Aged Black-and-Gold Howler Monkey Female (*Alouatta caraya*): A Sign of Reproductive Senescence?

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**Abstract**
Reproductive senescence patterns have been scarcely studied in Neotropical primates. The few studies available on the hormonal profiles of aging female monkeys indicate that the decline of ovarian function in nonhuman primates may resemble the hormonal events associated with the perimenopause in women. In this study, we explore a reproductive hormone profile of an aged black-and-gold howler monkey female (*Alouatta caraya*) from a wild population in northeastern Argentina and compare this profile with that of a cycling female in the same population. As part of a larger study, we recorded sociosexual behaviors in adult and subadult females belonging to two groups, and we collected urine \((n = 877)\) to determine the sex hormone profile of each female. These samples were analyzed using enzyme immunoassays for estrone conjugates and pregnanediol-3-glucuronide (PdG). We found differences in mean values of PdG between the younger (cycling) and the older female. These hormone values were lower in the older female, and she did not show any signs of cyclicity for either reproductive hormone. Our results show that the aging female in this wild population shows signs of ovarian senescence, indicated by low, acyclic levels of progesterone metabolites.

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Introduction

The menopause has been suggested to occur in many nonhuman primates, and while its timing and exact characteristics may differ between species, nonhuman primates could provide suitable models of human reproductive senescence [Walker and Herndon, 2008]. From a physiological perspective, the menopause may be defined as the cessation of ovarian steroid hormone secretion as a result of the depletion of oocytes and surrounding follicular apparatus [Johnson et al., 2004]. Several authors have suggested that a menopause exists in certain species of nonhuman primates. In apes and Old World species this process has been thoroughly studied, both in captivity and in wild settings [Van Wagenen, 1970; Gould et al., 1997; Johnson and Kapsalis, 1998; Shideler et al., 2001; Martin et al., 2003; Atsalis and Margulis, 2006; Downs and Urbanski, 2006; Emery-Thompson et al., 2007], highlighting that this is not a phenomenon unique to human primates [Flint, 1976; Pavelka and Fedigan, 1991]. Growing evidence from captive studies and from long-term studies in the wild indicates that postmenopausal nonhuman primate females are common (Semnopithecus entellus [Borries et al., 1991; Sommer et al., 1992]; Pan troglodytes schweinfurthii [Nishida et al., 2003]; Macaca mulatta [Small, 1984]; Papio anubis [Strum and Western, 1982]; Cercocebus albigena [Waser, 1978]), but their postreproductive lifespans are short compared to those of humans [Pavelka and Fedigan, 1991; Caro et al., 1995; Hawkes et al., 1998]. To characterize sex hormone profiles, the metabolites most commonly used in primates are estradiol, estrone conjugates (E1C), progesterone and pregnanediol-3α-glucuronide (PdG), depending on the species [Shideler et al., 1990, 1993; Lasley and Savage, 2007]. In several studies of nonhuman primates, urine samples were collected in order to evaluate hormonal profiles, estimation of the duration of the ovulatory cycle (DOC) and sexual behaviors, both in wild and captive status conditions (Cebus apella [Nagle and Denari, 1983; Carosi et al., 1999]; Saginus oedipus [Ziegler et al., 1993]; Callimico goeldii [Jurke et al., 1994]; Pithecia pithecia [Savage et al., 1995]; Callicebus moloch [Valeg gia et al., 1999]; Alouatta seniculus [Herrick et al., 2000]; Ateles geoffroyi [Campbell et al., 2001]; Callithrix geoffroyi [Mustoe et al., 2012]; P. troglodytes schweinfurthii [Emery-Thompson, 2005]; Pan troglodytes verus [Stumpf and Boesch, 2005]; Rhinopithecus roxellana [Yan and Jiang, 2006]; Colobus guereza [Harris and Monfort, 2006]; Gorilla beringei beringei [Habumuremyi et al., 2014]).

