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Genetic variability in two captive colonies of *Cebus apella paraguayanus* (primates: platyrrhini) from eastern Paraguay

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Abstract — The tufted capuchin, *Cebus apella* is a New World Monkey (NWM) species widely used in biomedicine. Its genetic variability has been assessed through various genetic tools. The present study offers a cytogenetic characterization of two captive colonies of Paraguayan specimens, in order to increase the current information on the role of cytogenetic polymorphisms in management of captive capuchins, specially useful for those employed as biomedical models. G-banding confirms all individual as *Cebus apella paraguayanus* (CAPp). C- banding shows heterochromatic polymorphisms already published for this species, and a paracentric inversion of the interstitial heterochromatin of pair 12, with members of this pair remaining of equal size in all the sample. A retrospective study of C-band polymorphisms in captive CAPp individuals shows a great number of heteromorphisms, considering not only presence/absence of a particular band, but also size and amount of heterochromatin. Therefore, two types of heterochromatic heteromorphisms have been observed (distributional, with a paracentric inversion but no apparent loss of heterochromatin, and quantitative, with a drastic reduction of one homologue's band, therefore on its total size) suggesting complex chromatin rearrangements for Paraguayan populations, and supporting the idea that *Cebus* is, among the NWM, perhaps the best model to analyze heterochromatin behavior.

Key words: Captive colonies; *Cebus apella*; Cytogenetics; Heterochromatin; Polymorphisms.

INTRODUCTION

The tufted capuchin, *Cebus apella* (Platyrrhini: Cebidae) is widely distributed along South America, ranging from 5° north to 30° south. The subspecies *Cebus apella paraguayanus* (CAPp) popularly known as "Ka'i Paraguay" has been reported from southern Brazil, southwestern Bolivia, eastern Paraguay up to the left bank of the Paraguay river and the southeastern portion of Argentinean provinces of Jujuy, Salta and probably Formosa (CABRERA 1957, MANTECÓN *et al.* 1984). Recently, its variability has been studied using various genetic tools, such as cytogenetics, electrophoretic allozyme patterns and molecular genetics (MUDRY 1990, SZAPKIEVICH *et al.* 1998, ASCUNCE *et al.* 2002). Cytogenetics of *C. apella* has been directly compared with man's, using almost all banding techniques, replication chronology and, more recently, ZOO-FISH (COUTURIER and DUTRILLAUX 1981, RICHARD *et al.* 1996, GARCÍA *et al.* 2000). A very large analogy among different karyotypes has been found and comparisons have not only been limited to the autosomes. We found at both mitotic and meiotic levels a sexual determination system like the human XY (MUDRY *et al.* 2001). This could be considered an advantage for the election of this species as a biomedical model. Genotoxic effects of physical mutagens have also been performed using *Cebus* as a valid experimental model (BORRELL *et al.*1998).

Cytogenetic variability of individuals belonging to two captive colonies of CAPp of known geographic origin is described in order to confirm its karyotype and establish any parameter that might be useful for further pedigree establishment or genetic variability assessment, since some of these animals are currently under mid and long term biomedical research protocols. All animals were treated according to the recommendations from the "Statement of

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Principles on the use of Non Human Primates with biomedical purposes" published by the International Primatological Society (IPS) (POOLE and SCHWIBBE 1993).

MATERIALS AND METHODS

All studied individuals are of Paraguaian origin, specifically from the departments of San Pedro and Encarnación. Captures were made using live trapping following conventional methods. Animals from Encarnación (one female and six males), are kept at the Centro Argentino de Primates (CAPRIM), at Corrientes province, Argentina. 5 females and 6 males, captured in San Pedro, belong to the Institute for Research on Health Sciences (IICS), from Asunción, Paraguay. Additional animals (N=22), also captive in CAPRIM at different times, were retrospectively analyzed for C-band heteromorphism. Whole blood (3-5ml) was obtained by venipuncture with disposable heparinized syringes from anesthetized (ketamine 22 mg/kg) animals. 0,5 – 1ml of every blood sample was cultured under standard conditions detailed elsewhere (MUDRY and SLAVUTSKY 1987). Metaphases were scored and treated for G and C bands using standard protocols (SEABRIGHT 1971, SUMNER 1972, modified). C-band polymorphism frequency was assessed by registering the absence of a particular heterochromatic band on one or both members of a chromosome pair.

