Association between Genomic Instability and Evolutionary Chromosomal Rearrangements in Neotropical Primates

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Abstract

During the last decades, the mammalian genome has been proposed to have regions prone to breakage and reorganization concentrated in certain chromosomal bands that seem to correspond to evolutionary breakpoints. These bands are likely to be involved in chromosome fragility or instability. In Primates, some biomarkers of genetic damage may be associated with various degrees of genomic instability. Here, we investigated the usefulness of Sister Chromatid Exchange as a biomarker of potential sites of frequent chromosome breakage and rearrangement in *Alouatta caraya*, *Ateles chamek, Ateles paniscus*, and *Cebus cay*. These Neotropical species have particular genomic and chromosomal features allowing the analysis of genomic instability for comparative purposes. We determined the frequency of spontaneous induction of Sister Chromatid Exchanges and assessed the relationship between these and structural rearrangements implicated in the evolution of the primates of interest. Overall, *A. caraya* and *C. cay* presented a low proportion of statistically significant unstable bands, suggesting fairly stable genomes and the existence of some kind of protection against endogenous damage. In contrast, *Ateles* showed a highly significant proportion of unstable bands; these were mainly found in the rearranged regions, which is consistent with the numerous genomic reorganizations that might have occurred during the evolution of this genus.

Key words: Platyrrhini, Sister Chromatid Exchange, chromosome evolution.

Introduction

Genomic instability in mammals has become a growing area of interest in evolutionary cytogenetics. Special attention was paid to the relationship between chromosomal instability and rearrangements, which are considered to occur in fragile regions (Ruiz-Herrera et al. 2006; Robinson and Ruiz-Herrera 2010).

Currently, it is accepted that chromosomal breakage is required before the occurrence of chromosomal rearrangements (Karran 2000; Liu et al. 2006). These rearrangements are generally unviable, but they may be stable if the genetic information remains balanced as is the case for reciprocal translocations or inversions. When these new chromosomal forms occur in the germ line, they may result in new species or polymorphic variants (Robinson and Ruiz-Herrera 2010). Different hypotheses have been proposed to account for the molecular mechanisms underlying this process, and although one of them was experimentally confirmed (Natarajan and Obe 1978; Natarajan et al. 1980; Nowak and Obe 1984), the topic is still controversial (Garcia-Sagredo 2008).

Many studies in different genera and species of nonhuman primates showed that the biomarkers fragile sites (FS) and intrachromosomal telomeric sequences (ITS) may be associated with different degrees of genomic instability (Azzalin et al. 2001; Ruiz-Herrera et al. 2002a, 2002b). Moreover, other researchers suggested that a large proportion of chromosome bands involved in evolutionary rearrangements would correspond to some fragile and heterochromatin regions (Kehrer-Sawatzki et al. 2005; Ruiz-Herrera A, Ponsá M, García F, Egozcue J, Garcia M. 2002; Ruiz-Herrera A, García F, Giulotto E, et al. 2005; Locke 2003). The frequency of Sister Chromatid Exchange (SCE) is a biomarker for the detection of symmetric exchanges between chromatids of the same chromosome known to occur naturally during normal DNA replication. Currently, the most accepted hypothesis

states that SCE arises through homologous recombination when Holliday junctions are resolved and DNA strands are exchanged at a given frequency (Wilson and Thompson 2007 and references therein). An association between the occurrence of SCE and chromosomal rearrangements implicated in the evolutionary process was suggested by the fact that the former is considered an indicator of genomic instability (Garcia-Sagredo 2008). Although both DNA repair and rearrangements take place in different contexts, they share, to some extent, the same underlying mechanisms.

The three genera of Platyrrhini of interest display large karyotypic diversity, including not only the chromosome formula but also types of rearrangements and amount of heterochromatin (Müller et al. 1999; Neusser et al. 2001; Stanyon et al. 2001; García et al. 2003; de Oliveira et al. 2005, 2012; Dumas and Mazzoleni 2017). Although the genus *Cebus* presents rearrangements of inversion-type, it has a highly conserved karyotype in terms of euchromatin compared with the common ancestor of Platyrrhini. These primates have only two chromosome numbers, $2n = 54$ and $2n = 52$, with the most important cytogenetic feature being the large proportion of constitutive extracentromeric heterochromatin (~15% of the genome in all the species examined) (García et al. 2000; Seuánez et al. 2005; Amaral 2008; Nieves et al. 2011). The karyotypes of *Ateles* species have a large number of inversions and fusions; this genus exhibits a limited range of variation in chromosome number, with $2n = 32$ in *A. paniscus* and $2n = 34$ in *A. chamek*. In contrast, the chromosome number in genus *Alouatta* (with 12 described species), ranges from $2n = 44$ in *A. seniculus* to $2n = 58$ in *A. pigra* (Steinberg 2011). This genus has a distinctive sex chromosome system in the male, originated from translocations involving autosomes and a sexual chromosome (Rahn et al. 1996; Mudry et al. 1998, 2001; Steinberg 2011).

