

Seasonal glucocorticoid production correlates with a suite of small-magnitude environmental, demographic, and physiological effects in mandrills

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Abstract

Objectives: The activation of the hypothalamic-pituitary-adrenal axis is a neuroendocrine response to external and internal changes that animals face on a predictable or unpredictable basis. Across species, variation in glucocorticoid production has been related to such changes. In this study, we investigated the predictable, seasonal sources of variation in the levels of fecal glucocorticoid metabolites (fGCM) in a large natural population of mandrills (*Mandrillus sphinx*) in Southern Gabon.

Materials and methods: Using five years of regular behavioral monitoring and hormone analyses performed on 1,233 fecal samples collected on 99 individuals of both sexes and all ages and General Linear Mixed Models, we studied the three main seasonal predictors of fGCM concentrations: (i) weather conditions, (ii) number of adult males, and (iii) female reproductive status. These three predictors all vary seasonally in mandrills.

Results: We first showed an increase in fGCM concentrations during the short dry season while controlling for other factors. Pregnant females, which include the large majority of adult females at this time of the year, mainly drove this increase, although a combination of other small-magnitude, season-related effects linked to climatic events and demographic changes also partly explained this seasonal trend. Indeed, fGCM concentrations increased with both low temperatures (and low rainfall) and high numbers of adult males present in the group. These seasonal changes, while correlated, held true throughout the studied years and when restricting our analyses to a given season. Finally, we found that older mandrills showed on average higher fGCM concentrations than younger ones and that medium-ranked females exhibited the highest levels of fGCMs.

Discussion: The observed patterns suggest that plasticity in mandrills' metabolism in the form of glucocorticoid production allows them to adjust to predictable changes in climatic, demographic and physiological conditions by mobilizing and redirecting energetic resources toward appropriate, calibrated seasonal responses.

KEYWORDS

fecal glucocorticoids, mandrill, noninvasive sampling, seasonality

1 | INTRODUCTION

Several theoretical models have been proposed to explain animals' short- and long-term physiological and behavioral responses to social and ecological changes. Prominent models include the "allostatic load

model" (McEwen & Wingfield, 2003) and its extension, the "reactive scope model" (Romero, Dickens, & Cyr, 2009). The former model originates from the biomedical literature (Sterling & Eyer, 1988) and proposes allostasis as an adaptive process for maintaining homeostasis through changes (McEwen & Wingfield, 2003). This model encompasses

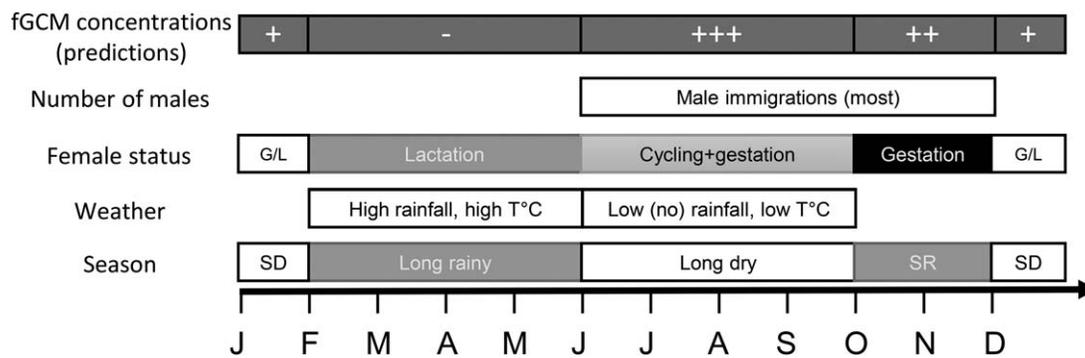


FIGURE 1 Schematic representation of seasonal variation of the different studied predictors across months. This schema represents general patterns of seasonal changes observed in the studied mandrills. SD: short-dry season; SR: short-rainy season; G/L: gestation or lactation; T°C: temperatures. The absence of box represents intermediary cases

the biological mechanisms allowing individuals to meet energetic demands related to predictable and unpredictable external and internal changes. The benefits of allostasis and the costs associated with adaptive responses, or allostatic load (i.e., a cumulative physiological burden; McEwen & Wingfield, 2003), result in different trade-offs in health and disease occurrence (Korte, Koolhaas, Wingfield, & McEwen, 2005). A state of allostatic overload is attained when the energetic demands outstrip those available (internally or from the environment), leading to pathologies and eventually death (McEwen & Wingfield, 2003, 2010). The reactive scope model goes beyond the allostatic load model by considering a broader range of physiological mediators (Romero et al., 2009; and see discussion in Crespi et al., 2013). A normal reactive scope of a mediator encompasses both the “predictive homeostasis” corresponding to individuals’ responses to predictable circadian and circannual changes and the “reactive homeostasis” in response to unpredictable or threatening changes. Homeostatic overload and failure are attained when these mediators reach critical values above or below, respectively, the normal reactive scope, again with possible pathological consequences (Romero et al., 2009).