RESULTS AND DISCUSSION

A modal number of 2n=54 for both sexes was recorded in 71% of the analyzed metaphases (N=211), with 29% of hypoploid cells (2n<54). General features for this species, such as the chromosomal formula (16SM+4M+32A, XY), the XX/XY sexual system, the secondary constriction of pair #22, and the G banding pattern are all coherent with the phenotype description, and correspond to *Cebus apella paraguayanus*. The analysis of C-bands showed the expected pattern for CAPp, with extracentromeric heterochromatin for chromosome pairs 4, 11, 12, 13 and 19. Polymorphisms of location and quantity of heterochromatin are present for pairs #11 and #12 as has been described elsewhere for *C. apella* from other geographic locations (MUDRY 1983, 1990, MUDRY *et al.* 1991). Additional CAPp from CAPRIM were included for polymorphism analysis of pair #12 and these animals showed only one interstitial band. Twelve of them showed a severe heterochromatin loss for one homologue, giving heterochromatic heteromorphism on 26.3 % over the total metaphases analyzed (Fig 1a). All IICS animals had bands on both homologues and thirty five of the eighty nine analyzed metaphases (39.3%) showed a second interstitial band on chromosome #12 as a result of a paracentric inversion (Fig. 1b). All specimens have large analogies to our previous findings as well as other published descriptions, except for chromosome #12 (MUDRY and SLAVUTSKY 1987 MATAYOSHI *et al.* 1987, MUDRY 1990, MUDRY *et al.*

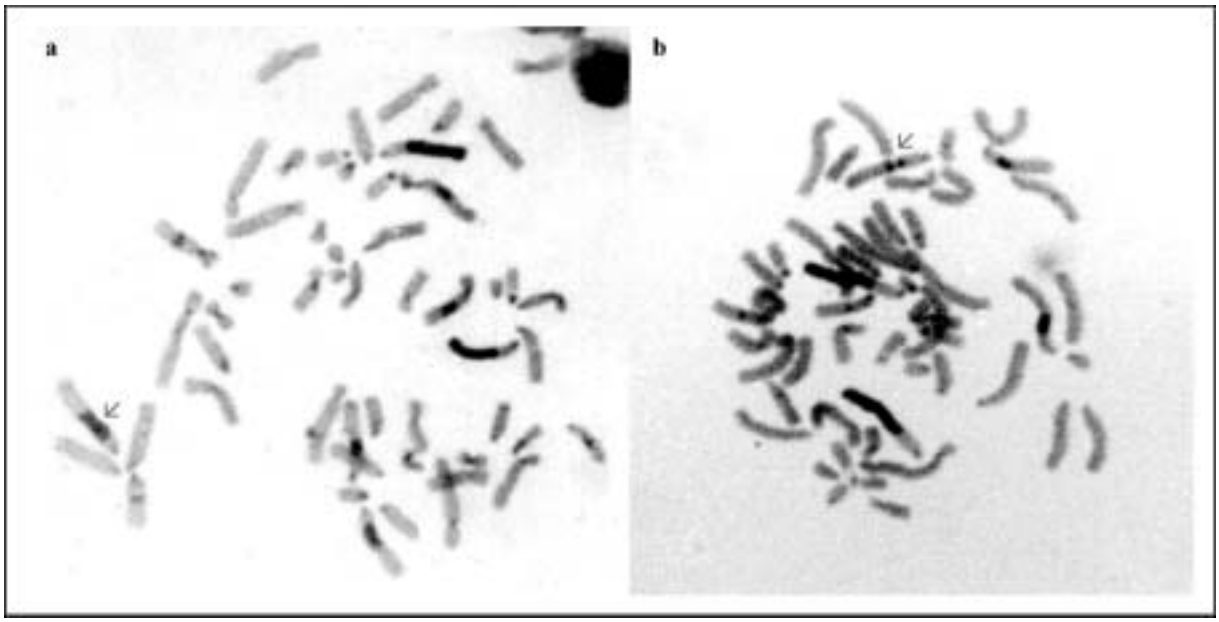

Fig. 1 — Polymorphisms for pair #12 of CAPp from Paraguay. 2a). Significative loss of heterochromatin in one homologue. 2b) Heterochromatic Paracentric Inversion.

Table 1 — Polymorphisms for chromosome #12 in *Cebus apella paraguayanus* from Paraguay

Source	Polymorphic individuals/Total	Metaphases scored		
		Total	Par. Inv.[1] (%)	Heterochromatic heteromorphism (%)
IICS	6/11	89	39.3	0
CAPRIM	12/22	80	0	26.3

[1] Paracentric Inversion

1991, SCHNEIDER *et al.* 1991, PONSÁ *et al.* 1995). Due to the great variability reported for this species, genetic evaluation of a captive colony is essential to establish a proper experimental design and to consider the future and projections of the colony, as well as to increase the available information to establish management parameters for zoos or other similar institutions (SZAPKIEVICH *et al.* 2002).

This species has been broadly used in many areas of biomedicine, and is considered a valuable experimental model, suitable for comparisons with other mammals, including humans (KHOSLA *et al.* 1997, NAGLE *et al*. 1989, PEACOCK *et al*. 1999, COLOMBO *et al*. 2001). As a complement to cytogenetic information, an electrophoretic analysis of 14 protein allozymes, using the same sample, resulted in two polymorphic loci: Transferrin (TF), showing two polymorphic alelles only for animals from the IICS (TF^1: 0.9; TF^2: 0.1), and Haptoglobin (HP) whose frequencies were, for the IICS population: HP^2: 0.18; HP^3: 0.82 and for CAPRIM: HP^2: 0.5; HP^3: 0.5. Both loci had the populational genotypes in a Hardy-Weinberg equilibrium (IICS: TF: $X^2 = 0.059$ p = 0.808; HP: $X^2 = 2.45$ p = 0.117; CAPRIM: HP: $X^2 = 0.34$ p = 0.56). Nei's Genetic Distance (D) among CAPp populations (D=0.006) is coherent with the genetic variability Index (H), and close to the expected value (SILVA *et al*. 1993). So, in the light of these findings, we suggest to rely more on cytogenetic data than electrophoretic patterns to find unique genetic features for CAPp.

