Cytogenetic Study of the Bolivian Monkeys: I. Preliminaly Report on Karyotypes of *Cebus. apella*, *Saimiri sciureus*, *Aotus azarae* and *Saguinus labiatus* 

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# ABSTRACT

Chromosome studies in four Bolivian primate species, Cebus apella, Saimiri sciureus, Aotus azarae and Saguinus labiatus, are carried out on G- and C-banding in 142 specimens.

Bolivian monkeys reveal the unique karyotypes or chromosomal variations. Cebus apella has 54 chromosomes (20 bi-arm and 32 acrocentric autosomes, submetacentric X-chromosome and acrocentric Y-chromosome) and 9 variant type chromosome (two pericentric inversions, two paracentric inversions and five C-band size variations). All four inversions are unique and are found only in Bolivian race. Saimiri sciureus has 44 chromosome (30 bi-arm and 12 acrocentric autosomes, submetacentric X-chromosome and acrocentric Y-chromosome). Aotus azarae has 50 chromosomes in female and 49 chromosomes in male. The difference of chromosome number between two sexes is due to the Y-chromosome translocation to autosome as reported in many litratures. Saguinus labiatus has 46 chromosomes (32 bi-arm and 12 acrocentric autosomes, submetacentric X-chromosome and acrocentric Y-chromosome) and show one pericentric inversion. Based on the karyotypes of Bolivian primates, a karyotypic evolution of platyrrhini is discussed.

# INTRODUCTION

The position of platyrrhine monkey karyotype in primate karyotypic evolution process has been clarified in the systematic works of Dutrillaux and his colleagues using many banding techniques (Dutrillaux, 1978a, b; Dutrillaux and Couturier, 1981, Dutrillaux et al., 1980). Their phylogeny of primate karyotype widely differs from that drawn with conventional (unbanded) karyotype (Chiarelli, 1980; Egozcue, 1975). Durtillaux (1979a) placed the karyotype of *Cebus capuchinus* midway between the karyotypes of *Microcebus murinus* (Prosimians) and *Pongo pygmaeus* (Hominoids) and suggested that *Cebus* has a karyotype not very different from simian ancestral karyotype. Karyotypes of New World Monkeys, however, are not generally conservative or stable. It is clear that *Aotus* (owl monkeys) and *Saimiri* (squirrel monkey) have experienced a rapid chromosomal evolution (Dutrillaux and Couturier 1981).

Though, only a few karyotypic studies of primates used many individuals (Ma et al., 1976; Freitas and Seuanez 1982), many intrageneric and intraspecific chromosomal variations were discovered in New World Monkey. Even *Cebus* and *Lagothrix*, which are thought to have the karyotypes similar to that of simian ancestor (Dutrillaux and Couturier, 1981), possess many intraspecific chromosomal variations (De Caballero et al., 1976; Cambefort and Moro, 1978; Garcia et al., 1978, 1983; Freitas and Seuanez, 1982). Intrageneric and intraspecific karyotypic variation were also reported in many other New World Monkey genera, such as *Saimiri* (Jones et al., 1973; Ma et al., 1974; Jones and Ma, 1975), *Callicebus* (Benirschke and Bogart, 1976; Minezawa and Valdivia, 1984), *Ateles* (Kunkel et al., 1980), *Callithrix* and *Saguinus* (Ardito, 1979). Especially, highly variable karyotypes of *Aotus* were well investigated, and the revised classification of *Aotus* splits this genus nine species from one species mainly based on karyotypic variation (Brumback, 1974, 1975; Reumer and De Boer, 1980; Ma, 1981; Galbreath, 1983; Hershkovitz, 1983).

These karyotypic variabilitys of platyrrhine monkeys are in a striking contrast to those of other primate groups. The karyotypes of *Macaca*, *Papio* and *Cercocebus* are very similar each other and possess only a few chromosomal changes among them (Dutrillaux et al., 1982). While, Prosimians, *Cercopithecus* and Hominoids probably have a similar karyotypic variability to that of platyrrhini. But types of karyotypic variations involved in karyotypic differrentiation within each taxonomic group, were largely different each other, e.g., centric fusion dominates in Prosimian, centric fission in *Cercopithecus* and pericentric inversion in Hominoids (Dutrillaux, 1979a). In platyrrhini group, tendency to accumulate one variation type seems to be not exist or a little if exist.

