

Hormonal Monitoring of Reproductive Status in Monogamous Wild Female Owl Monkeys (*Aotus azarai*) of the Argentinean Chaco

Eduardo Fernandez-Duque^{a, d} Kevin Burke^a Kelsi Schoenrock^{a, b}
Christy K. Wolovich^c Claudia R. Valeggia^a

^aDepartment of Anthropology and ^bBiological Basis of Behavior Program, University of Pennsylvania, Philadelphia, Pa., and ^cDepartment of Biology, MacMurray College, Jacksonville, Ill., USA; ^dCECOAL-Conicet, Corrientes, Argentina

Key Words

Primates · Platyrrhines · Monogamy · Progesterone · Estrogen · *Aotus* spp.

Abstract

The Neotropical owl monkeys (*Aotus* spp.) are a good model for evaluating the hypothesis that monogamy may arise if female reproductive cycles limit the mating potential of males. To evaluate this hypothesis, we first needed to assess the feasibility of using fecal sampling for monitoring the reproductive status of females. We collected fecal samples ($n = 242$, from 7 females) from wild adult *Aotus azarai* females in the Gran Chaco forests of Argentina during 3 years. Fecal estrone-1-glucuronide (E_1C) and pregnenadiol-3-glucuronide (PdG) tended to rise in parallel during the luteal phase. The average cycle length was 22 ± 3 days ($n = 5$ females, 10 cycles). We identified 2 conceptive cycles and characterized the E_1C and PdG profiles of 2 pregnancies. This report is the first of its kind on wild female owl monkeys. Despite the difficulties in sample collection and processing in the field and providing a species-specific validation in the laboratory, we show that fecal samples from *A. azarai* can be used for monitoring female reproductive status and function.

Copyright © 2011 S. Karger AG, Basel

Introduction

A traditional hypothesis for explaining the evolution and maintenance of monogamy in primates suggests that monogamy was a default social system imposed upon males when the spatial or temporal distribution of females made it difficult for

KARGER

Fax +41 61 306 12 34
E-Mail karger@karger.ch
www.karger.com

© 2011 S. Karger AG, Basel
0015-5713/11/0823-0143\$38.00/0

Accessible online at:
www.karger.com/fpr

Eduardo Fernandez-Duque
Department of Anthropology, University of
Pennsylvania, 431 University Museum, 3600 South Street
Philadelphia, PA 19104-6398 (USA)
Tel. +1 215 898 1072, E-Mail eduardof@sas.upenn.edu

single males to simultaneously defend access to more than one mate [Emlen and Oring, 1977; Rutberg, 1983; Van Schaik and Van Hoof, 1983]. In this theoretical context, it is proposed that social monogamy may arise if females exhibit synchronous reproductive cycles that limit the number of females that a male can fertilize. Even if not synchronous, it is possible that in a socially monogamous species with intensive care, the combination of pregnancy and postpartum amenorrhea generates a very brief period when females are receptive.

A thorough evaluation of a hypothesis that considers the reproductive cycles of females requires that demographic and behavioral information on reproductive events be collected together with adequate information on the females' hormonal status. The development of methods for the collection and processing of field samples over the last two decades [Ziegler and Wittwer, 2005] has allowed significant progress in the monitoring of the reproductive ecology of wild primates [Ziegler et al., 1997; Valeggia, 2007; Van Belle et al., 2009; Ziegler et al., 2009; Pappano et al., 2010]. The Neotropical owl monkeys (*Aotus* spp.) are a good model for evaluating hypotheses on the evolution of monogamy, but there have been to date no field studies to characterize the reproductive cycles of this small, sexually monomorphic, arboreal primate.

Owl monkeys are consistently found in small, socially monogamous groups composed of 1 adult heterosexual pair, 1 infant, and 1 or 2 individuals of smaller size [Fernandez-Duque, 2011]. In an Azara's owl monkey (*Aotus azarai*) population of the Argentinean Chaco, females produce 1 offspring a year, with the majority of births occurring between October and November [Fernandez-Duque et al., 2002]. Infants nurse intensively until 5 months of age, but they are still observed on the nipple when they are 8 months old [Rotundo et al., 2005; Huck and Fernandez-Duque, in press]. Male parental investment is unusually high in owl monkeys, with the male carrying the infant over 80% of the time [Fernandez-Duque et al., 2009]. The reproducing pair occupies a relatively small and exclusive territory (4–10 ha), and within their territory they forage primarily for fruits, but also obtain significant amounts of leaves, flowers and insects [Van der Heide et al., in press]. Social groups interact aggressively with other groups at territory boundaries or with individuals who, after dispersing from their groups, are ranging solitarily among territories.

