaletsky et al. 2003).

Such a process of gradual suppression of recombination between sex chromosomes, of PAR reduction, and formation of evolutionary strata has been documented in several animal and plant lineages (e.g (Nicolas et al. 2005; Zhou et al. 2014; White et al. 2015)). Why such a process occurred, however, is unclear. It has been proposed that sexually antagonistic mutations may have favoured the suppression of recombination (Rice 1987; Charlesworth et al. 2005). Theoretical models suggest that if there are male-beneficial/female-detrimental mutations in the PAR, genetically linking those mutations to the Y chromosome, through for example an inversion, will be selected. Some evidence supporting this

hypothesis has recently been found in guppies (Wright et al. 2017), but in many groups including primates where it has been proposed to explain sex chromosome evolution, evidence is lacking.

While previous work on primate sex chromosomes has focused on Haplorrhini (apes, Old and New World monkeys), we studied representatives of the other main primate lineage, the Strepsirrhini (lemurs and lorises). In strepsirrhines, female social dominance (FSD), in which females dominate males, is widespread and likely ancestral (Kappeler and Fichtel 2015; Petty and Drea 2015). FSD is associated with increased testosterone production in females, resulting in the masculinization of females, including aspects of their social behaviour and genitalia (Kappeler and Fichtel 2015; Petty and Drea 2015). Some species also have rather egalitarian social systems (Pereira and Kappeler 1997). In addition, sexual size dimorphism is virtually absent among strepsirrhines (Kappeler and Fichtel 2015; Petty and Drea 2015). This is in sharp contrast with haplorrhines, where sexual dimorphism is much more pronounced and male-biased (Plavcan 2004). We therefore hypothesized that if male-female differentiation and sexually antagonistic mutations were associated with the degree of X-Y recombination suppression, strepsirrhines should show evidence of less recombination suppression compared to haplorrhines. However, to date, very little is known about the sex chromosomes of strepsirrhines, except that strata 4 and 5 are missing in gray mouse lemurs (*Microcebus murinus*, (Glaser et al. 1999)) preventing previous tests of this hypothesis.

To identify the PAB of strepsirrhines, we used an approach relying on sequencing a male and a female at low/moderate depth, mapping the reads to a reference genome and computing the male:female depth ratio (Vicoso and Bachtrog 2011; Vicoso et al. 2013a; Vicoso et al. 2013b; Zhou et al. 2014). For autosomes, a M:F depth ratio of 1 is expected as males and females have the same number of autosomes. On the X chromosome, a ratio of 1 should indicate the PAR that is shared among sexes, a ratio of 0.5 the X-specific region as males have only one such region and females two, and the boundary between both would indicate the PAB. Using Illumina short-read sequencing technology, we sequenced a male and a female genome in seven species covering the main strepsirrhine lineages representing 65 My of evolution

(Pozzi et al. 2014): four Lemuriformes (aye-ayes, gray mouse lemur, red-bellied lemur, greater bamboo lemur) and three Lorisiformes (northern greater galago, senegal bushbaby, slow loris). The sequencing depth of each sample was between 11.8X and 39.1X (of human genome equivalent) with 78% of the samples being between 20X and 40X, i.e. moderate sequencing depth (Table S1). We then mapped the reads onto publicly available reference genomes of two strepsirrhines (using the human X to scaffold the strepsirrhine X chromosome) and computed a normalized M:F depth ratio to identify the X-specific region and the PAR on the X chromosome (see Methods).