The Cytogenetic study of New World Monkeys has at least two significances as described below.

a. As in the Case of *Aotus*, a cytogenetic study could distinguish a difference among externaly similar taxonomic group. And if the group apparently dissimilar, a karyotypic investigation could make clear a dissimilarity or a relationship among them from a different aspect and should give more natural phylogeny than that at present.

b. New World Monkeys live in relatively similar habitats and have many related species with rather different social structures, natural historys (for example, duration time from emergence of taxonomic group) and degrees of karyotypic evolution. Therefore, cytogenetic study of platyrrhine monkeys possibly offers an excellent model for a study of primate karyotopic evolution.

The current study carried out with Bolivian monkeys. Bolivia abounds in endemic species and/or subspecies of New World Monkeys. Primate fauna of this country is largely different between southern and northern area. therefore Bolivia is favor for cytogenetic study of primates. The authers report karyotypes of *Cebus apella*, *Saimiri sciureus*, *Aotus azarae* and *Saguinus labiatus*. This study aimes preliminarily to discuss karyotypic evolution of New World Monkey based on many samples.

# MATERIALS AND METHODS

Peripheral blood samples were taken from 4 species (142 individuals), 21 *Cebus apella* (including 12 females and 9 males), 75 *Saimiri sciureus* (14 females and 61 males) 43, *Aotus azarae* (27 females and 16 males) and 3 *Saguinus labiatus* (one females and 2 males). These samples were acquired through the courtesy of Mr. Jiro Ohnishi, Zoological Garden Supplier, Santa Cruz, Bolivia. The former three species were captured within a radius of 100 km from Santa Cruz. *S. labiatus* was trapped near Cobija, Pando and kept there.

The whole blood samples were cultured with RPMI-1640 containing PHA-M (without any serum) within 24 hrs. of collections, except for *S. labiatus*, at Instituto, Bioclinico Central

in Santa Cruz and fixed by the standard method. The blood samples of *S. labiatus* were collected at Cobija and cultured in Santa Cruz. The fixed samples were carried back to the Primate Research Institute, Kyoto University. The metaphase chromosomes were sequentially stained for standard Giemsa, and G- and C-band with ASG (Sumner et al., 1971) and BSG (Sumner, 1972) techniques. The chromosomes of *S. labiatus* were stained for only standard Giemsa because of its poorness of metaphase cell.

A minimum of 10 metaphases from each specimen were analysed under the microscope for counting the chromosome number and recording their gross morphology. If possible, more than two banded and unbanded karyotypes were prepared for each individual, and compared with other karyotypes previously reported.

# RESULTS

# a. Cebus apella

The genus Cebus comprises four different species (Mittermeier and Coimbra-Filho, 1981) and is widely distributed in the Central and South American continent. Wild population of *Cebus apella* and *C. albifrons* are presently found in Bolivia. *C. apella* is classified into tufted

#### Table 1. Karyotypes of the Bolivian monkeys.

	Number of specimen			Chromosomes			
Species	Male	Female	2 <i>n</i>	N-A	Α	Х	Y
Cebus apella	9	12	54	20-19	32-33	SM	Α
Saimiri sciureus	61	14	44	30	12	SM	Α
Aotus azarae	16	27	49-50	17-16	31-32*	SM	Tr
Saguinus labiatus	2	1	46	32-31	12 - 13	SM	A
Callicebus moloch**	7	6	50	18	30	SM	M

2n = diploid number; N-A = non-acrocentric chromosome; SM = submetacentric chromosome; M = metacentric chromosome; A = acrocentric chromosome; Tr = translacation.

\*including subtelocentric chromosomes;

\*\*Minezawa and Valdivia (1984).

	Nos. of			Autosomes				
Authors	Date	specimen	2 <i>n</i>	N-A	А	Х	Y	
Bender & Mettler	1958	2	54	24	28	А	А	
Chu & Bender	1961	2	54					
Bender & Chu	1963	2	54	26	26	А	А	
Chiarelli & Barberis	1966	1	54	22	30	SM	Α	
Torres de Caballero et al.*	1976	6	54	20	32	M	SM	
Cambefort & Moro*	1978	3	54	20	32	Μ	SM	
Garcia et al.*	1978	1	54	21	31	А	SM	
		1**	54	23	29	Α		
			54	22	30	Α		
			54	20	32	Α		
			54	18	34	A		
	1983*	3	54	20	32	SM		
Freitas & Seuanez*	1982	19	54	20	32	SM	SM	
		1	54	21	31	SM	SM	

Table 2. Chromosome studies in Cebus apella.