The aims of this work were to characterize the spontaneous occurrence of SCEs in *Alouatta caraya, Ateles chamek, Ateles paniscus*, and *Cebus cay*, and to assess the possible association between the localization of SCEs and the regions with rearrangements implicated in chromosome evolution. Our hypothesis is that the SCE frequency in each species is significantly higher in rearranged regions than in conserved regions.

Materials and Methods

Samples and Chromosome Preparations

Metaphase spreads for G-banding and Fluorescence Plus Giemsa (FPG) techniques were prepared from cultures of phytohemagglutinin-stimulated lymphocytes and fibroblasts of 9 adults of *Alouatta caraya* ($2n = 52$), *Ateles chamek* ($2n = 34$), *Ateles paniscus* ($2n = 32$), and *Cebus cay* ($2n = 54$) (table 1). These individuals were kept in captivity at different institutions in Argentina and Brazil. Their cells were harvested by standard procedures and classical cytogenetic characterization was performed for each specimen according to modified standard protocols (Steinberg, Nieves, Fantini, et al. 2014). Each monkey was identified taxonomically at the species level by analyzing metaphases treated by G-Wright and C-banding (20 spreads for each one) and using species-specific karyological patterns reported previously (Mantecón et al. 1984; Mudry et al. 1998; Nieves et al. 2005). Blood and biopsies were collected by trained veterinarians under sterile conditions.

Table 1
Information on the Specimens of *Alouatta caraya*, *Ateles chamek*, *Ateles paniscus*, and *Cebus cay*

Species	Number of Specimens	Sex	Origin	Biological Samples
Alouatta caraya	3	♂	REHM	Blood
		♀	REHM	Blood
		♀	REHM	Blood
Ateles chamek	3	♂	ZBA	Skin
		♂	ZBA	Skin and blood
		♂	ZBA	Skin and blood
Ateles paniscus	1	♂	CNP	Skin
Cebus cay	2	♂	ZBA	Skin and blood
		♀	ZBA	Skin and blood

ZBA, Buenos Aires Zoological Garden; REHM, Horco Molle Experimental Park; CNP, National Center of Primates, Belém, Brazil.

Sequential G-FPG Banding

G-banding with a modified Wright staining method (Mudry et al. 2011) was applied to all metaphases as follows: first, slides were maintained for one week at room temperature and in the dark (or, alternatively, for 5 h at 37 °C); then they were pretreated in 2xSSC for 2 min 30 s at 65 °C, washed with distilled water and covered with a 3: 1 Wright stain: Sørensen buffer (KH_2PO_4 and Na_2HPO_4 solutions at a 2: 1 ratio) in the dark for 2 min 30 s. After recording all the available metaphases, slides were destained and treated by the Fluorescence Plus Giemsa (FPG) technique to visualize SCEs (Martínez 2003; Mudry et al. 2011). Briefly, the slides were incubated with Hoechst 33258/2xSSC/distilled water for 20 min, irradiated with ultraviolet light for 35 min and rinsed in deionized water. Then, they were incubated in 2xSSC for 2 h at 60 °C, rinsed in distilled water, and counterstained with 4′, 6-diamidino-2-phenylindole (DAPI).

Image Acquisition and Processing

The G-banding and FPG images were analyzed on a Leica DMLB fluorescence microscope and photographed with a Leica DFC 340 FX camera. These were compared with the karyotypes already described for the species of interest. Image processing was performed with Adobe Photoshop 7.0 (Adobe

