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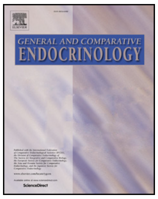

Fur color change and hormonal development in captive females of northern white-cheeked (*Nomascus leucogenys*) and buff-cheeked (*Nomascus gabriellae*) gibbons

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ABSTRACT

Gibbons of the genus *Nomascus* exhibit strong sexual dichromatism in fur color. Change of fur color in sub-adult wild *Nomascus* females is associated with the onset of puberty and the time of their dispersal. The variability in fur change may be influenced by social factors. In this study, we determined whether in captive females of crested gibbons begin reproductive maturity prior to dispersing and with association to their fur color. We collected 287 fecal extracts to analyze pregnandiol -3-glucuronide and 17β estradiol profiles of 4 sub-adult females (*Nomascus leucogenys* and *Nomascus gabriellae*) and 183 samples from their mothers, using enzyme immunoassays. The sub-adult females were monitored from 4 years of age. Their hormone profiles showed the onset of ovulatory cycling between 4.6 and 5.8 years. Based on the information about the estrogen influence to the secondary sex characteristic (fur color of female) the positive link between estrogen concentration and age of the sub-adult females was found. However, the amount of the estrogen can apparently be influenced by the presence of mother. If the mother was presented, the level of estrogen was higher than if the mother was missing. Our findings suggest that the probability of changing to beige fur color by the sub-adult females increased with increased age and if they were without mother. This initial study presents the maternal influence as a possible social factor affecting the fur color change of female offspring.

1. Introduction

Gibbons (family Hylobatidae), territorial primates living in Southeast Asia, are traditionally described as monogamous, but much greater flexibility exists in their social system (Sommer and Reichard, 2000; Barelli et al., 2008b; Reichard and Barelli, 2008). Polygyny in the *Nomascus* genus is associated with the type of their habitat and food sources (Bleisch and Chen, 1991; Jiang et al., 1994, 1999; Fan et al., 2006; Fan and Jiang, 2010; Huang et al., 2013; Guan et al., 2013, 2017).

The genus *Nomascus* is one of four genera, includes seven species (Thinh et al., 2010, 2011; Anandam et al., 2013), and has perhaps the most marked sex-specificity in pelage color of all gibbons. Fur color change in females is generally reported to be detectable after the onset of physical maturity (Mootnick and Fan, 2011; Hu et al., 2018).

Individuals are born with beige fur, but this slowly changes to black fur during their first year of life. While males maintain black fur throughout their lives, females change again during pubescence (Mootnick and Fan, 2011). Adult females have cream to golden fur, with a blackish crown patch or streak, and may have a white or black face ring depending on the species (Geissmann et al., 2000; Mootnick, 2006). Despite detailed knowledge of the sexual dichromatism in fur color in adult *Nomascus* gibbons, the timing of the fur color change in adolescent females is still unclear. The question remains whether or not the onset of the ovarian cycle really corresponds with the fur color change (Mootnick, 2006). Deputte and Leclerc-Cassan (1980) mention a possible delay in fur color change of two to three years after the onset of the regular ovarian cycle, but this study did not take into account composition within the group and the possible presence of another breeding/adult female. Geissman and Anzeberger (2009) describe the dominance of a 10-year-old female over a 7.5-year-old female living nearby in another cage. This younger female had black fur but full cycling, so this example could just indicate a possible context for fur color change prolongation and the presence of another female. In both cases, the authors point to the potential important influence of the social environment on the studied females.

The latest study from Burns and Judge (2016) gives us the opportunity to take a new look at the problem of fur color change. The authors describe this process in one 6.4-year-old *N. leucogenys* female removed from her natal group. The fur color change began around 6.5–7 years, before the arrival of the male. A similar situation was described by Burns et al. (2011), where a 6-year-old *N. leucogenys* female

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Table 1

Determination of the onset of ovarian cycle and color change in sub-adult females: an age of sampled females at the beginning of sample collection was 4 years.