We present here the results of a preliminary study of hormonal changes during the reproductive cycle in wild *A. azarai* females. We evaluated the feasibility of using fecal sampling for monitoring the reproductive status (e.g. pregnant, cycling) of wild *A. azarai* females. Owl monkeys do not show any visual signs of ovarian cycling [Bonney et al., 1979, 1980; Wolovich and Evans, 2007], therefore it is necessary to rely on changes in hormone levels to ascertain their reproductive status. To our knowledge, this is the first report of a characterization of female reproductive cycles from a free-ranging population.

Methods

Study Area and Population

The area of study is located in the Guaycolec Ranch, 25 km from the city of Formosa in the Argentinean Gran Chaco of South America (58°11' W, 25°58' S). The region consists of a matrix

of grasslands dotted with patches of forests and transected by gallery forests that grow along rivers. In the area, owl monkeys inhabit the gallery forests that grow along the Pilagá and Guaycolec Rivers, as well as the patches of forests dispersed in the grasslands. Approximately 20 social groups have been monitored, at least monthly, since 1997 providing background information on the timing of birth seasons, mating seasons and dispersal patterns [Fernandez-Duque et al., 2002; Fernandez-Duque, 2009].

Fecal Sample Collection and Processing

We collected 242 fecal samples from 7 reproductive females during 2005, 2008 and 2009. We collected samples, on average, every 3.5 days (range: 1–7 days) between February and August of each year. Two of the females were sampled in all 3 years, 1 female was sampled in 2005 and 2008, and the remaining 4 were sampled once in different years. Our sample collection and processing methods followed established protocols [Ziegler and Wittwer, 2005; Pappano et al., 2010]. Briefly, fecal droppings were collected as soon as the individual defecated; the specimens were transferred to a screwcap plastic tube, which was then labeled and placed in an insulated bag with a cold pack until processing, usually within a few hours. At the field station, we mixed individual samples thoroughly, and 0.1 g of fecal material was weighed and transferred to a tube containing 5 ml of a 1:1 ethanol:distilled water solution. Tubes were vortexed for 5 min and then centrifuged at high speed for 10 min to separate the solid material. A 2-ml aliquot of the supernatant was transferred to solid-phase extraction columns (Prevail C18 from Alltech®), which were then stored at -20°C until shipment to the Reproductive Ecology Laboratory at the University of Pennsylvania, where they were stored again at -20°C until processed.

Hormonal Assays

Once at the Reproductive Ecology Laboratory, samples were eluted from the solid-phase extraction columns with 2 ml of ethanol and evaporated in a 50°C water bath under a constant stream of air. Once dry, the samples were reconstituted in 1 ml of ethanol and stored at -20°C until assaying. On the day of the assay, samples were dried again and reconstituted in phosphate buffer and assayed for estrone-1-glucuronide (E_1C) and pregnenadiol-3-glucuronide (PdG) using enzyme conjugates and antisera provided by Coralie Munro at the Clinical Endocrinology Laboratory at UC Davis. The E_1C and PdG enzyme immunoassays were validated for owl monkey fecal samples by determining parallelism and accuracy. Serial dilutions of a pooled sample ($n = 4$) paralleled the standard curve with no statistically significant difference in slope. The intra- and interassay coefficients of variation for E_1C and PdG were 6.5 and 20.8, and 12.3 and 24.8%, respectively. Hormone values are provided as nanograms per gram of feces for E_1C and as micrograms per gram of feces for PdG.

Data Analysis

We used the criterion of progesterone-defined ovulation to identify ovulatory cycles. We considered changes in PdG levels of more than 50% above baseline as evidence that the cycle was ovulatory. Baseline levels were calculated as an average of the follicular phase levels for each individual. PdG levels were either consistently low or exhibited a clear peak, with few instances of hormonal profiles exhibiting intermediate progesterone activity. We estimated cycle length by two methods: (1) counting the days between two consecutive E_1C peaks (for females with more than 2 cycles, we computed the average cycle length) and (2) counting the days between the nadir (the lowest) points in the profile. We report both cycle length estimations. We estimated the possible conception window period by backcounting the published estimates of pregnancy duration in *Aotus* spp. (121–133 days [Wolovich et al., 2008]) from a known birth date [Ziegler and Sousa, 2002]. Then, the conceptive cycle leading to the pregnancy was identified by looking at the cycle that most closely preceded the window of conception. We calculated gestation length range as the number of days from the progesterone-defined ovulation day to the range of days for the birth of an infant. Hormonal values for pregnant females were identified by the same backcounting method.

