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Old female *Macaca assamensis* at the Rome Zoological Garden

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GENETIC CHARACTERIZATION AND PHYLOGENETIC RELATIONSHIPS BETWEEN THE *ATELES* SPECIES (ATELIDAE, PRIMATES) BY MEANS OF DNA MICROSATELLITE MARKERS AND CRANIOMETRIC DATA

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Key words: DNA Microsatellites, cranial traits, *Ateles*, mutation models, effective numbers, Latin America

Abstract

A genetic characterization and some phylogenetic conclusions were obtained by analyzing six DNA microsatellite markers in seven different *Ateles* taxa. Additionally, 38 skull measurements were applied on several of these Colombian *Ateles* taxa. The main results obtained were as follows. 1-Two *A. fusciceps robustus* populations, one from the Chocó (Colombian Pacific Coast) and other from the northern Atlantic area of Colombia, were clearly differentiated by means of the DNA microsatellites. 2-The taxa, which presented the highest and the lowest average number of alleles, were the northern Atlantic *A. fusciceps robustus* population and *A. geoffroyi*. Otherwise, *A. chamek* presented the highest average genetic diversity (heterozygosity), meanwhile *A. hybridus* showed the lowest one. 3-Three taxa samples were not in Hardy-Weinberg equilibrium, *A. fusciceps robustus*, *A. belzebuth* and *A. chamek*. 4-The markers AP68, AP74 and D5S117 show a considerably heterogeneity among the *Ateles* taxa analyzed. However, the values of G_{ST} and R_{ST} obtained for *Ateles* were much lower than those recorded for other Neotropical Primates, such as *Alouatta* and *Cebus*. 5-Only the *A. fusciceps robustus* population from Chocó showed evidence of a recent bottleneck. 6-The coalescence methods estimated the lowest effective numbers for *A. hybridus* and *A. geoffroyi*, which coincides with the *Ateles* taxa in the most dangerous situation. 7-Generally speaking, the mutation model in the microsatellites analyzed was uni-step. The unique marker showing a significant multi-step mutation model was AP74 for *A. fusciceps robustus*, *A. belzebuth* and *A. hybridus*. 8-The mutation rates per generation were different for all the microsatellites employed. 9-No species or sex associations were displayed by the constructed phenograms using 38 craniometric variables. 10-The variables mainly influenced by size were maximum frontal and minimum frontal width followed by basal height, bigonion width, auricular height and maximum transversal braincase width. The influence of cranial shape was very dispersed among all the analyzed variables.

Introduction

The spider monkeys of the *Ateles* genus are among the largest primates in the Neotropics along with *Alouatta*, *Lagothrix* and *Brachyteles* (STRIER, 1992). Unfortunately, endemic *Ateles* populations are currently being threatened by a number of events. For example, they are intensively hunted for food by indigenous people of di-

verse countries in Central and South America. Additionally, the traditional demand for spider monkeys as attractive animals in zoos and as pets has led to the development of an intense commercial traffic (legal and illegal) of this genus. As large primates, *Ateles* species have low reproductive rates and therefore, even a low hunting pressure could extirpate extensive populations of spider monkeys throughout their distribution range (COLLINS, 1999). KONSTANT et al. (1985) and ROSENBERGER and STRIER (1989) revealed that *Ateles* are extremely environmentally sensitive and are the first primates to disappear after small environmental changes are introduced in rain forests. Normally, these primates live in undisturbed areas within primary rain forests and are mainly arboreal. Furthermore, *Ateles* is primarily frugivorous and feeds largely on the mature, soft parts of a wide variety of fruits that provide more energy than leaves. VAN ROOSMALEN (1980) estimated that 82.9 to 90 % of the *Ateles* diet is composed of fruit. This diet requires *Ateles* to have extensive territories because the territories must encompass the non-uniform distributions of fruit. This is remarkable different from *Alouatta* that can in live in small patchy forests. High sensitivity to environmental change, low reproductive rate and large territories help to explain why many *Ateles* taxa are now seriously threatened and therefore need to be targeted for conservation. The *Ateles* taxa reported as either endangered, critically endangered or vulnerable are *A. geoffreyi azuerensis*, *A. g. frontatus*, *A. g. grisescens*, *A. g. panamensis*, *A. g. vellerosus*, *A. g. yucatensis*, *A. fusciceps fusciceps*, *A. f. robustus*, *A. belzebuth belzebuth*, *A. hybridus* and *A. marginatus* following the IUCN Mance-Lande categories (RYLANDS et al., 1997). After *Brachyteles* and *Leontopithecus*, *Ateles* is considered the most endangered genus of the New World Primates (MITTERMEIER et al., 1989). Effective conservation proposals for any taxa should include information about gene diversity levels, genetic heterogeneity, existence of recent historical bottlenecks, and the identity and quantity of the sub-taxa or evolutionary units that exist in the natural environment.

Unfortunately, there is disagreement in the literature over the systematics of *Ateles*, making it difficult to construct and present an effective conservation proposal for this taxon. The most commonly used classificatory scheme recognizes 4 different species (*A. geoffroyi* from Central America with 9 subspecies, *A. belzebuth* with three geographical discontinuous subspecies, *A. fusciceps* in the Pacific coast of Colombia and Ecuador with two subspecies and *A. paniscus* with two discontinuous subspecies) for a total of 16 taxa (KELLOG and GOLDMAN, 1944). More recently, other authors have used the same scheme (KONSTANT et al., 1985). Differences between species and subspecies of this classification were based almost entirely upon pelage characteristics. Conversely, other authors such as HERSHKOVITZ (1972) or HERNÁNDEZ-CAMACHO and COOPER (1976) supported a second classification scheme and have considered all *Ateles* to belong to one, wide-ranging variable polytypic species, *A. paniscus*, using heterochromatism as the main parameter responsible for the different coat color patterns observed at this genus. Nevertheless, other authors (SHEDD and MACEDONIA, 1991; JACOBS et al., 1995) have not supported the use of metachromatism to infer phylogenetic relationships among diverse Neotropical Primate taxa because the genetic and developmental systems that underlie the phenotypic expression of pelage traits may be different across primate species. Furthermore, pelage characteristics are not only variable within populations, but they may intergrade where population distributions overlap. For example,

intergradation occurs among *A. geoffroyi* and *A. fusciceps robustus* and *A. g. grisescens* at the interface of Colombia and Panamá (KELLOG and GOLDMAN, 1944). ROSSAN and BAERG (1977) located sympatric populations of *A. geoffroyi* and *A. fusciceps* in eastern Panamá, where both populations had hybridized to some extent along a contact zone. In addition, an *Ateles* specimen collected inside the territory of *A. fusciceps robustus*, (Catival, San Jorge river in Colombia) had a strong admixture of light-colored hairs on the back, similar to the *A. hybridus* phenotype (HERNÁNDEZ-CAMACHO and COOPER, 1976). Similarly, ELLIOT (1913) identified specimens with pelage characteristics of *A. belzebuth belzebuth* in the territory of *A. p. chamek* (Chamicuros in the Huallaga River in Perú). The authors of the current paper add further support with their observations of hybrids between *A. p. chamek* and *A. b. belzebuth* at the Loreto region in the Peruvian Amazon. In a third classificatory scheme based on cytogenetic analyses, diverse authors (GARCÍA et al., 1975; KUNKEL et al., 1980 and MEDEIROS et al., 1997) postulated that differences in the morphological chromosome pairs 5, 6 and 7 did not support the *Ateles* taxonomy proposed by KELLOG and GOLDMAN (1944). PIECZARKA et al. (1989) determined that *A. paniscus* possessed 32 chromosomes, while all the other *Ateles* taxa had 34 chromosomes, suggesting the first as a separate species from the others. On the other hand, GROVES (2001) stated that there were seven different species of *Ateles*, including *A. paniscus*, *A. belzebuth*, *A. chamek*, *A. hybridus*, *A. marginatus*, *A. fusciceps* (with two subspecies) and *A. geoffroyi* (with 5 susbspecies). FROEHLICH et al., (1991) used a fourth classificatory scheme based on discriminant analysis of cranial and dental morphologies. These authors classified *Ateles* in three different species, *A. paniscus, A. belzebuth* (which included *A. chamek* and *A. marginatus*), and *A. geoffroyi* (which included *A. fusciceps* and *A. hybridus*).

Given the establishment and use of multiple classification schemes it is imperative to determine the research criteria that provide accurate information about the evolutionary units within the *Ateles* genus in their natural environment. Only recently have some molecular approaches been carried that have focused on the mitochondrial DNA control region, the cytochrome c oxidase subunit II gene and the nuclear Aldolase A intron V gene (COLLINS, 1999; COLLINS and DUBACH, 2000 a,b, 2001). These studies determined that although variation in nuclear genes is less than in mitochondrial genes (at least in the genes they studied) the constructed phylogenies via these two types of DNA are primarily similar. These authors suggested the need for additional studies that included more variable nuclear DNA to test their preliminary findings. Therefore, we used 6 hyper-polymorphic STRPs (Short Tandem Repeat Polymorphisms) to analyze the genetics and evolutionary phylogenetics aspects of some *Ateles* populations. These kinds of markers are composed of short repetitive elements, one to six nucleotide base pairs in length. They are also randomly distributed, highly polymorphic, and are frequently inside the eukaryotic genomes. An additional and positive property of these markers is the small DNA quantity needed to carry out these molecular analyses (via PCR). The small sample size allows the investigator to use non-invasive procedures to sample wild animals and successfully examine population biology dynamics through the use of molecular genetic techniques (BRUFORD and WAYNE, 1993) as well as to establish gene linkage maps. It is important to determine phylogenetic relationships among diverse *Ateles* taxa with this proposed marker type in order to verify the

schemes proposed with other molecular markers. It is also of interest to compare the results obtained with DNA microsatellites with classical biometric studies. Therefore, the objectives of this study are to use nuclear DNA microsatellites and craniometrical biometric distances to determine *Ateles* systematics.

Material and Methods

A total of 181 blood, drops of blood and hair samples were collected and analyzed from 7 different *Ateles* taxa for six microsatellite markers (AP6, AP40, AP68, AP74, D5S117 and D8S165). The taxa analyzed were as follows: *A. belzebuth belzebuth* (n = 22; Colombia and Perú), *A. fusciceps robustus* (n = 65; Colombia), *A. chamek* (n = 40; Perú and Bolivia), *A. paniscus paniscus* (n = 2; Brazil), *A. fusciceps fusciceps* (n = 2; Ecuador), *A. hybridus* (n = 36; Colombia and Venezuela) and *A. geoffroyi vellerosus* (n = 14; Guatemala) (Table 1).

Table 1: Geographic origin, sample sizes and sources of the seven *Ateles* taxa analyzed in the current work. Also the type of biological material analyzed is indicated.

Species	**N**	**Origin**	**Geographic origin of the samples**	**Source**
Ateles belzebuth belzebuth	22	Colombia	**La Macarena (Meta**) (teeth) **Villavicencio (Meta)** (hairs) **Caquetá Amacayacu (Amazonas)** (hairs and bones)	M. Ruiz-García
		Perú	**Loreto (Peruvian Amazon)** (hairs)	
Ateles fusciceps robustus	65	Colombia	**Turbo(Antioquia)** (teeth and bones) **Colosó (Sucre)** (teeth and hairs) **Barranquilla (Atlántico)** (hairs blood and teeth) **Alto Sinú (Córdoba)** (hairs) **Darién (Chocó)** (blood) **Parque Nacional los Katíos(Chocó)** (teeth) **Rio Sucio(Chocó)** (blood)	M. Ruiz-García
Ateles fusciceps fusciceps	2	Ecuador	**Ecuadorian Chocó** (hairs)	A. Castellanos L. Albuja
Ateles hybridus	36	Colombia	**Pto. Wilches (Santander)** (blood) **Pto. Rico (Bolivar)** (blood) **Morales (Bolivar)** (teeth) **Catatumbo (Norte de Santander)** (hairs)	M. Ruiz-García J. Villavicencio
		Venezuela	**Maracaibo** (hairs)	A.E. Bracho

Species	**N**	**Origin**	**Geographic origin of the samples**	**Source**
Ateles chamek	40	Perú Bolivia	**Loreto (Peruvian Amazon)** (blood, teeth, hairs) **Noel Kempf National Park** (hairs)	M. Ruiz-García
Ateles paniscus paniscus	2	Brasil	**Manaos (Brazilian Amazon)** (hairs)	M. Ruiz-García
Ateles geoffroyi vellerosus	14	Guate-mala	**Petén** (hairs)	Connie Stelle

One of the molecular markers used in the current study, AP6 did not completely amplify in *Ateles* and it was therefore not used in the population genetic analyses. The PCR characteristics of the STRPs were as follows. The final PCR volume reaction, for DNA extracted from blood by means of the phenol-chloroform procedure was 25 µl, with 3 µl of MgCl2 3 µM, 2.5 µl of Buffer 10x, 1 µl of dNTPs 1µM, 1 µl of of each primer (forward and reverse; 4 pmol), 13.5 µl of H2O, 2 µl of DNA, and 1 Taq Polymerase unit per reaction (1 µl). For the PCR reactions with DNA extracted from hairs and blood drops by means of the Chelex resine, the overall volume was 50 µl, with 20 µl of DNA and twofold amounts of $MgCl_2$, Buffer, dNTPs, primers and Taq Polymerase. The PCR reactions were carried out in a Geneamp PCR System 9600 Perkin Elmer thermocycler. The temperatures used were as follows: 95 °C for 5 minutes, 30 cycles of 1 minute at 95 °C, 1 minute at the most accurate annealing temperature (57 °C for AP40, 50 °C for AP68 and 52 °C for the remaining markers), one minute at 72 °C, and 5 minutes at 72 °C. The amplification products were kept at 4 °C until used. The PCR amplification products were run in denaturant 6 % polyacrilamide gels within a Hoefer SQ3 sequencer vertical chamber. Gels migrated for 2-3 hours depending on marker sizes, and were then stained with AgNO3 (silver nitrate). Every sixth line in the gel contained molecular markers (ϕ174 cut with Hind III and Hinf I).

Population Genetics Analyses

Several population genetics statistics were estimated through the microsatellite genotypes obtained. The mean number of alleles per locus and the expected heterozygosity (H) (NEI, 1973) were calculated for the *Ateles* species studied and statistically analyzed with a student t test. The expected heterozygosity values were arcsign transformed prior to stastical analysis (ARCHIE, 1985).

The Hardy-Weinberg equilibrium (H-W E) and the genotypic disequilibrium for the *Ateles* species studied were estimated using several different strategies. The WEIR and COCKERHAM (1984)´s F (W-C F) and the ROBERTSON and HILL (1984)´s f (R-H f) statistics were used to calculate the degree of excess or deficit, of homo- and heterozygous within each one of the populations considered. To measure the exact probabilities of these statistics, the Markov chain method, with a 10,000 dememorization number, 200 batches and 10,000 iterations per batch, was used, following the Genepop v. 3.1 program (RAYMOND and ROUSSET, 1995). The H-W E was simultaneously analyzed by locus and species using Fisher´s method (RAY-

MOND and ROUSSET, 1995). The gametic disequilibrium among loci-pairs was studied by means of the Markov chains and the Fisher´s procedures with the same parameters as those used for the H-W E.

The genetic heterogeneity among the *Ateles* species was studied globally for each marker and for species pairs. The first strategy used the mean gene frequencies of the 5 microsatellites studied, exact tests with Markov chains, 10,000 dememorizations parameters, 200 batches, and 10,000 iterations per batch. The second strategy used the Wright F-statistics (WRIGHT, 1951) with the MICHALAKIS and EXCOFFIER (1996)´s procedure. The standard deviations of the F-statistics were calculated using a jackknifing over loci and the 95 and 99 % confidence intervals were measured by means of bootstrapping over loci. Two procedures were used to measure the significance of F_{st}. The first one used 10,000 randomizations of overall alleles sampled and assumed random mating within species by means of the G test (GOUDET et al., 1996). The second procedure used 10,000 randomizations of genotypes among species and did not assume random mating within species by means of the log-likelihood G test (GOUDET et al., 1996). The significance of F_{is} and F_{it} was also found by using 10,000 randomizations of alleles within samples and in the overall sample. Additionally, the gene diversity analysis of NEI (1973) was estimated to measure the gene heterogeneity between the *Ateles* species analyzed. These analyses are useful to determine which STRPs more clearly discriminate among the species of *Ateles* studied and to determine the degree of gene variability within each species studied relative to the whole genus. Possible theoretical gene flow estimates among the *Ateles* species studied were measured using the private allele model (SLATKIN, 1985; BARTON and SLATKIN, 1986).

Another population genetics analysis was focused on the detection of recent bottleneck events using the most recently derived theory generated by CORNUET and LUIKART (1996), and LUIKART et al. (1998). The species, which experienced a recent bottleneck, simultaneously decreases the allele number and the expected levels of heterozygosity. Nevertheless, the allele number (ko) is reduced faster than the expected heterozygosity. Therefore, the value of the expected heterozygosity calculated through the allele number (Heq) is lower than the obtained expected heterozygosity (He). For neutral markers, in a population in gene mutation drift equilibrium, there is an equal probability that a given locus has a slight excess or deficit of heterozygosity in regard to the heterozygosity calculated from the number of alleles. In contrast, in a bottlenecked population, a large fraction of the loci analyzed will exhibit a significant excess of the expected heterozygosity. To measure this probability, four diverse procedures were used as follows: sign test, standardized difference test, Wilcoxon´s signed rank test and graphical descriptor of the shape of the allele frequency distribution. A population, which did not suffer a recent bottleneck event, will yield a L-shape distribution (such as expected in a stable population in mutation-gene drift equilibrium), whereas a recently bottlenecked population will show a mode-shift distribution. The Wilcoxon´s signed rank test probably has its greatest power when the number of loci analyzed is low, such as in the current case. The BOTTLENECK program was used to test for historical bottlenecks.

A first approximation to estimate historical effective numbers was accomplished using a maximum likelihood estimate of θ (= $4N_e\mu$) following the original formulation of GRIFFITHS and TAVARÉ (1994). N_e is the historical effective number of the

species studied and μ is the mutation rate per generation. If the value of μ is known, N_e can be calculated, which will indicate historical patterns of reproduction and population size of *Ateles* species populations. The mutation rates within dinucleotide repetitions vary among species. For example, the mutation rates for humans, pigs, and rats were estimated to be around 5.6×10^{-4} (WEBER and WONG, 1993), 7×10^{-5} (ELLENGREN, 1995), and 1.5×10^{-4} (SERIKAWA, 1992), respectively. Thus, to obtain a wide range of feasibly effective numbers in the *Ateles* species analyzed, the mutation rates used in this study ranged from 5.6×10^{-4} to 7×10^{-5}. For a given value of θ, the likelihood of observing $\underline{k}$ alleles in a size sample of $\underline{n}$ is $L(k, n / \theta) = S^k_n \, q^k / [\theta (1 + \theta) (2 + \theta) \ldots..(n\text{-}1 + \theta)$, where S^k_n is a function of $\underline{k}$ and $\underline{n}$ (EWENS, 1979). The maximum estimate of θ is derived by using the expression $\theta = k / [(1/\theta) + (1/(1+\theta)) + (1/(2 + \theta)) + \ldots.. + (1/(n\text{-}1 + \theta))]$ and setting L to zero $(k, n / \theta)$. A general Monte Carlo procedure introduced by GRIFFITHS and TAVARÉ (1994) offered approximate probabilities of the different θ values of the functions obtained. The historical effective numbers were calculated from the approximated function with the highest probabilities.

The maximum likelihood procedure with a Markov chain recursion method (NIELSEN, 1997) was used to calculate the second historical effective number for each one of the *Ateles* species studied. Probable θ values were calculated using NIELSEN's (1997) model based on the equation $L(\theta) = P(\varpi / \theta)$, where ϖ is a vector. A one step determination mutation model that was typical of microsatellites was also adopted (NIELSEN, 1997). Mathematical expressions by OHTA and KIMURA (1973) and WEHRHAHN (1975) were used to calculate the probability that an allele chosen at random was $\underline{m}$ repetitions higher than other allele chosen at random. The recursivity of the coalescence theory was applied to obtain the likelihood functions of θ for samples of a determined size. The coalescence time between two alleles was exponentially distributed with a mean equal to 1 and the conditional number of mutations in each lineage followed a Poisson distribution with a mean of $\theta_t/2$. It is feasible to calculate the probabilities of observing an allele sample that was determined by recursion in the previous generations and by considering the allele genealogies of the sample and a sum of all the previous possible states. This calculation is completed by conditioning the last event through mutation or coalescence and by using a symmetric random walk of k-allele states that reflect barrier types. The probability $q(\varpi)$ of the sample is determined by the addition of all the previous possible states multiplied by the transition probability of these states relative to the current state. With the chosen mutation model (uni-step), this probability is $\theta(\varpi) = (\theta/(n + q - 1)) \Sigma (\nu_i + 1/(n)\ 1/2\ q(\varpi + \varepsilon_i - \varepsilon_j) + (n - 1)/(n + \theta - 1) \Sigma (n_j - 1)/(n - 1)\ q(\varpi - \varepsilon_j)$, where e_i is an unity vector which adds values equals to 1 to the entry of *i* in ϖ. This recursive procedure is determined with: 1) the probability that the last event before the present moment is a mutation and that a mutational or a coalescence event has occurred previously $(q/(n + \theta - 1)$, 2) the probability that a coalescence event happened after a previous mutation or coalescence has already occurred, 3) the probability that a mutation occurs in an *i* allele, given that a mutation occurred $((n\text{-}1)/(n+\theta\text{-}1)$, of $(n_i\text{-}1)/(n\text{-}1)$, and 4) the probability that two alleles belonging to the *j* state will be coalescent, given a coalescence event has occurred $((n_j - 1)/ (n - 1))$. When a mutation event has occurred in the *i* state, there is a 0.50 probability that the *i* state will change to a *j* state.