Study subject				
ZOO Species	Usti n. L. Czech Republic Northern white-cheeked gibbon *Nomascus leucogenys*		Liberec/Czech Republic and Asson/France Northern white-cheeked gibbon *Nomascus leucogenys*	Bratislava Slovak Republic Southern yellow-cheeked gibbon *Nomascus gabriellae*
Date of birth and Studbook Number (Brice, 2012)	13 Oct 2006; No 176	31 Aug 2008; No 181	18 May 2006; No 168	27 Feb 2007; No 144
Time of fecal collection	Dec 2010–Oct 2013	Aug 2012–Feb 2014	Jul 2010–Feb 2013	Mar 2011–May 2012
Number of collected samples	113	51	77	46
Hormonal stage and fur color at the age of 4 year	Non – cycling/black	Non – cycling/black	Non – cycling/black	Non – cycling/black
Mother presence	No	Yes	Yes	No
Age (years) in the time of the life change/ absence of the mother	3.7 separation from the mother	5.5 transfer	6.1 transfer	3.7 motheŕs death
Beginning of fur color change (grey-beige) in years	4.4	5.6	6.2	4.1
Age (years) and fur color in the time of *OC onset	4.9 grey-beige	4.8 black	5.8 black	4.6 grey-beige
Mean estrogen level ± SD (ng/g)	6.87 ± 6.09	3.81 ± 3.31	1.93 ± 1.71	1.75 ± 0.78
Mean *OC length in days ± SD progesterone and estrogen	27.1 ± 4.9 28.1 ± 4.8	24.0 ± 3.5 27.7 ± 5.2	24.6 ± 4.6 25.8 ± 5.3	26.3 ± 3.9 29.3 ± 1.5
Age (years) and place of final fur color change/beige	6.8 in the same facility	6.9 Lyon Zoo (France)	7.1 Asson Zoo (France)	4.9 in the same facility

* OC = ovarian cycle.

changed fur color after removal from the family group.

Mootnick et al. (2005) report that gibbons in captivity lack an incest avoidance mechanism. The authors presuppose that inbreeding may be a part of hylobatid flexibility in the social mating system. In this case, we could exclude the theory of inbreeding avoidance and reproductive suppression (Hamilton, 2004). However, reproductive suppression may take several forms. Reproductive suppression varies across taxa and results in endocrine inhibition (Wasser and Starling, 1988; Hamilton, 2004), or nonbreeding individuals physically capable of reproducing are influenced by behavior directed at them (Kutsukake and Nunn, 2006; Hager and Jones, 2009). In connection with this theory and with the limited housing space in captivity (possible close proximity to an adult/dominant female), we should also take into account this factor, which may influence the timing of fur color change.

The natural dispersal of sub-adult females in the wild is assumed to be around 6–10 years of age in all gibbons and is often associated with sexual maturity or the attainment of adult body size (Brockleman et al., 1998; Geissmann, 1995; Huang et al., 2013, Hu et al., 2018). Territory size and food abundance may influence the time of dispersal and the resulting number of individuals in the group. It seems that the flexibility of the social system with more than one female breeding is restricted to the genus *Nomascus* (Fan and Jiang, 2010). In the captivity, the time of removal of sub-adult individuals is variable and depends on the management system and facility dimension (personal unpublished data). Conclusions from Geissmann (1991) suggest an earlier sexual maturity in captivity at five years age.

Traditionally, the onset of the sexual maturity in females is connected with the beginning of a regular ovarian cycle, which is characterized by anogenital swelling during the cycle (Barelli et al., 2006; Dahl and Nadler, 1992; Cheyne and Chivers, 2006). The swellings in gibbons are not as exaggerated as in other catarrhine primates (Nunn, 1999); they are mainly limited to labial tissues (Cheyne and Chivers, 2006, Barelli et al., 2008a). Nevertheless, when comparing the swellings in *Nomascus* females with, for example, those in *Hylobates* females, we can note just faintly swollen pink labia or more often the absence of swellings. Therefore, swellings in this genus cannot be a precise indicator of the start of the ovarian cycle (personal unpublished data). Based on this finding, detection of sexual maturity through an analysis of sex hormones is preferred (Heistermann, 2010). Only two studies have quantified sex hormones in *Nomascus* females (Lukas et al., 2002; Rafacz et al., 2013) using fecal hormone analysis. Unfortunately, neither of these studies followed sub-adult females up to the onset of their puberty. Lukas et al. (2002) determined the ovarian cycle only in one 12-year-old *Nomascus leucogenys* female. Rafacz et al. (2013) analyzed adult females (four *N. leucogenys* and one *N. gabriellae*). However, both of these studies found that there were not any differences between the lengths of the ovarian cycle in *Nomascus* gibbons, as Geissmann and Anzenberger had described (2009). However, the relationship between sex hormones and fur color change still remains unclear.