The activation of the hypothalamic-pituitary-adrenal (HPA) axis is one neuroendocrine response to external and internal changes that animals face on a predictable or unpredictable basis. This system regulates many physiological and metabolic processes such as digestion, the immune system or energy storage and expenditure, and is therefore implicated in mediating biological transitions and trade-offs in life-history traits (Crespi et al., 2013). In particular, the mediators involved in the HPA axis, such as glucocorticoids (steroid hormones), divert animal’s internal resources toward physiological functions that bypass but also facilitate recovery following triggering events through physiological feedbacks (Sapolsky, Romero, & Munck, 2000). Across animal species, variation in glucocorticoid production has been related to different physiological states, life-history events and environmental challenges (see for review in primates: Beehner & Bergman, 2017; Higham, 2016). Social challenges, such as social ostracism or loneliness, social instability, or aggressive events have also been shown to impact glucocorticoid concentrations (e.g., Cavigelli & Caruso, 2015; Feng et al., 2016; Flinn & England, 1997; Sapolsky, Alberts, & Altmann,

1997). Glucocorticoid production therefore reflects the baseline activity of the HPA axis in relation to an animal’s energetic state that fluctuates on a predictable basis following daily, seasonal or circannual changes, but it also reflects the response to unpredictable challenging or life-threatening situations.

Here, we investigated the seasonal and predictable sources of variation in glucocorticoid concentrations both within and across members of a large natural population of mandrills (*Mandrillus sphinx*). We chose to restrict our analyses to these predictable changes to establish the predictive scope for mandrills or as a baseline for future studies related to the unpredictable events that may affect this species. As such, we evaluated three main sources of seasonal variation (Figure 1): (i) weather conditions implying inherent season-related differences either in food availability or in metabolic demands (or both), (ii) number of adult males that shows clear circannual patterns (Brockmeyer et al., 2015), and (iii) female reproductive status as reproduction is seasonal in this species (Setchell, Lee, Wickings, & Dixson, 2002). We evaluated fecal glucocorticoid metabolites (fGCM) in 99 mandrills of both sexes and all ages during a five-year period.

The relationships between fluctuating environments and glucocorticoids have been mainly studied in species experiencing dramatic and unpredictable changes in weather conditions characterized by severe droughts or other ecological disasters (Wingfield, 2013). The most challenging season regarding either food restriction or metabolic demands, both commonly associated to weather changes, has been associated with increased glucocorticoid production in several primates (e.g., ring-tailed lemurs *Lemur catta*: Cavigelli, 1999; yellow baboons *Papio cynocephalus*: Gesquiere et al., 2008; chacma baboons *Papio ursinus*: Weingrill, Gray, Barrett, & Henzi, 2004). Mandrills live in the dense Equatorial forests of Central Africa, from Southern Cameroon to Southern Congo (Abernethy, White, & Wickings, 2002). They experience therefore rather predictable climatic changes throughout the year because weather conditions vary little in equatorial environments. Gabon is, nonetheless, characterized by four different seasons, including two marked ones (the long dry and long rainy seasons). We predict that the season showing high food shortage and/or inclement weather, corresponding to the long dry season in the study site (Figure 1), is

associated with high fGCM concentrations, although we do not expect high amplitude of variation across seasons such as observed in species characterized by harsh and unpredictable environments.

Changes in group composition in group-living species may have profound impacts on the social organization and stability of the group as well as on animals' daily lives. For example, the immigration of new males, and more generally an increase in the number of males, often observed during the mating season, are generally associated with increases in male-male competition and overall aggression levels (Alberts, Sapolsky, & Altmann, 1992). These behavioral modifications have been shown to translate into dramatic events such as feticides and infanticides (yellow baboons: Zippel et al., 2017; langurs *Presbytis entellus*: Agoramoorthy, Mohnot, Sommer, & Srivastava, 1988; Hrdy & Hausfater, 1984), associated with rises in glucocorticoid production (e.g., Engh et al., 2006a). In mandrills, adult males are present in the group year-round, but seasonal changes are observed due to temporary immigration of adult males during the mating season (Brockmeyer et al., 2015), i.e. during the long dry season but also just after, during the short rainy season (Figure 1). This seasonal change in group composition may be regarded as predictable modifications in mandrills' daily lives because of the clear circannual patterns of male immigration events (Brockmeyer et al., 2015). We predict a positive relationship between the number of adult males and fGCM production in both males and females because male-male competition for reproduction is intense in this species (Setchell, Charpentier, & Wickings, 2005) and may therefore affect all group members.