Within the atelines, Campbell et al. [2001] characterized the reproductive endocrine profiles in female A. geoffroyi, where they collected urine samples from females in captivity in a first instance and, then, fecal samples from wild females [Campbell, 2000]. These authors reported a DOC of 20–23 days for this species [Campbell et al., 2001]. In particular, in Alouatta sp. there are few studies which explore levels of sex hormones and sexual behavior. Van Belle et al. [2008] conducted the first study in the genus on reproductive behavior and its relationship with sex hormones in 5 females belonging to two groups of wild A. pigra in Palenque National Park, Mexico; they estimated a DOC of 18.3 ± 1.4 days for this species. On the other hand, Herrick et al. [2000] conducted a study with 2 wild female red howler monkeys (A. seniculus), and they estimated a DOC of 29.5 ± 1.5 days for this species. In A. caraya females, the DOC (20 days, n = 1) was estimated for the first time from cytological profiles and vaginal scrapings of females in captivity [Colillas and Coppo,
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Later on, Kugelmeier et al. [2011] described for *A. caraya* females in captivity ($n = 4$) a DOC of $19.11 \pm 2.1$ days. Last, we could estimate a DOC of $17 \pm 2$ days ($n = 3$) in the wild, based on PdG values in the cycling females and considering that the DOC is estimated as the period between two consecutive ovulations [Raño, 2017].

Data on aging and reproductive senescence from Neotropical primates remain scarce; there are some long-term data available from capuchins [see review in Alberts et al., 2013]. Tardif and Ziegler [1992] described hormonal profiles of 6 aging captive females of 2 species of tamarins (*S. oedipus* and *S. fuscicolis*), and they found that females experienced a reduction in fertility at approximately 17 years of age, based on plasma and urinary hormone measurements. However, ovarian histology of older females suggests that large luteal masses continue to process steroids, despite the absence of normal folliculogenesis [Tardif and Ziegler, 1992]. This phenomenon distinguishes these animals from Old World primates and humans, although further research on reproductive senescence in New World primates is clearly needed. On the other hand, Strier and Ziegler [2005] measured fecal androgen (testosterone and dihydrotestosterone) and estradiol levels in 5 females of wild northern muriquis (*Brachyteles hypoxanthus*) at the Estacao Biologica de Caratinga/RPPN Feliciano Miguel Abdala, Minas Gerais, Brazil, to evaluate the hormonal bases underlying individual variation in the resumption of cycling and conception. They found a non-conceptive female who presented lower periovulatory androgen levels and substantially lower mean estradiol levels during her periovulatory periods than conceptive females (110 vs. >325 ng/g). This female was the oldest female sampled (approx. 17 years of age), and the authors explained that her significantly lower levels of sex steroids were possibly related to a decline in fertility that has been associated with aging in other nonhuman primates (e.g., tamarins [Tardif and Ziegler, 1992] and humans [Sherman et al., 1978]). And finally, Walker et al. [2009] examined the age-associated cytological changes in the ovaries of 24 squirrel monkeys (*Saimiri sciureus*), in particular the granulosa cell clusters, ranging in age from newborn to approximately 20 years, in captive settings. They found a significant age-related decline in the number of primordial follicles, and these findings indicate that reproductive senescence in female squirrel monkeys, as in other primates, involves the inexorable depletion of ovarian follicles.

In Argentina, *A. caraya* reaches the southernmost limit of the species and the genus [Cortés-Ortiz et al., 2015], with the most southerly of all living at 29°30′S, in flooded forest on islands in the Parana River [Brown and Zunino, 1994]. *A. caraya* females have interbirth intervals of 15 ± 3.6 months ($n = 28$) [Pavé et al., 2015]. Age at first reproduction is between 4 and 6 years [Kowalewski et al., 2017].

There are no published data on reproductive senescence in *Alouatta* species. We report the first results of hormonal profiles and associated behaviors in an aging (18–20 years old) free-ranging black-and-gold howler (*A. caraya*) female. For comparison, we compare her hormonal profile with that of a cycling female (5–7 years old) and discuss a possible explanation for this pattern. These results are part of a larger project which aims at understanding alternative female sexual strategies in howlers.
Methods

Study Area
We conducted this study in a semideciduous gallery forest in San Cayetano (27°30′ S, 58°41′ W), located in the basin of the Riachuelo River, in the northwestern part of the province of Corrientes, Argentina. The study area has 24 identified forest fragments that contain black-and-gold howler monkeys. Fragment size averages 9.24 ha, SD = 7.62 (n = 24 fragments), ranging in area from 1.4 to 29.3 ha. The average distance between fragments is 1,763.1 m, SD = 538.7 (n = 11 fragments) [Oklander et al., 2010]. The average annual temperature is 21.7°C, and rainfall is around 1,230 mm.

