

## Use of genetic and behavioral analysis in the captive management of night monkeys *Aotus azarae* (Platyrrhini: Cebidae)

Uso de análisis genéticos y comportamentales en el manejo en cautiverio del mono de noche *Aotus azarae* (Platyrrhini: Cebidae)

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### RESUMEN

Los jardines zoológicos proveen un recurso único y valioso para la investigación en primatología. El conocimiento sobre la aclimatación y el patrón de actividad en cautiverio de los monos de noche (*Aotus azarae*) es escasa. En este trabajo caracterizamos el presupuesto de actividad de un grupo de *Aotus azarae* en cautiverio en el zoológico de Córdoba, Argentina. El estatus taxonómico de los especímenes fue determinado mediante análisis cariológicos utilizando distintas variables cromosómicas. Los datos de comportamiento se colectaron mediante la técnica de barrido, registrándose 8 categorías comportamentales. La principal actividad del grupo fue descanso. El comportamiento social estuvo influenciado por el momento del día. El presupuesto de actividad obtenido permitirá establecer una línea base para futuros planes de manejo y enriquecimiento ambiental.

Palabras clave: (Mono de noche), (cautiverio), (cariología), (comportamiento).

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## SUMMARY

Zoological gardens provide a unique and valuable resource for primate research. Knowledge on acclimatizing and behavioral pattern of night monkeys (*Aotus azarae*) in captivity is scarce. Here, we aim to characterize the activity budget of a group of *Aotus azarae* housed at the Córdoba Zoo, Argentina. The taxonomic level of the specimens was confirmed by karyological analysis using different chromosomal variables. Behavioral data were collected via scan sampling, recording eight behavioral categories. The main activity of the group was resting. Social behavior was influenced by the moment of the day. The obtained activity budget will allow establishing a baseline for future management and environmental enrichment plans.

Key words: (Night Monkeys), (captivity), (karyology), (behavior).

## INTRODUCTION

Zoological gardens provide a unique and valuable resource for primate research. Topics related to conservation and welfare are the most studied, in particular those addressing the influence of the captive environment, handling and environmental enrichment on primates behavior<sup>26</sup>. Captivity imposes an environment that's vastly different to the one where wild animals have evolved<sup>6, 19, 24</sup>. The behavior of an animal is the main mean by which zoo personnel and visitors assess its psychological well-being<sup>6, 43</sup>. Ethological indicators are good measures to detect inadequate individual needs in captivity<sup>4</sup>. Behavioral monitoring of captive animals should serve as the foundation for better practices in animal management and welfare<sup>25, 49</sup>. Research undertaken in New World Primates (Platyrrhini) at zoological gardens provides valuable information that may be difficult to observe in the wild.

The presence of *Aotus azarae* in Argentinean zoological gardens is infrequent; therefore knowledge on acclimatizing and behavioral pattern is scarce. The genus *Aotus* (night monkeys) ranges widely through forested areas from Panama to the Argentinean Chaco<sup>2, 33, 50, 52</sup>. Night monkeys are small arboreal, sexually monomorphic primates and the only monkey with a nocturnal lifestyle in the Neotropics<sup>50, 51, 52</sup>. In the Argentinean Chaco *Aotus azarae* (= *Aotus azarai*) is cathemeral, showing diurnal as well as nocturnal activity, as opposed to

other species of the genus<sup>14, 10, 12, 40</sup>. This situation provides a good opportunity for studying their behavior during daylight. The animals live in small, socially monogamous groups<sup>50, 51</sup> consisting of an adult heterosexual pair, one infant and one or two juveniles<sup>15, 11, 52</sup>, although some adult individuals range solitarily until they find mates to establish a new territory<sup>13, 16</sup>.

The pattern of pelage coloration, traditionally used for taxonomy, is insufficient for proper captivity specimen characterization, particularly for the genus *Aotus*<sup>7, 8, 20</sup>. In this genus, not only species assignment is intricate, but also sex determination has proven highly difficult, both in captivity and in the wild (Baschetto, personal communication). Therefore, in captivity, genetic characterization of specimens becomes relevant for their correct taxonomic identification as well as for a proper management of the groups<sup>33, 36, 46, 48</sup>.

Little research has been done concerning the behavior of night monkeys (*Aotus* sp.) in captivity: intragroup social behavior and biparental care in *A. trivirgatus*<sup>50, 51</sup>; self anoint behavior in *Aotus* spp.<sup>53</sup>; behavioral interactions between parents and offspring in *A. trivirgatus griseimembra*<sup>9</sup>. The aim of this study is to characterize the activity budget of one group of *Aotus azarae* in captivity, whose species assignment was confirmed by karyological analysis.