RESULTS

Figure 1A-B shows the results for the gray mouse lemur. Using the human X chromosome to order the gray mouse lemur X scaffolds, we found that the scaffolds corresponding to human strata 4, 5 and PAR1 have a M:F depth ratio around 1 (Fig. 1B), indicating that these regions have remained pseudoautosomal in gray mouse lemurs and agreeing with older cytogenetic data (Glaser et al. 1999). The rest of the gray mouse lemur X is X-specific with a ratio close to 0.5. However, five regions in the X-specific region show an elevated ratio. Detailed analysis of these five regions showed that they are fragments of autosomes (see Text S1). It is not clear, however, whether this comes from contamination of the assembly of the X chromosome by autosomal scaffolds or if this has resulted from fusion of autosomal DNA fragments to the PAR during evolution, which are misplaced in the current assembly of the X chromosome. Only an improved assembly of the X chromosome in the gray mouse lemur could confirm one of these alternatives. With the fragmented assembly that is available our approach only reliably identifies the PAB, not the size of the PAR. If some autosomal material were translocated to the PAR, and thus enlarging it, it would not be possible to detect it with our approach. Despite these limitations, it is nonetheless clear that the regions homologous to strata 4, 5 and PAR1 of humans are still recombining in gray mouse lemur and that the PAB is still the same PAB as in the ancestor of all primates.

We repeated the same analysis for the other six species (Fig. 1C). For the lemurs, we used the gray mouse lemur reference genome for the mapping because it is the only available one, and for the lorises, we used the northern greater galago reference genome for the same reason (see Methods and Fig. S1 for the dot plot with the human X). Some species are quite distant from the species with the reference genome and mapping was consequently more difficult. This explains why in some cases the M:F depth ratio is more variable. The results of the aye-ayes analyses are especially noisy because of the large phylogenetic distance to the species with the reference genome (Fig. 1C). However, in all seven species studied here, the pattern is very similar. All studied strepsirrhines harbor a large pseudoautosomal region including the genes that are in PAR1, strata 4 and 5 in humans. We can therefore conclude that no recombination suppressing event has occurred in strepsirrhines since the origin of the group >65 millions years ago (Fig. 2).

Our findings are consistent with the hypothesis that recombination suppression between X and Y chromosomes is driven by sexually antagonistic mutations. However, the rate of strata formation is generally low: in primates two strata (4 and 5) were formed in apes and Old World monkeys, one was formed independently in New World monkeys (4') based on the species studied so far (Hughes et al. 2012, Cortez et al. 2014, and see Fig. 2) and our additional data found no new strata formation in strepsirrhines. This observation is consistent with our hypothesis, but could have happened by chance because of a low common rate of strata formation in both suborders. A statistical test of a common rate of strata formation (expressed in event per My) taking into account the respective times in the haplorrhine and strepsirrhine parts of the phylogenetic tree of the studied species is only marginally significant (binomial test, p=0.051 see Text S2). Because haplorrhines and strepsirrhines have different generation times, comparing rates on a generation-based timescale might however be more relevant. Rescaling time in generations to compare rates of strata formation (expressed in event per million generations) lead to a significantly higher rate in haplorrhines (binomial test, p=0.01 see Text S2), consistent with our hypothesis.

It is possible that the M:F read depth approach missed recently evolved strata in strepsirrhines. Recent strata are indeed more difficult to detect with the M:F read depth approach as sex chromosome divergence can be so low that both X and Y reads map onto the X chromosome and the ratio is close to 1 (Wright et al. 2017). To identify recent strata, we computed the male:female SNP density ratio, which is expected to more effectively detect the PAB when recent strata are present (Vicoso et al. 2013a; Wright et al. 2017). The M:F SNP density ratio is predicted to be 1 for the PAR, 0.5 for the old strata (using the same rationale as for the M:F read depth ratio) and to be increased (>1) for the recent strata due to accumulation of fixed X-Y differences (Wright et al. 2017). However, our analyses revealed no recent strata in the seven strepsirrhines species studied here (Figure S2).