Therefore, one of the aims of the present study was to determinate the age at which captive females of northern white-cheeked gibbons (*Nomascus leucogenys*) and buff-cheeked gibbons (*Nomascus gabriellae*) begin a regular ovarian cycle as the onset of reproductive maturity. We examined the onset of the ovarian cycle using an enzyme immunoassay (EIA) of fecal estrogen and progesterone metabolites. We also examined the beginning and the final time of fur color change in these females as well as important life changes (separation, transport, etc.). We predicted that the hormonal levels of estrogens, as hormones related to secondary sex characteristics (Dixson, 2012), would increase with age and with the presence of the mother (Jenner et al., 1972; Mitamura et al., 2000). Finally, we predicted that there would be a connection between the timing of the fur color change in sub-adult females and the presence of the mother based on previous studies (Deputte and Leclerc-Cassan, 1980; Geissmann and Azenberger, 2009) suggesting the influence of the social environment on the fur color change.

2. Material and methods

2.1. Study subject

The research involved the participation of five northern white-cheeked gibbon females and one buff-cheeked gibbon female in Czech (Liberec, Usti nad Labem), Slovak (Bratislava), and French (Asson) zoos (Table 1). At the beginning of the sample collection, all sub-adult females were four years old and had black fur. As a result of the possible variability in the onset of sexual maturity in captivity (Geissmann, 1991) and for the purpose of this study, we classified females from 4 to 6 years of age as sub-adults.

Two of the sub-adult females, studbook numbers (Std. Nos.) 181 and 168, were transferred during the study to another zoo. Sampling of Std. No. 181 was carried out in the period before this transfer, whereas for Std. No. 168 the sampling was carried out both before and after transfer to the new zoo (Asson). The reason was the late onset of the regular cycle (Table 1) in the origin zoo, so to confirm the regularity of the cycling, sampling continued in the new zoo.

All females in the study were born in captivity and reared by both genetic parents or only by the mother/father. The family from the Liberec Zoo consisted of a father and mother and their offspring. Pairs (of unrelated animals) formed the groups in the Asson Zoo and one group in the Usti nad Labem Zoo. Only the group in Bratislava Zoo comprised just a genetic father and his sub-adult daughter. A similar situation held for the second group in the Usti nad Labem Zoo, where the group was formed of a mother with her sub-adult daughter.

Each group of gibbons had permanent access to indoor and outdoor enclosures. The enclosures that were indoors and outdoors featured platforms at various heights, trees, and extensive rope systems. The gibbons were fed four/five times a day and water was available ad libitum. Their diet consisted of primate pellets, a mix of fruit and vegetables, and supplemented with browsing, seeds, legumes, or eggs.

The research conducted herein was approved by the Ethics and Animal Care Committee at the Czech University of Life Sciences Prague (protocol number: CZU/16-06) and was performed in accordance with the relevant guidelines and regulations. All zoological institutions have rigorous standards for animal welfare, and are accredited by the European Association of Zoos and Aquaria. The research adhered to the legal requirements of France and the Czech and Slovak Republics for the ethical treatment of nonhuman primates as well as in accordance with European Directive 2010/63/EU.

2.2. Fur color recording

Information about the beginning of fur color change and all life changes in the sampled females was obtained from individual records registered by each involved zoo. According to Mootnick and Fan (2011), there are three levels of fur color in sub-adult females: black, gray–beige, and beige. The beginning of fur color change (gray–beige) was defined as an occurrence of gray–beige hairs intermixed with black fur, especially well visible on the shoulders and the crown of the head.

2.3. Fecal sample collection

Collection of fecal samples from the four sub-adults (Table 1) and two adult females (Table 2) was carried out during 2010–2014 in the four zoos.