## METHODS

### Study site and subjects

The study subjects constituted a family group of four night monkeys (*Aotus azarae*) housed at the Zoological Garden of Córdoba, Argentina (31° 12.32' S; 64° 16.84' O). The group consisted of a male-female pair (age unknown) and their offspring, one female (3.5 years old) and one male (1.5 years old). Individuals were marked with different colour belts in order to recognize them. Previously to the behavioral study, karyological analyses were performed for correct species' assignment. Phenotype parameters (pelage coloration pattern) were considered following the most recent taxonomical revision<sup>33</sup>.

### Housing and husbandry conditions

The night monkeys were housed in a 78.7m<sup>3</sup> outdoor exhibit under natural conditions of photoperiod and temperature. The roof consisted of plastic panels that protect the monkeys from rain and sunlight. The substrate of the exhibit was soil; with some grass patches, little vegetation (*Lazo de amor*, *Chlorophytum comosum*) and some logs and stones on the ground. The enclosure was furnished with a central metal bar with a platform at the top and several branches and ropes interconnected. There were two refuges, one attached to a lateral panel, heated in winter with a lamp; and the other near the central bar. The subjects had free access to both refuges. Fresh water was available *ad libitum*.

The normal husbandry routine was performed by a single keeper, preferably during the morning, and consisted of the daily cleaning of the exhibit, provision of fresh food and water. The monkeys were fed once a day with a mix of 1 Kg of fruits (80%), vegetables, cereals (18%) and milky products (2%). They occasionally hunted insects.

### Karyological confirmation

In order to perform the genetic characterization of the specimens to attain a correct taxonomic assignment and a proper

management of the group, peripheral blood samples were collected from all the animals with previously heparinized disposable syringes (ABOTT HEPARINE). Lymphocyte cultures were performed<sup>5</sup> for 72 hours at 37 °C. Metaphase spreads were stained by G-Wright banding<sup>44</sup> and C-banding<sup>45</sup>. The mitotic index (MI %) was obtained considering the metaphases found among 1000 nuclei. At least 50 metaphase spreads were analyzed under optic microscope (1000X) to determine the diploid number (2N). At least 10 G-banded metaphases with the species 2N were photographed. Photomicrographs were taken using a Leica DMLB microscope equipped with a photographic camera MPS 30 using an Ilfor-pan F 50 ASA black and white slide film.

### Behavioral data collection and analysis

The group of night monkeys was observed from March to May 2005 for seven days, on a monthly basis. Two sampling periods were used to observe the behavior during the time the animals were most likely active (from 0900 to 1200 h and from 1600 to 1900 h), considering the daily routines of the institution. Each behavior was recorded using scan sampling method at 1-minute intervals<sup>1</sup> to determine the activity budget of the group. The behaviors were classified as resting, locomotion, foraging, BRPBE, social, abnormal, self-grooming and other activities (Table 1) and we calculated respective percentages for the frequencies and duration of the recorded activities. Kruskal-Wallis non parametric test was used to analyze the differences in the percentage of time allocated to each behavior of the general activity budget. Mann-Whitney *U* test was used to test the differences between the behavioral categories at the different moments of the day (morning and afternoon). The statistical analyses were performed using PASW Statistic 18 software. Statistical significance was assumed at  $\alpha < 0.05$ .

All research reported in this manuscript has met the appropriate national and institutional guidelines for the legal acquisition and use of laboratory animals and authorized study of wild animals. The authors also adhered to the Guide

for Care and Use of Experimental Animals, as promulgated by the Canadian Council of Animal Care, and to the American Society of Primatologists (ASP) Principles for the Ethical Treatment of non-human Primates. The work which took place in Argentina was done in accordance with Argentina's law.