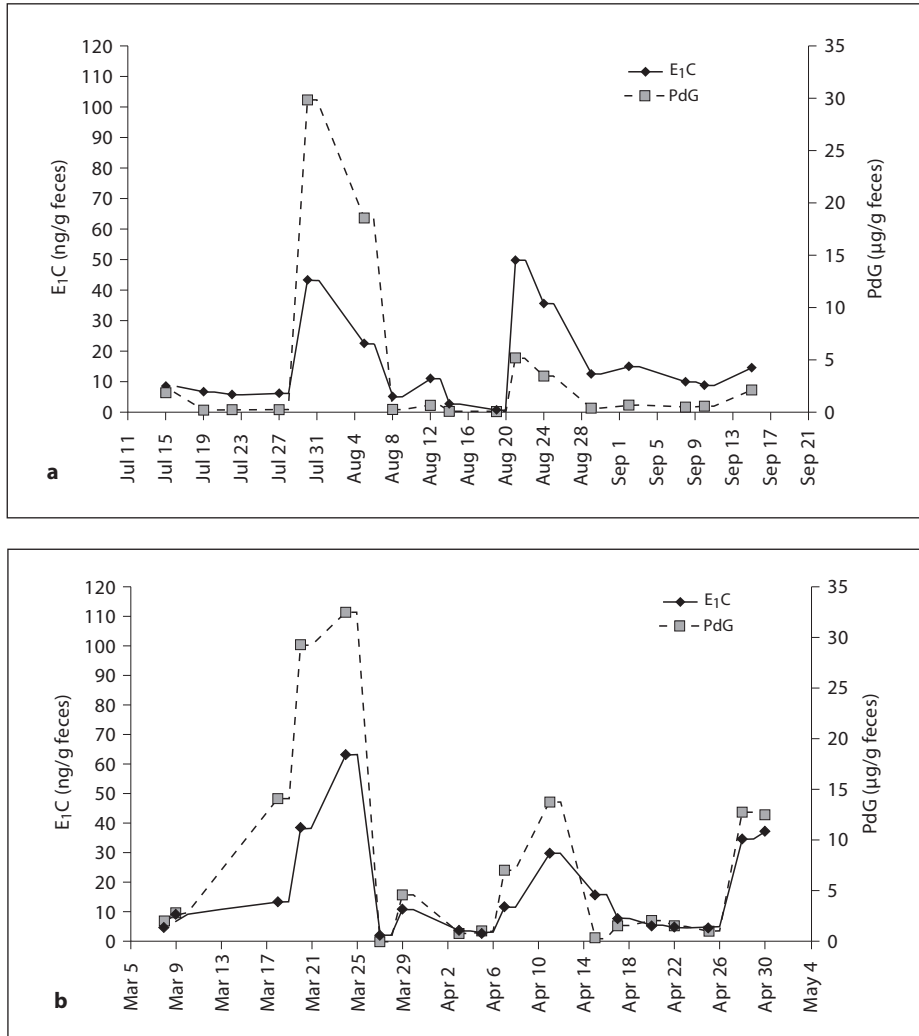


Fig. 1. a, b Fecal E₁C and PdG excretion profile of 2 adult females in 2 different years of data collection (**a** 2005, **b** 2008).

Results

Ovarian Cycles

Fecal E₁C and PdG followed a similar pattern, both rising almost in parallel during the luteal phase. Fecal E₁C, a conjugated form of estrone, did not show a follicular surge prior to ovulation. To illustrate a general pattern that was observed in all females, figure 1a shows E₁C and PdG changes in 1 female during a 2-month period. The hormonal profile of a second female who gave birth at the end of November 2007 is shown in figure 1b. The profile, which starts when she was approximately 3 months

Table 1. Average cycle length per female calculated as number of days between successive E₁C peaks (P2P) and number of days between successive nadir samples (N2N)