Fecal samples were collected once per week in ziplock bags using gloves to prevent contamination. Each sample with an approximate weight of 1–2 g was properly sealed and indicated with the date of collection and animal identification. Immediately after collection, it was frozen and stored at −20 °C until being transported to the laboratory.

Identification of the sample was a priority, so in the event that it was not possible to ensure 100% withdrawal from the individual, natural dyes such as carrots, beetroot, and blueberries, were used in the individual's diet (Fuller et al., 2010). It was always necessary to control the individual's intake of this diet.

Table 2
Length of the ovarian cycle in adult females.

Subject study – adult females (mothers)		
ZOO	Usti n. L. Czech Republic	Liberec Czech Republic
Species	Northern white-cheeked gibbon *Nomascus leucogenys*	
Date of birth and Studbook Number Brice (2012)	1986 in the wild ; **No. 74**	2 Sept 1993; **No. 109**
Time of fecal collection	Dec 2010–Feb 2014	Jul 2010–Apr 2012
Number of samples	134	43
Mean [*]OC length in days ± SD	26.40 ± 4.78	25.40 ± 4.96
progesterone and estrogen	26.65 ± 5.76	25.00 ± 4.82

[*] OC = ovarian cycle.

The number of samples per cycle was 4.7 ± 0.95 (mean ± S.E.) in all age categories. The time when each female was monitored encompassed at least three full potential ovulatory cycles. Tables 1 and 2 present additional detail about the number of samples from each individual and the time period of sampling.

2.4. Enzyme immunoassay (EIA)

To determinate the onset of the ovarian cycle, we analyzed the metabolite of pregnandiol -3- glucuronide (progesterone) and the metabolite of 17β estradiol (estrogen). Samples were collected within two hours of defecation. Only uncontaminated feces were put in a plastic ziplock bag that identified the individual by name and collection date and were stored frozen at −20 °C until hormone analysis. Sample collection was performed at least once per week in all females. For hormone analysis, 5 ml 80% methanol water was added to 0.5 g feces and vortexed for 1 min. Following extraction, the samples were centrifuged and 5 ml of the supernatant decanted and stored at −20 °C until the assay. For the detection of progesterone and estrogen metabolites in the feces, polyclonal progesterone (CL425), estrogen antibodies, and progesterone and estrogen conjugates (R522-2) designed by Coralie Munro (University of California, Davis, U.S.A.) were used. Hormone measurements in the fecal methanol extract were carried out using a microtiter plate enzyme immunoassay (F96, MaxiSorp, Number 44204, Nunc Company, Denmark). Based on the assay methodology described by Hodges and Heistermann (2011) for field endocrinology in primates, we had to determinate the appropriate antibody and conjugate dilution to ensure the absorbance of the control wells between 0.8 and 1.20 nm. According to the result of the check board titration, we selected the dilutions that showed a color intensity of about 1.0 nm leading to the most discriminating ratio within the assay range.

This resulted in selection of the following working dilutions: for progesterone: 1:1600 for antibodies and 1:800,000 for the conjugate, and for estrogen: 1:32,000 for antibodies and 1:100,000 for the conjugate. The sensitivity was 4.9 pg/ml for the progesterone metabolite and 0.15 pg/ml for the estrogen metabolite. Inter-assay coefficients of variability (CVs) were 10.7% for progesterone and 12.7% for estrogen, and intra-assay CVs were 7.8% for progesterone and 9.8% for estrogen. Cross-reactivity of the progesterone antibodies were 100% with progesterone, 55% with 5α-Pregnan-3,20-dione, 12.5% with 5ß-Pregnan-3ß-ol-20- one, 8% with 5ß-Pregnan-3,20- dione, 2.7% with 4-Pregnen-11ß-ol-3,20-dione, 2.5% with 5ß-Pregnan-3α-ol-20-one, and ≤0.1% with pregnanediol, androstenedione, and corticosterone. Cross-reactivity of estradiol-17ß antiserum was 100% with estradiol, 3.3% with estrone, 1.0% with testosterone and androstenedione, 0.8% with progesterone, and < 0.1% with cortisol and dihydrotestosterone.

For the final analyses, we used a Biotek automatic MTP reader

(Vermont, U.S.A.). All assays were performed in duplicate to calculate the CV and recalculate the amount of hormone metabolites at ng/g of feces.