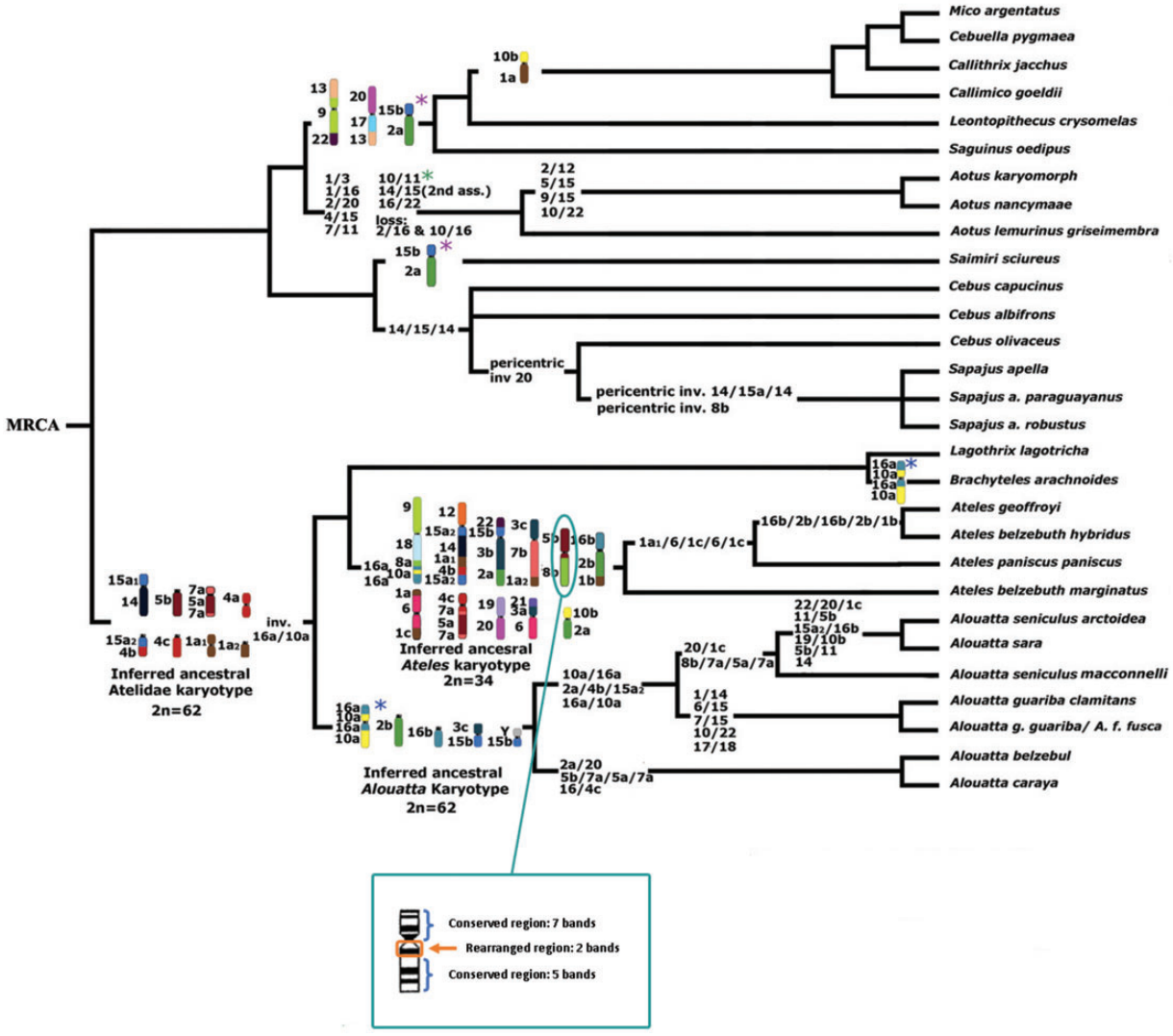

Fig. 1.—Partial phylogenetic tree of Atelidae and Cebidae, modified from Dumas and Mazzoleni (2017). This phylogeny shows chromosome syntenies characterizing each node. MRCA, Most Recent Common Ancestor of Atelidae and Cebidae. Box: Idiogram of chromosome #5 of *Ateles* spp., illustrating rearranged and conserved regions, and their respective number of G-bands.

Systems Inc.). For each specimen, we analyzed a minimum of 20 metaphases and quantified the SCEs per chromosome and per cell. Then, we determined the location of the SCEs at a subchromosomal level, using banding patterns obtained with the sequential G-band (400 band resolution)-FPG technique.

Reconstruction of Evolutionary Chromosomal Rearrangements Described for Platyrrhini

We analyzed literature data of chromosome evolution in Platyrrhini based on chromosomal rearrangements occurring within and between families and genera so far described (Medeiros et al. 1997; de Oliveira et al. 2002, 2005, 2012; Amaral 2008; Steinberg, Nieves, and Mudry 2014; Dumas and Mazzoleni 2017). The chromosomal rearrangements mentioned in the reviewed reconstructions were determined indirectly by analyzing human syntenies. This allowed us to identify the conserved and rearranged chromosomes in the three genera under study as determined in relation to the most probable common ancestor of Platyrrhini (fig. 1). Finally, we compiled a database of all available information on the number of SCEs and their location in both conserved and rearranged chromosome regions for each studied genus.