Female ID	Ovulatory cycles contributed n	Average P2P cycle length \pm SD days	Average N2N cycle length \pm SD days
Eva	4	22.5 \pm 2.9	22.2 \pm 1.3
Duquesa	3	21.3 \pm 3.1	22.7 \pm 2.3
Celina	2	25 \pm 1.4	24
Mony	2	24 \pm 0.0	25
Estrella	1	18	23
Overall average		22.2 \pm 2.7	24.0 \pm 1.6

Table 2. Mean values (\pm SD) of fecal E₁C and PdG characteristic of ovarian cycles and gestation

	Cycles n	Females n	Mean E ₁ C \pm SD ng/g feces	Mean PdG \pm SD μ g/ feces
Ovarian cycles				
Intercycle troughs	10	5	6.9 \pm 3.5	1.1 \pm 0.8
Postovulatory peak	12	5	49.9 \pm 14.1	18.1 \pm 9.1
Pregnancy				
First days		1	51.6 \pm 27.6	6.4 \pm 8.3
Midpregnancy		2	43.6 \pm 20.2	5.2 \pm 2.8

into the postpartum period, suggests 2 complete nonconceptive ovulatory cycles and a partial one at the end of the sampling period.

One female contributed 4 nonconceptive cycles, 1 female contributed 3 cycles, 2 females contributed 2 cycles each and 1 female contributed 1 cycle. The overall average cycle length was 22.2 \pm 2.7 days (range 18–25 days), when computed from E₁C peak to E₁C peak (n = 5 females, 12 cycles) and 24.0 \pm 1.6 days (range 20–26 days), when computed from nadir to nadir (n = 5 females, 10 cycles). The average cycle length per female is presented in table 1. Mean fecal E₁C and PdG levels characteristic of ovarian cycles and pregnancy are presented in table 2.

Conception and Gestation

We identified 2 conceptive cycles and we characterized the E₁C and PdG profile of 2 pregnancies. Figure 2a shows a possible window of conception around June 30, 2009, by a female who gave birth between October 30 and November 6. The estimated gestation period for this female is 120–126 days. The profile in figure 2b suggests a conception around August 8, 2005, for a second female who gave birth between December 20 and January 14. Given the relatively longer time between observations of this second female, we estimated her gestation length using information