## RESULTS

### Cytogenetic analysis

Different chromosomal variables were studied in order to confirm species status and the sex of the specimens. The chromosomal characterization of the four night monkeys was easily achieved due to a good mitotic index (MI %) presented in all the cultures performed with an average value of  $6.30 \pm 1.13$  %. The modal number was  $2N = 50, XX$  for the females and  $2N = 49, X_1X_2Y$  for the males confirming the sex distribution in the monogamous group (2 males and 2 females). From the 50 metaphases analyzed for each specimen, at least 70% showed the aforementioned modal numbers for each sex (Fig. 1A). The fundamental number (FN) was 74 for males and 73 for females. When G-banding technique was applied, the pattern observed in all the individuals analyzed was in agreement with the G-banding pattern previously described for *Aotus azarae* (Fig. 1B) showing the presence of a XX sex determination in the females and indicating a multiple sex determination system  $X_1X_2Y$  in the males. The C-banding pattern showed all centromeres as C+. The presence of two different interstitial bands in size and distribution was observed in 100% of the C-banded metaphases analyzed both in males and females (Fig. 1C). Therefore, all the chromosomal variables analyzed confirmed the unequivocal species and sex assignment.

### Behavioral analysis

The group of night monkeys was observed for 126 h, obtaining 26497 behavioral records from the four individuals during the study period (we did not take into consideration records where the individuals were not visible). The analysis of the activity budget showed that resting

was the most prevalent activity of the group, representing 55.21% of the activity budget. The monkeys were active during 44.79% of the observation day, where 33.73% of the time were engaged in BRPBE, 4.67% foraging, 3.31% in locomotion, 2.67 % in social behaviors, 0.32% self-grooming and 0.09% in other activities. Abnormal behaviors were not observed during the study period. The Kruskal-Wallis test showed significant differences in the percentage of time allocated to the different behavioral categories of the activity budget ( $X^2=24.760$ ,  $df= 6$ ,  $p=0.000$ ). According to U Mann-Whitney test, resting was the prevalent activity ( $Z= -2.309$ ;  $p= 0.021$ ), followed by BRPBE ( $Z= -2.309$ ;  $p= 0.021$ ). Foraging, Locomotion and Social behaviors were not significantly different within them ( $X^2=3.231$ ;  $p= 0.199$ ), but different from the rest of the behaviors analyzed ( $Z= -2.309$ ;  $p= 0.021$ ). Similarly, Self-grooming and other activities did not differ within them ( $Z= -0.292$ ;  $p= 0.77$ ), but they did from the rest of the behaviors ( $Z= -2.309$ ;  $p= 0.021$ ). When analyzing the behavioral categories at different moments of the day, we found significant differences in the percentage of the activity budget devoted to social behaviors ( $Z= -2.309$ ;  $p= 0.021$ ), which were highly exhibited during the afternoon (Fig. 2).

## DISCUSSION

In Mammals in general and in particular in Primates, cytogenetic characterization has proven of significant value for taxonomic determination<sup>29, 32, 34, 35, 36, 39, 17</sup>, which is critical for their conservation in the wild and in captivity. Incorrect assignment of species in captivity may lead to hybridization that in turn results in a reduced reproductive fitness<sup>7, 8, 20</sup>. For *Aotus*, 18 different karyotypes had been characterized, with diploid numbers ranging from 46 to 58, with a multiple sex determination system  $X_1X_2Y$  described for male specimens by meiotic studies<sup>18, 20, 21, 22, 23, 28, 30, 47, 42</sup>. In the present contribution, it was confirmed by mitotic karyology the species status of four *Aotus* specimens that constituted a monogamous