The GRIFFITHS and TAVARÉ (1994)´s procedure was applied to evaluate the likelihood functions based on the expression recursion which led to the calculation of q (ϖ) with a Monte Carlo method. The MISAT program (NIELSEN, 1997) was used to estimate the likelihood surfaces for θ. The 5 % confidence interval was calculated by multiplying the log likelihood of the maximum likelihood value by two. A grid size of 40 with a previous θ calculation and method of the moments (θ_0) in a mutation one-step model with 1,000,000 Markov chains was used. The θ value with the least negative log likelihood is the estimate of the θ maximum likelihood. From this value, N_e was calculated for each *Ateles* species studied. In addition, the largest possible multi-step mutation percentages (ranging from 0 to 0.5) were calculated through the maximum likelihood of θ by means of 3,000,000 Markov chains. We analyzed the different mutation rates possible that affected each one of the microsatellites for each *Ateles* species studied. We tested the hypothesis $\theta_1 = \theta_2 = \theta$ (the values of θ for two different microsatellites) using a likelihood ratio test with the expression -2 log $[L_1(\theta)L_2(\theta)]/[L(\theta_1,\theta_2)]$, following $\alpha\ \chi^2$ with one degree of freedom. A probability lower than $\alpha = 0.05$ indicates that both microsatellites have different mutation rates. Likewise, we measured if the multi-step mutation models' estimates were significant improvements over the uni-step mutation models within each *Ateles* species. The likelihood ratio of -2 log $[L(\theta, p = 0)/L(\theta, p)]$ was applied to the maximum likelihood obtained multi-step p percentage. Large samples have a value of χ^2 with one degree of freedom with the null hypothesis $p = 0$. A probability lower than $\alpha = 0.05$ indicates that the multi-step mutation percentage is significantly different from the uni-step mutation model and this last model is then rejected.

Craneometric Analyses

For the craniometrical data, 38 quantitative cranial traits were measured on 27 mature adult *Ateles* skulls representing all the taxa of this genus living in Colombia (Appendix 1). The distribution of skulls included: one *A. geoffroyi*, 3 *A. hybridus*, 8 *A. belzebuth*, 13 *A. fusciceps robustus* and 2 *Ateles* sp. In the first analysis carried out, the craniometric distances were obtained without using any type of standardization or transformation to determine the simultaneous impact of size and shape among the individuals analyzed. Different distance matrices (correlation, variance-covariance, and Manhattan distances; SNEATH and SOKAL, 1973; MARCUS, 1990) were calculated among the individuals analyzed. Each one of these procedures has different mathematical properties, which must be evaluated in order to determine the effects on the obtained results. The UPGMA algorithm was applied to each one of the matrices in order to construct a phenogram which showed the relationships among the skulls analyzed. The cophenetic correlation coefficient was calculated for each one of these trees. A strict consensus tree was used to analyze the degree of similarity among these diverse phenograms (ROHLF, 1982). Only the clusters present in all trees were included in this technique. A graphic matrix ("Minimum Spanning tree") was calculated among the individuals studied (GOWER and ROSS, 1969; ROHLF, 1970) in order to determine their phenotypic relationships. A Component Principal Analysis (PCA) of the standardized data was carried out to establish the relationships among the individuals and the influence of size and shape. Therefore, the same weight was given to all of the morphometric variables. A high and positive correlation of all variables with the first component usually denotes differences of

size among the individuals. Otherwise, the following components mainly describe the shape of the individuals. The connections between the second and third components were analyzed to determine the relationships among individuals that were exclusively based on shape.

Results

Private alleles and allele sets shared by several Ateles species

AP68: This marker globally showed six alleles for *Ateles* (Table 2). The population which presented a major quantity of alleles (5) was *A. fusciceps robustus* located in Antioquia, Sucre, Córdoba and Atlántico. An allele of 166 bp was only found in the two populations of *A. fusciceps* and an allele of 168 bp was only discovered in *A. belzebuth belzebuth* and in *A. hybridus*. It is also interesting to note that at this marker a 178 bp allele was presented in only one of the two *A. fusciceps* populations and in the sample of *A. geoffroyi vellerosus*.

Table 2: Alleles found in 8 *Ateles* populations. The alleles are indicate in base pairs (bp). * Private allele (= alleles found in one only population).

	Markers				
	AP68	**AP74**	**AP40**	**D5S117**	**D8S165**
Ateles belzebuth belzebuth	168, 174, 176 (3 alleles)	130, 132, 136*, 142*, 158, 162, 166 (7 alleles)	176 (1 allele)	145, 147, 149, 153, 155, 157 (6 alleles)	145, 147, 149, 155 (4 alleles)
Ateles fusciceps robustus – Chocó	166, 174, 176 (3 alleles)	150, 152, 154, 156, 158, 160, 162, 164*, 168 (9 alleles)	176 (1 allele)	145, 146, 147, 149 (4 alleles)	143, 145, 147, 151, 155, 157, 159 (7 alleles)
Ateles fusciceps robustus – Antioquia, Atlántico, Sucre, Córdoba	166, 172, 174, 176, 178 (5 alleles)	130, 132,134*, 138, 144, 148, 152, 154, 156, 158, 160, 162, 166, 168, 170* (15 alleles)	176 (1 allele)	145, 147, 149, 150, 155, 157 (6 alleles)	141, 143, 145, 147, 149, 151, 152*, 153, 155, 157* (10 alleles)
Ateles fusciceps fusciceps	174 (1 allele)	156, 158 (2 alleles)	176 (1 allele)	149, 155 (2 alleles)	----
Ateles hybridus	168, 172, 174, 176 (4 alleles)	130, 132, 148 (3 alleles)	176 (1 allele)	146, 147, 152*, 153, 157, 161* (6 alleles)	143, 145, 147, 148*, 151, 153 (6 alleles)

	Markers				
	AP68	**AP74**	**AP40**	**D5S117**	**D8S165**
Ateles chamek	172, 174, 176 (3 alleles)	130, 132, 144, 146*, 150, 152 (6 alleles)	174*, 176 (2 alleles)	141*, 143*, 146, 148*, 149, 150,153 (7 alleles)	137*, 139*, 141, 142*, 143, 145, 147, 149, 151 (9 alleles)
Ateles paniscus paniscus	176 (1 allele)	154, 160 (2 alleles)	176 (1 allele)	140* (1 allele)	----
Ateles geoffroyi vellerosus	176, 174, 178 (3 alleles)	150, 154, 156, 158, 160, 162 (6 alleles)	176 (1 allele)	149, 151*, 153, 157 (4 alleles)	143, 147, 149, 151 (4 alleles)

AP74: This marker presented the highest number of alleles for the microsatellites analyzed in *Ateles* (20 alleles) and the *A. fusciceps* population displayed the highest number of alleles (15). Several private alleles were detected such as a 164 bp allele in one of the *A. f. robustus* populations (Chocó), a 168 bp allele in both *A. f. robustus* populations, and a 170 bp in the second *A. f. robustus* population. Additionally, two private alleles were located in *A. b. belzebuth* (136 and 142 bp), while another private allele (146 bp) was only located in *A. chamek*. It should be noted that several *Ateles* taxa had relatively small-sized alleles compared with the majority of the alleles determined at this marker. This occurred for example in *A. hybridus*, *A. b. belzebuth*, *A. chamek* and the second population of *A. f. robustus* with 130 and 132 bp alleles. All the other *Ateles* taxa presented alleles that were larger than 150 bp (*A. paniscus paniscus*, *A. f. fusciceps*, *A. geoffroyi* and the first population of *A. f. robustus* (Chocó)). *A. hybridus* only presented the smallest alleles, whereas *A. b. belzebuth*, *A. chamek* and the second population of *A. f. robustus* simultaneously presented small and large sized alleles. The characteristic of allele size is fundamentally important to understand the biogeographic evolution of *Ateles*, as we will comment on in the discussion.

AP40: This marker was practically monomorphic and was therefore not useful in the determination of genetic structure within *Ateles* species or for the determination of possible dispersion routes. All species yielded an allele of 176 bp, with the exception of *A. chamek*, where one individual presented a 174 bp allele. Therefore, this microsatellite does not have any power to discriminate among *Ateles* species. Previously, RUIZ-GARCÍA et al. (2004) demonstrated a constrictive mutation or natural selection event on this marker.

D5S117: This marker yielded 15 alleles for *Ateles*. *A. chamek* was the taxa that had the largest quantity of different alleles at this marker (7). Several private alleles were discovered: *A. hybridus* presented two (152 and 161 bp), *A. chamek* showed three (141, 143 and 148 bp), *A. geoffroyi* had one (151 bp), and *A. p. paniscus* had one (140 bp).

D8S165: This marker also showed 15 alleles for *Ateles*. Again the first *A. f. robustus* population presented the highest number of different alleles (10). At this population, one private allele was determined (152 bp). Both *A. f. robustus* popula-

tions also included a common private allele of 157 bp. *A. hybridus* also presented an exclusive allele of 148 bp, whereas *A. chamek* showed three private alleles of 137, 139 and 142 bp. It is remarkably to note that the smallest size alleles determined were in the sample of this last species and that this species presented the second highest number of alleles at this locus after *A. f. robustus*.

Average number of alleles per locus and expected heterozygosity

With the exception of *A. fusciceps fusciceps* and *A. p. paniscus* (n = 2), the highest average allele number per locus occurred in the northern Atlantic *A. f. robustus* population ($n_A = 7.4 \pm 5.31$) (Table 3), followed by *A. chamek* ($n_A = 5.4 \pm 2.88$). *A. geoffroyi* had the lowest n_A value (3.6 ± 1.81). However, there was no significant difference between these extreme values ($t = 1.354$, 8 df, $P = 0.40$). The highest and lowest expected heterozygosity levels (h) occurred in *A. chamek* ($h = 0.752 \pm 0.193$) (Table 3) and *A. hybridus* ($h = 0.519 \pm 0.369$) respectively. There was no significant difference between values ($t = 1.185$, 8 df, $P = 0.51$).

Table 3: Average number of alleles per locus and mean expected heterozygosity for each *Ateles* taxa studied.

Species	**Average number of alleles per locus (n_A)**	**Mean Expected heterozygosity (h)**
Ateles belzebuth belzebuth	4.2 ± 2.38	0.565 ± 0.387
Ateles fusciceps robustus (total)	8.2 ± 6.09	0.601 ± 0.358
Ateles fusciceps robustus (Chocó)	4.8 ± 3.19	0.548 ± 0.294
Ateles fusciceps robustus (Antioquia, Bolívar, Sucre, Atlántico and Córdoba)	7.4 ± 5.31	0.661 ± 0.276
Ateles hybridus	4.0 ± 2.12	0.519 ± 0.369
Ateles chamek	5.4 ± 2.88	0.752 ± 0.193
Ateles geoffroyi vellorosus	3.6 ± 1.81	0.569 ± 0.360

Hardy-Weinberg equilibrium (H-W E)

There was homozygous excess in three species (*A. fusciceps robustus*, χ^2 = infinity 8 df, $P < 0.00000$; *A. belzebuth belzebuth*, $\chi^2 = 18.7$, 8 df, $P = 0.0167$; and *A. chamek*, χ^2= infinity, 8 df, $P < 0.00000$) (Table 4). For the first species, the markers showing homozygous excess were AP74, D5S117 and D8S165. However, if the two *A. fusciceps robustus* populations studied were analyzed separately, the first population (Chocó) was at H-W E at all of the markers studied separately or grouped together ($\chi^2 = 12.7$, 8 df, $P = 0.1223$). In contrast, all of the markers that were analyzed in the second population (Antioquia, Sucre, Córdoba and Atlántico) significantly deviated from H-W E (χ^2 = infinite, 8 df, $P < 0.000000$). Knowledge of this result is critical in order to understand some of the evolutionary events within *A. f. robustus*, as we will later discuss. The markers AP74 and D8S165 in *A. b. belzebuth*, and all four poly-

morphic markers in *A. chamek* showed significant homozygous excess. On the other hand, *A. hybridus* and *A. geoffroyi* did not have any microsatellites with significant deviations from H-W E. However, when the results were analyzed by locus taken simultaneously, none were in H-W E. This provides evidence of reproductive isolation among these populations. Similarly, there was no evidence of H-W E when all loci and all species were analyzed together (χ^2 = infinity, 8 df, P < 0.00000). Neither significant cases of gametic disequilibrium among microsatellite pairs were discovered within each species studied nor significant cases of gametic disequilibrium for each microsatellite pair across all species studied taken together were detected with the Fisher´s method.

Table 4: Deviations from Hardy-Weinberg equilibrium for all *Atetes* taxa studied with exact tests. F_{is}-W & C = F_{is} with the WEIR and COCKERHAM (1984) procedure. F_{is} – R & H = F_{is} with the ROBERTSON and HILL (1984) procedure. * Significant probabilities at p < 0.05. In all cases, the significant values were by homozygous genotype excess.

Species	**Marker**	**Hardy-Weinberg Equilibrium Test**		
		Probality value	**F_{is}-W & C**	**F_{is}- R & H**
A. fusciceps robustus	AP 68	0.266 ± 0.0025	-0.036	0.092
	AP 74	0.000 ± 0.0000*	0.384	0.181
	AP 40	--	--	--
	D5S117	0.000 ± 0.0000*	0.302	0.385
	D8S165	0.014 ± 0.0013*	0.149	0.120
All markers taken together (Fisher's method): χ^2= 8; df= 8; P= 0.000001*				
Ateles b. belzebuth	AP 68	0.0588 ± /	0.652	0.558
	AP 74	0.0387 ± 0.002*	0.411	0.286
	AP 40	--	--	--
	D5S117	0.8130 ± 0.0021	0.125	0.107
	D8S165	0.0476 ± /*	0.583	0.516
All markers taken together (Fisher's method): χ^2= 18.7; df= 8 ; P= 0.0167*				
Ateles hybridus	AP 68	1 ± /	0.066	0.050
	AP 74	0.5954 ± /	0.136	0.096
	AP 40	--	--	--
	D5S117	0.7256 ± 0.0023	-0.179	-0.143
	D8S165	0.2739 ± 0.0033	-0.070	-0.055
All markers taken together (Fisher's method): χ^2= 4.3; df= 8 ; P= 0.8321				
Ateles chamek	AP 68	0.0085 ± /*	0.461	0.579
	AP 74	0.0182 ± 0.001*	0.444	0.430
	AP 40	--	--	--
	D5S117	0.0000 ± 0.000*	0.673	0.755
	D8S165	0.0006 ± 0.000*	0.596	0.606
All markers taken together (Fisher's method): χ^2= 8; df= 8 ; P= High. Sign.*				

Species	**Marker**	**Hardy-Weinberg Equilibrium Test**		
		Probality value	**F_{is}-W & C**	**F_{is}- R & H**
Ateles geoffroyi vellerosus	AP 68	0.1715 ± /	0.355	0.220
	AP 74	0.0996 ± 0.0017	0.029	0.011
	AP 40	--	--	--
	D5S117	0.4667 ± /	0.273	0.250
	D8S165	--	--	--
All markers taken together (Fisher's method): χ^2= 9.7; df= 6 ; P= 0.1396				
All locus, all populations (Fisher's method): χ^2= 8; df= 32 ; P= High. Sign.**				

Genetic heterogeneity among Ateles taxa

Four of the five microsatellite markers that amplified nicely in *Ateles* showed significant genetic heterogeneity (AP68, AP74, D5S117 and D8S165; Table 5). Only AP40 was not efficient for discriminating among the seven *Ateles* taxa studied. The same analysis applied to species pairs revealed significant differences at AP68, AP74 and D5S117 (33, 62 and 60 % of comparison cases, respectively). Therefore, these last two microsatellites presented the highest power to discriminate the *Ateles* taxa studied (Table 5). *A. chamek* was significantly different from all other species at both AP68 and AP74. *A. hybridus* was also significantly different from other species at AP74. Four species (*A. fusciceps robustus*, *A. belzebuth belzebuth*, *A. hybridus* and *A. chamek*) differentiated from the others at D5S117. *A. f. robustus* and *A. belzebuth* were also differentiated from other species at D8S165.

Table 5a: Genetic heterogeneity for each marker studied. Only AP40 did not show significant heterogeneity between all the *Ateles* taxa studied. Df = degree of freedom.

Locus	**Probability-value ± S.E**
AP68	0.00000 ± 0.00000
AP74	0.00000 ± 0.00000
AP40	0.55991 ± 0.00599
D5S117	0.00000 ± 0.00000
D8S165	0.00000 ± 0.00000
Test combination (Fisher's method): χ^2 =4; df =10; P = 0.00001	

Table 5b: Genetic heterogeneity among *Ateles* species pairs for each one of the markers studied. A, S ,B, C, At = Population of *Ateles fusciceps robustus* from Antioquia, Sucre, Bolivar, Córdoba and Atlántico Departments of Colombia. *Significant heterogeneity ($P < 0.05$).

	***Ateles fusciceps robustus* Chocó**	***Ateles fusciceps robustus* A,S,B,C,At**	***Ateles belzebuth belzebuth***	***Ateles hybridus***	***Ateles chamek***	***Ateles geoffroyi vellerosus***	***Ateles paniscus paniscus***	***Ateles fusciceps fusciceps***
***Ateles fusciceps robustus* Chocó**								
AP68	---	0.00172*	0.00692*	0.00722*	0.00093*	0.02152*	---	0.32293
AP74	---	0.00000*	0.00002*	0.00000*	0.01837*	0.12310	0.39938	0.54870
AP40	---	---	---	---	0.22208	---	---	---
D5S117	---	0.01870*	0.01906*	0.00014*	0.00005*	0.00015*	0.00308*	0.05860
D8S165	---	0.01855*	0.06948	0.00397*	0.01934*	0.01666*	---	---
***Ateles fusciceps robustus* A, S, B, C, AT**								
AP68	---	---	0.19828	0.26439	0.00001*	0.12044	---	0.16180
AP74	---	---	0.19294	0.41610	0.00064*	0.00001*	0.13475	0.10838
AP40	---	---	---	---	0.05698	---	---	---
D5S117	---	---	0.00163*	0.00000*	0.00000*	0.00002*	0.00042*	0.21391
D8S165	---	---	0.82151	0.10517	0.00000*	0.00302*	---	---
Ateles belzebuth belzebuth								
AP68	---	---	---	0.83458	0.00013*	0.00426*	---	0.05300
AP74	---	---	---	0.00480*	0.03268*	0.00029*	0.10454	0.18252
AP40	---	---	---	---	0.28500	---	---	---
D5S117	---	---	---	0.04576*	0.00027*	0.16029	0.01302*	1.00000
D8S165	---	---	---	0.00547*	0.01424*	0.01168*	---	---
Ateles hybridus								
AP68	---	---	---	---	0.00096*	0.02041*	---	0.19422
AP74	---	---	---	---	0.00002*	0.00000*	0.00595*	0.00596*
AP40	---	---	---	---	0.28552	---	---	---
D5S117	---	---	---	---	0.00000*	0.10356	0.01970*	0.03229*
D8S165	---	---	---	---	0.10717	0.09352	---	---
Ateles chamek								
AP68	---	---	---	---	---	0.04814*	---	1.00000
AP74	---	---	---	---	---	0.00017*	0.09781	0.10084
AP40	---	---	---	---	---	---	---	---
D5S117	---	---	---	---	---	0.00570*	0.01150*	0.19456
D8S165	---	---	---	---	---	0.26708	---	---
Ateles geoffroyi vellerosus								
AP68	---	---	---	---	---	---	---	0.56507
AP74	---	---	---	---	---	---	1.00000	0.59867
AP40	---	---	---	---	---	---	---	1.00000
D5S117	---	---	---	---	---	---	0.14249	0.57123
D8S165	---	---	---	---	---	---	---	---

	***Ateles fusciceps robustus* Chocó**	***Ateles fusciceps robustus* A,S,B,C,At**	***Ateles belzebuth belzebuth***	***Ateles hybridus***	***Ateles chamek***	***Ateles geoffroyi vellerosus***	***Ateles paniscus paniscus***	***Ateles fusciceps fusciceps***
Ateles paniscus paniscus								
AP68	---	---	---	---	---	---	---	---
AP74	---	---	---	---	---	---	---	---
AP40	---	---	---	---	---	---	---	---
D5S117	---	---	---	---	---	---	---	0.33275
D8S165	---	---	---	---	---	---	---	---
Ateles fusciceps fusciceps								
AP68	---	---	---	---	---	---	---	---
AP74	---	---	---	---	---	---	---	---
AP40	---	---	---	---	---	---	---	---
D5S117	---	---	---	---	---	---	---	---
D8S165	---	---	---	---	---	---	---	---

Table 5c: Genetic heterogeneity per species pairs for all the DNA microsatellites studied simultaneosly. *Significant probabilities at $P < 0.05$. **Significant probabilities with a multiple Bonferroni test ($P < 0.002381$). Probability values obtained after 10.000 permutations.