Statistical Analysis

Following Mariani's (1989) approach to the statistical analysis of SCE, a Poisson test was used to evaluate association between the localization of SCEs and chromosomal regions with rearrangements involved in the evolution of each genus. All statistical analyses were performed with STATISTICA 10 (StatSoft Inc.). For each species, we identified the conserved

and rearranged regions, where we determined the type of chromosomal rearrangement and quantified the total number of chromosomal bands per haploid set from the G-banding pattern (see box in fig. 1). Then, we calculated the SCE frequencies for both regions.

Our data set is assumed to follow a Poisson distribution, with the number of events per unit of area corresponding to the number of SCEs per unit of genome. We considered each chromosomal band in the G-banding pattern as a unit of area because the genome length in base pairs of the studied species has not been determined yet.

Based on the G-band karyotype, we defined the number of bands per haploid set (N), the number of metaphases analyzed (S) and the total number of SCEs observed (E) for each species. The probability of finding 0 or more SCEs (h) randomly distributed at one of the N bands of the karyotype is:

$$a(h) = E/(2 \times S \times N)$$

The value of $a(h)$ is usually <1 because N is high but E is low. Since each band identifies a sample of $2S$ bands, the number of SCEs per band will follow a Poisson distribution with a mean $\mu = E/N = 2S \times a(h)$ and variance $\sigma^2 = 2S \times a(h) \times [1 - a(h)]$. Under this distribution, the probability of finding h or more SCEs at a band is:

$$p_h = 1 - \sum a_i$$

where $a_o = e^{-\mu}$ and $a_i = (\mu/i) \times a_{i-1}$.

If there are N bands in the karyotype, the expected number of bands showing 0 or more SCEs (h) is:

$$F_h = N \times p_h$$

If $F_h < 0.05$, then the probability of finding a band with h SCEs is $<5\%$. Thus, h is a value significant at the 5% level, and all bands in which h SCEs are found can be classified as unstable regions.

This statistical approach provides a simple and reliable tool not only to verify the nonrandom distribution of SCEs throughout the genome but also to determine the minimum number of SCEs required to consider a band as an unstable region (Mariani 1989).

Results

The absolute SCE frequencies per metaphase and per chromosome pair were calculated for each individual of each species.

Considering all metaphases, in *A. caraya* ($2n = 52$) the number of SCEs per chromosome pair ranged from 0 to 10 in the male and 0 to 9 in the females, with an average of 7.1 ± 3.6 SCEs/metaphase. The SCE frequencies per chromosome pair were considerably higher in *Ateles* than in the other species, ranging from 4 to 42 in *A. chamek* and 1 to 25 in *A. paniscus*, with average values of 6.7 ± 2.7 and 4.7 ± 2.1 SCEs/metaphase, respectively. In *C. cay* the SCE frequency per chromosome pair varied from 0 to 8 in the male and 0 to 7 in the female, with an average of 5.7 ± 2.6 SCEs/metaphase.

Table 2 shows published information on chromosomal rearrangements for *A. caraya*, table 3 for *A. chamek* and *A. paniscus*, and table 4 for *C. cay*. Subsequently, the SCEs located in each chromosomal region (rearranged and conserved) were quantified for each species.

Using the equations given in Materials and Methods, we determined the minimal number of SCEs necessary to classify a given band as significantly unstable for each species (see Supplementary Material). Table 5 shows the percentage of significantly unstable bands in rearranged and conserved regions obtained for each species. To emphasize interspecific differences, figure 2 illustrates the percentage of unstable bands in conserved and rearranged regions per species, relativized to the species having the largest percentage (i.e., *A. chamek*).

The male and females of *Alouatta caraya* showed differences in the proportion of significantly unstable bands between conserved and rearranged regions. The male had a larger proportion of significantly unstable bands in conserved regions (chromosome pairs # 3, 4, 6, 9, 12–18, 20, and X_1), while in the females these were mainly detected in rearranged regions (chromosome pairs # 1, 5, and 11) (see Supplementary Material).

In the two studied species of *Ateles*, SCE frequencies were higher in rearranged than in conserved regions. *Ateles chamek* had a large proportion of unstable bands in both rearranged and conserved regions and the SCEs were concentrated in chromosome pairs # 1, 2, 3, 4, 5, 6, 7, 9, and 12. *Ateles paniscus* showed a higher proportion of significantly unstable bands in rearranged regions, particularly in chromosome pairs # 1, 2, 3, 4/12, 5, 6, 7, 9, and Y (see Supplementary Material).