Study Groups and Behavioral Data Collection
From August 2011 to August 2012, we followed from sunrise to sunset two neighboring groups of black-and-gold howler monkeys. Group composition of “Se” was 1 adult male, 2 subadult males, 2 adult females, 1 juvenile male, 1 juvenile female, and 2 infants, and that of “Ta” was 2 adult males, 2 adult and 2 subadult females, 1 juvenile female, and 2 infants.

We followed each group every 2 consecutive days during 1 full year (we followed one group for 2 days, then the other, then back again, etc.) with the help of 4 trained field assistants. Individuals in our study groups were recognized by differences in body size, pelage coloration, scars, and artificial marks (ear tags and colored anklets) placed on howlers during a program of trapping and marking. To understand the behavioral and hormonal profile of an old female, we present also data belonging to a younger cycling female of the same study group “Ta.” The age category of these females was established following Rumiz’ [1990] criteria, and the specific age of each female was determined using our demographical database. In this regard we have collected demographic data on these groups since approximately 1982 [Rumiz, 1990; De Luycker, 1995; Agoramoothy and Lohmann, 1999; Kowalewski and Zunino, 1999, 2004; Zunino et al., 2007; Kowalewski et al., 2011, 2017], and we know the age of many individuals. In this case, the older individual was approximately 18–20 years (multiparous) old and the younger one 4–5 years old (nulliparous). The younger female was only used in order to note differences fromthe characteristic profile of the older female. During the period of study, the groups did not experience new male invasions, or nonresident females trying to get breeding positions, or the death of juveniles/infants within the group. The older female was not seen engaging in mating behaviors during the entire observational period (copulations, direct lingual gestures, or mate guarding).

Urine Sample Collection
Urine samples were obtained using a collection device made with a long pole and a plastic bag attached to one end. We located the group before they awakened and waited for the first morning void under each female, collected the urine, and transferred it from the collection device to plastic polypropylene tubes, labeled with the female’s name, the date, and time. These tubes were immediately placed in a container with ice packs [Hodges and Heistermann, 2003]. Upon return from the field, we measured the specific gravity value (SG) for each urine sample with an Atago 4410 PAL-10S Digital Hand-Held Pocket Urine Specific Gravity Refractometer prior to storage in the freezer [Anestis et al., 2009]. Samples were obtained for 2 consecutive days every 2 days per female. A total of n = 94 and n = 80 urine samples were collected from the older and younger female, respectively. Samples were kept at –20°C until shipment on dry ice to the Penn Reproductive Ecology Laboratory of the Department of Anthropology at the University of Pennsylvania, where they were stored at –20°C until analysis.

Assays of Hormone Profiles
Samples were analyzed for E1C and PdG following established protocols [Shideler et al., 1990; Munro et al., 1991; Valeggia et al., 1999]. Hormone values were corrected by specific gravity to account for differences in hydration status [Anestis et al., 2009]. Samples were analyzed in duplicates. Intra- and interassay coefficients of variation for the E1C enzyme immunoassay averaged 6.75 and 3.77%, respectively. For the PdG enzyme immunoassay, intra- and interassay coefficients of variation averaged 7.03 and 5.82%, respectively.
Results

The ovarian steroid profiles differed between the older female and the younger female (Fig. 1). The older female presented a somewhat erratic pattern of hormone release, without indication of cyclicity, and overall lower levels of progesterone metabolites. In contrast, the younger female showed a characteristic profile of a cycling adult female [Raño et al., 2013]. PdG, a metabolite of progesterone, defines the luteal phase of the ovulatory cycle and its rising levels during the luteal phase are indicative of ovulation; the curves of PdG in the profile of the younger female (Fig. 1) indicate several ovulatory cycles and the valleys generated by E1C indicate the beginning and end of each ovulatory cycle.