Genomic rearrangements with structural changes that do not imply numerical variations, such as inversions, are frequently observed in Platyrrhini, and are proposed as the second mayor force driving the possible chromosomal speciation processes (COUTURIER and DUTRILLAUX 1981, MATAYOSHI *et al*. 1987, MUDRY 1990, PONSÁ *et al*. 1995 GARCÍA *et al.* 2000). However, data about pericentric or paracentric inversions are ambiguous. If they remain as balanced polymorphisms among populations, with neutral or adaptive characteristics and little or no impact in the next generations, they are not regarded as relevant in evolution, because they are unlikely sources of postmating isolating mechanisms. Previous findings from our research group (MUDRY and SLAVUTSKY 1987) suggested intraindividual polymorphisms, based on pericentric inversions for chromosomes #2 (25% inv2) and #4 (36% inv4). Other lower frequency intraindividual rearrangements, such as a paracentric inversion for chromosome #7 and a t(3p1.1-1.3;4p) were also referred. This report, along with our current findings, might sustain the statement of an unusually high chromosomal variability for Paraguayan populations, in both euchromatic and heterochromatic distribution. Regarding heterochromatic paracentric inversions, as the one described in the current work for chromosome #12, other authors have reported them in pairs # 4 and # 17 for captive individuals with unknown origin (PONSÁ *et al.* 1995).

Inversions seem to be relevant for *Cebus* speciation processes, and have been proposed to explain the main karyotypic differences between *Cebus apella* (CAP) and *Cebus capucinus* (CCA) (CARLA CAMPÁ and STANYON 1992). Apparently, inversions of pairs # 6, 8 and 10 for CAP originated in pairs #6, 13 and 22 of CCA, respectively.

Among other NWM species, pericentric inversions have also been proposed to explain genomic conservation without evident involvement of highly repeated sequences, but with a possible relationship with other forms of DNA, such as highly repetitive or multidispersed sequences, detectable with several restriction enzymes. *Ateles* shows at least 5 inversions that would be essential to explain some of the homeologies among *A.paniscus, A.hybridus* and *A.belzebuth* (MEDEIROS *et al*. 1997; MORESCALCHI *et al.* 1997). The involvement of heterochromatin addition in NWM speciation may be significant. Despite the lack of evidence for reduced hybrid fertility between cytotypes heterozygous for the amount and location of heterochromatic blocks (KING 1987), the genus *Cebus* is, among the NWM, perhaps the best model to analyze heterochromatin behavior, considering the diversity of heterochromatic distributions and polymorphisms, observed through different genetic techniques (GARCÍA *et al*. 1995, PIECZARKA *et al*. 1996, TORRES *et al*. 1998). Its karyotype combines an even distribution of euchromatin with extremely unusual amounts of heterochromatin. C bands for CAP show a greater number of heteromorphic pairs than the other species, considering not only presence/absence of a particular band in any member of

the chromosome pair, but also size and amount of heterochromatin for each band. Pair #11 appeared as heteromorphic in 55% of our captive population, with the outcome of a notorious size difference between homologues and demonstrating that the polymorphism results from heterochromatin loss and not from reshuffling. However, this explanation is not suitable for polymorphisms in chromosome #12, because members of the pair remain of equal size in all the sample. Therefore, the different heterochromatic heteromorphisms observed (distributional, with a paracentric inversion but no apparent loss of heterochromatin, or quantitative, with a drastic reduction of one homologue's band, therefore on its total size) allows a suggestion of highly complex chromatin rearrangements for Paraguayan populations of this species. Experimental data for CAPp suggests a biased distribution of a genomic instability parameter, the Sister Chromatid Exchange frequency (SCE, data not shown) in certain chromosomal bands; these bands have, in turn been associated to Fragile Sites and/or Evolutionary Informative Points by other authors (GARCÍA HARO 2001, MARTINEZ 2003). If so, the 12q2.2 paracentric inversion could be regarded as marker for a potentially labile site, and expect to find at this same site the expression of other instability markers, as well as associations between other heterochromatic polymorphisms and genomic landmarks.

Genetic polymorphisms, even if not informative for speciation processes, might be useful indicators of genetic susceptibility. This could gain additional value when referred to captive animals used for biomedical research, as is the case for CAP. The IICS sample considered is currently being used as an experimental model for Chagas disease. So, a correct individual characterization and pedigree follow-up is important to monitor natural genetic polymorphisms in the colony and include them as constants within our animal model, to diminish background noise obscuring the analysis (AUSMAN *et al.* 1985). On the other hand, marker loci, if defined, can be of great value if associated with physiological parameters, or with any other feature within the same chromosome that could shed light on the disease processes.

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