DISCUSSION

Evidence for the sexually antagonistic mutations hypothesis has been found in other organisms. In guppies, populations exhibiting stronger sexual dimorphism seem to have more strata (Wright et al. 2017). In the brown alga *Ectocarpus*, sexual dimorphism is extremely low and as expected sex chromosomes are homomorphic, with a small non-recombining region, despite being very old (Ahmed et al. 2014). It should be noted, however, that other forces might be driving the process of strata formation in some lineages. In ruminants, the PAB seems to have moved through a process of erosion due to DNA repeats accumulation (Van Laere et al. 2008). In *Microbotryum violaceum*, a fungus having mating types instead of sexes and where sexually antagonistic mutations are absent, strata have nonetheless been found on the mating type chromosomes (Branco et al. 2017). Thus, sexually antagonistic mutation may not be a ubiquitous explanation of strata formation in all organisms.

Although sexual dimorphism is generally low in strepsirrhines, there are some differences among species in this lineage, with the genus *Eulemur* exhibiting the most pronounced sexual dimorphism (Petty and Drea 2015). In these species, including the red-bellied lemur (*Eulemur rubriventer*), which was analysed here, males and females exhibit striking sexual dichromatism, i.e. they differ in pelage

colouration (Rakotonirina et al. 2017). The red-bellied lemur showed no more evidence for recombination suppression than the other species studied here. Sexual dichromatism may imply sexually antagonistic mutations. The antagonism might have been solved not through Y-linkage but instead through sex-biased expression for example (Ellegren and Parsch 2007). Similarly, in Emu (*Dromaius novaehollandiae*), sex chromosomes are homomorphic and sexual dimorphism is thought to be have evolved mainly through sex-biased expression (Vicoso et al. 2013b). Future research could focus on sex-biased expression in strepsirrhines to test these ideas.

METHODS

Research plan. To examine whether strepsirrhine sex chromosomes have undergone less recombination suppression compared to haplorrhines, we selected strepsirrhine species that would maximise the representation of this group's diversity, and that were also readily accessible. We then sequenced a male and female of each species and mapped the obtained male and female reads to a reference X chromosome. The male to female depth ratio was then computed along the length of the X chromosome and the PAB was identified as the boundary between zones with a ratio of one (indicative of the PAR) and zones with a ratio of 0.5 (indicative of the non-recombining region).

Sampling. We selected seven species covering as much phylogenetic diversity of Strepsirrhini as possible (see Table S1). Both infra-orders (Lemuriformes and Lorisiformes) are equally represented. A male and a female individual were sampled for all species (except *Otolemur garnetti*, the northern greater galago, for which sequence data from a female individual were retrieved from NCBI, see Table S1). Blood samples of *Eulemur rubriventer* (red-bellied lemur) and *Prolemur simus* (greater bamboo lemur) were collected from living animals at Zoo de Lyon on EDTA blood collection tubes to avoid coagulation. Hair samples (with follicles and roots) of the female *Daubentonia madagascarensis* (aye-aye) were collected from a

living animal at Zoo Frankfurt. Samples of *Microcebus murinus* belong to the Brunoy laboratory (UMR7179, France; agreement E91-114-1 from the Direction Départementale de la Protection des Populations de l'Essonne): the biopsies were obtained from muscle tissues after the animals' natural death. Tissues samples of a male *Daubentonia madagascariensis*, and samples of *Galago senegalensis* (Senegal bushbaby), *Nycticebus coucang* (slow loris) and of a male *Otolemur garnetti* were obtained from the tissues and cryopreserved cells collection of the National Museum of Natural History (MNHN, Paris, see Table S1).

DNA extraction and sequencing. DNA from *Eulemur rubriventer*, *Prolemur simus* and female *Daubentonia madagascariensis* were extracted using two different Macherey Nagel kits. Blood sample were treated with NucleoSpin Blood Quickpure kit. Hair samples were treated with NucleoSpin DNA trace kit after a mechanical crushing of hair bulbs. DNA from the tissues and cells samples (for other species) was extracted using the DNeasy Blood and Tissue kit (Qiagen) following the manufacturer's instructions. DNA was stored at -20° C and sent onto dry ice to the sequencing platform.