\*Studies with chromosome banding;

\*\*Different cell line found in the same specimen.

N-A = Non acrocentric chromosome; A = Acrocentric chromosome; SM = Submetacentric chromosome; <math>M = Metacentric chromosome.

group and is distributed in southern area of Bolivia and *C. albifrons* belongs to untufted group and is found in northern area. In the current study, only the samples of *C. apella* were obtained. All the specimens (21 samples) have a diploid number of 54 chromosomes as reported in the litratures (Table 1 and Table 2). Of the autosomes of almost all specimens, 20 chromosomes are bi-arm and 32 chromosomes are acrocentric. The X-chromosome is submetacentric and the Y-chromosome is acrocentric (Fig. 1).

Two types of pericentric inversions are found in two chromosomes (Nos. 6 and 12). The variant No. 6 chromosome is found in 4 individuals in heterozygotic state. The variant No. 12 is observed in the other individual in heterozygotic condition. Therefore, these five monkeys have 19 bi-arm and 33 acrocentric autosomes. The variations in chromosomes which were found in many reports are also observed as shown in Table 3.

The G- and C-banded karyotypes of 6 individuals are prepared and nearly agree with those of Freitas and Seuanez (1982). With G- and C-band staining, seven other chromosomal variations are also discovered in 6 individuals. Two of these variations are paracentric inversion, the chromosome numbers 11 and 13, and the other five are variations of C-band size, the chromosome Nos. 5, 11, 12, 13 and 20 (Fig. 2, Table 3).

Though Cambefort and Moro (1979) also studied three male *Cebus apella* of Santa Cruz, Bolivia, they reported that the karyotype of this species agree with those of De Caballero et al.



Fig. 1. Standard G- (above) and C-band (below) karyotype of Cebus apella.

Table 3. The occurrence of	of c	hromosomal	variations	observed	in	Bolivian	Cebus	apella*
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Chromosome	Nos.	Phenotype					
pair	observed	+/+	+/v	$\mathbf{v}/\mathbf{v}$	$+/v_2$		
6**	21	18	3				
11**	21	6	11	4			
12**	21	20	1				
5***	6	4	2				
11***	6	5	1				
12***	6	4	2				
13***	6	2	2		2		
20***	6	3	2	1			

\*See Fig. 2; \*\*Observed with conventional staining; \*\*\*Observed with G- and C-staining; + = Common type; v,  $v_2 =$  Variant type.



Fig. 2. G- (above) and C-band (below) chromosomes of *Cebus apella*. Common (right) and variant (left).

(1976) and the chromosomal variation is no or little. The karyotype of this species in De Caballero et al. (1976) was as 20 bi-arm and 32 acrocentric autosomes, metacentric X-chromosome and submetacentric Y-chromosome. From this description, the morphology of sexchromosomes are different between two observations of Bolivian *Cebus apella*. Of the nine variants described above, the variation of C-band size was observed commonly through wide area of their habitat and also in congeneric species of Cebus (De Caballero et al., 1976; Garcia et al., 1978, 1983; Koiffmann and Saldanha, 1981; Freitas and Seuanez, 1982). Whereas, the other inversion variants were probably unique or endemic and largely different from those of Brasilian *Cebus apella* (Freitas and Seuanez, 1982).

### b. Saimiri sciureus

Most recent authors recognized either one or two species in the genus Saimiri, S. sciureus and S. oerstedii (Cabrera, 1958; Cooper, 1968; Napier, 1967; Mittermeier and Comibra-Filho 1981). The former species is widely distributed in South America and the latter is Centtal America. The Bolivian *Saimiri sciureus* largely different from *S. sciureus* of other regions even more than *S. oerstedii* (Thorington, 1976 in Mittermeier and Coimbra-Filho, 1981) and classified as *S. s. boliviensis* by Cabrera (1958). Several regional difference of squirrel monkey were found in their coat color and karyotype (Jones et al., 1973; Ma et al., 1974; Jones and Ma, 1975). Whereas, those characters were said as individual variables in some reports (Ariga et al., 1978; Ferrari et al., 1980: Mittermeier and Coimbra-Filho, 1981).