Both sexes of *C. cay* showed a low proportion of unstable bands, all of which were concentrated in conserved regions. These bands were found in chromosome pairs # 1, 2, 5, 11, 15, and 16 of the male, and in # 1, 11, 17, and 18 of the female (see Supplementary Material).

Discussion

Comparative cytogenetic analysis proved to be useful to determine chromosome homeologies and to identify changes leading to the evolution of karyotypes. In this regard, FISH and derived techniques allow a more detailed study of genomic reorganizations in mammals from an evolutionary perspective. In the context of primate evolution, the analysis of homologous synteny blocks have contributed to the reconstruction of chromosome rearrangements based on an inferred ancestral karyotype. This same approach has been

Table 2

Summary of Chromosomal Rearrangements Described for Each Chromosome Pair of *Alouatta caraya*

Chromosome Pair	Rearranged/Conserved Chromosome	Type of Rearrangement	SCEs
1	Rearranged region 1	Fusion 1a/5b	2.5
1	Rearranged region 2	Fusion 5b/7a/5a/7a	7
1	Conserved	–	1
2	Conserved	–	4.5
3	Conserved	–	4
4	Rearranged region 1	Fission–fusion 16b/4c	0.5
4	Conserved	–	4.5
5	Rearranged region 1	Inversion 16a/10a	4.5
5	Conserved	–	0
6	Conserved	–	4.5
7	Conserved	–	1.5
8	Conserved	–	3
9	Conserved	–	2.5
10	Conserved	–	0
11	Rearranged region 1	Fusion 2a/20	7.5
11	Conserved	–	1
12	Conserved	–	5
13	Conserved	–	5.5
14	Conserved	–	4.5
15	Conserved	–	5
16	Conserved	–	4.5
17	Conserved	–	8.5
18	Conserved	–	4.5
19	Conserved	–	2.5
20	Conserved	–	5
21	Conserved	–	2.5
22	Conserved	–	2.5
23	Conserved	–	1.5
24	Conserved	–	6
25	Conserved	–	0.5
X_1	Conserved	–	6
X_2[a]	Rearranged region 1	Translocation 7-Y	0
X_2[a]	Conserved	–	0
Y_1[a]	Rearranged region 1	Translocation Y-15b/3c	1
Y_1[a]	Conserved	–	0
Y_2[a]	Rearranged region 1	Fission 15b/3c	2
Y_2[a]	Conserved	–	0

NOTE.—The last column gives the average frequency of Sister Chromatid Exchanges (SCEs) for all the individuals analyzed. References: de Oliveira et al. (2002), Steinberg, Nieves, and Mudry (2014), and Dumas and Mazzoleni (2017).

[a]The chromosomes of males (with a multiple sex chromosome system $X_1X_2Y_1Y_2$).

used to identify unstable chromosome bands involved in evolutionary reorganizations (Ruiz-Herrera A, García F, Mora L, et al. 2005). Moreover, in the human genome Ruiz-Herrera et al. (2006) have reported the presence of bands indicative of chromosome instability that may accumulate evolutionary breakpoints, which are colocalized with intrachromosomal telomeric sequences (ITS) corresponding to remnants of ancestral chromosomal rearrangements.

If, as mentioned earlier, genomic instability is a requirement for the occurrence of stable rearrangements, then SCE frequency combined with other cytogenetic techniques emerges as a valuable tool to establish the relationship between genomic regions with differential stability and stable rearrangements. In our study focused on Neotropical primates, SCE showed high sensitivity and specificity for the detection of regions prone to break and rearrange. Thus, it proved to be a valuable genomic instability biomarker which provides information to be used in comparative evolutionary studies.

In *Alouatta caraya*, the chromosome pairs #11 and #17 showed the highest frequencies of SCEs. In pair #11, SCEs were more frequent than expected in the band showing a rearrangement involving fusion, which is consistent with the considerable amount of evolutionary rearrangements that are

Table 3

Summary of Chromosomal Rearrangements Described for Each Chromosome Pair of *Ateles* spp