A DNAg library was constructed for each sample using Illumina kits (TruSeq nano LT for Hiseq 2500 and 3000 sequencing). Paired-end sequencing was conducted using an Illumina Hiseq 2500 (2 x 125 bp) or 3000 (2 x 150 bp) with 1 or 2 individuals per lane at Genotoul, the INRA sequencing platform in Toulouse. Sequences were all found to be of high quality and without contamination. Sequence data and coverage are shown in Table S1. All the data generated in this study is available at NCBI (project # PRJNA482296).

Chromosome assembly. Reference X chromosomes were not available for any species and genome assemblies were only available for two species that were 1) closely related to, or the same as the species being studied, and 2) assembled to an extent that it would be possible to construct a *de novo* X chromosome. These were *Microcebus murinus* (gray mouse lemur) and *Otolemur garnettii* (northern greater galago).

De novo X chromosomes were constructed for these species using scaffolds from whole genome assemblies on NCBI, which were selected, ordered and oriented against the human X chromosome. This was achieved using SynMap, an online software pipeline within the CoGe toolkit (Lyons and Freeling 2008; Lyons et al. 2008)that identified putative homologous genes between potential X scaffolds and the human X chromosome with a blast comparison (Altschul et al. 1990) using the Last algorithm (a variant of Blastz; (Schwartz et al. 2003)). An algorithm within the SynMap pipeline then identified a colinear series of homologous genes between potential X scaffolds and the human X chromosome as regions of synteny, and these were arranged in order accordingly. The relative gene order DAGChainer option was used, with a maximum distance of 20 genes between two matches and a minimum of five aligned pairs of genes. The human X chromosome reference was sourced from the GRCh37.p13 Primary Assembly on NCBI (Reference Sequence: NC_000023.10).

As the results of some of the analyses in this study required normalisation using an autosome from the corresponding species, a reference autosome was constructed using the same process. In this case, the human chromosome four was used to construct a *de novo* chromosome four for *Microcebus murinus* and *Otolemur garnettii*, which was selected for its similar size to the X chromosome).

Read mapping. Male and female reads for each species were aligned separately to their most closely related *de novo* X chromosome using Bowtie version 2-2.2.7 (Langmead et al. 2009). The reads were then sorted according to their position on the *de novo* X chromosome using Samtools version 1.3.1 (Li et al. 2009; Li 2011).

Coverage analysis. Read depth was calculated for each sex at each position from the mapped reads on the *de novo* X using Samtools. The coverage for each sex was then normalised by dividing the depth at each position by the mean coverage depth for that species and sex on an autosome (chromosome four). The ratio of normalised male to female coverage was then calculated at each position and the data was summarised as a sliding window average using a window size of 150 kb sliding at increments of 10 kb.

This data manipulation was performed using AWK version 4.1.3. Scripts for the entire coverage analysis pipeline (suitable for compute clusters using Torque job scheduling) are available on GitHub (https://github.com/rylanshearn/sex-read-depth).

SNP density analysis. To detect potential areas that may have stopped recombining between strepsirrhine X and Y chromosomes relatively recently, the difference in male to female SNP diversity was also examined for all species. For each sex of each species, SNPs were called from the mapped reads using Samtools mpileup and then converted to profiles using sam2pro version 0.8 from the mlRho package (Haubold et al. 2010). Average SNP density was then calculated at each position following (Wright et al. 2017) as the sum of SNPs divided by four, and one was added to allow for a Log2 transformation. The male to female SNP density was then calculated at each site as Log2(average male SNP density) – Log2(average female SNP density). This calculation was performed using AWK version 4.1.3 and R version 3.4.0. Scripts for the SNP density analysis are also available on the GitHub repository (https://github.com/rylanshearn/sex-read-depth).