Finally, in mammals, including in semi-free ranging female mandrills (Setchell, Smith, Wickings, & Knapp, 2008), elevated glucocorticoid levels have been observed in pregnant females (e.g., Pepe & Albrecht, 1995), presumably as an adaptive physiological response allowing fetuses to grow appropriately (Keller-Wood & Wood, 2001). Most female mandrills give birth from November to March in the study population (MJEC, unpublished data). The large majority of pregnancies are therefore observed during the period spanning from the long dry season to the short dry season (Figure 1). We predict that female reproductive status impacts fGCM production, with pregnant females showing higher concentrations compared to other females. In turn, we should observe an overall decrease in fGCM production in females during the birth season (Figure 1). Climatic variables, the number of adult males, and female reproductive status were all highly correlated, we therefore performed three alternative models to disentangle their effects on fGCM concentrations.

2 | MATERIALS AND METHODS

2.1 | Ethical statement

This study complies with ethical protocols approved by the CENAREST institution (authorization number: AR0024/16/MESRS/CENAREST/CG/CST/CSAR). This research adhered to the legal requirements of Gabon and to the American Society of Primatologists principles for the ethical treatment of nonhuman primates.

2.2 | Study site and subjects

Mandrills form promiscuous multi-male multi-female groups all year-long (Brockmeyer et al., 2015). Females are philopatric and form a typical matrilineal society in which maternal and paternal kin are preferentially associated with each other (Charpentier, Peignot, Hossaert-McKey, & Wickings, 2007). Males emigrate from their natal group at puberty (Abernethy et al., 2002) but their subsequent fate is unknown. Dominance rank is maternally inherited in females (Setchell et al., 2002) and competitively acquired in males (Setchell, Wickings, & Knapp, 2006b). Alpha male tenure is generally short in the study population, typically lasting only a few months, with some periods characterized by frequent changes in the alpha position (MJEC, unpublished data).

In January 2012, a long-term field project (Mandrillus Project: www.projetmandrillus.com) was set-up to study the only social group of habituated, free-ranging mandrills. This population ranges in the Lékédi park and its vicinity, near Bakoumba village in Southern Gabon, and originates from 65 captive-born mandrills housed at CIRMF (Centre International de Recherches Médicales de Franceville, Gabon) and released into the park on two occasions (36 individuals released in 2002 and 29 in 2006; see for details: Peignot et al., 2008). From 2002 to 2012, mandrills were supplemented with bananas and monkey chow in limited quantities to supplement their natural diet. The first years post-release, food was provided three to four times a week but the frequency was decreased rapidly to occasional supplementations (on average once a week). In early 2012, this supplementation occurred about once or twice a month and finally stopped in April 2012, at the beginning of this study.

As early as 2003, wild adult males were seen mating with reproductive females of the group. At the end of this study (November 2016), the group consisted of about 145 individuals, including more than 85% wild born animals. All adult individuals, but only about half of the infants and juveniles, can be individually recognized. The study group occupies a home area of less than 1,000 ha, exceeding the park's boundaries (Brockmeyer et al., 2015). Moreover, other wild mandrill groups are known to live in the park and its vicinity but in five years of continual presence, we never witnessed intergroup encounters, albeit wild solitary males are observed in the home range of the study group from time to time.

2.3 | Fecal collection and hormone analysis

We estimated cortisol production (the main glucocorticoid hormone produced in primates; Palme, 2005; Palme, Rettenbacher, Touma, El-Bahr, & Möstl, 2005) using fecal extracts. Noninvasive fecal samples provide useful improvements compared to blood samples. In addition to the ease of sampling and the limited impact on animals' daily lives, levels of fecal glucocorticoid metabolites (fGCM) may reflect the cumulative secretion of circulating native hormones over a certain period (several hours/days). Concentrations in fGCM are therefore buffered, at least partly, against pulsatile production of glucocorticoids that may possibly mislead interpretations of the socio-ecological challenges animals may undergo (Touma & Palme, 2005). The drawback of measuring

fGCMs is that native glucocorticoids are often present in limited quantities in the feces of most vertebrates, albeit cortisol enzyme immunoassays (EIA) have been shown to perform well in several species of primates following experimental physiological stimulation of the HPA axis (Heistermann, Palme, & Ganswindt, 2006).