In the current study all specimens show the diploid number of 44 as reported previously (Table 1). Of autosomes of all specimens, 30 chromosomes are bi-arm and 12 chromosomes are acrocentric. The X-chromosomes is submetacentric and the Y-chromosome is acrocentric.

G- and C-band karyotype of Bolivian *Saimiri* is almost the same as that of Leticia one (Jones and Ma, 1975). Cambefort and Moro (1978) discovered heteromorphism (probably reciprocal translocation between two different chromosomes) in one of the two Bolivian squirrel monkey (from Santa Cruz) that they examined. On the other hand, we don't observe any karyotyoe which deviates from the autosome composition of "Leticia" type as is the case of Ariga et al. (1978). Based on C-band staining, Dev et al. (1982) reported interstitial C-band variation in Bolivian squirrel monkeys. Lau and Arrighi (1976) also suggested the existence of C-band variants and N-band variants. Though the present Bolivian samples also reveal the variations of C-band size and of size in secondary constriction which corresponds to N-band (Fig. 3), the screening for C-band of this species in insufficient. So we shall report C-band variant in detail separately.

# c. Aotus azarae

Genus *Aotus* is the most problematic genera in New World Monkey classification in recent years. Most authors of the past two decades (Cabrera, 1958; Napier and Napier, 1967; Hershkovitz, 1977; Mittermeier and Coimbra-Filho, 1981) have recognized a single wide ranging species. On the other hand, recent cytogenetic studies have indicated that *Aotus* have many karyotypic variations which differ each other among many regional populations (Brumback, 1974, 1975; Reumer and De Boer, 1980; Ma, 1981). On the Cytogenetic base, Brumback (1974, 1975) demanded a revision of this genus. Hershkovitz (1983) reexamined this genus and concluded to split genus *Aotus* from one to nine species.

In the current study, all 43 specimens reveal the karyotype so called Bolivian type which is identical to "type VI" of Ma et al. (1976). The diploid number of this species is 50 in female and 49 in male. The difference in the chromosome number between male and female is due to the Y-chromosome translation to autosome. Of the autosome, 12 chromosomes are meta or submetacentric and 36 chromosomes are acro or subtelocentric in female. Male karyotype has 35 acro or submetacentric automosomes instead. The X-chromosome is submetacentric

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16	17	18	19	20	21	

Fig. 3. Standard G- (above) and C-band (below) karyotypes of Saimiri sciureus.

(Fig. 4). In many subtelocentric chromosomes, short arms are completely heterochromatic and variable in size. At present, we designate here acro- or subtelocentric chromosome, because subtelocentric chromosomes include "true-" (with euchromatic short arm) and "pseudo-" subtelocentric (with heterochromatic short arm) chromosomes. Qualification and/or quantification of these short arms, however, are difficult, so we do not discuss further at present.

The chromosome number 2 show polymorphism like in other reports (Ma et al., 1976;



Fig. 4. Standard G- (above) and C-band (below) karyotype of Aotus azarae.

Cambefort and Moro, 1978). The common type chromosome No. 2 is submetacentric and variant type No. 2 is also submetacentric but more metacentric. The variant type of No. 2 chromosome is observed in only heterozygitic condition in 4 of 24 animals which can be scored their morphology.

#### d. Saguinus labiatus

Red chested tamarin, Saguinus labiatus, inhabits in area between Rio Madeira and Rio Prus. S. labiatus was positioned at the most primitive species of the mystax group by Hershkovitz (1977), at least with respects to facial depigmentation and whisker development. He recognized two subspecies, S. l. labiatus and S. l. thomasi, and Bolivian red chested (moustached) tamarin seems to be S. l. labiatus from his description.

The chromosome number of S. l. labiatus is 46. The bi-arm chromosome number is 32 (one female and one male) and 31 (one male). Accordingly, the acrocentric chromosome number is 12 and 13, respectively. The X-chromosome is submetacentric and the Y-chromosome is acrocentric (Fig. 5). The difference in chromosome composition seems to be due to pericentric inversion occurred on one chromosome of the Nos. 7-9 pairs (Fig. 5).