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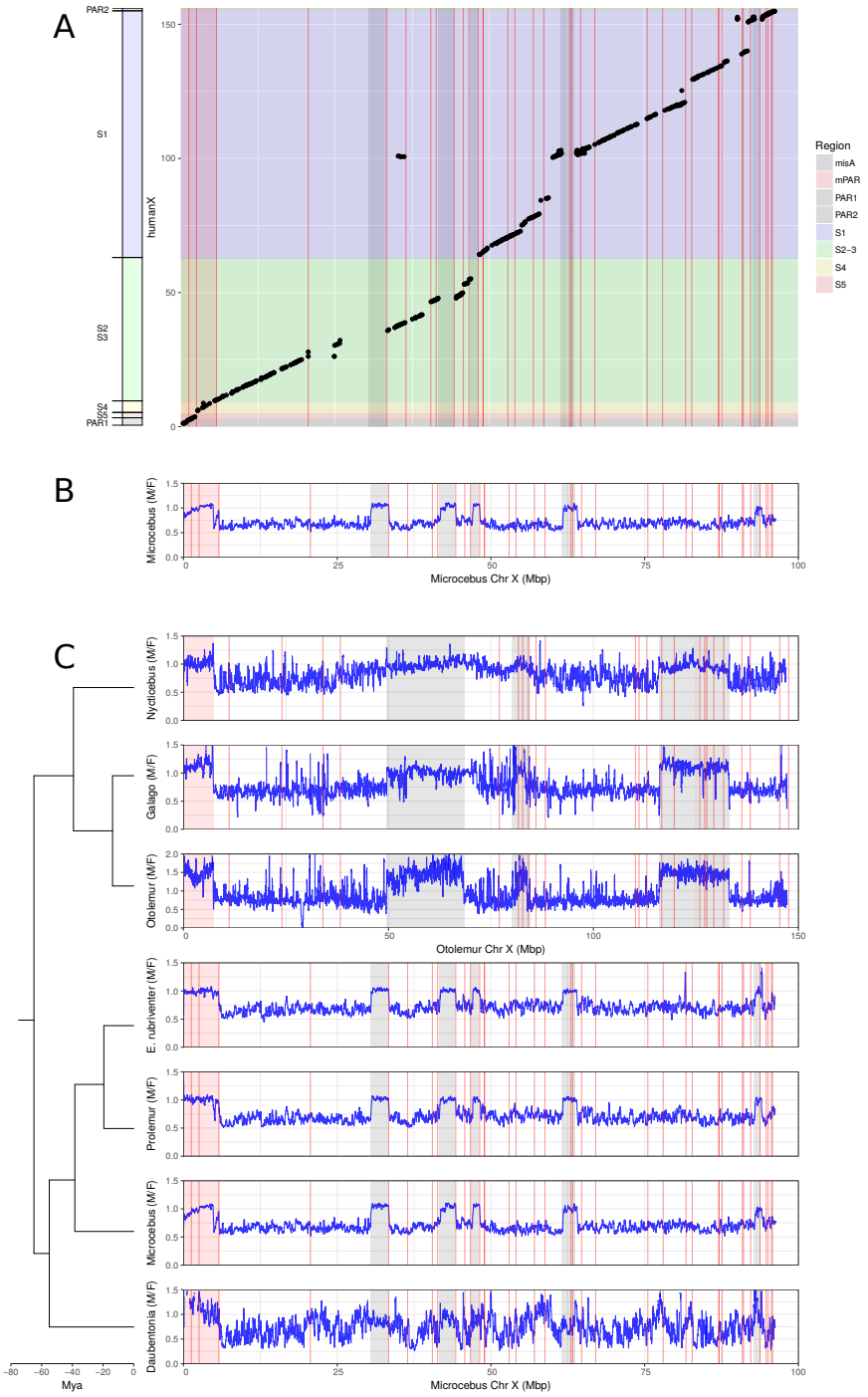

Fig. 1. Identification the PAB in seven strepsirrhine species. (A) synteny plot of the human and gray mouse lemur X chromosomes. The human X was used to order the gray mouse lemur scaffolds (see Methods). Black dots represent orthologous genes between the human and gray mouse lemur X chromosomes. Strata in humans using (Skaletsky et al. 2003; Hughes and Rozen 2012) definition of strata are shown (note that old strata have been split into smaller strata in (Pandey et al. 2013)). (B) M:F read depth ratio along the gray mouse lemur X chromosome. (C) M:F read depth ratio for all seven strepsirrhine species. See Text S1 for the detailed analysis of the regions with elevated M:F coverage ratio shown in grey.