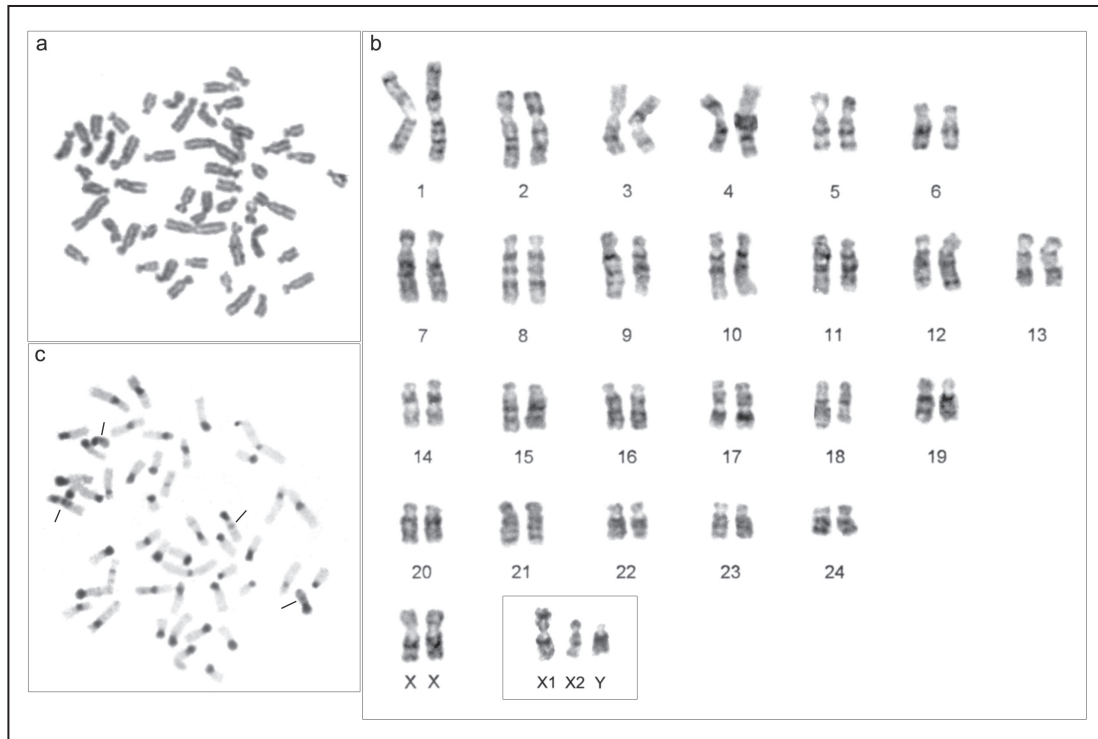


Fig. 1. Genetic characterization of the specimens analyzed. a) Male standard metaphase of *Aotus azarae*. b) G- banded metaphase of an *Aotus azarae* female. c) C-banded metaphase of a *Aotus azarae* female. The lines indicate interstitial C+ bands.

Caracterización genética de los especímenes analizados. a) Metafase con tinción estándar de un macho de *Aotus azarae*. b) Metafase con tinción de bandas G de una hembra de *Aotus azarae*. c) Metafase con tinción de bandas C de una hembra de *Aotus azarae*. Las líneas indican bandas C+ intersticiales.

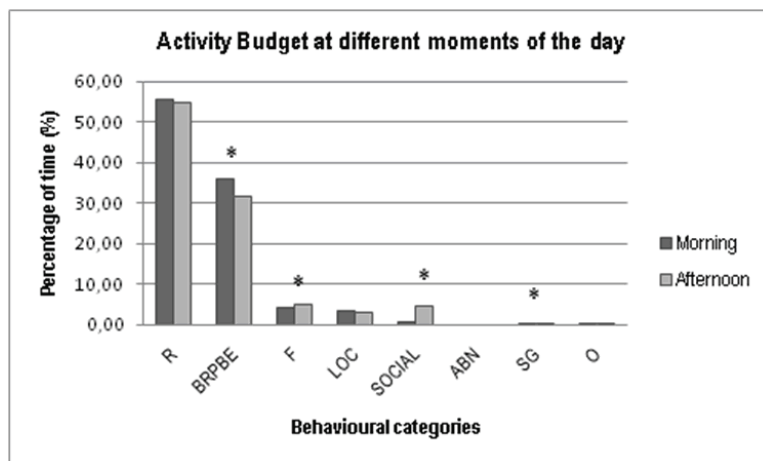


Fig. 2. Group activity budget of *Aotus azarae* at different moments of the day at Zoo Córdoba, Argentina. Behavioral categories: R= resting; BRPBE = behaviors related to the physical-biological environment; F= foraging; Loc= locomotion; Social= social behaviors; ABN= abnormalities; SG= self-grooming; OA= other activities. Categories with asterisks (\*) identify a significant difference between moment of the day ( $p < 0.05$ ).

Presupuesto de actividad grupal de *Aotus azarae* en diferentes momentos del día en el Zoo de Córdoba, Argentina. Categorías comportamentales: R=descanso; BRPBE=comportamientos de relación con el ambiente físico-biológico; F=forrajeo; LOC=locomoción; SOCIAL=comportamiento social; ABN=anormalidades; SG=autoacicalamiento; OA=otras actividades. Categorías con asteriscos (\*) identifican una diferencia significativa entre momentos del día.