Species	***Ateles fusciceps robustus***	***Ateles belzebuth belzebuth***	***Ateles hybridus***	**Ateles *chamek***	***Ateles geoffroyi vellerosus***	***Ateles paniscus paniscus***	***Ateles fusciceps fusciceps***
Ateles fusciceps robustus	---	0.02850*	0.00010**	0.00010**	1.00000	0.84730	0.76700
Ateles belzebuth belzebuth	---	---	1.00000	1.00000	1.00000	0.36570	0.44000
Ateles hybridus	---	---	---	1.00000	0.99900	0.36320	0.13210
Ateles chamek	---	---	---	---	0.74050	0.40310	0.03460*
Ateles geoffroyi vellerosus	---	---	---	---	---	0.59940	0.00010**
Ateles paniscus paniscus	---	---	---	---	---	---	0.00010**
Ateles fusciceps fusciceps	---	---	---	---	---	---	---

Other results were collected upon analysis of the two *A. f. robustus* populations separately. For example, at AP68 the first *A. f. robustus* population significantly differed from the second population as well as the remaining *Ateles* taxa with the exception of *A. f. fusciceps*.

In contrast, the second *A. f. robustus* population did not significantly differ from *A. belzebuth belzebuth*, *A. hybridus* and *A. geoffroyi*. Both *A. f. robustus* populations, also significantly differed at AP74. The first populations was also significantly different from *A. b. belzebuth*, *A. hybridus* and *A. chamek* but not from *A. f. fusciceps* nor *A. geoffroyi*. Again, in contrast, the second *A. f. robustus* population did not differ from *A. b. belzebuth* or *A. hybridus*, did it did differ from *A. geoffroyi*. At D5S117, both *A. f. robustus* populations significantly differed. Also, both populations were significantly different from the remaining *Ateles* taxa studied with the exception of *A. f. fusciceps*. Both *A. f. robustus* populations significantly diverged at D8S165. The first population diverged from *A. hybridus*, *A. chamek* and *A. geoffroyi*, while the second one did not differentiate from *A. hybridus*. Therefore, globally, both *A. f. robustus* populations were dramatically different from each other with the first differentiated from *A. b. belzebuth* and *A. hybridus*, whereas the second one was comparatively similar with these taxa. The relationships of *A. geoffroyi* with the two *A. f. robustus* populations were variable depending on the markers used. When the theoretical gene flow was measured among all the *Ateles* taxa via the private allele method of BARTON and SLATKIN (1986), the value was considerably small (Nm = 0.688), which supports that these *Ateles* taxa are globally isolated reproductive units.

The expected gene diversity at the global genus (H_t = 0.675) was slightly lower than the same value obtained for other Neotropical primates such as *Alouatta* and *Lagothrix* (Table 6 and 7). The average gene diversity within each *Ateles* taxa studied was only slightly lower than the previous statistic (H_s = 0.604), which revealed that each *Ateles* taxa on average has a relatively high level of total gene diversity at the global genus. Although the genetic heterogeneity was significant (G_{st} = 0.105-0.123, depending on the method of calculation), it was relatively lower than that obtained in other Neotropical primate genera. The global F-statistics jackknifing over loci values (F_{it} = 0.318 ± 0.04, F_{is} = 0.256 ± 0.039) showed a significant homozygous excess at the global level of genus as well as within the species studied. Meanwhile, the significant but relatively small F_{st} value (0.084), indicates that the microsatellite divergence within the *Ateles* genus is limited because the divergence time among species is small (recent speciation) or some kind of constrictive natural selection is acting upon all or a fraction of the microsatellites studied. The significant homozygous excess supports that the major parts of the populations studied are reproductively isolated from each other. Furthermore, the Wahlund effect may have helped produced homozygous excess inside some taxa in agreement with H-W analyses. All individual values of F_{is} were significant for a Bonferroni adjusted level of α' = 0.00143 with the exception of AP68 and AP40. Also, all the F_{it} statistics were significant with the exception of AP40. For F_{st}, the situation was the same when an exact G-test, random mating within samples and 10,000 randomizations of alleles overall samples (only AP40 was not significant) were used. On the other hand, there was no significant heterogeneity in AP40 and D8S165 when 10,000 randomizations, non-random mating and a log-likelihood G test were used.

Table 6: Nei´s gene diversity analysis for five Microsatellite markers for all *Ateles* taxa studied. H_o = Observed heterozygosity; H_s = Average expected heterozygosity within species; H_t = Global expected heterozygosity for all the genera taken as at all. D_{st} = absolute genetic differentiation between the *Ateles* species studied. G_{st} = Relative genetic differentiation between the *Ateles* species regard to the total gene diversity found at the genera. H_t´, D_{st}´ and G_{st}´ are the same statistics corrected by sample size. G_{is} = deviations from Hardy-Weinberg equilibrium within species.

Markers	**H_o**	**H_s**	**H_t**	**D_{st}**	**D_{st}'**	**H_t'**	**G_{st}**	**G_{st}'**	**G_{is}**
AP68	0.302	0.472	0.583	0.110	0.132	0.605	0.189	0.219	0.361
AP74	0.674	0.827	0.902	0.075	0.088	0.915	0.083	0.096	0.185
AP40	0.100	0.091	0.098	0.007	0.009	0.100	0.076	0.093	-0.103
D5S117	0.603	0.825	0.911	0.086	0.100	0.925	0.094	0.108	0.270
D8S165	0.657	0.804	0.880	0.076	0.095	0.899	0.087	0.106	0.294
Overall	0.449	0.604	0.675	0.071	0.085	0.689	0.105	0.123	0.256

Table 7a: Estimation of the Wright F-Statistics for each one of the microsatellites analyzed.[1]Assuming Random Mating within simples (G-test 10.000 randomisations). [2]Not assuming Random Mating (log-likelihood G-test). Except certain cases for AP40, all other cases are significant.

Locus	**F_{it}**	**P-value**	**F_{is}**	**P-value**	**F_{st}**	**P-value[1]**	**P-value[2]**
AP68	0.235	0.0001	0.145	0.0333 -0.0208	0.106	0.0001	0.0001
AP74	0.393	0.0001	0.332	0.0001	0.092	0.0001	0.0010
AP40	0.237	0.0552 -0.0195	-0.289	0.9999	0.408	0.0557 -0.0207	0.0181 -0.0143
D5S117	0.364	0.0001	0.302	0.0001	0.089	0.0001	0.0001
D8S165	0.253	0.0001	0.212	0.0001	0.052	0.0001	0.0174
Overall loci	0.317	0.0001	0.253	0.0001	0.085	0.00010	0.0001

Table 7b: Average F-statistics values by means of jackknifing over populations for each marker.

Locus	**F_{it}**	**F_{is}**	**F_{st}**
AP68	0.146 ± 0.233	0.054 ± 0.214	0.082 ± 0.065
AP74	0.397 ± 0.028	0.402 ± 0.113	0.024 ± 0.127
AP40	0.383 ± 0.297	-0.605 ± 0.234	0.759 ± 0.396
D5S117	0.370 ± 0.077	0.302 ± 0.085	0.098 ± 0.031
D8S165	0.191 ± 0.125	0.156 ± 0.123	0.039 ± 0.020

Table 7c: Average F-statistics by means of jackknifing over loci and 99% confidence interval by means of bootstrapping.

	F_{it}	F_{is}	F_{st}
Jakknifing over loci	0.318 ± 0.040	0.256 ± 0.039	0.084 ± 0.013
Bootstrapping over loci 99 % confidence interval	0.235 – 0.391	0.128 – 0.324	0.058 – 0.120

Recent bottlenecks, historical effective numbers and microsatellite mutation models

There was no evidence of a recent bottleneck for any of the species studied, at least not for the small number of loci analyzed (Table 8). The Step-Wise mutation model did support the occurrence of an event for *A. f. robustus* that was the reverse of a bottleneck (T_2 = -2.5, P = 0.00621). This could indicate a recent population expansion, gene flow among different gene pools or Wahlund effect within the sample studied of this species. Therefore, the analysis was repeated with the two *A. f. robustus* populations separately. The findings for the first population (Chocó) were unique because the analyses (the standardized differences test and the Wilcoxon test) of the infinite mutation allele and the step-wise mutation models' results supported that the population had experienced a recent bottleneck. In contrast, the standardized differences test with the step-wise mutation model applied to second *A. f. robustus* population supported an event opposite to a recent bottleneck. This along with other results, provides evidence that this *A. f. robustus* population has experienced gene flow with other *Ateles* taxa as will be discussed later.

Table 8: Recent bottleneck analysis employing the Cornuet and Luikart (1996) procedure for all *Ateles* taxa studied. I. A. M = Infinite Allele Mutation Model. S. M. M = Step-Wise Mutation Model. K_o = Number of alleles found. H_e = Expected heterozygosity measured from allele frequencies. H_{eq} = Expected heterozygosity from the number of alleles determined. S.D. = Standard Deviation for the last measure. Prob = Probability of significant differences among H_e and H_{eq}. * Significant difference at P < 0.05 corresponding to a recent bottleneck event. ** Significant differences at P < 0.05 corresponding to some event contrary to a recent bottleneck. Only *A. fusciceps robustus* from Chocó showed some trends to cross a recent bottleneck. A, B, S, C, At = Population of *Ateles fusciceps robustus* from Antioquia, Bolivar, Sucre, Córdoba and Atlántico Departments of Colombia.

	Under the I.A.M					**Under the S.M.M**		
	Ko	**He**	**Heq**	**S.D.**	**Prob**	**Heq**	**S.D.**	**Prob**
Ateles fusciceps robustus								
AP68	5	0.552	0.492	0.167	0.4362	0.638	0.099	0.1676
AP74	17	0.850	0.846	0.052	0.4270	0.911	0.015	0.0040**
AP40	1	---	---	---	---	---	---	---
D5S117	7	0.739	0.614	0.138	0.1718	0.752	0.064	0.3280
D8S165	11	0.857	0.755	0.091	0.0556	0.855	0.031	0.4686

	Under the I.A.M					Under the S.M.M		
	Ko	**He**	**Heq**	**S.D.**	**Prob**	**Heq**	**S.D.**	**Prob**
SIGN TEST: I.A.M, P= 0.127; S.M.M, P= 0.195 STANDARDIZED DIFFERENCES TEST: I.A.M, T_2= 1.236, P= 0.108; S.M.M, T_2= -2.500, P= 0.006** WILCOXON TEST: I.A.M: Probability (one tail for H excess)= 0.031*; S.S.M: Probability (one tail for H excess)= 0.968 NORMAL L-SHAPED DISTRIBUTION								
***Ateles fusciceps robustus* (Chocó)**								
AP68	3	0.681	0.435	0.154	0.0212*	0.494	0.138	0.0436*
AP74	9	0.905	0.850	0.047	0.0470*	0.881	0.026	0.1742
AP40	1	---	---	---	---	---	---	---
D5S117	4	0.746	0.560	0.135	0.0270*	0.632	0.100	0.0800
D8S165	7	0.889	0.807	0.059	0.0136	0.844	0.036	0.0634
SIGN TEST: I.A.M, P= 0.120, S.M.M, P= 0.116 STANDARDIZED DIFFERENCES TEST: I.A.M, T_2= 2.777, P= 0.002*, S.M.M, T_2= 2.347, P= 0.009* WILCOXON TEST: I.A.M: Probability (one tail for H excess)= 0.031*, S.S.M: Probability (one tail for H excess)= 0.031* NORMAL L-SHAPED DISTRIBUTION								
***Ateles fusciceps robustus* (A, B, S, C, At)**								
AP68	5	0.505	0.504	0.163	0.4226	0.644	0.099	0.0900
AP74	15	0.789	0.834	0.057	0.1724	0.901	0.018	0.0002**
AP40	1	---	---	---	---	---	---	---
D5S117	6	0.734	0.578	0.146	0.1144	0.712	0.075	0.4624
D8S165	10	0.846	0.747	0.090	0.0720	0.842	0.034	0.4692
SIGN TEST: I.A.M, P= 0.467; S.M.M, P= 0.540 STANDARDIZED DIFFERENCES TEST: I.A.M, T_2= 0.685, P= 0.2466; S.M.M, T_2= -3.650, P= 0.0001** WILCOXON TEST: I.A.M: Probability (one tail for H excess)= 0.093; S.S.M: Probability (one tail for H excess)= 0.906 NORMAL L-SHAPED DISTRIBUTION								
Ateles belzebuth belzebuth								
AP68	3	0.307	0.473	0.142	0.2344	0.525	0.130	0.1262
AP74	7	0.825	0.824	0.052	0.4296	0.854	0.035	0.1804
AP40	1	---	---	---	---	---	---	---
D5S11	6	0.850	0.775	0.069	0.1034	0.813	0.046	0.2478
D8S165	4	0.758	0.666	0.094	0.1824	0.706	0.077	0.3204
SIGN TEST: I.A.M, P= 0.455; S.M.M, P= 0.518 STANDARDIZED DIFFERENCES TEST: I.A.M, T_2= 0.452, P= 0.325; S.M.M, T_2= -0.515, P= 0.303 WILCOXON TEST: I.A.M: Probability (one tail for H excess)= 0.437; S.S.M: Probability (one tail for H excess)= 0.906 NORMAL L-SHAPED DISTRIBUTION								

	Under the I.A.M					Under the S.M.M		
	Ko	**He**	**Heq**	**S.D.**	**Prob**	**Heq**	**S.D.**	**Prob**
Ateles hybridus								
AP68	4	0.458	0.540	0.140	0.2632	0.619	0.104	0.0880
AP74	3	0.384	0.414	0.161	0.4146	0.474	0.144	0.2650
AP40	1	---	---	---	---	---	---	---
D5S117	6	0.858	0.774	0.069	0.0522	0.814	0.046	0.1522
D8S165	7	0.853	0.779	0.071	0.0940	0.825	0.043	0.2938
SIGN TEST: I.A.M, P= 0.538; S.M.M, P= 0.538 STANDARDIZED DIFFERENCES TEST: I.A.M, T_2= 0.743, P= 0.228; S.M.M, T_2= -0.277, P= 0.390 WILCOXON TEST: I.A.M: Probability (one tail for H excess)= 0.156; S.S.M: Probability (one tail for H excess)= 0.562 NORMAL L-SHAPED DISTRIBUTION								
Ateles chamek								
AP68	3	0.579	0.394	0.167	0.1376	0.459	0.152	0.2394
AP74	6	0.864	0.827	0.048	0.2908	0.848	0.036	0.4596
AP40	2	0.333	0.436	0.112	1.0000	0.453	0.113	0.4608
D5S117	8	0.852	0.789	0.068	0.1448	0.840	0.037	0.4714
D8S165	9	0.874	0.850	0.046	0.3616	0.881	0.026	0.3400
SIGN TEST: I.A.M, P= 0.254; S.M.M, P= 0.643 STANDARDIZED DIFFERENCES TEST: I.A.M, T_2= 1.072, P= 0.141; S.M.M, T_2= 0.116, P= 0.453 WILCOXON TEST: I.A.M: Probability (one tail for H excess)= 0.078; S.S.M: Probability (one tail for H excess)= 0.406 NORMAL L-SHAPED DISTRIBUTION								
Ateles geoffroyi vellerosus								
AP68	3	0.588	0.429	0.156	0.1784	0.487	0.142	0.2782
AP74	6	0.835	0.796	0.061	0.3802	0.827	0.044	0.6198
AP40	0	---	---	---	---	---	---	---
D5S117	4	0.867	0.835	0.033	0.5304	0.840	0.033	0.6014
D8S165	2	0.500	0.546	0.075	1.0000	0.553	0.077	1.0000
SIGN TEST: I.A.M, P= 0.275; S.M.M, P= 0.362 STANDARDIZED DIFFERENCES TEST: I.A.M, T_2= 0.988, P= 0.161; S.M.M, T_2= 0.517, P= 0.302 WILCOXON TEST: I.A.M: Probability (one tail for H excess)= 0.062; S.S.M: Probability (one tail for H excess)= 0.093 NORMAL L-SHAPED DISTRIBUTION								

A first simulation coalescence method was used to determine the likelihood θ value (= $4 N_e \mu$) and to estimate long historical effective numbers. Two extreme mutation rates that were previously determined for dinucleotide microsatellites (5.6×10^{-4} and 7×10^{-5}, respectively) were used. The first *A. f. robustus* population had the highest effective number showing values that oscillated from 9,132 to 73,054 individuals

(Fig. 1). The second *A. f. robustus* population (Chocó) presented lower effective numbers than the first population (5,946-47,570 individuals). However, when both populations were studied simultaneously, these values were incremented (11,887-95,097 individuals), which supports that these two *A. f. robustus* populations have different gene pool compositions. This finding is probably due to the second population having a mixed influence. *A. hybridus* (3,310-26,481 individuals) and *A. geoffroyi* (3,560-28,480 individuals) had the lowest effective numbers while other *Ateles* species showed intermediate values.

The second coalescence method was also used to determine the historical effective numbers in the *Ateles* taxa studied (Table 9). The long effective numbers were very similar to those obtained with the previous method except for the magnitude of the effective numbers in *A. belzebuth*. This second method offered effective numbers for this species that were higher than the first method. The two species with the lowest effective numbers were again *A. hybridus* (2,642-21,137 individuals) and *A. geoffroyi* (2,900-23,199 individuals). An advantage of the second method is that it calculates θ for a multi-step mutation model and determines the most precise and significant multiple mutation percentage for each microsatellite analyzed as well as determines significant differences among the mutation rates of the microsatellites studied.

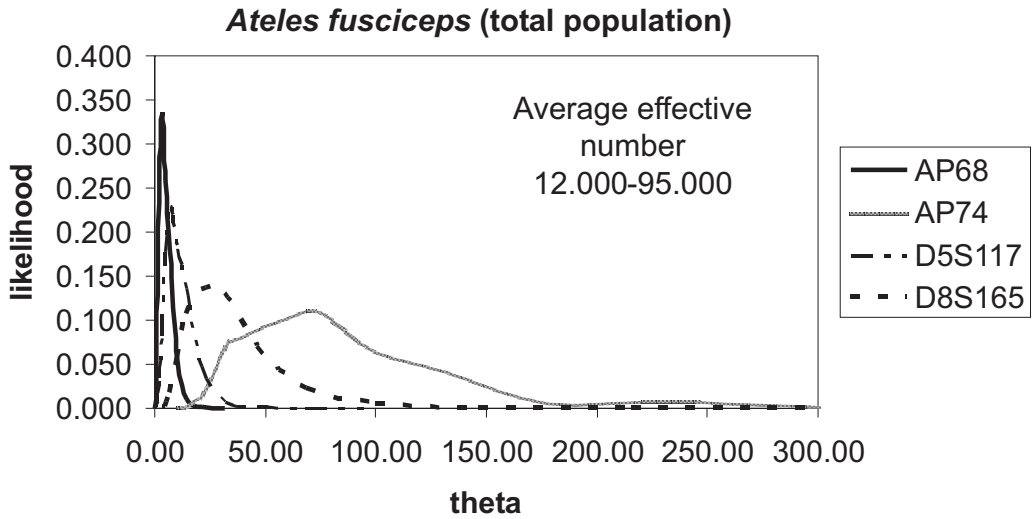
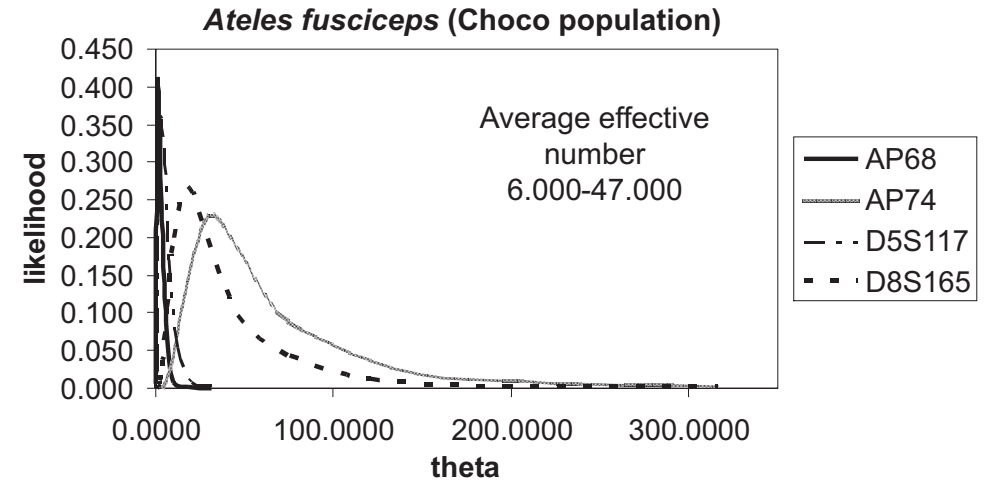
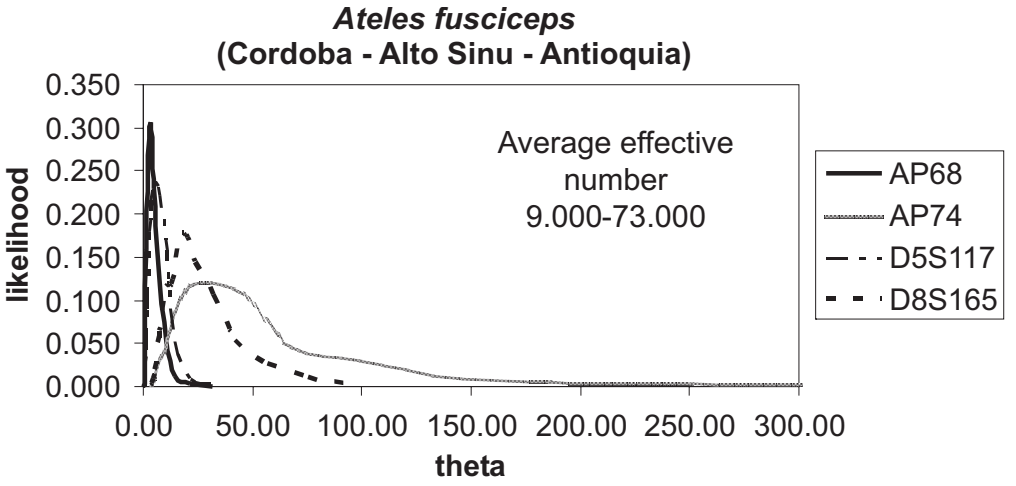
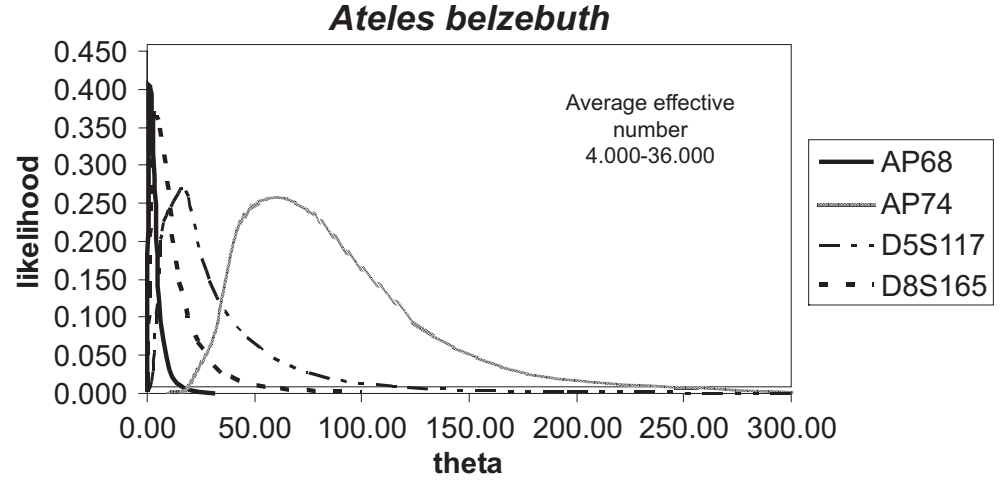
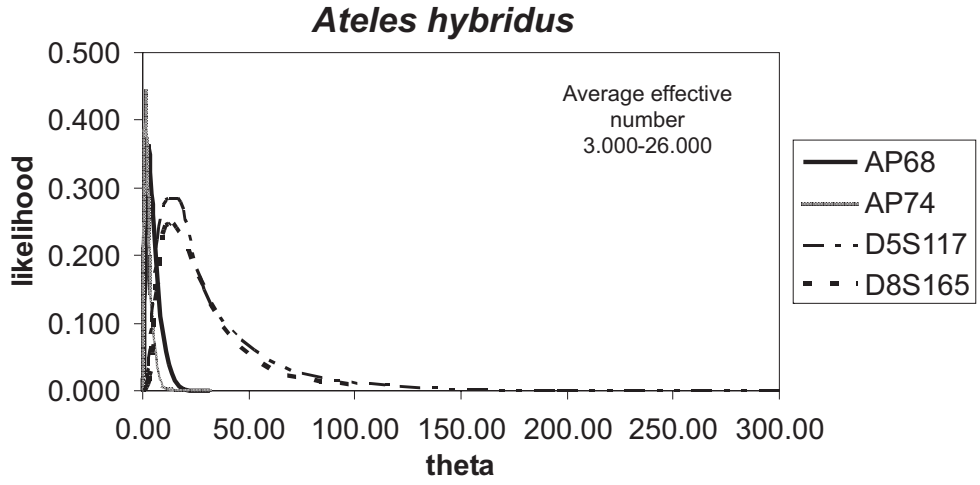