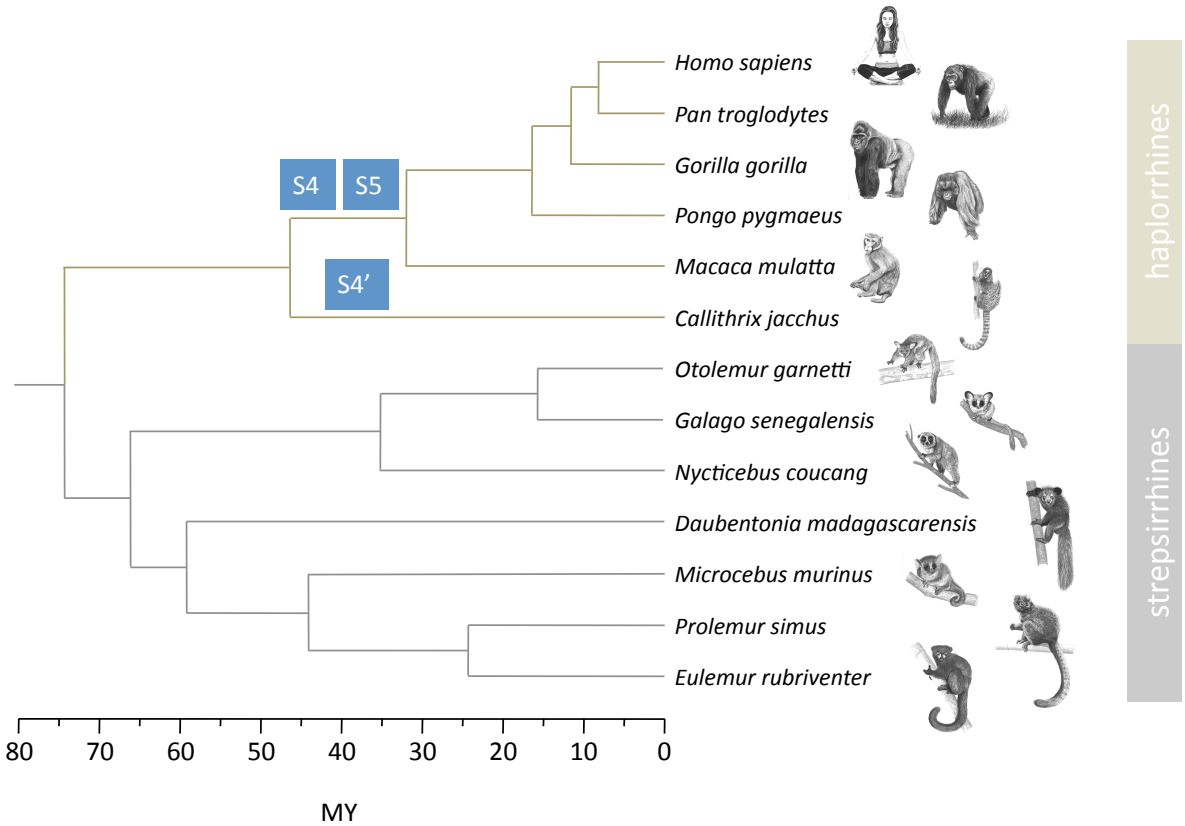

Fig. 2. Strata formation in primates. Data on strata in haplorrhines are from (Lahn and Page 1999; Skaletsky et al. 2003; Ross et al. 2005; Hughes and Rozen 2012; Hughes et al. 2012; Cortez et al. 2014); data on strepsirrhines are from this study. The phylogenetic tree and divergence times are from (Horvath et al. 2008; Pozzi et al. 2014). Drawings of primates have been prepared by Philippe Faivre.