2.5. Ovarian cycle detection

The length of a regular ovarian cycle was defined as the number of days from the first elevated progesterone = PdG (pregnandiol -3- glucuronide) and estrogen = E2 (17β estradiol) metabolite value until the first elevated progesterone and estrogen metabolite value of the next cycle (Geismann and Anzeberger, 2009; Rafacz et al., 2013). We considered cycles consecutive if peaks occurred within 20 days of one another based on the results from Geissmann and Azenberger (2009), where the mean cycle length in *N. gabriellae* females was 21.1 ± 1.2 days. We excluded from our calculation of cycle length any peaks that occurred < 20 days apart. We then calculated the average cycle length over all individuals. Hormonal analyses of the adult females (mothers) sampled at the same time as the young females/daughters were used as a reference value for ovarian cycle length (Table 1).

2.6. Statistical analyses

2.6.1. Fur color change of sub-adults versus estrogen level and/or presence of the mother

The data was analyzed with SAS (SAS Inst. Inc. Cary, NC, version 9.4) using a generalized linear mixed model (PROC GLIMMIX). The results were considered statistically significant when $P \leq 0.05$.

The fitted models included daughter identity nested by mother identity as a random effect to account for the use of repeated measures on the same individuals. The variables age of female nested by presence of the mother tested the effect on the estrogen level of the sub-adult females and the probability of fur color change from black to beige.

The models were tested using the Akaike information criterion to increase the power of the statistical tests.

3. Results

3.1. Ovarian cycle detection

The reference value or ovarian cycle length in the adult females was 25.91 ± 4.00 days based on Pdg metabolites and 25.83 ± 4.41 days based on E2 metabolites (Table 2) and the average ovarian cycle length in the sub-adult females was 25.50 ± 3.70 days based on Pdg and 27.37 ± 3.87 days based on E2 (Table 1).

3.1.1. Age at ovarian cycle onset

Table 1 presents the age at the time of regular ovarian cycle onset in individual sub-adult females and their ovarian cycle length similar to adults (Table 2).

3.2. Fur color change of sub-adults versus estrogen level and/or presence of the mother

Fig. 1 depicts the variability in the estrogen levels by mother presence. The estrogen level was significantly affected by the age of the sub-adult females, but differently by whether or not the mother was present ($F_{2,282} = 11.07$; $P < 0.001$). With increasing female age, the level of estrogen increased. Sub-adult females residing with their mothers showed higher estrogen levels than females without a mother did (Fig. 1). The variability in estrogen levels by mother presence is shown in Fig. 2. The mean estrogen levels of sub-adult females grouped by either mother‘s presence or absence are shown in Table 1.

The probability of beige fur in the sub-adult females was affected by the age of the females and by whether the mother was together with the sub-adult female or absent from the facility (F2,285 = 30.95; $P < 0.001$). With increasing age of the sub-adult female, the probability having beige fur in sub-adult females without the presence of the mother increased. On the other hand, if the mother was still present, the probability of changing fur color did not increase (Fig. 3).

4. Discussion

This study investigated the timing of the onset of sexual maturity in three *N. leucogenys* females and one *N. gabriellae* female. The onset of a regular ovarian cycle in these sub-adult females confirmed the earlier sexual maturity in the captivity (Geissmann, 1991). This suggestion is also in agreement with Melfi and Marples (2000) explaining earlier sexual maturity as a result of the human care provided to the animals (energy-rich food at a faster rate). In addition, changes in the social environment could lead to earlier maturity (Burns and Judge, 2016; Geissmann, 1991). The present data showed similar ovarian cycle length as that found by Rafacz et al. (2013) using fecal progesterone analysis and minimal variation within ovarian cycle length between adult and sub-adult females.

Within the period of sample collection, two females (Std. Nos. 181 and 168) in black fur started regularly cycling before their transfer (at 4.8 and 5.8 years of age), but they changed the fur color right in the new zoo. A recent study from Corley et al. (2017) confirmed cycling in sub-adult owl monkeys (*Aotus azarae*) before their dispersal, even when they lived in their natal group with reproductive adults. These results indicate that sexual maturation is not strongly associated with dispersal. Taking into account differences between these two primates, we can still see a certain similarity to our study. In contrast, we found another study about wild *Hylobates lar* where the females did not show cycling prior to their dispersal (Barelli et al., 2007). We can suppose that there are many more potential environmental factors that could influence the ovarian cycle, such as an excess of high energy food in captivity.