From April 2012 to November 2016, we collected and extracted a total of 1,588 fecal samples during daily monitoring of the population or during punctual trapping sessions (see for details: Galbany et al., 2014). The entire fecal bolus was collected on each occasion and following defecation of unambiguously identified animals or directly from the anal tract of anesthetized individuals. Samples were stored in collection tubes for a few hours before storage or extraction in the field lab. Indeed, from April 2012 to August 2013, fecal samples were first frozen at -20°C before subsequent extraction and EIA analyses. Starting in August 2013, samples were immediately extracted on the day of collection. These later extracts were then stored at -20°C until being assayed.

Extractions occurred as follows: 5–15 g of frozen or fresh fecal material were sampled from thoroughly homogenized fecal boluses (using lab wooden sticks) and placed into a 2 ml Eppendorf tube and dried into a dry bath (60°C) for 24–72 hr depending on the humidity of the samples and of the ambient air. Dried material was ground to remove as much as possible vegetal particles. We weighted 0.18 g of fine and dried fecal powder and added 1.8 ml of ethanol to the powder. Samples were then thoroughly vortexed for 30 min. Mixtures were centrifuged for 15 min at 5,000 rpm and 1 ml of supernatant was then pipetted and placed into another Eppendorf tube and dried in a dry bath (60°C) during 12 hr. Dried samples were then stored at -20°C until assay.

Samples were thawed in batches just before performing EIA analyses. We added 100 μl of 96% ethanol diluted at 1/10 with assay buffer and 400 μl of assay buffer to thawed samples. The mixture was left on the bench for 15 min and then vortexed for 10 min. To remove small particles, we then centrifuged the samples for 1 min at 3,000 rpm. Finally, 50 μl of the mixture were deposited on manufactured EIA kits (DetectX – Cortisol Enzyme Immunoassay kit - Arbor Assays) to measure immunoreactive cortisol (as per: Setchell et al., 2008) along with standard samples to obtain a standard curve. We followed the protocol as proposed by the manufacturer. Microtiter plates were coated with a mouse antibody and a cortisol-peroxidase conjugate was added to standards and wells. The following cross reactants were used in the assay and calculated at the 50% binding point: cortisol (100%), dexamethasone (18.8%), prednisolone (1-dehydrocortisol) (7.8%), corticosterone (1.2%), cortisone (1.2%) progesterone ($<0.1\%$). Sensitivity of the assay was 17.3 pg/ml and the limit of detection was determined as 45.4 pg/ml. Linearity was determined using serial dilutions of two human urine samples and the average recovery was 96.7%. Intra-assay and inter-assay precisions were determined using 20 replicates or duplicates run in 10 assays (resp.) of three human samples. Coefficients of variation (CV) were 8.8% (intra-assay precision) and 8.1% (inter-assay precision). Also, several mammalian species were assayed with these kits by the manufacturer. Cortisol values ranged from 2.48 to 27.22 pg/mg across species. The optical density of the assayed samples

was read using a microplate photometer with a 450 nm filter (Multiskan FC).

2.4 | Analytical validation

First, we calculated parallelism using four serial dilutions of five mandrill's fecal samples (Supporting Information Table S1a). The slope of the standard curve ($\%B/B_0 = f(\log[\text{cortisol}])$) was compared to the slope of the antibody binding using an ANCOVA (SAS v4). Slopes were significantly parallel (estimate = 0.065; $t = 3.30$; $p = .004$). Second, we measured accuracy by mixing different dilutions of different concentrations of the same five fecal samples (Supporting Information Table S1b). When plotting the expected concentrations of mixes with the actual concentrations, we found an accuracy of 96.5%. Finally, we estimated the precision of our analyses by measuring CV of one sample run four times on a same plate, on five different plates. Intra-assay CV was 4.3% and inter-assay CV was 11.0%. All samples assayed were run in duplicates and samples with a CV above 10% were discarded from subsequent analyses. Samples with a concentration lower than 50 pg/ml but higher than 4,000 pg/ml were discarded to avoid lack of sensitivity of the assay or to avoid considering data points out of the acceptable range as proposed by the manufacturer, respectively. With these restrictions, we obtained reliable fGCM concentrations for 1,233 fecal samples collected on 49 females ($N = 711$) and 50 males ($N = 522$) aged 0.5–22.4 years (1–118 samples collected/id; mean \pm SD: 12.5 ± 16.0 ; samples were uniformly collected across individuals of different ages except for individuals aged 19 years and older for whom samples were rare). During the course of the study, one female was over-sampled; we re-ran all our analyses excluding this individual. Because results did not change (not shown), we decided to keep this particular individual in all the following analyses.