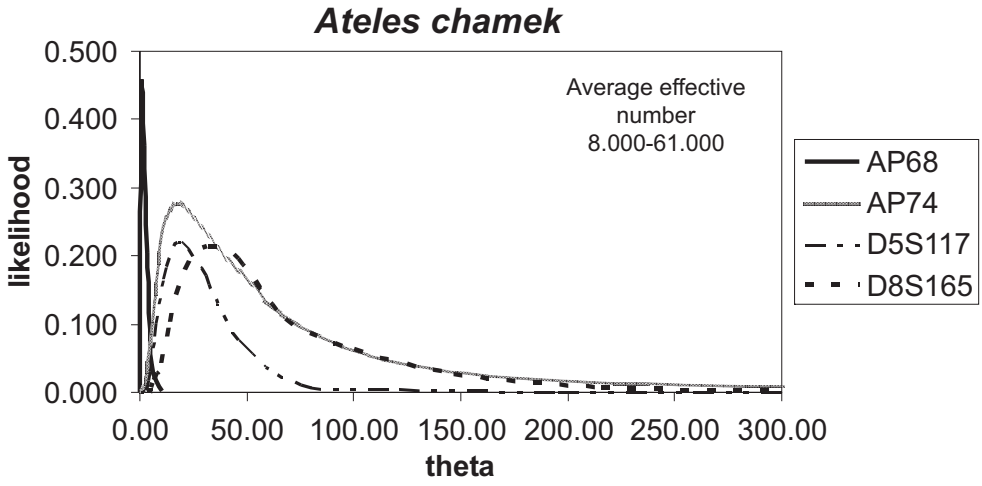
Fig. 1: Coalescence simulation to obtain the most likelihood θ value for four DNA Microsatellites in seven *Ateles* populations. Throughout the most likelihood values of θ, the average effective numbers were estimated using two probable mutation rates (5.6×10^{-4} and 7×10^{-5}, respectively).


Ateles fusciceps (Choco population)
likelihood
0.450
0.400
0.350
0.300
0.250
0.200
0.150
0.100
0.050
0.000
Average effective number 6.000-47.000
AP68
AP74
D5S117
D8S165
0.0000
100.0000
200.0000
300.0000
theta


Ateles fusciceps
(Cordoba - Alto Sinu - Antioquia)
likelihood
0.350
0.300
0.250
0.200
0.150
0.100
0.050
0.000
Average effective number 9.000-73.000
AP68
AP74
D5S117
D8S165
0.00
50.00
100.00
150.00
200.00
250.00
300.00
theta


Ateles belzebuth
likelihood
0.450
0.400
0.350
0.300
0.250
0.200
0.150
0.100
0.050
0.000
Average effective number 4.000-36.000
AP68
AP74
D5S117
D8S165
0.00
50.00
100.00
150.00
200.00
250.00
300.00
theta


Ateles hybridus
0.500
0.400
0.300
0.200
0.100
0.000
likelihood
Average effective number 3.000-26.000
AP68
AP74
D5S117
D8S165
0.00
50.00
100.00
150.00
200.00
250.00
300.00
theta


Ateles chamek
0.500
0.400
0.300
0.200
0.100
0.000
likelihood
Average effective number 8.000-61.000
AP68
AP74
D5S117
D8S165
0.00
50.00
100.00
150.00
200.00
250.00
300.00
theta

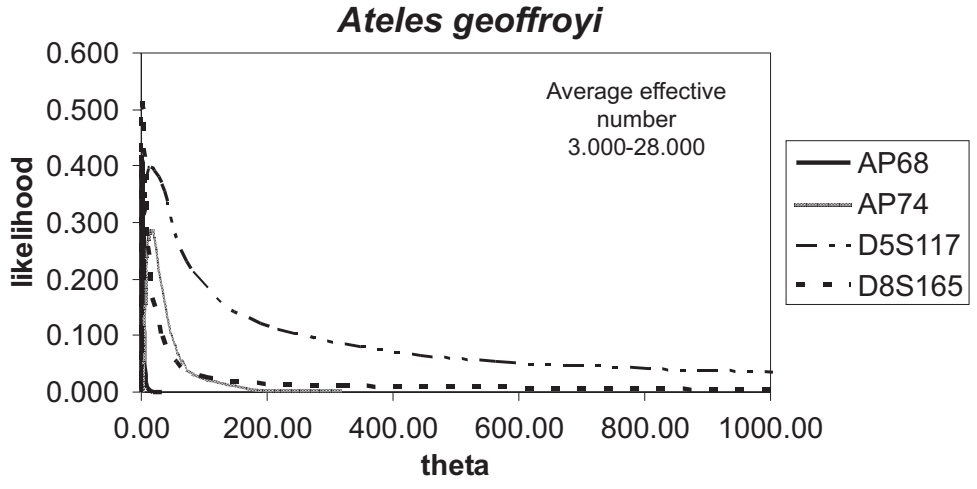

Ateles geoffroyi
0.600
0.500
0.400
0.300
0.200
0.100
0.000
likelihood
Average effective number 3.000-28.000
AP68
AP74
D5S117
D8S165
0.00
200.00
400.00
600.00
800.00
1000.00
theta

Table 9: Estimation of θ (= 4 $N_e\mu$) for different mutation models (uni-step and multi-step) affecting to the Microsatellite markers studied. Percentages of multiple mutations and their respective significances are shown. Also possible effective numbers are shown for two possible mutation rates per generation ($Ne^1 = 5.6 \times 10^{-4}$ and $Ne^2 = 7 \times 10^{-5}$). * Siginificant values at $P < 0.05$. A, B, S, C, At = The *A. fusciceps robustus* population from Antioquia, Bolivar, Sucre, Córdoba and Atlántico Departments from Colombia. *Ateles hybridus* and *Ateles geoffroyi* showed the lowest effective numbers.

Markers	**UNI-STEP MODEL**			**MULTI-STEP MODEL**			
	θ_0	θ	**ln likelihood**	θ	**ln likelihood**	**multiple mutation%**	χ^2
***Ateles fusciceps robustus* (Chocó)**							
AP86	8.507	2.5096	-12.3055	1.7015	-12.2219	20	0.1670
AP74	19.030	36.3478	-19.3931	25.5006	-21.6175	5	4.4480*
D5S117	1.181	1.8068	-6.50539	1.8069	-6.5071	0	0.0010
D8S165	13.758	18.4360	-15.8981	13.2078	-16.8726	5	1.9489
	10.619	14.775	Ne^1=6.596	10.5542	Ne^1=4.712	7.5	
	± 7.618	± 16.303	Ne^2=52.768	± 11.333	Ne^2=37.693	± 8.66	
***Ateles fusciceps robustus* (A, B, S, C, At)**							
AP86	1.504	3.30166	-18.0948	3.1588	-16.2754	0.075	3.6387
AP74	96.789	65.3327	-106.6797	92.9177	-120.4659	0.075	27.5723*
D5S117	2.615	4.24917	-17.2144	4.00075	-17.1448	0	0.1391
D8S165	7.643	16.7767	-23.6038	10.2418	-25.3913	0	3.5751
	27.1377	22.4150	Ne^1=10.007	27.5814	Ne^1=12.313	3.75	
	± 46.5109	± 29.2634	Ne^2=80.053	± 43.6718	Ne^2=98.505	± 4.33	
Ateles belzebuth							
AP86	1.882	2.1647	-9.6803	1.0918	-8.2923	0.35	2.7759
AP74	88.133	76.2353	-31.6831	51.1173	-27.0697	0.10	9.2267*
D5S117	9.192	10.5704	-12.2485	17.5561	-11.6891	0	1.1187
D8S165	7.939	4.6048	-10.0032	4.6048	-10.1035	0	0
	26.7865	23.3938	Ne^1=10.444	18.5925	Ne^1=8.300	11.25	
	± 41.0220	± 35.4042	Ne^2=83.549	± 22.809	Ne^2=66.402	± 16.52	
Ateles hybridus							
AP86	1.545	3.0977	-11.7281	1.4832	-11.4878	0.225	0.4805
AP74	5.483	3.18	-15.8055	1.0966	-9.1562	0.45	13.2985
D5S117	15.700	6.123	-16.2723	9.1060	-16.0074	0	0.5297
D8S165	5.368	11.2727	-11.7853	14.3325	-11.3314	0	0.9078
	7.024	5.9183	Ne^1=2.642	6.5046	Ne^1=2.904	16.875	
	± 6.0665	± 3.8369	Ne^2=21.137	± 6.3902	Ne^2=23.230	± 0.21554	
Ateles chamek							
AP86	0.814	1.4768	-7.2371	1.5541	-7.2289	0	0.0164
AP74	45.864	30.9580	-16.5841	44.0291	-16.4725	0.025	0.2232
D5S117	6.593	8.2088	-12.5021	6.3297	-12.3658	0	0.2725
D8S165	0.828	1.4236	-6.5843	1.4236	-6.5876	0	0.0065
	13.5247	10.5167	Ne^1=4.695	13.3341	Ne^1=5.953	0.625	
	± 21.730	± 13.995	Ne^2=37.560	± 20.590	Ne^2=47.622	± 1.25	

Markers	UNI-STEP MODEL			MULTI-STEP MODEL			
	θ_0	θ	ln likeli-hood	θ	ln likeli-hood	multiple mutation%	χ^2
Ateles geoffroyi vellerosus							
AP86	0.828	1.4236	-6.5843	1.4236	-6.5876	0	0.0033
AP74	5.769	11.5673	-10.7442	9.9231	-10.8019	0	0.1154
D5S117	5.6	9.10	-5.8580	9.632	-5.8704	0	0.0247
D8S165	4.5	3.8925	-4.3529	4.32	-4.3748	0	0.0439
	4.1743 ± 2.3007	6.4958 ± 4.6550	Ne^1=2.900 Ne^2=23.199	6.3247 ± 4.1604	Ne^1=2.824 Ne^2=22.588	0	

A. geoffroyi and *A. hybridus* had the lowest (0 %) and highest (16.88 %) multiple mutation percentages respectively and no species had overall average values that were significantly different from the uni-step mutation model (0%). Only AP74 deviated from the uni-step mutation model in the two *A. fusciceps robustus* populations (Chocó -5 % with χ^2 = 4.45, 1 df, P < 0.05, and Northern Atlantic -7.5 % with χ^2 = 27.572, 1 df, P < 0.001, respectively), in *A. belzebuth* (10 % with χ^2 = 9.23, 1 df, P < 0.01) and in *A. hybridus* (45 % with χ^2 = 13.29, 1 df, P < 0.01). No other microsatellite nor other *Ateles* species deviated from the uni-step mutation model. In fact, the average q calculated with the uni-step mutation model for each *Ateles* species was very similar to the average q calculated with the multi-step mutation model. Therefore, no important differences in effective numbers were detected with a uni-step mutation model (easier to calculate compared to a multiple mutation model), at least not in *Ateles* with the microsatellites included in this study. Table 10 shows the differences in mutation rates among microsatellite pairs in each one of the *Ateles* species studied for the uni-step and the multi-step mutation models. These models had the exact same results for the *A. f. robustus* population of Chocó. All of the microsatellites yielded significantly different mutation rates from each other. For the Chocó population, the order of microsatellites that depicted decreasing mutation rates was AP74 > D8S165 > AP68 > D5S117. The results of the uni-step and for the multi-step mutation models were identical for the Northern Atlantic *A. f. robustus* population (Antioquia, Sucre, Córdoba, Atlántico). There were some minor differences between these two *A. f. robustus* populations. For example, the microsatellite mutation rate in decreasing order for the Northern Atlantic population was AP74 > D8S165 > D5S117 = AP68, with the last two values not statistically different. The global *A. f. robustus* sample had the same trends as that of the Northern Atlantic *A. f. robustus* population. For the *A. belzebuth*, there were no differences between the uni-step and the multi-step mutation models, as in the previous cases. The decreasing order of mutation rates for this species was: AP74 > D5S117 > D8S165 = AP68, with no significant difference between the two last markers. Therefore, several dynamic differences in mutations were detected between *A. fusciceps* and *A. belzebuth*. Different mutation patterns were observed for *A. hybridus* compared to the previous species. For example, the mutation rates of microsatellites D5S117 and AP74 were not significantly different with the uni-step model, but D5S117 was significantly greater than AP74 with the multi-step mutation model. The mutation rate ranking (D8S165 > D5S117 > AP74 > AP68) was also different from the previous mentioned species. For *A. chamek*, similar results were obtained for both mutation models. The

mutation ranking order was: AP74 > D5S117 > D8S165 > AP68, and was more similar to *A. belzebuth* than to *A. hybridus*. Finally, for *A. geoffroyi*, the results for both mutation models were similar. The mutation ranking order (AP74 > D5S117 > D8S165 = AP68) was identical to that discovered in *A. belzebuth*, relatively similar to that detected in *A. fusciceps*, but dissimilar to that found in *A. hybridus*. Perhaps the mutation rate rankings provide phylogenetic signatures that can be used to differentiate among *Ateles* species, such as in this case, where *A. hybridus* is different from the other *Ateles* species studied?

Table 10: χ^2 differences in the mutation rates per generation between the *Ateles* taxa studied for uni-step (1) and multi-step (2) mutation models for all possible microsatellite pairs. *Significant Differences at $P < 0.05$. Most of the mutation rates comparisons are significant. A, B, S, C, At = The *A. fusciceps robustus* population from Antioquia, Bolivar, Sucre, Córdoba and Atlántico Departments from Colombia.

	MARKERS			
	AP68	**AP74**	**D5S117**	**D8S165**
AP68				
Ateles fusciceps robustus Chocó	---	[1] 14.17* [2] 18.79*	11.60* 11.42*	7.18* 9.30*
Ateles fusciceps robustus (A, B, S, C, At)	---	177.16* 208.38*	1.76 1.73	11.01* 18.23*
Ateles belzebuth	---	44.00* 37.55*	5.13* 6.79*	0.64 3.62
Ateles hybridus	---	8.15* 4.66*	9.08* 9.03*	0.11 0.31
Ateles chamek	---	18.69* 18.48*	10.53* 10.27*	1.30 1.28
Ateles geoffroyi vellerosus	---	8.31* 8.42*	4.46* 4.42*	1.45 1.43
AP74				
Ateles fusciceps robustus Chocó	---	---	25.77* 30.22*	6.98* 9.48*
Ateles fusciceps robustus (A, B, S, C, At)	---	---	178.93 206.64*	166.15* 190.14*
Ateles belzebuth	---	---	38.86* 30.76*	43.35* 33.93*
Ateles hybridus	---	---	0.93 13.70*	8.04* 4.35*
Ateles chamek	---	---	8.16 8.21*	19.65* 19.76*
Ateles geoffroyi vellerosus	---	---	9.77* 9.86*	12.78* 12.85*

	MARKERS			
	AP68	**AP74**	**D5S117**	**D8S165**
D5S117				
Ateles fusciceps robustus Chocó	---	---	---	18.78* 20.73*
Ateles fusciceps robustus (A, B, S, C, At)	---	---	---	12.77* 16.49*
Ateles belzebuth	---	---	---	4.49* 3.17
Ateles hybridus	---	---	---	8.97* 9.35*
Ateles chamek	---	---	---	11.83* 11.55*
Ateles geoffroyi vellerosus	---	---	---	3.01 2.99

Craniometric data

The first craniometric data collected were not standardized and the UPGMA algorithm was applied to the obtained correlation matrix. No species or sex associations were displayed by the constructed phenogram. The Manhattan distance was also applied to the non-standardized data (Fig. 2a) resulting in the detection of two large clusters. In the first large cluster there were two *A. f. robustus* (both from the northern area of Colombia), two *A. hybridus*, one *A. geoffroyi* and one *A. belzebuth*. The second large cluster was comprised of one *A. hybridus*, 11 *A. f. robustus* (from different northern and Pacific Colombian areas), 7 *A. belzebuth* and two *A.* spp., with no apparent trend. A small cluster composed of three *A. f. robustus* females from Chocó region and a cluster of two *A.* spp. were also detected. No associations by sex were determined.

Application of the UPGMA phenogram with the Manhattan distance to the standardized data matrix, provided findings (Fig. 2b) that were similar to those obtained from the non-standardized data matrix. This analysis presented the highest cophenetic correlation coefficient ($r = 0.837$, $t = 6.971$, $p = 0.0000$, 1,000 permutations, one tail probability p [random z > observed z] = 0.001). A strict consensus phenogram with correlation, variance-covariance and Manhattan distances applied to the standardized data showed a large cluster comprised of several *A. f. robustus* individuals from different points, two *A. hybridus* and two *A. belzebuth* from the Meta Department (Eastern Llanos). A second cluster decomposed into seven different associations resulting in only 3 significant associations. One association grouped four *A. f. robustus* (three females and one male) from the Chocó area with one *A. hybridus* from the Bolivar area. Another association clustered two *A. f. robustus* females from the Antioquia region while a third association was composed of two *A.* spp. The consensus coefficients of this phenogram were as follows: Colles index, $c = 0.6$; Mickevich index, $m = 0.2189$ and Schuh & Farris index, $sf = 91$.

The minimum spanning tree with the correlation coefficient showed striking relationships among the following paired individuals: one *A. belzebuth* (Caquetá Department) and one *A. f. robustus* (Sucre); one *A. hybridus* female (Bolivar) and one *A. f. robustus* female (Antioquia); two *A. belzebuth* (one from La Macarena and other from Caquetá area), and two *A. belzebuth* (from the Caquetá and Meta Departments).