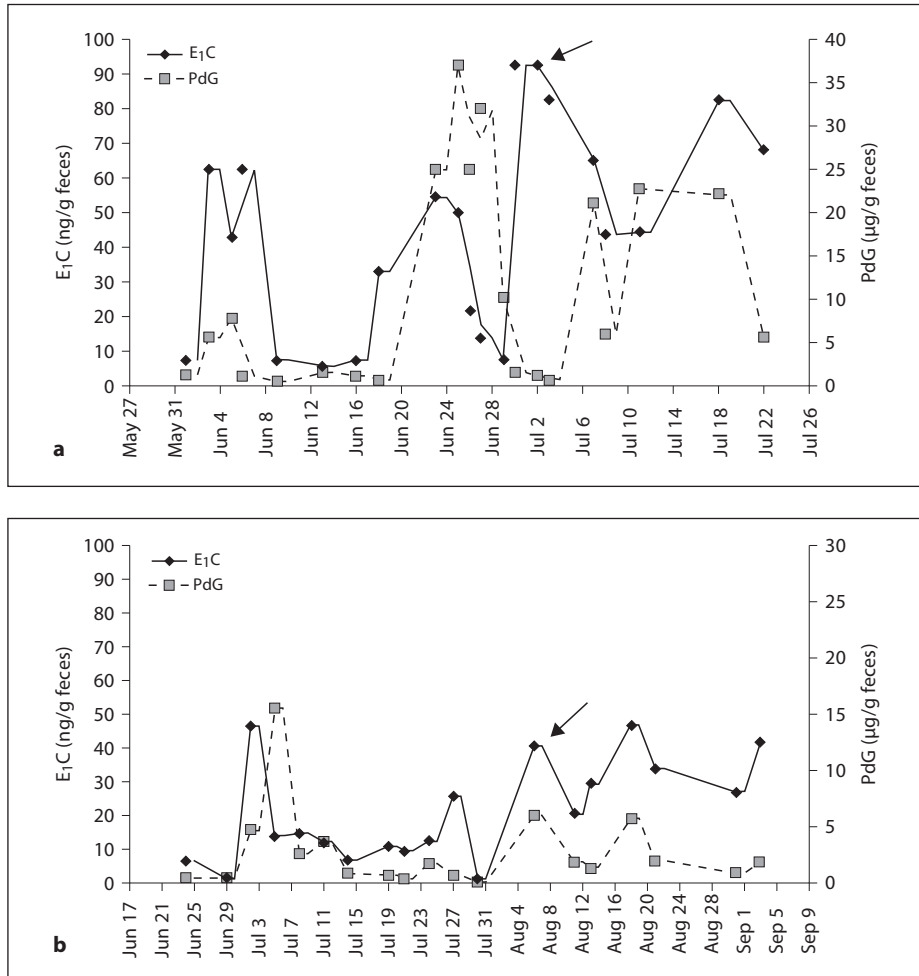


Fig. 2. a, b Fecal E₁C and PdG excretion profile of 2 adult females in 2 years (**a** 2009, **b** 2005) showing conceptive cycles. Arrows indicate the possible timing of conceptions, based on back-counting from the approximate date of birth of an infant.

on the developmental stage of the infant when first observed. This resulted in an estimated gestational length of approximately 132–140 days.

Two females were well into their pregnancies during the time when they were sampled (fig. 3). In both cases, fecal E₁C levels show a slight, but sustained increase during pregnancy, while PdG remains at basal levels. The first female (fig. 3a) gave birth on September 18; counting back 133 days from the birth date, this profile covers from approximately day 46 through the last day of pregnancy. The second female (fig. 3b) gave birth on October 6, and, thus, this profile covers from approximately day 46 through day 113 of pregnancy.

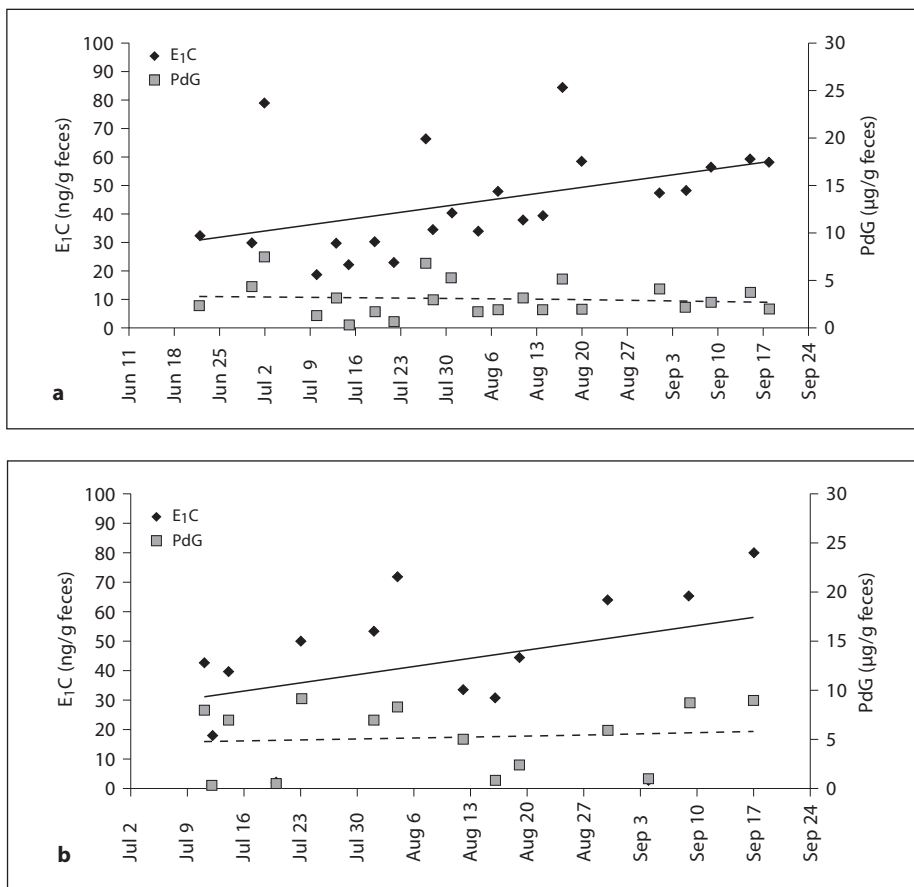


Fig. 3. a, b Fecal E₁C and PdG excretion profile of 2 adult females during pregnancy in 2005.