The chromosome number of S. labiatus is the same as S. mystax and other Saguinus species but different from these species in chromosome composition. Saguinus spp. has basically 30



Fig. 5. Standard conventional Giemsa stained karyotype of Saguinus labiatus.

bi-armed and 14 acrocentric autosomes, submetacentric X-chromosome and metacentric Ychromosome, though *Saguinus* group show many variation (Ardito, 1979). *S. mystax* was known to have 30 or 34 autosomes, submetacentric X-chromosome and acrocentric Y-chromosome (Benirschke and Brownhill, 1963; Anderson et al., 1967). Possively, the karyotypes of *S. labiatus* observed here is within the range of variation reported in *S. mystax*.

#### DISCUSSION

Presently, five species of Bolivian monkeys, *Cebus apella*, *Saimiri sciureus*, *Aotus azarae*, *Saguinus labiatus* and *Callicebus moloch*, were known their karyotypes (this study; Cambefort and Moro, 1978; Dev et al., 1982; Minezawa and Valdibia, 1984). When compared the karyotypes of Bolivian monkey with that of related species and subspecies from other areas, the uniqueness in their karyotypes could be recognized.

a. The standard karyotype of species is different from that of its relatives: A. azarae, C. moloch donachophilus and probably S. labiatus.

b. The standard karyotype is the same as its relatives but have the Bolivian types of chromosomal variations: *C. apella* and *S. sciureus*.

Only a few karyotypic studies of New World Monkeys based on individuals of which the collection site was clarified. Further only export place or country were described in almost studies. Therefore, the possibility that the chromosomal variations observed in Bolivian monkeys are distributed widely in the surrounding area is undeneiable. Whereas taxonomical studies recognized many endemic species and/or subspecies of primates in Bolivia. So the authors thought the unique karyotypes observed in Bolivian primate species as endemic characters.

Two theory, "centripetal dispersal" (Hershkovitz, 1963) and "refuge" (Kinzey, 1982) were presented for explaining a differentiation of Neotropical Primates. As Bolivia had at least two regugia, "Inambari" and "Guapore" (Kinzey, 1982) and have Amazonian tributarys, Rio Beni, Rio Mamore and Rio Madre de Dios, Bolivia should become one of the good study area to pursuit a cause of speciation of Platyrrhini.

Viewed New World Monkeys from the range of chromosomal variations in congeneric species, the current five species are divided into at least two groups.

a. Large karyotypic differences exist among congeneric species: Aotus and Callicebus.

b. Karyotypic difference among congeneric species is not large: Cebus, Saimiri and Saguinus. The difference between two groups probably have a correlation with an amount of intrapopulational variation, but this corrlation is weak and indirect. Though Callicebus showed very large intrageneric difference (2n = 20-50), Bolivian titi, C. moloch donacophilus, have little chromosomal variation (Minezawa and Valdivia, 1984). On the other hand, Cebus show almost the same standard karyotype among congeneric species but have many chromosomal variations within a regional populations (this study; Freitas and Seuanez, 1982). Chromosomal variations. However, variants of heterochromatic region, which were known as highly variable character in human and capuchin population, were sacrecely found as a marker of speciation or differentiation in this case.

What kind of conditions should determine a chromosomal variability and a karyotypic evolution of Platyrrhine monkeys? Three conditions, social structure, history of taxonomic group and genetic variability, are discussed here in relation to karyotypic evolution.

A lot of ecological and social studies of New World Monkeys were reported and offered many data about the group size and the population density of the five species discussed here and their relatives (Table 4). Aotus and Callicebus occures in family group (adult pair and their offspring). Saguinus are found in a one male group (one male, 2-3, females and their offspring). On the other hand, Saimiri and Cebus show a multi male group and have larger group size than the former three genera. Naturaly, these groups are not closed and are involved in regional breeding populations. So, it is also important to know the population density of genus under discussion. Estimates of population density in Cebus have ranged from as low as 5 to as high as 55 per square km. The range of density in Saimiri were 16-528/km<sup>2</sup>, in Callicebus were 2-450/km<sup>2</sup>, in Aotus were 2-150/km<sup>2</sup> and in Saguinus were 7-10/km<sup>2</sup>. A correlation between group size and population density is not high and even within a species, a large variation in estimated population density are existent among regions. As seen in Table 4, the genus with large group size tend to have small karyotypic differences among congeneric species and/or conspecific subspecies and vice versa. The similar tendency were also suggested between gibbon and baboons by Marks (1982). However, the number of species discussed here is too small and socio-genetical interaction among groups are still largely obscure. Therefore, the authors point out only a possibility of a correlation between group size and chromosomal evolution.