A R Principal Component Analysis showed that the first component (size) explained 50.62 % of the total variance, while the top three components explained 70.74 % (size and shape) of the total variance (Fig. 3). The first component clearly differentiated six individuals from the remaining samples analyzed (one *A. belze-*

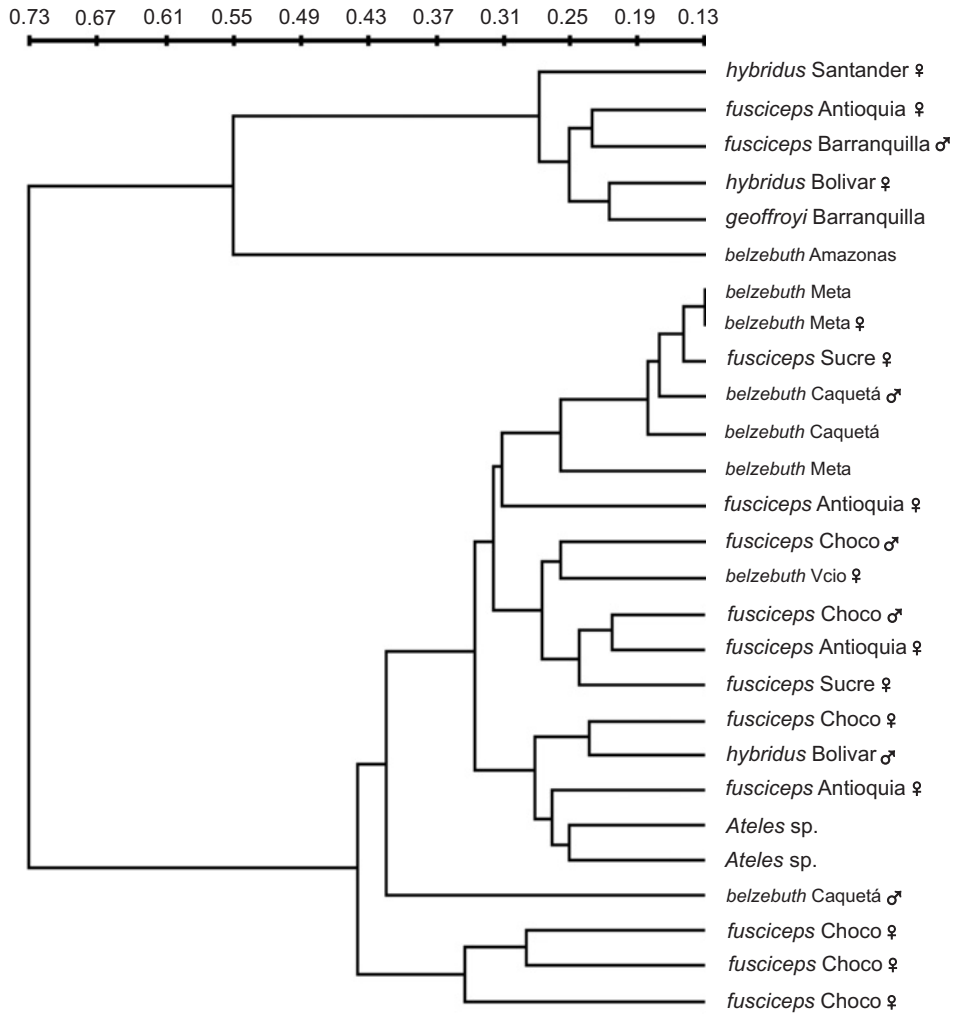


Fig. 2a: UPGMA phenogram with the Manhattan distance applied to non-standardized data of 27 *Ateles* skulls by using 38 cranial, mandible and dental measurements.

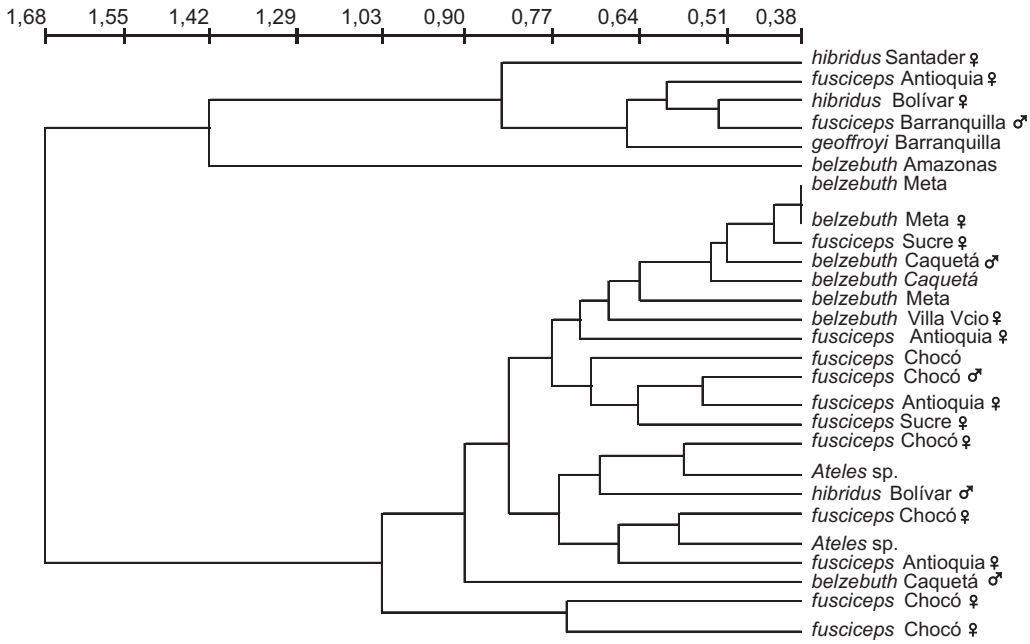


Fig. 2b: UPGMA phenogram with the Manhattan distance applied to standardized data of 27 *Ateles* skulls by using 38 cranial, mandible and dental measurements.

buth from Amacayacu, Amazon; one *A. f. robustus* from Barranquilla, Atlántico Department; one *A. hybridus* from Santander Department, one *A. hybridus* from Bolivar, one *A. f. robustus* from Turbo, Antioquia and one *A. geoffroyi* from Barranquilla). There were two examples in which the component shape clearly differentiated an individual from the remaining analyzed skulls (one *A. belzebuth* from Caquetá Department and other *A. belzebuth* from Amacayacu in Amazon).

A Q Principal Component Analysis supported that the variables mainly influenced by size were maximum frontal width and minimum frontal width followed by basal height, bigonion width, auricular height and maximum transversal braincase width. The influence of shape on the biometric variables studied (second and third components) was dispersed among all the analyzed variables. The craniometric variables more related with shape differences among the *Ateles* taxa were the lengths of the upper and lower first molars, the foramen magnum length and the symphisis height. The first component explained only 20.19 % of the total variance, while the first three components together only explained 45.41 %. Therefore, there was no single or small group of biometric variable(s) that could explain a large fraction of the skull differences found in the diverse species and individuals of *Ateles* studied. This supports that the biometric variables studied did not present extreme differences among the *Ateles* taxa analyzed and therefore do not have the same power as DNA microsatellites to discriminate *Ateles* individuals belonging to different geographical taxa.

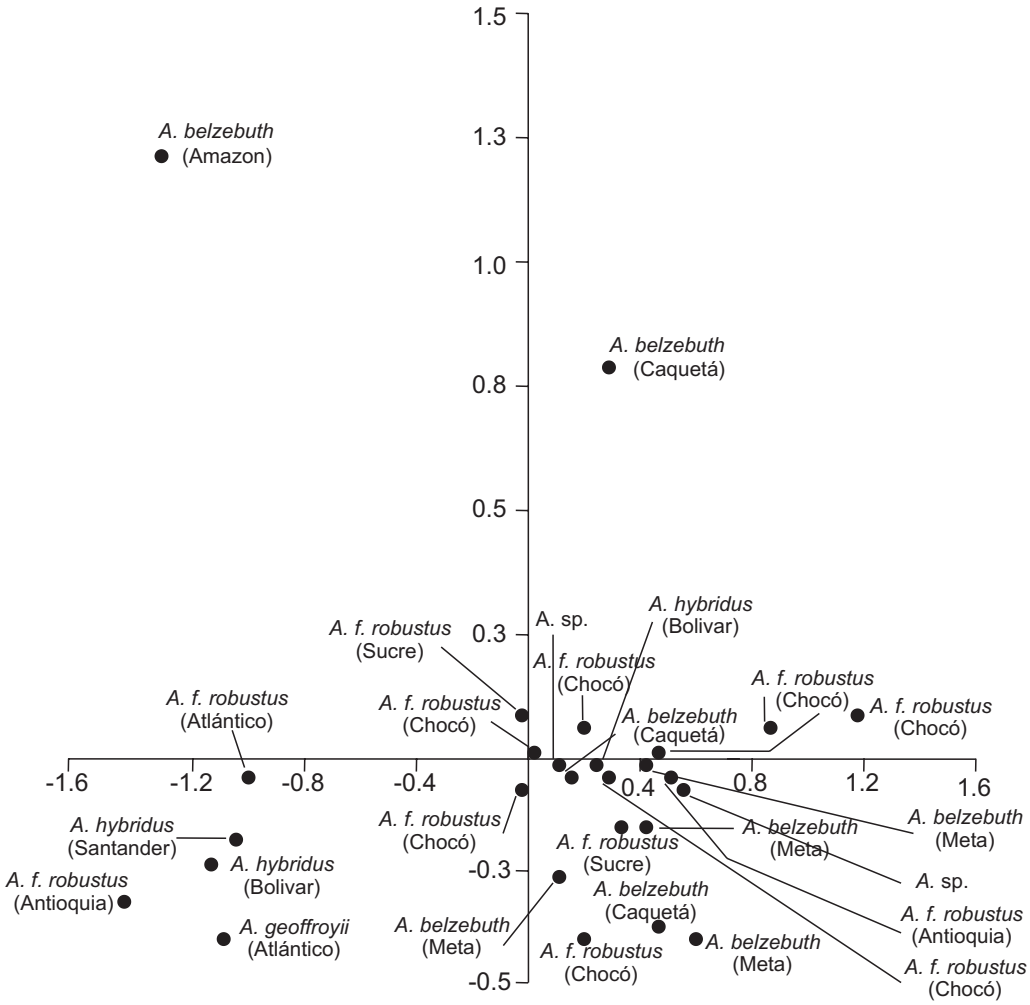


Fig. 3: R Principal Component Analysis with the standardized data of 27 *Ateles* skulls by using 38 cranial, mandible and dental measurements.

Discussion

Microsatellite genetic variability in Ateles and phylogenetics of this genus

One interesting result determined in this study was the detection of some private alleles in the seven *Ateles* taxa analyzed. The AP68, AP74, D5S117 and D8S165 markers showed these private alleles in most of the taxa that were exhaustively sampled. This result has two important implications. First, many of the *Ateles* exhibited in zoos are from unknown origins and sometimes many animals are hybrids. The existence of private microsatellite alleles could be a valuable tool that would help to correctly discriminate the *Ateles* taxa and the hybrids that are usually located in zoos and other institutions. Second, the existence of private alleles could be

a determinant tool to reconstruct the phylogenetics relationships within the *Ateles* genus. For example, AP74 showed a wide range of allele sizes. One species, *A. hybridus*, basically only presented small sized alleles (130-132 pb), whereas *A. belzebuth*, *A. chamek* and the northern Atlantic *A. f. robustus* population yielded these same alleles in addition to some larger sized alleles. However, other *Ateles* taxa only showed large sized alleles (*A. f. fusciceps*, *A. paniscus*, *A. geoffroyi* and the Chocó Pacific *A. f. robustus* population). This supports that *A. hybridus* descended from one of the first three taxa but not from any of the latter four taxa.

The northern Atlantic *A. f. robustus* population presented the largest average number of alleles per locus followed by *A. chamek*. However, the current estimated population size of the first population is considerably smaller (about 1,000 to 3,000 individuals and about 0 to 4 refuges, following the CONSERVATION ASSESSMENT AND MANAGEMENT PLAN, 1992; KONSTANT et al., 1985). This study provides the first evidence that the large number of alleles detected in *A. f. robustus* is the result of natural hybridization of an original *A. f. robustus* population located at the left side of the Magdalena river occupying part of the Antioquia, Bolivar, Sucre, Córdoba and Atlántico Departments. In fact, this *A. f. robustus* population presented the largest number of alleles at three of the four polymorphic microsatellite markers used (AP68, AP74 and D8S165). *A. chamek* had the largest number of alleles for the remaining locus (D5S117). In agreement with other molecular marker studies, *A. geoffroyi*, presented the lowest average number of alleles, although this value was not significantly different from other species. For instance, COLLINS and DUBACH (2000a) determined very low levels of intrapopulational genetic mitochondrial sequence variation for all the *A. geoffroyi* that they studied.

A. chamek and the second population of *A. f. robustus* showed the first and second highest expected levels of heterozygosity, while *A. hybridus* showed the lowest. This last finding agrees quite well with the fact that only 100 to 1,000 individuals of this species still exist in the wild. If the northern Atlantic *A. f. robustus* population, which is probably mixed with *A. hybridus*, is excluded from the results, then *A. chamek* has the highest gene diversity levels (expected heterozygosity and average number of alleles). The estimated size of the wild population for this species is greater than 10,000 animals and is considered to be in a safe status according to the CONSERVATION ASSESSMENT AND MANAGEMENT PLAN (1992). This is a remarkable result because this could be the origin of all other *Ateles* species studied herein (COLLINS and DUBACH, 2000 a,b).

A. chamek was the species which presented the highest levels of gene sequence diversity at the mitochondrial DNA and at the aldolase A intron gene (COLLINS and DUBACH, 2000a,2001). This agrees quite well with the fact that *A. chamek* was the species with the highest microsatellite gene diversity, thus suggesting *A. chamek* as the ancestral spider monkey clade. MEDEIROS et al. (1997) also concluded that *A. chamek* could represent the ancestral karyotype for *Ateles*. The finding of the 6b chromosome in *A. chamek* provided an important element to the identification of the population of this species as the ancestor within this genus. DUTRILLAUX et al. (1986) found that 6b corresponded to 2 chromosomes of *Lagothrix* and *Brachyteles*. Therefore, since the 6b form only occurs in *A. chamek*, *Lagothrix* and *Brachyteles* it is interpreted as the ancestral form of *Ateles*. They speculated that a migration eastward formed *A. marginatus* and a second migration leading north-westward to the

Magdalena River valley formed *A. hybridus*. In regard to the second migration, microsatellite results support the possibility that *A. chamek* was the ancestral species and that *A. belzebuth* derived from it. In turn, *A. belzebuth* originated at the Magdalena River valley from the northern Atlantic *A. f. robustus* populations (this is consistent with the 6d chromosome) which in turn originated the Chocó Pacific *A. f. robustus* population which originated the Ecuadorian *A. f. fusciceps* population. There were no significant differences among *A. f. fusciceps* individuals, at least not in the few analyzed in the current study. *A. hybridus* could have originated directly from *A. belzebuth*, or from the Magdalena River valley and Atlantic *A. f. robustus* population. From a chromosome point of view, both origins are possible because *A. hybridus* has 6a, 6c, 7b and 14b chromosomes, while 7b is found in *A. f. robustus* and 6c is found in *A. belzebuth*.

The existence of private alleles and high levels of gene diversity support that *A. chamek* is a full species as has been mentioned previously (ANDERSON, 1997 and SALAZAR-BRAVO et al., 2003). This was also supported with morphometric (FROEHLICH et al., 1991) and cytogenetic data (MEDEIROS et al., 1997) and therefore *A. chamek* is not a subspecies of *A. paniscus* (*A. paniscus chamek*) as it was traditionally claimed by KELLOG and GOLDMAN (1944). In addition, our microsatellites results revealed a strong connection between *A. chamek* and *A. belzebuth belzebuth* which is also supported by COLLINS and DUBACH's (2000 a,b, 2001) molecular results. They demonstrated a strong phylogenetic relationship among *A. chamek* and several *A. belzebuth* subspecies. Therefore, *A. chamek* could also be the origin of the *A. b. belzebuth* populations studied in Perú and Colombia.

Furthermore, COLLINS and DUBACH (2000a) supported the existence of four Ateles species based on mitochondrial DNA sequence variation in cytochrome c oxidase subunit II (COII) and the mitochondrial control region. The first species, *A. belzebuth*, had a distribution range from the western and southern Amazon Basin and from the Guyana Highlands to the Andes, part of the northern Amazon as well as the south-eastern Llanos in Colombia and Venezuela. Our microsatellite data confirmed an important connection between *A. chamek* and *A. belzebuth belzebuth*, although Wahlund effect was detected as was mentioned previously. COLLINS and DUBACH (2001) studied DNA sequence variation of the aldolase A intron V nuclear genomic region and determined that one *A. chamek* sequence was associated with an *A. belzebuth marginatus* sequence and separated from other *A. chamek* sequences. Disappointingly, no *A. b. marginatus* were sampled and therefore DNA microsatellites were not applied to this taxon. However, the phylograms obtained by these authors with that nuclear gene were considerably less robust compared to those constructed from the mitochondrial DNA genome due to a low sequence variation in the nuclear gene studied.

Nonetheless, the nuclear microsatellites studied in *Ateles* did not present a lower nuclear gene diversity than that obtained for other genera, such as *Alouatta, Lagothrix* and *Cebus* (RUIZ-GARCÍA et al., 2004, 2006a, unpublished data), and thus are not in agreement with the low level of aldolase A intron V sequence variation detected by COLLINS and DUBACH (2001). Recall that this nuclear gene only showed 10% of the variation at mitochondrial COII gene and only 6% of the mitochondrial control region. The second species, *A. paniscus*, was comprised of haplotypes from the northeastern area of the Amazon Basin in Surinam, Guiana and

Brazil. The analysis of the aldolase A intron suggested that the *A. paniscus* haplotypes form a unresolved trichotomy with *A. chamek* and with the rest of the *Ateles* haplotype at the base of the phylogenetic tree. In addition, *A. paniscus* represents the basal clade for the mitochondrial phylogenies which complements the fact that this taxon among all of the Ateles species is the most similar to the *Ateles* ancestor type (COLLINS and DUBACH, 2000a). The small sample size of *A. paniscus* collected in the current study is insufficient to test this conclusion. The third species, *A. hybridus*, was located along the right Magdalena River, the eastern bank of the lower Cauca river, the departments of Magdalena, Bolivar, Antioquia, Cesar and Guajira and at least to the northern departments of Caldas and Cundinamarca. There is an additional population in the Catatumbo River basin of North Santander Department and a second population in the northeastern piedmont in Arauca Department and the diverse areas around Maracaibo Lake in northwestern Venezuela. In agreement with the mitochondrial data results, our nuclear DNA microsatellite data support that this last population is possibly a full species.

It could be derived from *A. belzebuth* or from the second *A. f. robustus* population by founder effect, although founder effect affects these kinds of markers less than other markers such as isoenzymes or plasma proteins. However, the possibility that this population arose because of founder effect and genetic drift, does provide an explanation of why COLLINS and DUBACH (2000a) found different relationships among the *A. hybridus* clade and the other *Ateles* clades. For instance, the parsimony analysis of the control region did not relate *A. hybridus* with any other clade, whereas the distance-based analysis clustered *A. hybridus* with *A. geoffroyi*/*A. f. robustus* with a bootstrap support of 65 %. In contrast, the combined mitochondrial gene neighbor-joining analysis placed *A. hybridus* next to the clades of *A. b. chamek*/*A. b. marginatus* and *A. geoffroyi*/*A. f. robustus*. The differential relationship of this species could be the result of genetic drift, just as the average number of alleles per locus data showed. Although, only two *A. paniscus* samples were studied, they did not reveal any special relationship with *A. hybridus*. This finding is in agreement with the mitochondrial and the aldolase A gene results. Thus, microsatellites provide some data that do not support the link among *A. paniscus* and *A. hybridus* as was proposed by MEDEIROS et al. (1997) and based on chromosomal analysis (both taxa shared the 7b chromosome). The similarities of chromosome pair 7 between *A. paniscus* and other trans-Andean *Ateles* forms are not compatible with the microsatellite results. However, this chromosome could be polymorphic in *A. belzebuth* and in other *Ateles* individuals not yet studied and the apparent relationship among *A. paniscus* and the trans-Andean *Ateles* could be spurious. In fact, COLLINS and DUBACH (2000a) showed that the haplotypes of *A. hybridus* did not exhibit the 25-base pair control region deletion that occurs in all haplotypes of *A. paniscus*. Microsatellites did not reveal any evidence that connected *A. hybridus* and *A. geoffroyi* which agrees with the neighbor-joining cladogram for the aldolase A intron studied by COLLINS and DUBACH (2001). On the other hand, KUNKEL et al. (1980) detected the same 6c and 7b chromosomes in *A. geoffroyi* and *A. hybridus*. COLLINS and DUBACH (2000 a,b) only found a small amount of evidence for the inclusion of *A. hybridus* and *A. geoffroyi* in the same cluster when they used mitochondrial genes as FROEHLICH et al. (1991) had revealed previously with morphometrics. Nevertheless, the maximal genetic distances between *A. geoffroyi* and *A.*

hybridus were lower at the aldolase A gene than the distances between *A. geoffroyi* and the Chocó populations of *A. f. robustus* (COLLINS and DUBACH, 2001). FROEHLICH et al. (1991) used morphometric analysis to support that *A. hybridus* and *A. fusciceps* were conspecifics. Although the centroid distances between both taxa showed that they were related, there were also differences between them. We believe that, at least, the northern *A. f. robustus* population is related with *A. hybridus* (due to the limited hybridization between them) but they could be two different taxa such as karyotype analyses have revealed (KUNKEL et al., 1980). The fourth species was *A. geoffroyi*. The mitochondrial results placed the two former species *A. geoffroyi* (Central America) and *A. fusciceps*, (Pacific Colombia and Ecuador and northern Colombia) in the same group. The aldolase A gene region also clustered two *A. geoffroyi yucatanensis* haplotypes with two *A. f. robustus* haplotypes but with a very low bootstrap support of 48 %. Therefore, the aldolase A gene provided minor support for the inclusion of both the Central America *A. geoffroyi* and the South American *A. fusciceps* as one species. FROEHLICH et al. (1991) concluded in their morphological variation study that *A. geoffroyi* and *A. fusciceps* belonged to the same species. The coat colors of the two taxa are distinct overall, with the general trend of the production of darker pelages at the extremes of the Central American Isthmus (KONSTANT et al., 1985). Thus, it could be that the *A. fusciceps* individuals are completely dark. However, our microsatellite study revealed strong differences especially between *A. geoffroyi* and *A. hybridus*, but also between *A. geoffroyi* and *A. fusciceps robustus*. Both of the *A. f. robustus* populations had significant divergences from the *A. geoffroyi* sample analyzed. Therefore, microsatellite results supported statistical significant differences among *A. f. robustus*, *A. geoffroyi* and *A. hybridus*. The Chocó Pacific *A. f. robustus* population diverged clearly from the second *A. f. robustus* population. However, the first population was practically identical to the few *A. f. fusciceps* individuals studied from Ecuador as previously cited. Thus, the Colombian and the Ecuadorian Pacific *A. fusciceps* populations that were previously classified as two different subspecies now seem to make-up a single gene pool for the microsatellite studied. The second *A. f. robustus* population was clearly more related with *A. hybridus*, while this last population did not show specific resemblances with *A. geoffroyi*. On the other hand, either of the two Colombian *A. f. robustus* populations could have originated *A. geoffroyi*. Some specific differences between mitochondrial and microsatellite results could be due to the maternal mode of inheritance of mitochondrial DNA. This (mitochondrial DNA) could be distributed in maternally isolated lineages, although the populations could be related by male gene flow. Nonetheless, similar nuclear and mitochondrial phylogenies are expected with both molecular markers because spider monkeys are widely reported to show female dispersal (MOORE, 1993). A congruity test (ILD test) indicated that the nuclear and the mitochondrial trees obtained by COLLINS and DUBACH (2001) were congruent ($P = 0.476$). Perhaps the different mutation models that affect mitochondrial DNA and microsatellites provide an explanation for the different results obtained for these two types of DNA.