2.5 | Biological validation

To biologically validate our protocol and the data obtained, we checked that the fecal samples collected during the trapping sessions showed, on average, higher fGCM concentrations than the samples collected outside these sessions. Trapping sessions were defined as periods spanning from the two days following the first day of trapping to the two days following the last day of trapping in order to take into account a time-lag between glucocorticoid production and excretion. Based on the residuals obtained from the full statistical model taking into account the major sources of variance in fGCM production (see below), we found that fecal samples collected during trapping sessions were, on average, 2.8 times more concentrated than fecal samples collected outside these periods. This pattern suggests that fGCM values obtained using our protocol give indications about variations in the external conditions individuals may experience.

2.6 | Climatic conditions

Gabon is characterized by four seasons, with two marked long rainy (Feb-May; across the study period min-max monthly temperatures: 23.2 – 25.2°C ; min-max monthly rainfall: 0.08–0.32 mm) and dry (Jul-

Sep; across the study period min-max daily temperatures: 21.4–23.8°C; min-max daily rainfall: 0–0.07 mm) seasons and two short rainy (Oct–Nov; across the study period min-max daily temperatures: 23.1–24.3°C; min-max daily rainfall: 0.03–0.23 mm) and dry (Dec–Jan; across the study period min-max daily temperatures: 23.1–24.3°C; min-max daily rainfall: 0.08–0.15 mm) seasons. Climate data have been collected since early 2012 using a weather station (Davis Vantage PRO2 radio) located in Bakoumba village nearby the park. The station automatically records several variables (e.g., precipitation, temperature, humidity) every 30 min. We averaged the recorded temperatures (in °C) and amount of rainfall (in mm) for every month of the study. Note that we did not consider cumulative rainfall because of slight variation in the number of recorded data across months possibly slightly biasing this measurement. Monthly cumulative rainfall and average rainfall were highly correlated. Finally, regarding rainfall, there were missing data from November 2014 to June 2015 because of a mechanical problem with the weather station. We excluded these months in the models considering rainfall as an explanatory variable (see below).

2.7 | Demographic and reproductive data

We performed daily behavioral observations of the studied individuals over the entire study period, whenever possible (when the group was visible). We used daily censuses to obtain reliable information about the presence of individuals and retrieved the total number of adult males present in the group a given month. We considered a male as present when it was observed for more than a day that month. Note that these immigrating males may stay for varying periods of time in the group, from a few days to a few years (Brockmeyer et al., 2015). Census data also allowed retrieving regular information about females' reproductive status. Menstrual cycles of mature females were evaluated by visual inspection of their perineal skin: females were considered cycling when they presented a visible sexual swelling of any size around the ano-genital area (Dixon, 1983). Gestation was recorded based on the presence of a particular pink and swollen tumescence (Setchell et al., 2006a) that was generally visible two months following impregnation (MJEC, pers. obs.). In addition, subsequent births provided an additional way to infer the timing of receptivity by subtracting the average length of gestation (175 days; Setchell et al., 2002) from birth dates. Lactating females were those females with infants younger than six months of age because after this 6-month period, females start cycling again, indicating reduced lactation.

2.8 | Individual characteristics

In this study, we considered individual characteristics such as sex, age and dominance rank as possible confounding factors because they have been all shown to impact glucocorticoid production in primates and other mammals (e.g., sex: Tilbrook, Turner, & Clarke, 2000; age: Pavitt, Pemberton, Kruuk, & Walling, 2016; rank: Cavigelli & Caruso, 2015). Dates of birth of captive-born individuals were exactly known while the age of wild-born animals were either known due to daily monitoring or were estimated using general condition, and for some of

them, patterns of tooth eruption and wear (Galbany et al., 2014). Age was therefore exactly known for 26 individuals and estimated for 73 others. For 50 of these latter animals, however, the error made was estimated to be less than a year. Errors possibly larger than a year mainly concern immigrating adult males.