Based on knowledge about the influence of estrogen on secondary sex characteristics (Berkovitsch and Ziegler, 2002; Dixson et al., 2005; Wallen and Zehr, 2004), which includes the female's fur color, we analyzed and detected ovarian cycles also using an estrogen metabolite. The results were used to determine whether estrogen increases with the age of the female or is also influenced by the presence of the mother. Previous studies have found a positive link between estrogen concentration and the age of sub-adult females (Jenner et al., 1972; Mitamura et al., 2000). However, they mentioned that the amount of estrogen can apparently be determined by the presence of the mother. They found the level of estrogen higher in the case of mother presence. A possible reason is the lack of maternal care during adolescence (Coplan et al., 1996). Our original data from the estrogen analysis distributed by the age of the sub-adult females (in the same age range) and the presence or absence of the mother pointed to a different trend. The data collected from daughters with mothers indicate a tendency for higher estrogen level with increasing age (the onset of sexual maturity). On the contary to previous study (Coplan et al., 1996), the sub-adult females without a mother point out a higher estrogen level variability. Such results could be be a reaction to stress factors (loss of the mother, early weaning), but this is somewhat speculative and further research is necessary.

Our next findings suggest that the probability of beige fur in sub-adult females increased both with increased age and if their mother was absent. The results showed differences in the beginning and length of the fur color change, in contrast to a certain homogeneity of the ovarian cycle.

In two females (Std. Nos. 181 and 168) which were transferred to another zoo, the duration of the fur color change was around one year. This can be compared with Std. No. 176, who was separated early from her mother at the age of 3.7 but lived at the same zoo, where the fur color change took almost 2.5 years. This female started to change her fur color when she was living with an unfamiliar male but was still in

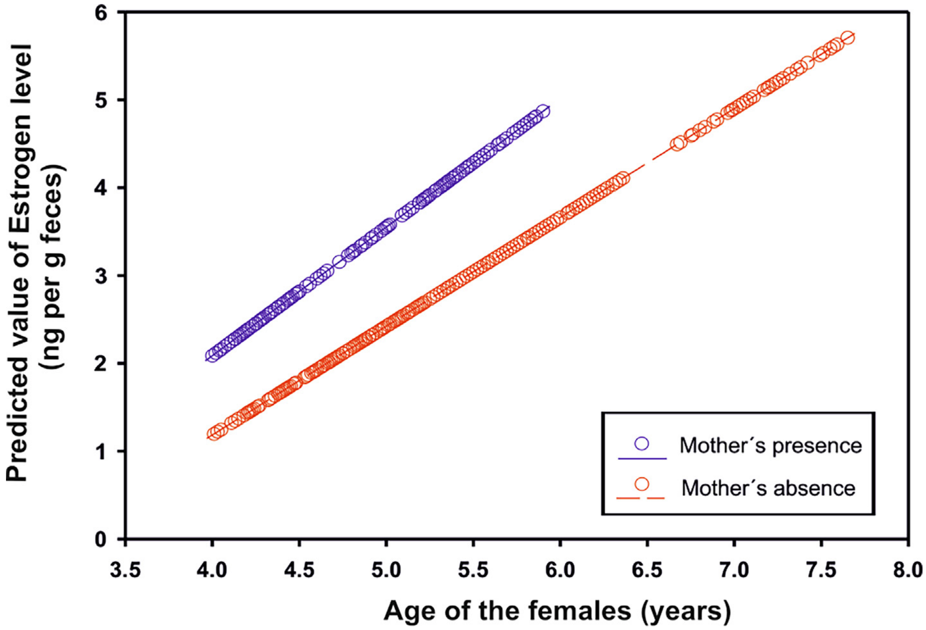

Fig. 1. Relationship between estrogen level of sub-adult females, their age and mother's presence.

proximity to her mother and cycling regularly. This suggests that something other than endocrine suppression may be necessary to fully explain the timing of fur color change in crested gibbons.