Chromosome Pair	Rearranged/Conserved Chromosome	Type of Rearrangement	SCEs *A. chamek*	SCEs *A. paniscus*
1	Rearranged region 1	Fission–fusion 9-18/8a	5.3	11
1	Rearranged region 2	Fusion 18/8a-16a/10a	13.3	17
1	Rearranged region 3	Inversion 16a/10a	5.0	9
1	Conserved	–	11.0	15
2	Rearranged region 1	Fusion 12/15a	8.0	6
2	Rearranged region 2	Fusions 15a/14-1a-4b/15a	14.0	23
2	Conserved	–	10.3	25
3	Rearranged region 1	Fusion 22/15b	4.7	7
3	Rearranged region 2	Fusion 15b-3b/2a	3.3	9
3	Rearranged region 3	Fusion 3b/2a	12.7	15
3	Conserved	–	5.3	6
4	Rearranged region 1	Fusion 3c/7b	8.3	6
4	Rearranged region 2	Translocation 1a and fusion 1a/7b	11.0	13
4/12[a]	Rearranged region 3	Fusion 13/3c	10.3	17
4	Conserved	–	9.0	8
5	Rearranged region 1	Fusion 5b/8b	13.7	11
5	Conserved	–	11.0	17
6	Rearranged region 1	Fusion 16/2b-1b	11.0	12
6	Conserved	–	5.7	9
7	Rearranged region 1	Translocation 1a y 6	7.7	9
7	Rearranged region 2	Fusion 6/1c	5.3	23
7	Conserved	–	10.0	1
8	Conserved	–	5.7	6
9	Rearranged region 1	Fusion 4c-7a/5a/7a	9.0	11
9	Conserved	–	2.3	8
10	Rearranged region 1	Translocation 6 and fusion 6–21/3a	10.3	5
10	Conserved	–	13.7	23
11	Conserved	–	8.0	15
12[b]	Rearranged region 1	Inversion 13	2.3	–
12[b]	Conserved	–	8.3	–
13	Conserved	–	4.3	9
14	Conserved	–	3.0	14
15	Conserved	–	3.3	4
16	Conserved	–	7.0	4
X	Conserved	–	1.3	6
Y	Rearranged		5.3	9

NOTE.—The last column gives the average frequency of Sister Chromatid Exchanges (SCEs) for all the individuals analyzed. References: de Oliveira et al. (2005) and Dumas and Mazzoleni (2017).

[a]The chromosome pair belongs to *Ateles paniscus* only, which was originated from the fusion of the ancestral chromosomes #4 and #12.

[b]The chromosome pair belongs to *Ateles chamek* only.

likely to have occurred in this pair in different species of *Alouatta* (Steinberg 2011). The pair #17, which corresponds to the chromosome #20 in the study of de Oliveira et al. (2002) because they adopted a different criterion for ordering the karyotype, shares homeology with the human chromosomes #14 and #15, indicating that, at least, it would have been derived from a rearrangement of the ancestral Platyrrhini karyotype (Dumas and Mazzoleni 2017). The high SCE frequency in pair #17 suggests that the rearranged region is unstable (fig. 2). Compared with the male, the females of *A. caraya* showed a significantly higher frequency of SCEs in the pair #1, especially in regions with fusions as primary rearrangements. This may be related to the homeology shared by the pair #1 with eight chromosomal segments of other species of the genus *Alouatta* (Steinberg 2011). However, the difference between sexes may be explained by the small sample size. Overall, the sex chromosomes of both sexes of *A. caraya* exhibited a low SCE frequency (table 2). Notwithstanding this, the male showed a high instability in conserved regions, which is in agreement with the presence of multiple sex chromosome systems in the males of *Alouatta* species (Steinberg 2011). A hypothetical explanation is that sex chromosomes are protected at the structural or epigenetic level, given their evolutionary importance.

Table 4

Summary of Chromosomal Rearrangements Described for Each Chromosome Pair of *Cebus cay*

Chromosome Pair	Rearranged/Conserved Chromosome	Type of Rearrangement	SCEs
1	Conserved	–	5.5
2	Conserved	–	4.5
3	Conserved	–	2
4	Conserved	–	2.5
5	Conserved	–	4
6	Rearranged region 1	Pericentric inversion 14/15	0
6	Rearranged region 2	Pericentric inversion 14/15/14	0.5
6	Conserved	–	0
7	Conserved	–	2
8	Rearranged region 1	Pericentric inversion 8b	0.5
8	Conserved	–	1
9	Conserved	–	0.5
10	Rearranged region 1	Pericentric inversion 20	1
10	Conserved	–	0
11	Conserved	–	7
12	Rearranged region 1[a]	Pericentric inversion 12	1.5
12	Conserved	–	3
13	Conserved	–	2.5
14	Conserved	–	2
15	Conserved	–	2.5
16	Conserved	–	3
17	Conserved	–	6
18	Conserved	–	4.5
19	Conserved	–	1.5
20	Conserved	–	0
21	Rearranged region 1[a]	Pericentric inversion 17	0.5
21	Conserved	–	0.5
22	Conserved	–	1
23	Conserved	–	1
24	Conserved	–	0.5
25	Conserved	–	0.5
26	Conserved	–	0.5
X	Conserved	–	1
Y	Conserved	–	0

NOTE.—The last column gives the average frequency of Sister Chromatid Exchanges (SCEs) for all the individuals analyzed. References: Amaral (2008) and Dumas and Mazzoleni (2017).