Discussion

We have presented the results of a preliminary, yet solid first attempt at characterizing the reproductive biology of free-ranging owl monkeys. The primary contribution of this technical report is showing the feasibility of characterizing the reproductive biology of wild *A. azarai* females, including the detection of ovarian function, conception and pregnancy. As seen in other Neotropical primates [Shideler et al., 1994; Ziegler et al., 1996b], fecal PdG and E₁C, metabolites of progesterone and estrogen, respectively, appear to cycle synchronously in *A. azarai*. During a cycle there is a peak in both fecal PdG and E₁C, and there appears to be no preovulatory rise in fecal E₁C during the follicular phase. Thus, fecal estrone conjugates appear to reflect luteal function.

Our determination of cycle length using the number of days between E₁C peaks and nadirs generated estimates which were close to the one described for captive *A.*

azarai (25 days [Wolovich et al., 2008]), and in the highest end of the range of other *Aotus* spp. [Fernandez-Duque, 2011]. Estimates of gestation length, based on visual inspection of the profiles, were within the range obtained from captive animals (133 days [Hunter et al., 1979]; 121 days for *A. azarai* [Wolovich et al., 2008]). However, it is worth noting that fecal E₁C and PdG levels do not rise above cycling levels as seen in urinary hormone profiles. We are unable to provide an explanation for this finding, but it is likely that this pattern is related to steroid conjugation and/or excretion dynamics during pregnancy.

Although we found good evidence of ovarian cyclicity using glucuronide conjugates of estrone and pregnanediol, the synchronous cycling of these metabolites, together with the lack of a clear rise following conception, prompts us to suggest that future studies using fecal samples to monitor reproductive cycles should include the analysis of free estrone and progesterone. It is also important to highlight the complex picture of reproductive hormone metabolism and excretion reported for Neotropical monkeys [Ziegler et al., 2009]. Each species, even within the same genus, seems to differ in the way and the timing these steroid hormones are metabolized in the liver (and other organs) and then excreted in feces [Ziegler et al., 1996a].

Given the technical nature of this report, we deem it important to discuss the difficulties and limitations of our study so that researchers interested in using fecal samples for monitoring reproductive function in *Aotus* spp. in the field may avoid some of the pitfalls we have encountered and can capitalize on our experience. Collecting small fecal pellets from free-ranging small arboreal monkeys represents the first challenge. However, to date, it is the only available noninvasive biological medium since collecting blood or salivary samples would require capturing the animals, and collecting urinary samples is not feasible because the urine stream seldom reaches the ground. Second, collecting fecal samples is extremely time consuming, requiring field assistants to wait several hours for the focal monkey to defecate. This results in a trade-off between frequency of collection and sample size. Do we sample a few individuals quite frequently or increase the sample size at the cost of reducing the frequency of collection? In our case, the target frequency was to collect 3–4 samples per week per female. This was not always feasible given the difficulties in locating the monkeys, finding the feces of the target individual on the ground and sometimes missing the defecation bout. It is then possible that our sampling may have resulted in missed hormonal peaks and some error in the calculation of cycle and pregnancy length.

In a comparative perspective, the reproductive characteristics of female owl monkeys we have described conform to the general pattern described for other cebid taxa. Quite interestingly, many aspects of the reproductive biology of owl monkeys are strikingly similar to the ones reported for titi monkey females [Valeggia et al., 1999]. Females in both genera give birth to singletons once a year, following an almost identical gestation period (*Callicebus* 128.6 ± 4.4 days, range 122–135 [Valeggia et al., 1999]). The length of the ovarian cycle (22–24 days) and the typical profile of reproductive hormones are also similar in the two taxa. In nature, both genera live in small groups consisting of a monogamously mated pair and younger animals [Fernandez-Duque, 2011; Norconk, 2011] and males of both genera provide obligate and intensive paternal care [Fernandez-Duque et al., 2009]. Are the similarities in their reproductive biology driving the equally similar patterns of socially monogamous relationships and biparental care? Could data on reproductive biology offer

some insights to the current debate on the phylogenetic relatedness of these two genera [Schneider and Rosenberger, 1996]?

Although we are still far from understanding the evolution and maintenance of monogamy in owl monkeys, the data presented here contribute to a larger attempt at evaluating the hypothesis that the spatial and temporal distribution of female reproductive opportunities may regulate social monogamy. The hormonal profiles we have presented provide evidence that the monitoring of reproductive cycles can, and should, be part of any attempt at examining the mechanisms regulating monogamy in owl monkeys.