The shortest period of fur color change was recorded in a female *N. gabriellae* who lost her mother at the age of 3.7. A very similar situation was observed in the Mulhouse Zoo (France) for a southern white-cheeked gibbon (*Nomascus siki*). In this group of *Nomascus siki*, after the loss of the adult female (mother), the daughter's fur color appeared to change within half a year (Lefaux, personal communication). The same situation was observed in the Fuengirola Zoo (Spain) in one 5-year-old *N. gabriellae* female. Her fur color started to change immediately after her mother was transferred to another institution (Recuero, personal communication). This last example was determined to be very similar also to our Std. No. 181.

Although, it seems that the mother's presence can suppress fur color change in sub-adult females over some time, our results do not provide clear evidence as to whether or not this situation is time-limited or not. For comparison with our results, we have found only studies showing a connection between possible suppression and minimizing female reproductive competition (Beehner and Lu, 2013; Burns et al., 2011; Shuker, 2009), but these studies described only individual young females in a different situation and/or without the data on hormone profiles. Only Geissmann and Azenberger (2009) noted the dominance of an older *N. gabriellae* female (10 years old) over younger female (7.5 years old), but they were unrelated. The younger female had not fully completed her fur color change and unfortunately there was no previous record about the beginning of the fur color change.

We can just theoretically suppose a possible connection between fur color change in sub-adult female *Nomascus* gibbons and the dominant influence of the mother and her territorial behavior (Barelli et al., 2008a,b; Brockleman et al. 1998). On the other hand, studies from the wild also describe the presence of two unrelated adult females in a group (Jiang et al., 1999; Fan et al., 2006; Fan and Jiang, 2010; Guan et al., 2013, 2017) with both of them breeding, singing synchronously, or feeding and grooming. Compared to the situation in captivity, we can suppose an effect from the mother due to the limited space and potential reproductive monopoly of the breeding female. In addition, the daily animal care system in the zoo could influence social interaction and behavior in a gibbon family (Burns and Judge, 2016). Interestingly, a key factor in our study was the social environment, not the estrogen

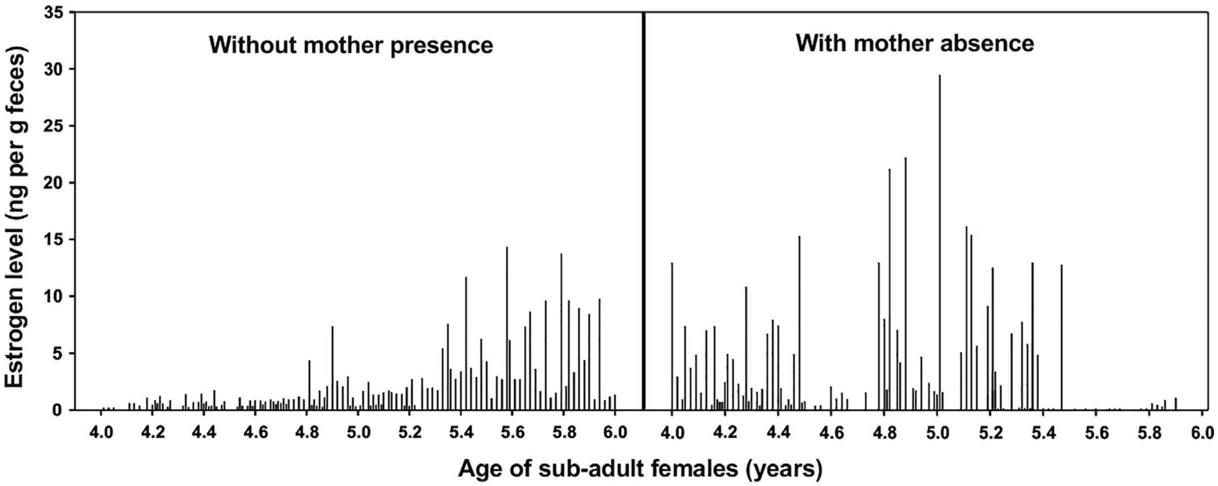

Fig. 2. Distribution of estrogen level of sub-adult females by the age of the females and by the situation if the mother was present or absent. Original data of estrogen levels used for GLLM, was distributed by the age of the females (in the same age range from 4 to 6 years) and the mother presence.