[a]Chromosomal rearrangements identified in this study.

Although the available literature does not provide any relationship between rearranged regions in *A. caraya* and the Platyrrhini ancestor, it may seem that rearranged regions in Y_1 and Y_2 sex chromosomes are prone to breakage and rearrangement. Furthermore, in *A. caraya*, we observed a considerable difference in the instability of conserved regions between male and females. Further analysis is required to shed light on the possible reasons for this result.

Both species of the genus *Ateles* showed higher chromosome instability than did the other genera under study. Particularly in *A. chamek*, we detected a large proportion of bands showing a high degree of instability, both in conserved (86.3%) and rearranged (83.3%) regions. Average SCE frequency was lower in *A. paniscus* than in *A. chamek*, but SCE distribution was highly associated with chromosomal rearrangements implicated in the evolution of this genus. Most of the rearranged regions (86.2%) showed a significantly high instability (table 5). This result is in line with the several chromosomal rearrangements accumulated during the evolution of *Ateles* genome (fig. 2). Moreover, the different percentage of unstable regions recorded for this genus may be due to the drastic reduction in the chromosome number occurring in the ancestors of Atelidae ($2n = 62$) and *Ateles* ($2n = 34$), which implies an important genomic reorganization. In both *A. chamek* and *A. paniscus*, SCE frequency in the chromosome pair #8 was noticeably lower than expected from random (see Supplementary Material), possibly because the nucleolar organizer regions (NOR) are located in this pair. These regions would confer protection against breakage and chromatid exchanges, as we previously

Table 5

Minimum Number of Sister Chromatid Exchanges (SCEs) for Which a Given Band is Considered to be Statistically Unstable, and Percentage (%) of Significantly Unstable Bands in Rearranged and Conserved Regions

Species	Minimum Number SCEs	% Unstable Bands in Rearranged Regions	% Unstable Bands in Conserved Regions	*P* Value
Alouatta caraya ♂	5	18.18	51.44	0.044
Alouatta caraya ♀	5	50.00	19.93	0.008
Ateles chamek ♂	14	83.33	86.25	0.033
Ateles paniscus ♂	9	86.21	50.63	0.047
Cebus cay ♂	4	0.00	33.45	0.022
Cebus cay ♀	4	0.00	19.80	0.020

NOTE.—Values were considered statistically significant at the $P < 0.05$ level.

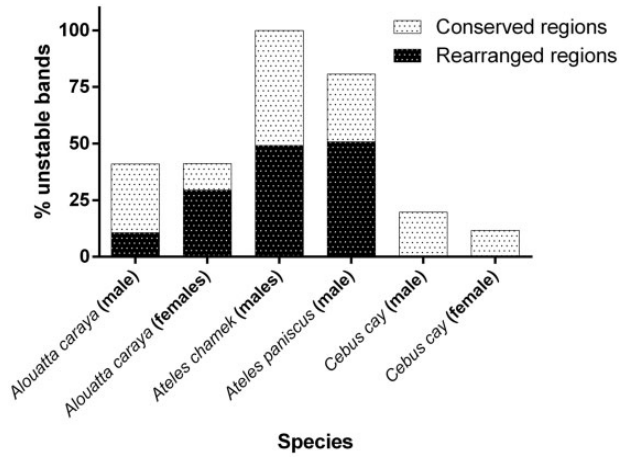

FIG. 2.—Percentages (%) of significantly unstable bands in conserved (black dots on white background) and rearranged (white dots on black background) regions in *Alouatta caraya, Ateles chamek, Ateles paniscus*, and *Cebus cay*. Results for males and females of *A. caraya* and *C. cay* are shown separately. For comparative purposes, values were relativized to those obtained for *A. chamek*, which were assigned to be 100% because this species had the highest percentage of SCEs.

proposed for the sex chromosomes of *A. caraya*. It is worth mentioning that in both species the Y chromosome showed the highest degree of instability among the smaller chromosomes of the complement. The SCE frequency in the chromosome Y was higher in *A. paniscus* than in *A. chamek*, in agreement with the study of Fantini et al. (2016), who used interspecies comparative genomic hybridization (iCGH) analysis in *Ateles* sp.; these authors reported that the different species exhibited rearrangements involving the Y chromosome, with higher frequencies in *A. paniscus*. These results, together with the frequent spontaneous occurrence of SCEs observed in our study, suggest that genome instability is much higher in *Ateles* than in the other studied genera.