The apearance of unique characters within a taxonomic group is not independent of time. The time, when the first ancestor of platyrrhini emerged, is not clear. It is clear from fossil record that Ceboidea and Carithricidae had been already differentiated 25–35 milion years before, and that 12–14 milion years ago the ancestral species of almost all modern platyrrhine monkeys were existed (Suzaley and Delson, 1979). Immunological molecular study told

Range			
Genus	Group size	Population density per sq·km	
 Cebus	2-150	5-55	Freese & Oppengeimer (1981)
Saimiri	10-550	16-528	Baldwin & Baldwin (1981)
Aotus	2-30	2-150	Wright (1981)
Saguinus	2-20	7–10	Hershkovitz (1977)
Callicebus	2-6	2-450	Kinzey (1981)

Table 4. Summary of Group size and Population density.

that almost all taxonomic group of New World Monkeys were already differentiated 15–20 milions years ago (Sarich and Cronin, 1980). These two studies are agree well on the point that adaptive radiation of platyrrhini occur relatively narrow range of time scale. Therefore, large difference in the scale of karyotypic evolution (Dutrillaux, 1979a) suggests that the rates of chromosomal evolution in different population are not constant among New World Monkeys.

The genetic study of population will give an important information of population structure (Nozawa et al., 1982). No natural population of New World Monkeys was studied genetically. Bruce (1977) reported that index of the genetic variability (21 loci) of six species of imported Platyrrhine monkeys. The observed averaged proportion of individuals heterozygous per locus ( $\overline{H}$ ob) were 0.000–0.053 and the proportion of polymorphic loci were 0.053– 0.259. The higher value of both indexes in *Aotus* might be because of large interregional differnce of this genus. The range of both indexes in Platyrrhine monkeys, excepts  $\overline{H}$ ob *Aotus*, were within the range of that in *Macaca* and *Papio* which are known having stable karyotype (Dutrillaux, 1982; de Grouchy, 1982). Consequently, the relation between electrophoretic variability and chromosomal variability might be weak.

Phylogenetic relationship of New World Monkey drawn from morphological (Perkins and Meyer, 1980; Rosenberger, 1981; Setoguchi, 1983), molecular (Dene et al, 1976; Sarich and Cronin, 1980; Bruce, 1977) and Cytogenetic studies (Dutrillaux, 1979a; Chiarelli, 1980) show rather different topography each other, even use similar technique (Fig. 6). Therefore,



The topography of platyrrhini phylogeny drawn from morphological studies (1–3), molecular evolution studies (4–6) and Cytogenetical studies (7–8). A = Callithrichidae; A = Callithrix; B = Saguinus; C = Leontopithecus; D = Cebuella; E = Callimico; F = Saimiri; G = Cebus; H = Aotus; I = Callicebus; J = Allouatta; K = Pithecia; L = Cacajao; M = Chiropotes; N = Lagothrix; O = Ateles; P = Brakyteles. 1. Rosenberger, (1981) 2. Setoguchi (1983) 3. Perkins & Meyer (1980); 4. Sarich & Cronin (1980); 5. Dene et al. (1976); 6. Bruce (1977); 7. Chiarelli (1980); 8. Dutrillaux (1979) modified.

it is suggested that those character should evolved independently. The phylogenetic tree drawn from karyotypic studies would widely differ from the topographies of morphological and molecular phylogeny even after the sufficient accumulation of karyological data. Similar tendency in Old World Monkey was also presented by Marks (1982). In New World Monkey, however, each taxonomic group show more independent direction and evolutionary tempo of karyotypic change than in Old World Monkeys.

So far, most genetic study of Platyrrhini have been carried out using imported monkeys and have ignored the sampling site. To clarify the karyotypic evolution mechanism of Platyrrhine monkeys, we must study (cyto-) genetically a popultion structure of New World Monkeys in field, as well as clarify a stability of karyotype itself in laboratory.

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