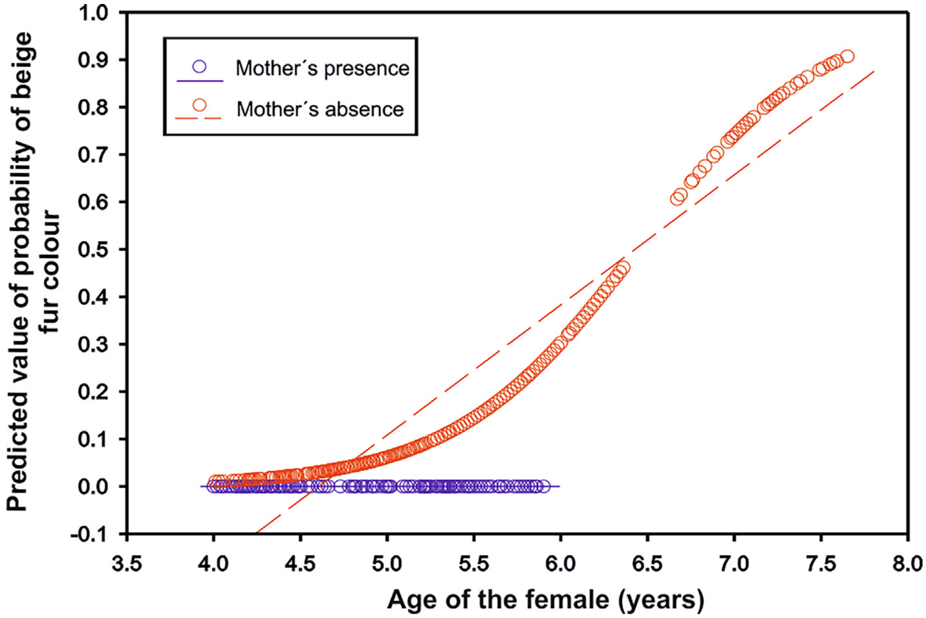

Fig. 3. Relationship between fur color change, their age and mother's presence.

level.

What other factors could be involved in the timing of the fur color change? Geissmann and Hulftegger (1994) found skin glands in gibbons and also some accumulated steroid hormones, so a role in olfactory communication is likely. A specific gibbon's vocalization with reference to vocal development of the "great call" in daughters (Merker and Cox, 1999; Koda et al., 2013) could contribute to fur color change. The point at which the daughter achieves a full-fledged vocal female pattern could be crucial in relation to the limited territory in captivity. It is obvious that the variable breeding environment in zoos affects animal behavior. Although in the present study there was no evidence of ovulation cycle suppression, increased maternal aggression towards daughters (Burns and Judge, 2016) and group composition and parental reproductive status (Burns et al., 2011) could be influencing factors.

Based on the results of the present study, additional links are available, such as cortisol and thyroid hormone analysis, for further investigation. The correlation between cortisol and estrogen levels and the influence on reproduction is well described in certain other primates, including humans (Coe et al., 1988; Ziegler et al., 1995; Cameron, 1997; Bouma et al., 2009), as is the neurological function of thyroid hormones in reproductive physiology and during maturity (Aleandri et al, 1996).

We recognize that the conclusions of this work are based on relatively small sample sizes for the four individuals studied. Furthermore, more comparative work is needed to understand the extent of potential factors influencing the fur color change.

Although further research of coherent factors is needed, this study, thanks to its long-term measurements, shows a new trend towards understanding the phenomenon of fur color change in *Nomascus* gibbons.

5. Conclusions

In summary, our study has proven the earlier age of sexual maturation in *Nomascus* females in captivity that Geissmann (1991) had hypothesized. Estrogen levels increased with the age and were more pronounced in sub-adult females when their mother was present. The fur color varied depending on female age and the mother's presence. Although estrogen level should impact fur color change, it was not conclusively confirmed in this study. Further investigation with a broader data set is necessary to clarify the importance of estrogen as a possible factor associated with fur color change in *Nomascus* gibbons. In addition, including the investigation of all social/stress and communication factors could be beneficial.

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