Hardy-Weinberg equilibrium

Three out of five *Ateles* samples, where H-W E tests were performed, showed no-existence of this equilibrium. This was the case of *A. f. robustus*, *A. belzebuth* and *A.*

chamek. In the first taxa, two different populations were sampled, one from the Pacific Colombian area of Chocó and the other from the Magdalena river valley and Atlantic coast of northern Colombia (Antioquia, Sucre, Bolivar, Córdoba and Atlántico). Both populations taken together showed a remarkable homozygous excess which was produced by Wahlund effect (= population subdivision). This means that each one of these *A. f. robustus* populations represented a different gene pool. When each population was tested separately, neither one deviated from H-W E. This reveals that these populations were composed of animals of different gene pools, where some were mixed and others were not. Therefore, Wahlund effect is also inside of the northern *A. f. robustus* population and the western cordillera of the Andes and the Cauca River were not barriers to gene flow between *A. f. robustus* and *A. hybridus* such as was claimed by COLLINS and DUBACH (2000a).

The deviation of *A. belzebuth* from H-W E could also be due to Wahlund effect because the samples studied covered a wide range of the distribution of this species in Colombia and Perú. However, the individuals sampled were dispersed across both countries and the samples could not be associated to any singular population. Therefore, we could only affirm that there is genetic heterogeneity within the distribution range of *A. belzebuth* in Colombia and Perú. COLLINS and DUBACH (2001) determined that the maximal genetic distance for the aldolase A and mitochondrial genes within *A. belzebuth* was the highest they had encountered among all of the *Ateles* taxa that they studied. They concluded that this was a very diverse species that could possibly harbor susbspecies that might be detected with more intensive sampling of individuals from a larger geographic area. COLLINS and DUBACH (2000b) commented that Pleistocene refugia could be an important element to produce genetic diversity among different geographical areas within the *A. belzebuth* distribution but that it was insufficient to result in speciation for this species. A more thorough and intensive sampling across this area must occur in the future, in order to be able to determine the precise limits of these diverse and different gene pools within *A. belzebuth*. Wahlund effect could also provide an explanation for the deviation from H W E by *A. chamek*, because the samples were obtained across different points of the Peruvian Amazon (Amazon, Napo and Ucayali rivers) as well as the Bolivian Amazon (Mamoré and Beni rivers). The two species which did not present any evidence of deviations from H W E were *A. hybridus* and *A. geoffroyi*. The samples of the second species were collected from a specific geographical point but the samples of the first species were obtained across the species distribution range. DEFLER (2003) distinguished two possible subspecies of *A. hybridus*, *A. h. hybridus*, from the major part of the geographical range of this species, and *A. h. brunneus* from along the lower Cauca and Magdalena Rivers in the departments of Bolivar, Antioquia and Caldas. Animals of the second subspecies have different coat colors from those of the first subspecies. However, in the current study we did not find evidence of molecular differences between individuals of *A. hybridus* from both geographical areas. Therefore, at a molecular level we could not differentiate between these two possible *A. hybridus* susbspecies.

Genetic heterogeneity

Of the five microsatellites that amplified nicely (in this study) for *Ateles*, AP40 was the only one not useful in discriminating among different taxa or helpful in establishing the genetic structure parameters of populations.

The two *A. f. robustus* populations were highly divergent for all the polymorphic markers in this study. This reveals that these two populations of *A. f. robustus* presented considerable genetic divergence and it could be interesting to analyze possible taxonomic differences between these two populations. The three microsatellites which showed the most outstanding divergence between these two populations were AP68, AP74 and D8S165. It is interesting to note that for AP68, the Pacific population of *A. f. robustus* did not present differences with *A. f. fusciceps* but did show noteworthy differences with the other *Ateles* species. In contrast, the second *A. f. robustus* did not present significant differences with *A. belzebuth*, *A. hybridus* or with *A. geoffroyi*. The first *A. f. robustus* population also did not show significant differences with *A. f. fusciceps* nor with *A. geoffroyi* for AP74. Once more, the second *A. f. robustus* population did not show significant differences with *A. belzebuth* and *A. hybridus* but, it did show significant differences with *A. geoffroyi.* For D8S165, the first *A. f. robustus* population showed significant differences with *A. hybridus*, *A. geoffroyi* and *A. chamek*, whereas the second population presented remarkable differences with *A. hybridus* but not with *A. geoffroyi*. Several ideas can be generated from these results. First, the Pacific *A. f. robustus* population was very similar with the *A. f. fusciceps* samples from Ecuador. Therefore, these two populations could represent the same gene pool, or that the separation between the populations is rather recent.

Nevertheless, the Pacific *A. f. robustus* population was not only extremely divergent from the second *A. f. robustus* population, but it was also extremely divergent from *A. belzebuth* and *A. hybridus*. In contrast, the second *A. f. robustus* population was very similar with *A. belzebuth* and with *A. hybridus* populations. This could be interpreted as follows. As it was discussed previously, *A. chamek* was the taxon that presented the highest level of expected heterozygosity and therefore it could be the ancestral origin of some of the other *Ateles* taxa studied herein. When geographical vicinity is considered, the descended species should be *A. belzebuth*. The current results support that *A. belzebuth* crossed the eastern Andes cordillera and originated the second *A. f. robustus* (Antioquia, Sucre, Bolivar, Córdoba, Atlántico) population. We have evidence that *A. belzebuth* crossed the eastern Andes Cordillera. Brother APOLIMAR MARIA (1913) recorded a specimen from the Tolima Department in the upper Magdalena Valley which was practically identical to *A. belzebuth* individuals that inhabited the eastern piedmont of the eastern Andes. We have even located *Ateles* bones from the Huila Department in various Colombian museums on the other side of the eastern Andes Cordillera (RUIZ-GARCÍA, unpublished results). HERNÁNDEZ-CAMACHO and COOPER (1976) mentioned that a precedent existed for a zoological passage from east of eastern Andes cordillera into the upper Magdalena valley. Several species of Primates such as *Cebus apella, Lagothrix lagotricha lugens* and *Saimiri sciureus cassiaquerensis*, as well as other vertebrate species, have crossed this passage. It is also possible that *A. belzebuth* originated *A. hybridus* via a different route. As we had mentioned earlier our microsatellite data revealed that *A. hybridus* was completely differentiated. However, there is evidence of introgression of *A. hybridus* in the second *A. f. robustus* population, which is clearly visible at the allele composition of AP74. This introgression is probably recent and is only in one part of the current population's (Antioquia, Sucre, Córdoba and Atlántico *A. f. robustus*) distribution and is compatible with the Wahlund effect

described in the H-W E section. This *A. f. robustus* population provided the origin of the *A. f. robustus* in the Colombian Pacific. The Central American species, *A. geoffroyi*, herein represented only by one subspecies, could have originated indistinctly from either of the two *A. f. robustus* populations as previously claimed. For instance, AP68 and D8S165 revealed that there was similarity between the second *A. f. robustus* and *A. geoffroyi* populations analyzed in this study. AP74 revealed extreme similarities among the Pacific Colombian *A. f. robustus* and *A. geoffroyi* populations. In contrast, D5S117 differentiated both *A. f. robustus* populations from the *A. geoffroyi* sample.

The estimated value of gene flow among the different *Ateles* taxa was very small, which provides strong support that the taxa studied are different reproductive units, even though two of the analyzed populations seem to share genetic characteristics (second *A. f. robustus* population and *A. hybridus*). Although there is evidence of hybridization among different *Ateles* populations as we reported in the introduction, the current *Ateles* distribution and its habitat restriction translate into a critical need for primary and terra firma forest. This fact could limit gene flow among *Ateles* populations (ESTRADA and COATES-ESTRADA, 1988; AQUINO and ENCARNACION, 1994).

However, we can not discount the possibility that animals with different karyotypes produce fertile offspring (MEDEIROS et al., 1997). These authors suggested that *A. geoffroyi* and *A. hybridus* could interbreed and produce fertile offspring, although we have not found evidence of this occurrence with microsatellites. Although, KUNKEL et al. (1980) and MEDEIROS et al. (1997) speculated that *A. f. robustus* could be reproductively isolated from *A. geoffroyi* and *A. hybridus* because of the differences in chromosome pairs (5 and 6), we have evidence of hybridization between *A. f. robustus* and *A. hybridus.* ROSSAN and BAERG (1977), also determined that there was hybridization between *A. f. robustus* and *A. geoffroyi panamensis* and they noted the location of a hybridization zone. Also, recall that no river has formed a barrier among *Ateles* species with the exception of the lower Amazon and possibly some of the black-water rivers draining the Guianan highlands (COLLINS and DUBACH, 2000b).

The genetic heterogeneity analysis revealed a global expected gene diversity of 0.675. This value was slightly lower than that obtained in other neotropical primate genera, such as *Alouatta*, which showed a value of 0.727 with the same microsatellites (RUIZ-GARCÍA et al., 2006b). The levels of genetic heterogeneity for the seven *Ateles* taxa were 0.105-0.123 for G_{st}, 0.084 for F_{st} and 0.251 for R_{ST}. These values were considerably lower than those obtained for the same markers in *Alouatta* (0.189-0.259 for G_{st}, 0.26 for F_{st} and 0.621 for R_{ST}) and in *Cebus* (0.197-0.311 for G_{st}, 0.34 for F_{st} and 0.764 for R_{ST}) (RUIZ-GARCÍA et al., 2006b, and RUIZ-GARCÍA, unpublished results). These findings could have two interpretations: 1) Temporal divergence among the *Ateles* taxa studied is considerable lower than in these other genera. or 2) some selective or constriction mutation is acting upon a fraction of the DNA microsatellites studied in *Ateles.* RUIZ-GARCÍA et al. (2004, 2006a) had previously detected selective and mutational constriction of some of these microsatellites in other Neotropical primate species, but in no case was the constriction to the extent as that detected in *Ateles.* GARZA et al. (1995) also detected constraints on allele size microsatellite evolution among humans and chimpanzees. Also, we suggest

that a certain fraction of microsatellite loci mutations produce alleles with identical sizes but with different internal structures (GARZA and FREIMER, 1996).

The divergence time between *Ateles* species was 3.59 Ma following COLLINS and DUBACH (2000 a,b). We assumed that this value was potentially correct and used it to estimate the average microsatellite mutation rate per generation for *Ateles*. The following equations by SLATKIN (1995) were used in the calculation:

$t_F = 4N_e (F_{ST}/(1 - F_{ST}))$ and $t_R = 4N_e (R_{ST}/(1 - R_{ST}))$, where t is the number of generations elapsed from the separation of the taxa analyzed and N_e is the total effective number. We obtained average F_{ST} and R_{ST} values for *Ateles* of 0.084 and 0.251. By using F_{ST} and R_{ST} with a mutation rate of 5.6×10^{-4} (low extreme), the divergence times obtained were clearly an underestimation of the divergence times with mtDNA (95,734-349,842 years, respectively, against 3.59 Ma, COLLINS and DUBACH, 2000 a,b). Also, if both F_{ST} and R_{ST} were used with the mutation rate per generation of 7×10^{-5} (high extreme), the divergence times were still lower than that obtained with mtDNA sequences (765,853 years ago and 2.8 Ma, respectively). These results support that R_{ST} rather than F_{ST} based values are more similar to the expected divergence times obtained with other markers such as we determined for *Alouatta* (RUIZ-GARCÍA et al., 2006a). Nevertheless, the average mutation rate per generation in *Alouatta* is almost equal to 7×10^{-5} which corresponds to a divergence time of 6.6 Ma. This mutation rate value is much higher than that required for a divergence time of 3.59 Ma in *Ateles*. To obtain mutation rates per generation with F_{ST} and R_{ST} that are compatible with 3.59 Ma of separation, the average mutation rate per generation in *Ateles* would need to be 2.49×10^{-6} and 9.09×10^{-6} respectively. Therefore, as we previously claimed, the microsatellite mutation rates in *Ateles* are lower than in *Alouatta* probably because of higher mutation or selective constriction occurrences in *Ateles*.

Bottlenecks, coalescence effective numbers and mutation models

The bottleneck analysis of both *A. f. robustus* populations together, as a single large population, detected a recent and significant deviation from equilibrium that was the opposite of a bottleneck. This supports inconsequential mutation-gene drift, Wahlund effect, expansions or partial gene flow regarding this population. Nevertheless, when the *A. f. robustus* populations were analyzed separately, the Chocó Pacific population was shown to have crossed a recent bottleneck. However, this population seems extremely small with less than 500 individuals. An analysis of the northern Atlantic *A. f. robustus* population again showed significant negative results agreeing quite well with the fact that a fraction of this population was probably recently mixed with *A. hybridus*, such as it was demonstrated in other analyses. It is interesting to note that two of the most endangered *Ateles* taxa, such as *A. hybridus* and *A. geoffroyi* did not present any evidence of recent bottlenecks. This could mean that the number of DNA microsatellites used was insufficient to detect recent bottlenecks in these species or that their number has always been small since their origin by founder effect.

The different coalescence methods that were used provided very similar effective numbers. Both methods showed that the second *A. f. robustus* population presented the highest effective numbers. Furthermore, the methods provide evidence of gene admixture involving *A. hybridus* and therefore probably overestimate the real effec-

tive number of this *A. fusciceps robustus* population. The estimated wild population of this taxa is about 1,000-3,000 individuals, which is extremely low compared to the effective number obtained. In addition, both coalescence methods revealed that *A. hybridus* and *A. geoffroyi* were the species presenting the lowest historical effective number. Uniquely, the discordance between the methods supported that the second procedure clearly showed that the numbers were higher than the first one for *A. belzebuth*.

Previously, RUIZ-GARCÍA (2003) using different theoretical models determined possible effective numbers of some of these *Ateles* taxa with a smaller sample size. For instance, estimates of average heterozygosity with the infinite allele and the step-wise mutation models showed values of 1,189 to 6,772 for *A. belzebuth*, from 997 to 5,334 for *A. hybridus* and from 1,486 to 9,242 for *A. f. robustus* (total population). These values are within the lower limits for the coalescence estimates obtained herein. The estimate ranges with the WERHARHN (1975)´s procedure on these same species were 25,882 to 92,436, 6,885 to 24,588 and 32,163 to 114,868, respectively. All of these values are relatively similar to those obtained in the current study. Therefore, sufficient evidence exists in favor of the present historical effective numbers.

It should be noted that there are no important multiple mutation percentages that affect the microsatellite evolution studied herein. Only AP74 had a significant multiple-step mutation model in several of the *Ateles* species including *A.f robustus* (two populations), *A. hybridus* and *A. belzebuth*. The species *A. hybridus* showed the highest multiple-step percentage (16.88 %) and the most differentiated microsatellite mutation rate order. The evidence that the founder effect could modify these mutation evolution parameters at the microsatellite level during the formation of this species (*A. hybridus*) is important because it supports that it is a true species. Since the effective numbers obtained with the uni and multiple-step mutation models were extremely similar, the selection of any one particular mutation model to be used in the estimation of effective numbers in *Ateles* is not important.

Craniometric data

The craniometric data were not clearly differentiable with or without standardized data of the 27 Colombian *Ateles* skulls analyzed by species, gender or by geographical origin. Only a few, small clusters were maintained, such as 3 *A. f. robustus* females from Chocó. The clear separation of the two *A. f. robustus* populations that was revealed with the microsatellite data was not similarly obtained with the biometric skull data. However, the unique Colombian *A. geoffrensis* skull studied was clustered with some *A. hybridus* specimens and agrees with biometric skull results obtained by FROEHLICH et al. (1991). Nevertheless, this phylogenetic link was not supported with the DNA microsatellites analyzed. The size of the skull was helpful in the discrimination of some individuals from the remaining specimens studied, but this variable was not helpful for the discrimination among species, gender or by geographical origin. The variables with the greatest discrimination power by size were maximum frontal width, minimum frontal width followed by basal height, bigonion width, auricular height and maximum transversal braincase width. Although these were the craniometric variables most efficient in the discrimination among *Ateles* individuals they were not as effective as microsatellites. In contrast to these current

results, other Neotropical primate cranial studies have clearly differentiated gender and species (DE FREITAS BURITY et al., 1997).

The current results could provide some insights for the conservation of *Ateles*, at least, in the determination of how many evolutionary units exist in the wild. Recall that *Ateles* along with *Alouatta* and *Lagothrix*, is one of the primary food sources for many indigenous tribes (MITTERMAIER et al., 1989; BODMER et al., 1994). Unfortunately, due to their slow reproductive rates, this genus could easily be eliminated from extensive forested areas. Also, the reduced number of chromosome pairs in *Ateles* could be related to its high degree of ecological specialization (CHU and BENDER, 1962). For instance, some molecular mutation traits of the microsatellite evolution in *A. hybridus* are unique, but the critical situation of this taxon may result in their disappearance at the species and genus (*Ateles*) levels. Recall, that according IUCN (RYLANDS et al., 1997), this taxon is considered to have a 20 % chance of extinction in the next 20 years.

The accurate determination of genetic relationships and biogeographical patterns is dependent on the collection of additional samples of different *Ateles* taxa and a greater incorporation of microsatellites types in studies. Current efforts for the conservation of *Ateles* could be hampered without further information regarding molecular speciation mechanisms.

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Appendix 1

Cranial mandible and dental measurements (38) analyzed in 27 *Ateles* skulls from Colombia.

1-Maximum Transversal Width
2-Zygomatic Width
3-Superior Facial Height
4-Total Facial Height
5-Nasal Width
6-Bigonian Width
7-Auricular Height
8-Greatest Skull Length
9-Nasal Height
10-Minimum Postorbital Width
11-Maximum Postorbital Width
12-Lower Face Length
13-Base Face Length
14-Basal Height
15-Palate Length
16-Palate Width
17-Foramen Magnum Length
18-Foramen Magnum Width
19-Symphisis Height
20-Maximum Length of Mandible
21-Mandibular body Height between P_1 and P_2

22-Mandibular body Height between M_1 and M_2
23-Mandibular body Height between M_2 and M_3
24-Mandibular Branch Width
25-Mandibular Branch Height
26-Biauricular Breadth
27-Upper Canine Length
28-Lower Canine Length
29-Upper Canine Breadth
30-Lower Canine Breadth
31-Upper Molar Length
32-Lower Molar Length
33-Upper Molar Breadth
34-Lower Molar Breadth
35-Maximum Biorbital Width
36-Orbital Height
37-Opistion-Nasal Spine-Opistion Distance (subnasal prognathisme)
38-Ectoconion-Nasion-Ectoconion Distance

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SOCIAL RELATIONSHIPS IN A FREE-RANGING GROUP OF BONNET MACAQUES IN TAMIL NADU, INDIA.

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Key words: Bonnet macaque, India, social behavior, grooming, sexual interactions

Abstract

Social relationships in bonnet macaques (*Macaca radiata*) are reflected by extensive affiliative and agonistic interactions between all individuals of different age, sex, dominance rank, and kinship groups. Adult females form strong linear dominance hierarchies with their daughters occupying ranks just below their mothers, which is typical among various Cecopithecine species. A habituated group of bonnet macaque (*Macaca radiata diluata*) in the village of Surakkadu near the town of Sirkali (Nagapattinam District, Tamil Nadu State, south India), was observed to record data on social behaviour. Females mainly initiated more affiliative behaviours than males. There were significant inter-sexual differences for grooming behaviour, grooming invitation and huddling and females involved more in those interactions. However, males displayed more homosexual behaviours such as touching genitals and presentation while females seldom mounted each other.