Our results lay the necessary methodological foundation to develop studies that can examine if the temporal distribution of reproductive opportunities may function as an effective female strategy to limit the male's reproductive potential. Given the information we have presented, supported by information from captivity, it seems that owl monkey females may have a pregnancy of 4.2–4.5 months, wean their infants at approximately 6 months and have a cycle length of 22–24 days. In *A. azaraei*, which shows such a narrow seasonal reproductive pattern [Fernandez-Duque et al., 2002; Fernandez-Duque, 2012] we should expect a window of only a few ovulatory cycles in each year. However, we did find a case in which the female started cycling 3 months after giving birth (March) and did not conceive until 4 months later, raising the possibility of several nonconceptive cycles. This is a possibility that should be explored in future studies. Females may be further limiting the males' opportunities by relatively high levels of same-sex aggression in females that may prevent other reproductive females from joining the group. Finally, females may be reducing the opportunities that males have of seeking other females by requiring a relatively prolonged courtship prior to mating, as well as by synchronizing their reproductive cycles with other females. The possible ovulatory suppression of daughters in the group, the synchronicity of females' reproductive cycles, a demand of a prolonged courtship and female-female aggression may limit the male's access to many females, therefore promoting a monogamous system.

We hope that the information provided here will be applicable to the understanding of the reproductive characteristics of other *Aotus* species, as well as to a broader understanding of the reproductive biology of other New World primates.

Acknowledgments

Financial support during all these years came from the Wenner-Gren Foundation, the L.S.B. Leakey Foundation, the National Geographic Society, the National Science Foundation (BCS-0621020), the University of Pennsylvania Research Foundation and the Zoological Society of San Diego. Thanks to all the students, volunteers and assistants who helped us collect the data. We also thank Mr. F. Middleton, Manager of Estancia Guaycolec, and Ing. Alfredo Casaretto (Bellamar Estancias) for the continued support of the Owl Monkey Project. The Ministerio de la Producción, Subsecretaría de Ecología and Recursos Naturales and Dirección de Fauna from Formosa Province provided permits to conduct the research. The Owl Monkey Project has had continued approval for all research presented here by the Formosa Province Council of Veterinarian Doctors, the Directorate of Wildlife, the Subsecretary of Ecology and Natural Resources and the Ministry of Production. At the national level, the procedures were approved by the National Wildlife Directorate in Argentina and by the IACUC committees of the Zoological Society of San Diego (2000–2005) and of the University of Pennsylvania (2006–2010).