Social dominance ranks were obtained using outcomes of approach-avoidance interactions ($N = 8,072$ interactions) and calculations of normalized David's Scores (David, 1987) because of the associated high performance in retrieving actual hierarchies (e.g., Gammell, De Vries, Jennings, Carlin, & Hayden, 2003; Hemelrijk, Wantia, & Gygas, 2005). Dominance rank is mostly linear in both males and females but cannot be compared between sexes; we therefore analyzed males and females separately. Indeed, contrary to males, females' rank is stable throughout their lives and maternally inherited (Setchell et al., 2002). We divided adult females into three categories based on their dominance ranks (calculated using David's Scores) across the entire study period (high-ranking, medium-ranking, low-ranking). In subadult (>8 years) and adult males (>10 years; see Setchell, Lee, Wickings, & Dixon, 2001 for definitions of age categories), a monthly dominance rank (David's Scores) was attributed to the studied males. We also considered monthly three classes of ranks. The alpha male was considered as the only high-ranking male, the next three males in the hierarchy were considered as medium-ranking males and all other males were considered as low-ranking males. This classification was based on our experience of the social relationships observed among males. The alpha male displays clear behavioral characteristics distinct from other males: he is generally frequently associated with females, especially with those from the dominant matriline (MJEC, pers. obs.). He also undergoes constant behavioral challenges from other males, generally those ranking just below him. Finally, males lower than the fourth position are generally more (spatially and socially) peripheral to the group. Note that we studied only the impact of dominance rank in subadult and adult males because juvenile (1–2 years) and adolescent (3–7 years) males follow their mother's rank before acquiring their own rank, which is essentially age-dependent at these young ages (MJEC, pers. obs.).

2.9 | Statistical analyses

We performed General Linear Mixed Models (LMM; proc GLIMMIX, SAS v4) to study the relationships between fGCM concentrations and different sets of predictors. We ln-transformed fGCM concentrations to achieve normality. Homoscedasticity of the data was also verified. In a first model (model 1 in Supporting Information Table S1), we considered seasonal and individual's predictors based on the full data set composed of 1,233 fecal samples. We considered as fixed effects: the season of sampling (class variable with four modalities: long rainy, long dry, short rainy and short dry seasons), the sex (class variable with two modalities) and the age of the sampled animals (continuous variable). The second and third models (models 2–3 in Supporting Information Table S1) were based on two restricted data sets: one composed of subadult and adult males ($N = 270$) and one comprising all females ($N = 711$) in order to test for possible effects of dominance rank (class

variables with three modalities each: high-ranking, medium-ranking and low-ranking individuals), while still controlling for the above predictors.

Because we observed pronounced variations in fGCM concentrations across the four seasons of sampling (see Results), we explored the possible environmental, demographic and physiological factors possibly underlying these seasonal effects. Climatic variables, the number of adult males, and female reproductive status all varied seasonally, possibly impacting fGCM production. However, these variables were also highly inter-correlated. Consequently, in a first set of models (the "climatic models"; models 4a and 4b in Supporting Information Table S1; $N = 1,233$ and 995 resp.), we replaced the class variable "season" by climatic data, while still controlling for individual's age and sex. In this set of models, we alternatively tested for an effect of either the monthly average temperatures or the monthly average rainfall (two continuous variables) because these two variables were highly correlated with each other ($R^2 = 0.54$).

In a second set of models (model 5 in Supporting Information Table S1; $N = 1,233$), we tested for an effect of the number of males present in the group a given month (continuous variable), again controlling for animal's age and sex. Finally, we restricted the full dataset to reproductive females aged 3 years and older (model 6 in Supporting Information Table S1; $N = 578$) to test for possible effects of female's reproductive cycle (class variable with four categories: pregnant, lactating and cycling females, and females that were neither pregnant, lactating nor cycling). In this last analysis, we controlled for female's age and dominance rank. Because pregnant females showed the highest fGCM concentrations (see results and discussion), we re-ran the above models excluding these females to ensure that these effects were not driven by them. We further tried to disentangle effects related to the number of males from those due to weather conditions by running different additional models in particular seasons.