In both sexes of *C. cay*, the average SCE frequency was substantially lower compared with the other two genera; the SCEs were mainly located in conserved regions, of which <20% were significantly unstable (fig. 2). Our results on *C. cay* and those using genome instability biomarkers other than SCE frequency (Borrell et al. 1998; Mudry et al. 2011) indicated that SCEs and FS shared the same chromosomal location. This supports the proposal that chromosome organization involves not only obvious bands (e.g., G-banding patterns) but also nonvisible bands which can be partially evidenced by biomarkers such as FS, ITS, and SCE. The latter bands are especially prone to breakage and are located in chromosome regions conserved during primate evolution (Ruiz-Herrera A, García F, Giulotto E, et al. 2005).

The fact that *Cebus* presents a highly conservative karyotype compared with the most recent common ancestor of Platyrrhini explains the high instability (i.e., the high tendency to break and reorganize) in conserved regions. Among significantly unstable chromosome pairs, #11 showed the highest SCE frequency, most of which were located at the interface between the euchromatin and heterochromatin, suggesting that it is an unstable region. Pairs # 4, 6, 12, 13, 17, and 19 also showed high SCE frequencies in eu-heterochromatic junctions. These results are in disagreement with those of Mudry et al. (2011), who found a significantly higher SCE frequency within the blocks and a nonsignificant SCE frequency between the two types of chromatin.

A higher genomic instability at eu-heterochromatic junctions has been previously reported for other mammalian models, such as the Indian muntjac (Carrano and Wolff 1975; Carrano and Johnston 1977). In addition, an increased frequency of FS and ITS in heterochromatin regions or at eu-heterochromatic junctions has been observed for different species of primates (Meyne et al. 1990; Garagna et al. 1997; Azzalin et al. 2001; Wijayanto et al. 2005; Mudry et al. 2007; Ruiz-Herrera et al. 2008; Dumas et al. 2016). These results suggest an evolutionary relationship between heterochromatin distribution and regions or loci associated with chromosome breakage, such as FS and ITS. Heterochromatin has been assumed to have a genome stabilizing role through the induction of chromatin compaction and the protection against endogenous damage (Vinogradov 1998; Nieves et al. 2017). Therefore, the reduced SCE frequency in *C. cay* would be due, at least partially, to the large proportion of heterochromatin in its genome (the

heterochromatin of *Cebus* species so far analyzed represents between 5% and 13% of the genome) (Nieves et al. 2017).

Our results provide evidence of higher genomic instability in karyotypes with numerous chromosomal rearrangements and are in agreement with studies that used instability biomarkers other than SCE (Sutherland and Richards 1999; Fundia et al. 2000; Ruiz-Herrera A, García F, et al. 2002; Ruiz-Herrera A, García F, Giulotto E, et al. 2005). Thus, both species of *Ateles*, with numerous chromosomal rearrangements implicated in the evolution of this genus, showed a large degree of genomic instability; this is particularly the case for *A. paniscus*, which is one of the most derived species (Medeiros et al. 1997; de Oliveira et al. 2005) In contrast, *C. cay* showed a considerable low genomic instability, which is consistent with its highly conserved karyotype compared with the ancestor of Platyrrhini. In regard to *A. caraya*, we found that its genomic instability and average number of chromosomal rearrangements were intermediate between those in the species of *Cebus* and *Ateles* under study.

In brief, through the use of SCE, we assume that genomes with rearrangements of evolutionary significance might provide evidence of association between genomic instability and a higher occurrence of structural chromosomal rearrangements. However, the analysis of a larger number of species belonging to a genus with a large occurrence of rearrangements (e.g., *Ateles*) may be needed to provide a more robust interpretation.

Finally, in all four species studied here (mainly *Cebus*), the use of SCE led us to characterize a large proportion of conserved regions as significantly unstable. This suggests the need to include other biomarkers such as FS and ITS to detect instability. Our approach represents a first step toward the identification of regions repeatedly involved in the chromosome evolution of the different Platyrrhini lineages.

Supplementary Material

Supplementary data are available at *Genome Biology and Evolution* online.

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