There were differences in the reproductive hormone levels between the younger female and older female as well. Overall, the cycling female presented higher average values of both hormones (PdG\textsubscript{younger female} = 348.6 ± 418.6 ng/SG, PdG\textsubscript{aging female} = 117.5 ± 108 ng/SG, E1C\textsubscript{younger female} = 59,169.5 ± 30,073.5 ng/SG, E1C\textsubscript{aging female} = 53,797.6 ± 22,023.2 ng/SG) (Fig. 1, 2).
Discussion

Among mammals, it remains unclear whether reproductive cessation during senescence is a byproduct of the inevitable process of aging or whether it is an adaptation to natural selection pressures that favors mothers who help their offspring to rear more of their own offspring or offer some other reproductive advantage [Peccei, 2001]. Our data show that the older female pattern in this wild population shows signs of ovarian senescence, indicated by low, acyclic levels of progesterone metabolites. This is clear when the older female profile pattern was compared to the younger female profile pattern. This could be the result of (a) suppression of ovulatory cycles due to some physiological stress (social, nutritional, etc.). We can affirm that, in general, the older females have higher parasite richness [Kowalewski, pers. commun.] and are less efficient at processing food. Tarazona et al. [2002] suggested that susceptibility to parasitic infection increases with age through the process of immunosenescence, an age-specific deterioration in the efficiency of the immune system [Zohdy, 2012]. Also, teeth after the age of 12 years are almost lost (i.e., enamel, crown) possibly related to the leafy diet. We need to continue analyzing the results from this older female and others. Or, the other possibility is because of (b) the general aging, as described for macaques (M. mulatta [Johnson and Kapsalis, 1995, 1998]), olive baboons (P. anubis [Packer et al., 1998]), and Hanuman langurs (Presbytis entellus [Sommer et al., 1992]). In fact, Packer et al. [1998] argue and support the idea that menopause, per se, is a function of aging. The timing, however, depends upon the female lifespan and the duration of infant dependency.

Although some studies have emphasized an age-related decline in the fertility of captive chimpanzees which supports the occurrence of menopause, Emery-Thompson et al. [2007] did not find evidence for the hypothesis that chimpanzees routinely experience menopause in the wild. Similar results were reported in mountain gorillas (Gorilla gorilla gorilla), which support the occurrence of menopause in captivity [Atsalis and Margulis, 2006], but its absence in the wild [Robbins et al., 2006]. In orang-
utan species (*Pongo pygmaeus*), Knott [1996] found evidence of significantly lower urinary estrone conjugate levels and the absence of mating, suggesting that energy imbalance had significant effects on fecundity. At any rate, wild orangutans [Knott, 1999] and chimpanzees [Emery-Thompson, 2005] produce significantly lower levels of ovarian hormones during their menstrual cycles than do their captive counterparts.

Our study has some limitations. First, given that this report is based on 1 single aged female, generalizations should be made with caution. However, because of the scarcity of data coming from the field, we considered it important to report these findings. Second, long-term, longitudinal hormonal data are very difficult to obtain in the field, and, thus, we cannot indicate how long the old female had been in a reproductive senescence period and whether this period was longer than 1 interbirth interval. But we can affirm that during the study period she did not have infants or parental care of infants or juveniles in the group. However, this is the first time that a case of reproductive senescence in a howler monkey has been reported, which complements data presented in other atelines such as *Brachyteles* [Strier and Ziegler, 2005]. In this regard, the estrogen conjugate values we found in the aging female followed a similar pattern to those found in the *Brachyteles* aged female. Strier and Ziegler [2005] support the idea that this case of senescence may therefore be related to declines in fertility that have been associated with aging.

Finally, we can conclude that we found differences between these two females in endocrine characteristics of reproduction; in particular, these results may provide evidence for reproductive senescence in this species. These results are important with respect to understanding the relationship between aging and female reproductive status in this species and other species of South American primates. It is also important to highlight that the interpretation of this case of reproductive senescence in a Neotropical primate species is not simple and unique, and we still have to explore other factors that contribute to variation of the female lifespan when we consider their development through the life cycle [Strier and Ziegler, 2005].

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**Statement of Ethics**

The study complied with the current laws and permission of the USA and Argentina (22421 and 666/97), and adhered to the *Folia Primatologica* principles for the ethical treatment of non-human primates.

**Disclosure Statement**

The authors declare that no conflicts of interest exist in relation to this work.
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