In all the aforementioned models, we controlled for possible confounding effects of the time elapsed between collection and extraction of the fecal samples (continuous variable in days) and of trapping sessions that occurred throughout the years (class variable with two modalities: collected during trapping sessions or outside trapping sessions). Depending on the model considered, these two variables differently impacted fGCM concentrations: the longer the time between collection and extraction, the higher the concentrations, and samples collected during trapping sessions also tended to show higher concentrations. For clarity's sake and because the purpose of this study was to examine individual and environmental predictors, we always kept these two predictors in all final models but did not present the associated statistics. Additionally, preliminary analyses showed neither a quadratic effect of individual's age nor an effect of the time of collection (i.e., morning vs. afternoon) on fGCM concentrations. We therefore did not keep these possible confounding factors in final models. Finally, we considered an individual's identity nested within the year of sampling as a random effect and further considered all first-order interactions. We kept the full models excluding only nonsignificant interaction terms ($P > 0.05$). When a class variable was found significant or marginally significant ($P < 0.10$), we performed post-hoc tests based on differences of least squares means.

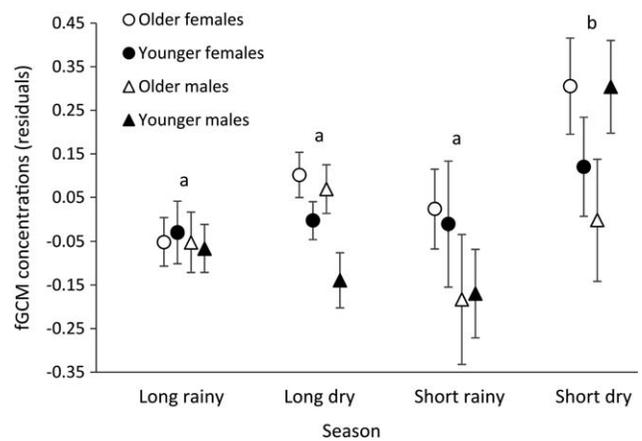


FIGURE 2 Seasonal variation in fGCM concentrations in female and male mandrills of different ages. For the sake of clarity, individuals were considered either as younger or as older than average for their sex. Averaged residuals (\pm SEM) are displayed per season, sex, and age. Residuals were obtained from the full model excluding the variables of interest (season of sampling and individual's age and sex). Note that none of the first interaction terms were found significant but we combined the three significant or marginally significant effects (Table 1) in one single figure for ease. Significant differences ($p < .01$ in all instances) across seasons (sexes and ages pooled) are indicated using different letters

3 | RESULTS

3.1 | Individual predictors

Overall, females ($1,189 \pm 32$ pm/ml) tended to produce on average more fGCMs than males ($1,041 \pm 33$ pm/ml; Figure 2; $p = .052$) and individuals older than the average age attributed to the sample donors (>10.0 years) also showed generally higher concentrations of fGCM than younger individuals (≤ 10.0 years; Table 1a), with the exception of young males that seemed to produce particularly high concentrations of fGCM during the short dry season (Figure 2). None of the first-order interaction terms considered in the analyses were, however, found to be significant. When considering females and adult along with subadult males separately, we further found that dominance rank impacted fGCM concentrations in females only (Table 1): medium-ranking females showed the highest average concentrations of fGCM ($1,374 \pm 86$ pm/ml) compared to low-ranking ($1,110 \pm 42$ pm/ml) or high-ranking ($1,236 \pm 55$ pm/ml) females (Figure 3). In adult and subadult males, we did not find such an effect related to dominance rank, and their age also did not impact fGCM concentrations (Table 1).

3.2 | Seasonal predictors

In the overall model based on all 1,233 samples, we further found that fGCM concentrations fluctuated with the season of sample collection (Table 1): on average, males and females of all ages excreted the highest concentrations of fGCM during the short dry season ($1,344 \pm 87$ pm/ml compared to $1,102 \pm 24$ pm/ml in all three other seasons pooled together; Figure 2). When replacing the season variable with different seasonal predictors as described above, we first found that

TABLE 1 Effects on individual fGCM concentrations in mandrills. Estimates, F and p values are displayed as well as the sample size of each model

	All individuals (N = 1,233)			Adult and subadult males (N = 270)			All females (N = 711)		
	Estimate	F	p	Estimate	F	p	Estimate	F	p
a. In all individuals									
Season	LR: -0.28 LD: -0.21 SR: -0.31	6.10	<.001	LR: -0.15 LD: 0.01 SR: -0.17	1.04	.38	LR: -0.28 LD: -0.16 SR: -0.22	3.21	.023
Sex	F: 0.11	3.78	<i>.052</i>	-	-	-	-	-	-
Age	0.01	4.10	.043	0.02	0.98	.32	0.01	3.51	<i>.062</i>
Dominance rank	-	-	-	HR: 0.10 LR: 0.06	0.38	.68	HR: -0.30 LR: -0.26	4.61	.010
	All individuals (N = 1,074)			All females (N = 552)					
	Estimate	F	p	Estimate	F	p			
b. Excluding gestating females									
Season	LR: -0.22 LD: -0.16 SR: -0.30	3.73	.011	LR: -0.14 LD: -0.05 SR: -0.18	0.95	.41			
Sex	F: 0.07	1.50	<i>.22</i>	-	-	-			
Age	0.01	3.01	<i>.083</i>	0.01	2.18	<i>.14</i>			
Dominance rank	-	-	-	HR: -0.28 LR: -0.28	3.79	.023			