Introduction

Social relationships in bonnet macaques (*Macaca radiata*) are reflected by extensive affiliative and agonistic interactions between all individuals of different age, sex, dominance rank, and kinship groups (SINHA, 2001). Adult females form strong linear dominance hierarchies with their daughters occupying ranks just below their mothers, which is typical among various Cecopithecine species. Interestingly, bonnet macaque females rarely form coalitions against more dominant individuals that might reflect the acceptance of a rigid dominance hierarchy among females (SINHA, 2001). However, many individuals seem to be able to develop complex social strategies like tactical deception, and use them in various social contexts such as competition for food, access to mate and allo-grooming (SINHA, 2003).

Grooming relationships among bonnet macaque females are relatively intense, and mothers with newborn infants usually attract more attention than those without infants (KOYAMA, 1973; ALI, 1981). Besides, the higher frequency of unreciprocated grooming of subordinate individuals by dominant females seems to be a unique feature of the bonnet macaque. Nonetheless, pattern of grooming between females can vary according to different ecological or stressful conditions (SINHA, 2001; RAM et al., 2003). Adult males also form linear dominance hierarchies and it appears to be more distinct and unstable than those amongst females. Male hierarchies are maintained through direct aggression and therefore depend on individual's age and body conditions (Fig. 1). Unrelated males often form coalitions against other males, and sometimes use male infants as potential buffer against aggression from other males (SIMONDS, 1965; SUGIYAMA, 1971). However bonnet macaque males appear to show greater signs of tolerance towards other males, as evident in affili-

ative rituals, which are believed to reduce tension and to peacefully display and reinforce dominance rank. Furthermore, males groom each other at rates comparable to those of females (SIMONDS, 1965; ALI, 1981; SUGIYAMA,1971; SILK, 1994). This study focussed on a group of wild bonnet macaques in India to understand the social relationships.

Fig. 1: Display of sharp canines by an adult male leader of the study group (Photo: G. Agoramoorthy).

Methods

Two subspecies of Bonnet macaques have been morphologically recognized in India: the northern *M. radiata radiata*, and the southern *M. r. diluta*. Between February and May 2001, a habituated group of bonnet macaque (*Macaca radiata diluata*) in the village of Surakkadu near the town of Sirkali (Nagapattinam District), which is located 270 km south of Chennai city (Tamil Nadu State, south India), was observed to record data on social behaviour. The wildlife populations including bonnet macaques have been monitored in the study site since 1995 (AGORAMOORTHY and HSU 2001, 2002a,b, 2005; AGORAMOORTHY et al., 2000). The habitat of the group was a mosaic of fields planted with crops such as rice and beans, swamps (during the rainy season), grassland and pastures. The area occupied by the macaque group was about 2 km² with a large river Uppanar crossing nearby (AGORAMOORTHY et al., 2000). The group had a composition of 21 individuals including 4 adult males, 6 adult females, 6 juvenile males, 2 juvenile females, and 3 infants. All

adults were identified based on physical characteristics, and juveniles were classified in two categories depending on their estimated age (AGORAMOORTHY et al., 2000).

Data presented here account for 30 days of behavioural observation with a total of 225 hours of observations. Sampling periods were distributed during the day from 6.30 am to 6.00 pm, covering 7.5 hours of data collection each day. All occurrences of social interactions were recorded using 30 min focal animal sampling technique (LEHNER, 1996). Monkeys were identified as initiator – if they actively performed the behaviour or receiver – if they received it. Events such as playing, huddling and social grooming were considered to be finished when they were interrupted either by a 10 sec period or by an inter-specific interaction. For grooming, duration was recorded in seconds by noting beginning and finishing time. Behaviours were recorded on observation sheets along with other information such as the identity of individuals involved in the interactions, date, time of day, and weather conditions.

Affinity indexes were calculated for each pair based on the total of their encounters. A status hierarchy was set up according to dominance index that was calculated for each individual on the basis of agonistic and submissive interactions:

Dominance index = (% (agonistic initiated / agonistic received)) / (submissive initiated / submissive received).

For the analysis of relations between grooming and dominance, the status of each monkey was assessed in regards to the status of other monkey in the pair. The mean duration of a grooming bout for each pair was calculated by dividing the total time spent grooming by the total number of bouts for each pair. Independent sample Student *t*-tests as well as paired sample Student *t*-tests were used for analyses. When requirements were not met for these tests, their non-parametric counterparts were used –Wilcoxon rank test for matched and independent samples. Correlation analyses were performed through Kendall test. The *p* value was fixed at 0.05 for bilateral tests. Statistical analyses were performed using SAS software (2000).

Results and discussion

A total of 3429 affiliative interactions were observed during this study. Females initiated more affiliative behaviours than males ($t= 2.31$; $p< 0.05$). There were significant inter-sexual differences for grooming behaviour ($t= 5.58$; $p= 0.001$), grooming invitation ($t= 3.15$; $p< 0.02$) and huddling ($t= 3.87$; $p= 0.01$) and females involved more in those interactions (Fig. 2). However, males displayed more homosexual behaviours such as touching genitals ($t= 7.18$; $p< 0.001$ for received behaviours only) and presentation ($t= 3.95$; $p< 0.01$) while females seldom mounted each other (Fig. 2). Females initiated more affiliative behaviours toward females than males ($Ws= 0$; $p= 0.015$). Males initiated affiliative interactions more frequently toward females than vice versa ($t= 3.8$; $p< 0.01$). Besides, females initiated affiliative behaviours more frequently toward infants than males ($Ws= 10$; $p< 0.01$; Fig. 3). In contrast, males initiated affiliative actions more frequently toward older juveniles (6 months to 3 years) than females ($Ws= 11$; $p= 0.02$; Fig. 3). Inter-sexual interactions through affinity scores revealed a significant correlation between sexual and affiliative interactions ($T= 0.31$; $p= 0.03$).

Involvement in sexual interactions varied in terms of dominance rank and there was more variation within males than females. The F-*max* test for homogeneity of

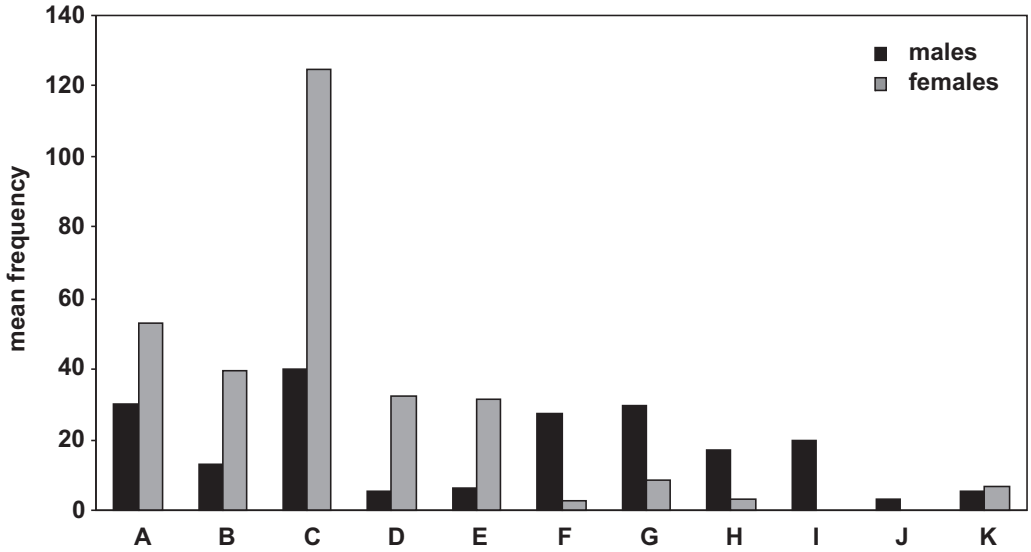

Fig. 2: Affiliative behaviours displayed by adult bonnet macaques. (A) contact, (B) solicitation to grooming, (C) allo-grooming, (D) huddling, (E) hugging, (F) play, (G) presentation, (H) touching genitals, (I) homosexual mounting, (J) greeting, and (K) mouth sniffing.

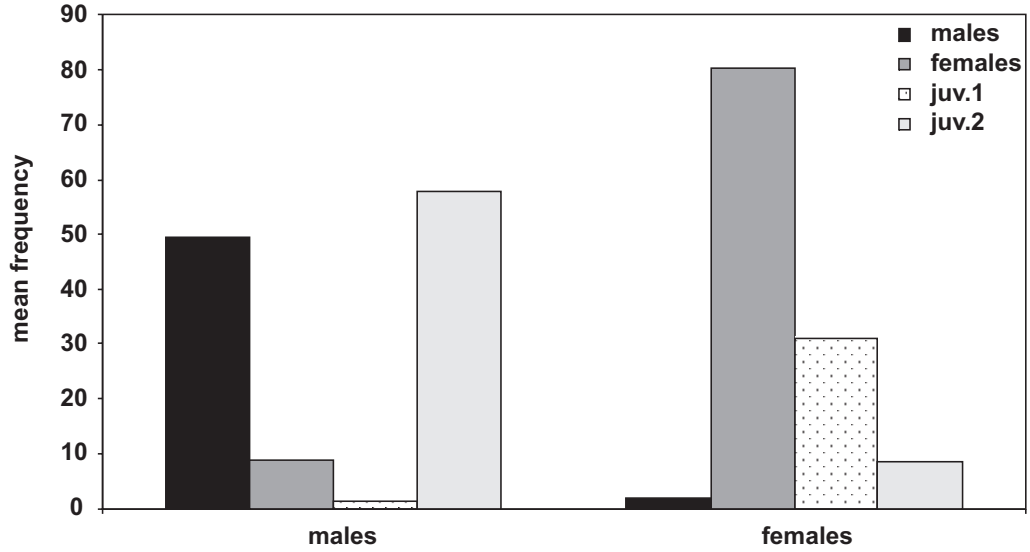

Fig. 3: Direction of affiliative behaviours displayed by adult bonnet macaques.

variance showed a significant difference between variances within sex categories (F=12.68 in males; > 7.76 in females). Analysis at the level of dyads gave interesting results-mixed dominant dyads (dominant males with dominant females) were in-

volved in more sexual interactions than any other type of dyad (dominant vs. crossed: t= 2.97; p< 0.01; dominant vs. subordinate: t= 2.31; p< 0.05; Fig. 4). Correlation analysis on mixed pairs showed a significant association between sexual and affiliative affinity scores (T= 0.31; p= 0.03). The mean duration of grooming bouts was significantly longer when directed from subordinate to dominant individuals than when directed from dominants to subordinates (t= 2.34; p= 0.03) and the trend was more obvious in females (t= 2.05; p= 0.059) than in males (t= 1.46; p= 0.2).

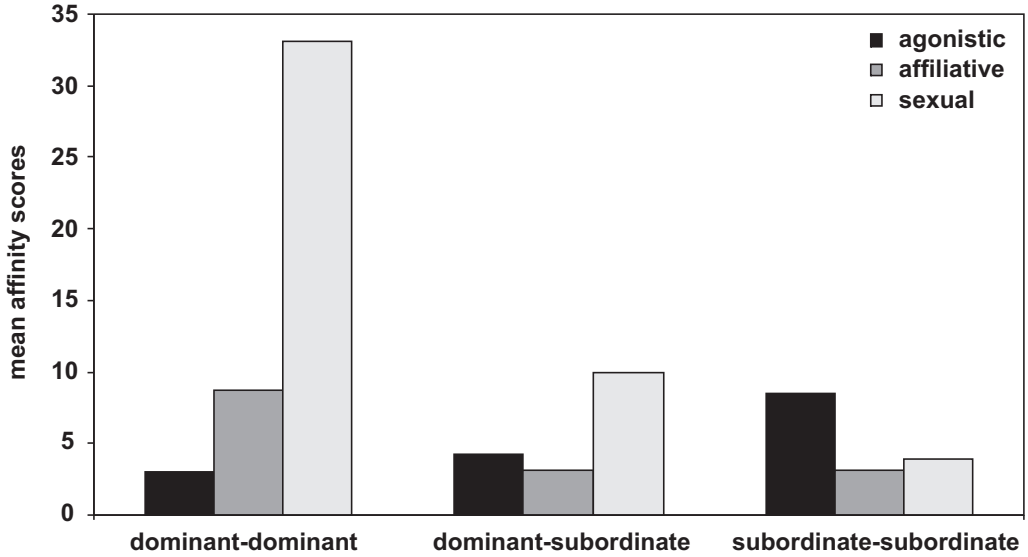

Fig. 4: Social relationships in different dyad types among bonnet macaques. Three types of dyad could be defined according to the status of the monkeys involved: dominant dyad (dominant-dominant), crossed dyads (dominant-subordinate) and subordinate dyads (subordinate-subordinate).

Females engaged significantly more often than males in allo-grooming, and the dominance rank was relevant to grooming only in females. Grooming bouts appeared to be significantly longer when directed to the dominant individual of the pair. These findings support an earlier study where grooming among adult males was less common than females, and grooming in male bonnet macaques appeared to be unrelated to rank (SUGIYAMA, 1971). As a matter of fact, female grooming was related to rank and grooming relationships could play an important role in regulating other aspects of females' social life. For example, among captive bonnet macaques, low ranking females obtained temporary protection from aggression by grooming high ranking females (SILK, 1982). Yet, it was not clear whether the risk of being harassed was also reduced outside the grooming context, so more data both from the wild and in captivity are needed to address this issue.

The prevalence of particular behaviours in a given sex class suggests that males and females differed in the way they lead affiliative relationships with individuals of their own sex class. Males did not groom as much as females and they were more in-

volved in homosexual behaviours. Indeed, homosexual behaviours lasted only for a few seconds whereas grooming or huddling sessions lasted more than half an hour. It follows that females of this group could be regarded as more closely associated through affiliative interactions than males and it supports the hypothesis that affiliative interactions between males could be used to peacefully maintain hierarchical relationships rather than to establish long lasting bonds (SUGIYAMA, 1971; RASMUSSEN, 1983).

Our study also showed that relationships between opposite sex were mainly based on sexual interactions. In addition, the correlation we observed between sexual and affiliative affinity indicates that pairs that showed more affiliative relationships were likely to involve in sexual interactions. Along with the general tendency that males initiated more sexual interactions than females, we observed that males also initiated more affiliative behaviours toward females. Considering that the proximate outcome of affiliative interactions is a decrease in tension, our observations suggest that males' endeavour to gain access to mate is not only reflected by obvious sexual behaviours but also by efforts to preserve a context facilitating sexual interactions. Behaviour of pairs that associate in non-sexual contexts may achieve a higher degree of coordination than others, thereby increasing the probability of fertilisation as suggested for the baboons (RASMUSSEN, 1983). Dominant females also showed more participation in sexual interactions, but the variation between individuals of that class was significantly lower than variation within males. In addition, our study showed that dominant mixed pairs (a dominant male with a dominant female) were involved more frequently in sexual relationships than any other type of pair, which is similar to earlier studies of bonnet macaques (GLICK, 1980; SILK et al., 1981).

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APPLIED PRIMATOLOGY IN ZOOS: HISTORY AND PROSPECTS IN THE FIELD OF WILDLIFE CONSERVATION, PUBLIC AWARENESS AND ANIMAL WELFARE.

Gippoliti S

"*Immediately I was struck by the physical magnificence of the huge jet-black bodies blended against the green palette wash of the thick forest foliage*"

Dian Fossey – Gorilla in the mist, 1983

"*They didn't look very attractive*, *with their squared-off muzzles*, *their dull hair*, *and their red hindquarters. That hamadryas baboons can be beautiful I experienced only later in Ethiopia when*, *after hesitating at length*, *a hundred of them descended from their sleeping rock in the morning*."

Hans Kummer – In Quest of the Sacred Baboon, 1995

Key words: zoological gardens, primates, animal welfare, conservation education

Abstract

A short historical overview of primate keeping is offered showing how each generation of zoo managers tried to improve standards according to prevailing scientific knowledge. This historical approach to primate husbandry may help to identify areas of concern about current management and husbandry techniques, often obscured by the current overenthusiastic rhetoric on modern zoo exhibits. Examples are given of issues potentially of great welfare significance deserving more scientific investigation, such as behavioural properties of diets, indoor/outdoor housing, climatic factors, species-specific social structure, birth control and intraspecific communication. Given ambiguity in defining 'animal welfare', it is argued that zoos should give priority to emphasizing the conservation message of their primate exhibits.

Introduction

In recent years, there has been a growing tendency to consider the last two decades as a new era in the management of zoo animals, and primates in particular (WALLIS, 1997). The great influx of scientific research into zoo primate management has been considered sufficient to coin the term 'applied primatology' to indicate the developments in the sector beginning from the 1980s (MAPLE and FINLAY, 1989). At the same time, a shift of zoos' interest from public entertainment to wildlife conservation has been universally reported (e.g. Rabb, 1994), reinforcing a clear-cut view of zoo history. A more thorough look at the specialised literature shows the existence of several areas of concern, ranging from nutrition to breeding programmes and public response to new exhibit styles (e.g. KAUMANNS et al., 1998/1999;

KAUMANNS et al., 2000; HYSON, 2000). Although it would be foolish to overlook the somewhat drastic changes occurring in the exhibition criteria of the major zoos or the increasing success in captive breeding, self-originating claims by contemporary zoos to be 'primate Edens' seem quite exaggerated, or, at least, premature. In particular, there is often assumed to be a strong correlation between 'naturalistic' (generally meaning 'without bars') enclosures and improved animal welfare (MELFI and FEISTNER, 2002). However, there is confusion between the public perception of how animals respond to 'naturalistic' habitats and the actual response of the animals themselves (ROBINSON, 1999). Furthermore, neglecting the long history of primate keeping in zoos might cause the loss of precious knowledge of great utility in furthering current zoos' goals and animal welfare. This is particularly important as scientific knowledge is translated into legislation about zoos and animal welfare, but most studies on animal welfare and environmental enrichment originated in laboratories whose living standards are, on average, lower than in zoos (cf BUCHANAN-SMITH et al., 2004).

The present paper is intended to briefly review the history of primates in zoos, to outline the many fashions in primate-keeping and to identify those aspects in zoo primate exhibits and management that are crucial to achieve zoos' multi-faceted goals.

A short overview of primate zoo exhibit history

A comprehensive history of zoo primate-keeping has yet to be written. However, knowledge of the developments of some of the world's major zoos might furnish enough information about the general trends in primate management history. The foundation of the Menagerie at the *Jardin des Plantes* of Paris in 1793 is usually considered the birth date of scientifically-managed zoos, i.e. those directed by a scientific staff with the stated purpose of contributing to public education and acquiring knowledge of the animal world (HANCOCKS, 2001a). In 1828 the Menagerie of the Zoological Society of London was founded. The great contribution to scientific knowledge achieved by these two early zoos is sharply in contrast with the recent introduction of science in primate management advocated by MAPLE and FINLAY'S (1989) definition of 'applied primatology'. Furthermore, a technical bulletin of the German zoo directors, *Der Zoologische Garten*, has been published ever since 1859, and technical innovations have been continuously introduced during the long history of zoos. Glass panels to separate apes from visitors, for example, were introduced in Frankfurt as early as 1871 (RAWLINS, 1979). In Paris the original 'singerie' opened in 1837 consisted of a big rounded outdoor cage connected to several smaller indoor cages. In the 'singerie' of the Royal Turin Zoological Garden, ten small indoor cages were connected to the large, glass-covered outdoor cage (MASCHIETTI et al., 1990), thus greatly limiting the opportunities of outdoor access to all primates, but in December 1871 hamadryas baboons were held outside in the carnivore section at -7°C (GIPPOLITI, 1997). In 1864 London Zoo opened a new glass-house style Monkey House with only indoor accommodation for the animals, an approach which subsequently became widespread in Europe at the time. The technical premise of such a choice was the unsuitability of the European climate for tropical animals, including primates. The deleterious effects of this kind of accommodation on the animals' skel-

etal growth were evidenced by Bland Sutton (1884). Peter Chalmers, from 1903 Secretary of the Zoological Society of London, wrote "The most fatal type of housing for any mammal or bird is confinement in the interior of a warmed house without free access to open air… The first requisite is free access to open air, the next is light, space and cleanliness…. For all mammals and birds, steady exposure to an even temperature is unnatural and unhealthy; change is a necessary condition for viability and longevity" (cited in HUXLEY, 1981). However, already in 1875 Munster Zoo had a monkey house in which the animals could move as they pleased from outside to inside all the year round through specially designed metal doors (Hancocks, 1971). On empirical grounds, the importance of the climatic factor was also challenged by Carl Hagenbeck, a unique figure of animal trader, trainer and zoo man. With the aim of reducing costs and exhibiting animals in more natural settings, Hagenbeck was a strong supporter of the 'acclimation' of tropical animals to temperate climates. His monkey houses were simple, unheated buildings always provided with outdoor cages. In the period 1912-1913, Hagenbeck and his team created in Stellingen (Hamburg) and in Rome the first naturalistic, bar-less exhibits for primates (Fig. 1). In Rome Zoo's original 'Monkey Village' (1912), Indian temples and living plants, including two hundred-year-old pine trees, were surrounded by a dry moat. The macaques, mainly *Macaca mulatta*, were kept outside year-round, and went to sleep high in the trees at night (KNOTTNERUS-MEYER, 1925). Although this kind of open exhibit became widespread in zoos around the world, their use was restricted to the more robust, terrestrial baboons and macaques. Their utilisation was often abandoned after problems encountered with diseases and social conflicts, such as those described in the pioneering work of ZUCKERMAN (1932).