**Table 1.** Behavioral repertoire recorded from *Aotus azarae* group housed at Zoo Córdoba, Argentina.  
Repertorio de comportamientos observados para el grupo de *Aotus azarae* en cautiverio en el Zoo de Córdoba, Argentina

| Behavior   |       | Definition  |
|--|-------|---|
| Resting  | R     | Subjects are not moving (includes sleep, lie and sit). Apparent inactivity.   |
| Locomotion   | Loc   | Moving from one place to another (includes walking, jumping, running, climbing).  |
| Foraging   | F     | Activities directly related to the acquisition and intake of food or fluid (includes forage, stalk, eat, chew and drink). |
| Behaviors related to the physical-biological environment | BRPBE | Includes vigilance and exploration.   |
| Social   | S     | Non aggressive social interaction between two or more individuals (includes grooming and play).                           |
| Abnormal   | ABN   | Subjects display abnormal activity (includes self-mutilation, stereotyped locomotion, pacing and weaving)                 |
| Self grooming  | SG    | Self inspection of different parts of their bodies.   |
| Other activities   | OA    | Includes fights, defecation and other activities.   |

group in exhibition. The modal and fundamental number, as well as the G-banding and C-banding patterns allowed to identify these individuals to belong to *Aotus azarae* (= *Aotus azarai*) in agreement with the so-called Karyomorph 5<sup>47</sup>. The presence all centromeres C+ was verified and one submetacentric chromosomal pair with a more conspicuous q interstitial C+ band, a putative pair #9, and another heteromorphic submetacentric pair with a smaller q interstitial C+, a putative pair #8 were observed following previous descriptions<sup>30, 31</sup>. In contrast with other C-banding descriptions with terminal C+ bands or full p arm C+<sup>27, 23, 38</sup> no telomeric C+ bands were observed in the analyzed specimens. Other *Aotus* species showed the presence of extracentromeric C+ bands, associated with Y/autosome rearrangement<sup>37, 38</sup>. Meanwhile only centromeric heterochromatic bands were described in *A. trivirgatus*<sup>27</sup>. The correct identification of the taxonomic level and sex determination of the individuals housed at zoological gardens is of great importance for their proper management in captivity.

Given that night monkeys housed at Zoo Córdoba have diurnal activity besides the widespread nocturnal activity characteristic in this species, it was possible to analyze their

behavior in the diurnal segment. Among the different behavioral categories, resting was the most prevalent. Similar observations were made on free-ranging *Aotus* in Formosa<sup>3</sup>. However, although the classification of behaviors differs, Arditi<sup>3</sup> observed that locomotion represented the 15.6% of the observation time, while in this study the mean percentage is lower (3.31%), probably due to the restricted space and captivity itself. Other behavioral category, defined as behaviors related to the physical and biological environment (BRPBE), which includes vigilance and exploration behaviors, represented the second most prevalent category (33.73%) and was highly exhibited during the morning, although it was not significant. This could be related mainly to the presence of their zookeeper during that period and the regular presence of unfamiliar visitors, although further analysis is needed to confirm this. Even though only social behavior was significantly higher in the afternoon, foraging and self-grooming behaviors were also observed to increase during the afternoon, probably associated to their characteristic crepuscular activity.

Concerning social behaviors, grooming was observed primarily on periods of regrouping and resting. Grooming between the adult pair had rarely been seen; however, adult grooming

of juveniles was more frequent. The adult female provides the major bouts of grooming to the juvenile male. Play was an important social behavior between the adult male and his offspring. The play behavior was observed particularly between the adult and juvenile males, and in some occasions the juvenile female (sibling) joined them, while the adult female rarely participated. Further evidence of play behavior came from studies in a captive group<sup>50, 51</sup> and in free-ranging groups<sup>41, 52</sup>, where the subadult, the juvenile and the adult male (sire) of the group engaged in the play, described as tag, pounce and retreat and rough and tumble<sup>50</sup>. Wright<sup>50, 51</sup> also observed the adult female (dam) rarely plays in captivity.

No behavioral pathologies or unsocial conducts related to captivity were observed during the study period. The absence of pathologies and the observation of species-appropriate behaviors are considered as good parameters of a proper behavioral adjustment to captivity conditions<sup>4</sup>. The activity budget will allow establishing a baseline to plan enrichment routines and include them in the daily management routine of the species, as well as it contributes to the knowledge of night monkeys acclimatizing in captivity.

## CONCLUSION

First, (1) the cytogenetic characterization enabled the correct identification of the taxonomic level of the individuals, (2) the group activity budget was fairly similar to the activity budget of wild primate groups; they spent an average of 55.21% of their time resting, followed by BRPBE, foraging, locomotion, social, self-grooming and other activities. (3) Social behavior was influenced by the moment of the day. (4) The activity budget of night monkeys in captivity will allow establishing a baseline for future management and environmental enrichment plans.

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