References

- Bonney RC, Dixson AF, Fleming D (1979). Cyclic changes in the circulating and urinary levels of ovarian-steroids in the adult female owl monkey (*Aotus trivirgatus*). *Journal of Reproduction and Fertility* 56: 271–280.
- Bonney RC, Dixson AF, Fleming D (1980). Plasma-concentrations of oestradiol-17-beta, estrone, progesterone and testosterone during the ovarian cycle of the owl monkey (*Aotus trivirgatus*). *Journal of Reproduction and Fertility* 60: 101–107.
- Emlen ST, Oring LW (1977). Ecology, sexual selection, and the evolution of mating systems. *Science* 197: 215–223.
- Fernandez-Duque E (2009). Natal dispersal in monogamous owl monkeys (*Aotus azarai*) of the Argentinean Chaco. *Behaviour* 146: 583–606.
- Fernandez-Duque E (2011). The Aotinae: social monogamy in the only nocturnal anthropoid. In *Primates in Perspective* (Campbell CJ, Fuentes A, MacKinnon KC, Panger M, Bearder SK, eds.), pp 140–154. Oxford, Oxford University Press.
- Fernandez-Duque E (2012). Studies of captive and wild owl monkeys. *International Zoo Yearbook* vol 46.
- Fernandez-Duque E, Rotundo M, Ramírez-Llorens P (2002). Environmental determinants of birth seasonality in night monkeys (*Aotus azarai*) of the Argentinean Chaco. *International Journal of Primatology* 23: 639–656.
- Fernandez-Duque E, Valeggia CR, Mendoza SP (2009). The biology of paternal care in human and non-human primates. *Annual Review of Anthropology* 38: 115–130.
- Huck MG, Fernandez-Duque E (in press). Building babies when dads help: infant development of owl monkeys and other primates with allo-maternal care. In *Building Babies: Proximate and Ultimate Perspectives of Primate Developmental Trajectories* (Clancy K, Hinde K, Rutherford J, eds.). New York, Springer.
- Hunter J, Martin RD, Dixson AF, Rudder BCC (1979). Gestation and interbirth intervals in the owl monkey (*Aotus trivirgatus griseimembra*). *Folia Primatologica* 31: 165–175.
- Norconk MA (2011). Sakis, uakaris and titi monkeys: behavioral diversity in a radiation of primate seed predators. In *Primates in Perspective* (Campbell CJ, Fuentes A, MacKinnon KC, Panger M, Bearder SK, eds.), pp 122–139. Oxford, Oxford University Press.
- Pappano DJ, Roberts EK, Beehner JC (2010). Testing extraction and storage parameters for a fecal hormone method. *American Journal of Primatology* 72: 934–941.
- Rotundo M, Fernandez-Duque E, Dixson AF (2005). Infant development and parental care in free-ranging *Aotus azarai azarai* in Argentina. *International Journal of Primatology* 26: 1459–1473.
- Rutberg AT (1983). The evolution of monogamy in primates. *Journal of Theoretical Biology* 104: 93–112.
- Schneider H, Rosenberger AL (1996). Molecules, morphology and platyrrhine systematics. In *Adaptive Radiations of Neotropical Primates* (Norconk MA, Rosenberger AL, Garber PA, eds.), pp 3–19. New York, Plenum Press.
- Shideler S, Savage A, Ortuno A, Moorman E, Lasley B (1994). Monitoring female reproductive function by measurement of fecal estrogen and progesterone metabolites in the white-faced saki (*Pithecia pithecia*). *American Journal of Primatology* 32: 95–108.
- Valeggia C (2007). Taking the lab to the field: minimally invasive techniques for monitoring reproductive hormones in population-scale research. *Population Development Review* 33: 525–542.
- Valeggia CR, Mendoza SP, Fernandez-Duque E, Mason WA, Lasley B (1999). Reproductive biology of female titi monkeys (*Callicebus moloch*) in captivity. *American Journal of Primatology* 47: 183–195.
- Van Belle S, Estrada A, Ziegler TE, Strier KB (2009). Sexual behavior across ovarian cycles in wild black howler monkeys (*Alouatta pigra*): male mate guarding and female mate choice. *American Journal of Primatology* 71: 153–164.
- Van der Heide GWE, Fernandez-Duque E, Iriart D, Juárez C (in press). Do forest composition and fruit availability predict demographic differences among territorial owl monkey (*Aotus azarai*) groups? *International Journal of Primatology*.
- Van Schaik CP, Van Hoof JARAM (1983). On the ultimate causes of primate social systems. *Behaviour* 85: 91–117.
- Wolovich CK, Evans S (2007). Sociosexual behavior and chemical communication of *Aotus nancymaae*. *International Journal of Primatology* 28: 1299–1313.
- Wolovich CK, Evans S, French JA (2008). Dads do not pay for sex but do buy the milk: food sharing and reproduction in owl monkeys (*Aotus* spp.). *Animal Behaviour* 75: 1155–1163.
- Ziegler TE, Sousa MBC (2002). Parent-daughter relationships and social controls on fertility in female common marmosets, *Callithrix jacchus*. *Hormones and Behavior* 42: 356–367.
- Ziegler TE, Wittwer DJ (2005). Fecal steroid research in the field and laboratory: improved methods for storage, transport, processing, and analysis. *American Journal of Primatology* 67: 159–174.

- Ziegler TE, Santos CV, Pissinatti A, Strier KB (1997). Steroid excretion during the ovarian cycle in captive and wild muriquis, *Brachyteles arachnoides*. *American Journal of Primatology* 42: 311–321.
- Ziegler T, Snowdon C, Abbott D, Scheffler G, Wittwer D, Schultz-Darken N (1996a). The metabolism of reproductive steroids during the ovarian cycle in two species of callithrichids, *Saguinus oedipus* and *Callithrix jacchus* and estimation of the ovulatory period from fecal steroids. *Biology of Reproduction* 54: 91–99.
- Ziegler TE, Strier KB, Van Belle S (2009). The reproductive ecology of South American primates: ecological adaptations in ovulation and conception. In *South American Primates. Comparative Perspectives in the Study of Behavior, Ecology, and Conservation* (Garber PA, Estrada A, Biccamarques JC, Heymann EW, Strier KB, eds.), pp 191–210. New York, Springer.
- Ziegler TE, Wegner FH, Snowdon CT (1996b). Hormonal responses to parental and nonparental conditions in male cotton-top tamarins, *Saguinus oedipus*, a New World primate. *Hormones and Behavior* 30:287–297.