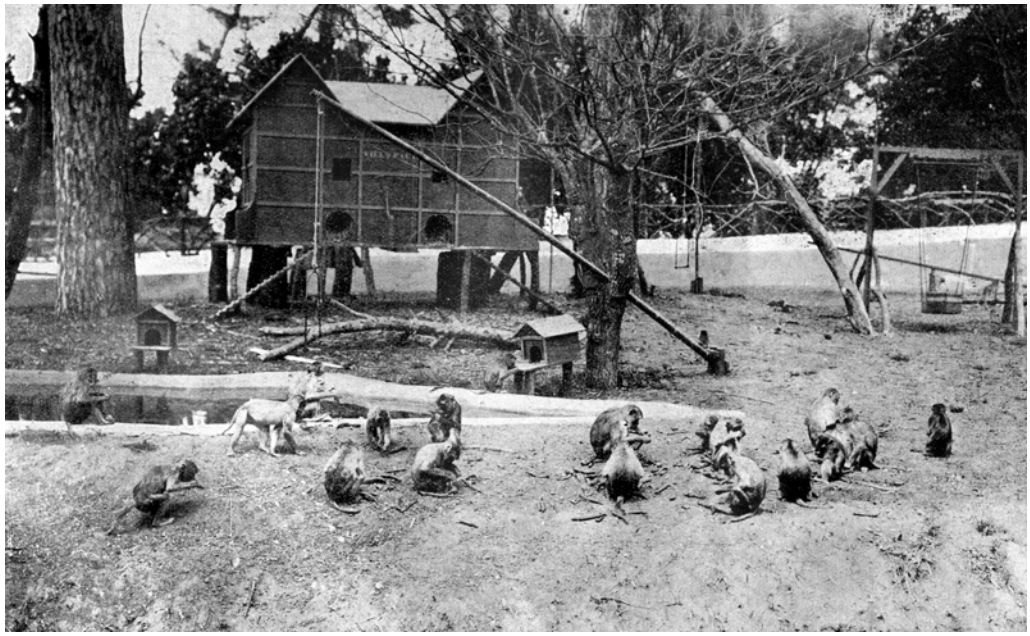

Fig. 1: Macaques in the Monkey Village of Rome Zoo, realised in 1912.

The sanitary epoch

Knowledge of problems encountered in those years is essential to understanding the rise of the modernistic-hygienist style in zoo architecture in the last Century. In 1932, for instance, a family of chimpanzees, including the first captive-bred one to be reared successfully in Europe, was killed by TB at the Rome Zoo (D'ALESSANDRO and GIPPOLITI, 1996). Hygienic developments were positively accepted by Hediger, the founder of zoo biology, who emphasized the need for functionality in cage design instead of romantic attempts to recreate the wild (HEDIGER, 1950). Hediger greatly opposed another development in zoo management after World War II, i.e. the preparation of standard diets for zoo animals (HEDIGER, 1965). It is interesting to note that, while zoo design has now universally condemned the barren hygienic cage style, much less attention seems devoted to the effects on welfare and physiology of the now widespread commercial zoo diets, despite a few cautionary papers on the subject (STOLLER et al., 1989; SCHWITZER and KAUMANNS, 2001).

However, going back to exhibit design, "sanitary modernism" coupled with zoos' desire for "postage-stamp" style collections resulted often in highly restricted and deprived habitats for primates in zoos. Particular attention was devoted to apes' accommodation and care. Before World War II, they were often kept permanently indoors, with no access to water, all food boiled and peeled, and no bedding material was provided for nesting (CRANDALL, 1964; STEMMLER-MORATH, 1968). Yet Desmond Morris and Caroline Jarvis, while introducing a special section on Great Apes in the first volume of the *International Zoo Yearbook* in 1960 (a classical reference on scientific zoo-husbandry for almost half a century now) say "Methods of exhibiting and caring for great apes in captivity have improved tremendously during the past twenty years". In fact, several modern monkey or great ape houses were built in those years, often copying one another. New primate and ape houses made of glass, concrete and steel, sometimes without outdoor accommodation, were first opened in Philadelphia in 1958 and successively in Frankfurt, Berlin, Antwerp, Basle, Zurich etc. Apart from hygienic factors, there was often little improvement until the 1970s. Scientists made proposals to improve the situation of captive apes, particularly chimpanzees, as field work on these animals increased (KORTLAND, 1961; REYNOLDS and REYNOLDS, 1965). In the 1950s outside enclosures with water moats were created for great apes in continental-climate zoos, for instance at the Bronx Zoological Park, where the male gorilla Makoko drowned in 1951. This event inaugurated a list of tragedies still continuing in our days (PATEROK, 2004), highlighting the conflict between aesthetically pleasant open exhibits and animal welfare. Chimpanzees generally achieved more success; in 1956 Chester moved its group onto islands surrounded by a water moat (MOTTERSHEAD, 1960). In the Arnhem Zoo, a large colony of chimpanzees was assembled and a complex outdoor habitat, including living trees, created (VAN HOOFF, 1973). In the sixties special houses were created to exhibit, through an inversion of the day-night regime, active nocturnal animals including primates (MORRIS, 1965). Although these 'night houses' are now losing popularity, the high artificiality of this kind of captive habitat suggests that more research should be directed toward the assessment of welfare among primates in zoos' night houses (SCHULZE, 1998). Interestingly, good results have been reported with owl monkeys of the genus *Aotus* kept in naturally lighted cages (WALTER and BROWN, 2004). Among the few early examples of more natu-

ralistic habitats, gibbons were introduced to islands surrounded by water first by the French ornithologist Delacour in his own park at Clères in 1926 (DELACOUR, 1933) and later in 1934 at the new Paris Zoo in Vincennes and at Rome. For decades, however, many improvements in primate keeping were introduced by practitioners running their own private zoos, and thus far from the scientifically-managed collections. Foremost I would mention the late John Aspinall for having introduced so many crucial aspects in the management of zoo gorillas, mainly utilising available information from field studies and recognising the need to adopt an individualistic attitude in the care of these animals. His 'gorillariums' are nothing more than large cages able to hold a large social group of gorillas, with deep litter of oat straw and a roof open to the weather and cobwebbed with brachiating bars (ASPINALL, 1986). Despite being one of the two most successful breeding colonies in the world, Aspinall's functionalist model has been largely ignored by the zoo world, now launched upon the landscape immersion architecture.

Landscape immersion

A number of criticisms have been made of the old zoo exhibits by zoologists, psychologists and landscape architects (MORRIS, 1964; SOMMER, 1972; COE, 1985). The rise of environmental issues in developed countries made still more evident the fact that, leaving apart objective welfare criteria for zoo animals, the message of hard architecture in zoos was one of anthropocentrism and superiority of humans over wildlife (SOMMER, 1972). To reverse the situation, the landscape immersion approach was used extensively in the Seattle Zoo master plan developed by HANCOCKS, JONES and COE in 1976 (GOLD, 1997; HYSON, 2000). Later, a manifesto for a naturalistic way to present and manage animals in zoos appeared authored by the Seattle team of biologists (HUTCHINS et al., 1984). It is interesting to note however that one of the most excellent attempts to simulate a landscape immersion experience with captive primates preceded this paper by many years. In the sixties, the Miami Monkey Jungle allowed the public access to a one and a half hectare tropical rain forest including several species of South American primates, such as the delicate red uakari *Cacajao calvus* (FONTAINE and DUMOND, 1977). On the other hand is a fact that several of the costly large indoor 'tropical rain forest' facilities developed in the US since the eighties appear not substantially different from the old bare cages of the past, as the naturalistic experience (i.e. living plants) is mostly limited to the public pathway (HANCOCKS, 2001a,b). This may be due to the extremely high construction costs of this kind of exhibit (RUSSELL and WEST, 1997) and lack of space. Furthermore, even when spacious and naturalistic outdoor exhibits are available, animals may spend most of the twenty-four-hour day in smaller indoor holding cages that are almost perfect replicas of the old barren cages (HANCOCKS, 2001b), and this may lead to stressful situations (AURELI and DE WAAl, 1997). Despite the importance of this aspect of the life of zoo animals, the issue does not appear to attract much scientific interest. However, a number of European institutions, such as the zoos of Apenheul (NL), Jersey (GB) and Rheine (GER), have been able to create fine outdoor naturalistic exhibits without the exaggerated costs of zoos in North America (MAGER and GRIEDE, 1986; REDSHAW and MALLINSON, 1991; SALZERT, 1989).

A look at the present without rhetoric: are we really approaching Eden in zoos?

While 'animal welfare' is becoming an autonomous and respectable issue, many crucial aspects of the management of wild animals in zoos seem to receive scanty attention by researchers. Only recently, for instance, have the effects of high temperature and insulation on gorilla behaviour received attention (STOINSKI et al., 2001) despite its known basic importance for animals. Coupled with the preference of people to visit zoos in the hottest part of the day and of the year, it is no surprise that visitors often lament seeing only inactive animals. Perhaps, it is necessary for zoos to increase the opportunity to see animals when they are more active, for instance in late afternoon, even changing their opening hours and daily husbandry regimes.

Furthermore, modern naturalistic exhibits are intrinsically more prone to escapes and present more 'dangers' to the animals (e.g. water moats). This makes it absolutely necessary to lock animals in the indoor holding quarters for the night, so that most animals spent only the hottest hours in the large, often inadequately shaded, naturalistic enclosures. Although there are no studies on this aspect, primates seem to benefit from the free access to outside enclosures when climatic conditions allow it, even to the point of refusing food by not entering indoor cages in the evening during the good season (GIPPOLITI, pers. obs. on chimpanzees).

How functional are naturalistic exhibits? Recent studies on the western lowland gorilla highlighted the importance of arboreality and frugivory for this species and of swampy areas (*bai*) for feeding and social behaviour (DORAN and MCNEILAGE, 1998; PARNELL and BUCHANAN-SMITH, 2001). All too often even the best 'naturalistic' enclosures lack these crucial features, including nest building opportunities for apes (PRUETZ and MCGREW, 2001), that significantly reduce the educational potentialities of these exhibits. The functionality of the exhibit (for instance for arboreal monkeys) may be enhanced through the inclusion of artificial structures (trees, lianas etc.) accurately inserted in an 'ecologically representative' habitat with natural soil, real trunks and living plants, as has been done in Atlanta (CHANG et al., 1999). As stated by FORTHMAN QUICK (1984), hidden mechanical feeding apparatus can encourage arboreal locomotion in primates without damaging the naturalistic impressions of these exhibits. Further, a compromise must be found between the need to protect some living vegetation and the need of the animals to interact with it. However, especially in northern climates, the transformation of outdoor naturalistic exhibits to 'living habitats' will necessarily require a change in the strict routines imposed on most captive primates, allowing longer access and greater functionality (feeding) of the outside area at least in the good season. Actually, it is not rare to see gorillas and orangutans not using outdoor areas, probably for a number of reasons such as lack of furnishings, stimuli such as feeding, shade etc. (GIPPOLITI, pers. obs.).

Another matter receiving little interest is the effects of 'social impoverishment' on the activity of zoo animals. Even successful species in captivity, such as the threatened lion-tailed macaque *Macaca silenus*, are known to be held in sub-optimal social conditions that can lead to negative consequences for the long-term survival of the species in captivity (KAUMANNS et al., 2001). More generally, the old traditional primate houses allowed at least visual and acoustic contacts and communication among several – closely related – species housed in rows of tiny and barren cages. This kind of 'social enrichment' (although probably sometimes quite stressful

for the more delicate species) is today declining as fewer and fewer species are exhibited by a single zoo and each cage is visually and acoustically isolated from the next. In some modern exhibits this is counteracted by creating multi-species assemblages resembling those polyspecific associations that occur in the wild (Hardie et al., 2003) or by allocating several enclosures to more than one social unit of the same species, as has been done with gorillas in Atlanta (MAPLE, 1993), geladas in New York (DOHERTY, 1991) and orang-utans in Apenheul (JENS, 2001).

If training through positive reinforcement represents a cognitive enrichment for captive primates (Shepherdson, 2003), its origin goes back to Hagenbeck and many others who recognized the importance of mental occupation especially for apes (HAGENBECK, 1909; STEMMLER-MORATH, 1968). Consequently, the value of former chimpanzee 'tea parties' and similar shows should perhaps be reconsidered at least from the point of view of animal welfare, even if they are counterproductive from a conservation and educational perspective.

Closely related to social factors is the problem of birth control. An ever increasing number of primate species are subject to population management that requires birth control through chemical or surgical methods. Not only may this be a problem in the management of captive populations of threatened species (DE VLEESCHOUWER et al., 2000), but, considering the importance of parental care in primates, it is inevitable that breeding suppression should produce negative consequences on group stability and on the typical expression of some behavioural patterns in captive primates (PRICE, 1997; De VLEESCHOUWER et al., 2003). Again, Hediger's opinion on this matter was very clear: "For the majority of surplus zoo animals, the best solution would be a kind of cropping, the humane selective elimination of those animals that can no longer be maintained. Of course, for the zoo biologist, who seeks to preserve healthy living species, this is a tragic way…. This method, however, is certainly preferable to surgical or medicinal castration, which would force the animal into an unnatural lifestyle" (HEDIGER, 1982). It is thus necessary that breeding control be implemented giving attention not only to population management factors but also to each species' social system.

While the nutritional value of food for captive animals has traditionally received great attention (RATCLIFFE, 1966; MANGILI, 1970; WACKERNAGEL, 1977), its behavioural properties have been greatly neglected (but see HEDIGER,1965 and ADESSI et al., 2005). It should be noted, however, that old diet routines were more varied than is usually thought. According to KNOTTNERUS-MEYER (1925) the macaques in Rome were fed three times a day. At 8.00 am they received tea with bread and milk, at 12.00 carobs, maize, rice and different kinds of leaves and grasses, plus figs, cherries and fennel seasonally. The last meal at 5.00 pm (6.00 in summer) included onions, apples, potatoes and chestnuts, all whole and boiled. It is known that food availability affects the sociality of the different primate species as of most other organisms (WRANGHAM, 1980), and that the same species can show a different social structure as a response to different environments and food availability. Therefore, a greater attention to food presentation to meet the particular needs of each species may be crucial to maintain its typical social organization and increase the educational relevance of the exhibit. The positive effect has been demonstrated of providing whole rather than chopped fruits to medium-sized primates (SMITH et al., 1989). Feeding schedules in zoos are often highly predictable. In a

study on the effects of unpredictable feeding in a laboratory colony of *Macaca arctoides*, WAITT and BUCHANAN-SMITH (2001) argued against the usefulness of adding such variables to the husbandry schedule. However, the fact that the studied macaques received only a piece of fruit in the morning may explain how delays in the afternoon meal could create a stressful situation due to severe hunger. Thus it is likely that in the more varied and appropriate zoo husbandry schedule, adding some levels of unpredictability may be really advantageous for the animals. Much remains to be done concerning techniques such as scattering food like seeds in the substrate to increase foraging time (ANDERSON and CHAMOVE, 1984). This appears a proper enrichment for semi-terrestrial species such as baboons *Papio* spp., many macaques *Macaca* spp. and the green monkey *Chlorocebus aethiops* (the species most commonly kept in laboratories), but it is completely inadequate for the many arboreal species found in zoos. Habituation to move and forage on the ground has negative consequences on the survival of captive-bred reintroduced primates (CASTRO et al., 1998; BRITT et al., 2001). But even if they will never be released into the wild, zoo primates living mostly on the ground greatly reduce their educational potential and will possibly negatively affect visitors' response to the exhibit and to the conservation message (GIPPOLITI, 2000). Contrary to public belief, arboreal primates may be better suited to mesh enclosures that provide them more usable vertical space (GOLD, 1997). This issue certainly deserves more attention from researchers and designers (e.g. ZIMMERMANN and FEISTNER, 1996). More traditional cages may allow arboreal primates to be fed on the top mesh and exhibit proper locomotion behaviour (BRITT, 1998), so it should be worthwhile to try to reconcile the functionality of a mesh roof with a naturalistic, fence-less exhibit.

Negative effects of visitors on the behaviour of zoo primates, particularly on tamarins (GLATSTON et al., 1984), may be linked to the small size and reduced height of the cages in which these minuscule monkeys are usually kept (Fig. 2a,b).

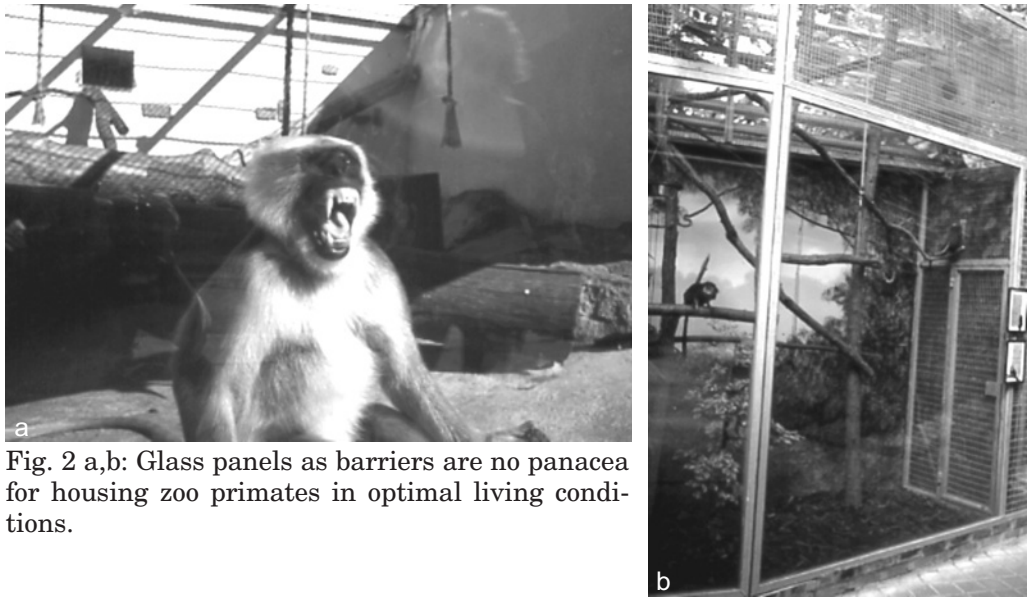

Fig. 2 a,b: Glass panels as barriers are no panacea for housing zoo primates in optimal living conditions.

These negative effects can be easily counteracted by providing larger and higher cages (WORMELL and BRAYSHAW, 2000). However, species of Cercopithecidae and in particular of the genus *Cercocebus* may be highly susceptible to human audiences (MITCHELL et al., 1991), although the effects on fertility and social relationships are not well understood. It is important that zoo design recognize species-specific responses to visitors and try to ameliorate such problems.

Conclusions

A critical review of zoo primate husbandry history does not allow the identification of a clear-cut demarcation in philosophies and techniques around the 1980s, as is often argued. It is true, however, that at that time the environmental crisis and increasing attention to animal welfare in national legislations required greater involvement in these fields by zoos. Considering how little we still know about 'animal welfare' and how to measure it (DAWKINS, 1998; CHANG et al., 1999; LITTLE and SOMMER, 2002; HOSEY, 2005), priority should be accorded to exhibit designs (Fig. 3) and husbandry regimes which enhance the intuitive conservation message of zoos (LINDBURG and COE, 1995). This should be possible if zoo primates are given the opportunity to transmit the same wonderful experience primates in the wild are able to send (see the two quotations from Diane Fossey and Hans Kummer printed at the beginning of this article). While reintroduction to the wild appears a secondary goal for zoos nowadays (HUTCHINS and CONWAY, 1995), the maintenance of appropriate behavioural repertoires and patterns in the captive populations (VAN HOFF, 1986; RABIN, 2003) respond to the educational goals and conservation ethics of what a modern zoo should be. Only recently has the importance of habitat size dependent factors in wildlife conservation begun to be fully appreciated (i.e. BAILLIE et al., 2000). Concurrently with their conservation mission, zoos should plan their exhibits not only to satisfy animal needs but also to highlight the need for large areas of undisturbed landscape if we really want to save biodiversity. In fact, well-kept and 'happy' primates in small zoo exhibits may lead visitors to overlook the importance of area size for the conservation of biodiversity (GIPPOLITI and SPERANZA, 2005). Popular exhibits such as those allowing a closer contact with primates (e.g. WEBSTER, 2000) should be critically reviewed in the light of the dangers posed by tourism (i.e. transmission of disease) to free-ranging primates (WALLIS and LEE, 1999; WOODFORD et al., 2002). Actually, it is time for zoos to recognize that if they want to serve as serious conservation organizations, they need to consider not only opportunities but also constraints imposed by this new role. While using twenty-first-century attitudes to judge zoo history is obviously inappropriate, knowledge of the historical developments in primate husbandry and an assessment of current husbandry routines is often overlooked. Research on environmental enrichment in laboratories highlighted some issues (e.g. the effects of inanimate objects) while some basic aspects such as the effects of diet and feeding routines, outdoor housing and variable temperatures have been incredibly overlooked. In agreement with a more holistic natural history-based approach to animal welfare and environmental enrichment proposed by NEWBERRY (1995) and MELLEN and MACPHEE (2001) more focused scientific investigations of zoos' current husbandry may greatly benefit our understanding of animal welfare problems and how to overcome them.

Fig. 3: Gorillas in a beautifully landscaped exhibit at the Paignton Environmental Park.

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Cover photo: Old female *Macaca assamensis* at the Rome Zoological Garden. This female lived for more than 31 years in a traditional zoo cage, establishing the record longevity for the species in captivity (S. Gippoliti).

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