Note that the estimates associated to season (LR: long rainy, LD: long dry, SR: short rainy) were set to the short dry season; the estimate associated to sex (F: females) was set to males; the estimates associated to dominance rank (HR: high-ranking, LR: low-ranking) was set to middle-ranking individuals. Significant effects are shown in bold and trends in italics.

climatic variables substantially impacted fGCM concentrations in both sexes when considered in combination with individual's age (Table 2): both lower rainfall and lower temperatures were associated with higher fGCM concentrations in older than average individuals only (Figure 4). Second, the number of adult males present in the group also positively

correlated with fGCM production (Table 2, Figure 5). Finally, female's reproductive status overall slightly impacted fGCM concentrations (Table 2). When considering pairwise comparisons, pregnant females appeared, however, to excrete the highest average concentrations of fGCM (1,362 ± 69 pm/ml; Figure 6) compared to all other stages of female's reproductive cycle (pooled together: 1,070 ± 38 pm/ml).

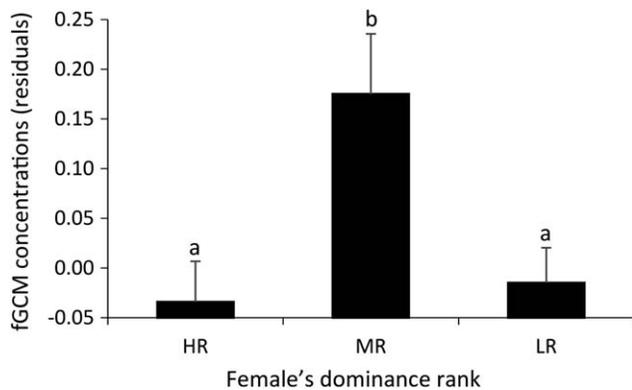


FIGURE 3 Female's dominance rank and fGCM concentrations. Averaged residuals (±SEM) are displayed per class of rank (HR: high-ranking females, N = 272; MR: medium-ranking females, N = 83; LR: low-ranking females, N = 356). Residuals were obtained from the full model excluding the variable of interest (female's rank). Significant differences (p < .01 in all instances) across classes of rank are indicated using different letters

3.3 | Disentangling multi-collinearity effects

When we removed pregnant females from the analyses, the main results remained unchanged, except that males and females no longer exhibited differences in fGCM levels (Table 1b). We still observed the highest fGCM values during the short dry season, but only when considering males and females together (Table 1a,b). Finally, we did not observe any major deviations from the models based on the three sets of seasonal predictors, except that the effect of the number of adult males was observed in combination with age (Table 2b): older individuals alone showed higher fGCM concentrations when the number of males increased than younger animals.

In order to disentangle possible collinear effects of climate and of the number of adult males present in the group, we studied the latter during the two seasons where the variance in the number of males was the highest across the five years of sampling (i.e., long dry and short rainy seasons). During these two seasons, the number of males significantly or marginally correlated positively with fGCM concentrations in

TABLE 2 Seasonal predictors of fecal cortisol concentrations in mandrills

			Estimate	F	p
a. In all individuals					
Climatic models	Model with averaged temperatures (N = 1,233)	Sex	F: 0.10	3.38	.066
		Age	0.25	4.82	.028
		Temperature	0	0	.95
		Age*Temperature	−0.01	4.39	.036
	Model with averaged rainfalls (N = 995)	Sex	F: 0.10	3.09	.079
		Age	0.03	10.92	.001
		Rainfall	0.76	1.11	.29
		Age*Rainfall	−0.14	4.81	.029
Demographic model	(N = 1,233)	Sex	F: 0.11	3.84	.050
		Age	0.01	3.64	.057
		N° adult males	0.02	4.11	.043
Physiological model	(N = 578)	Age	0.01	0.59	.44
		Dominance rank	HR: −0.40 LR: −0.35	6.35	.002
		Reproductive status	P: 0.23 L: 0.10 NC: 0.03	2.59	.052
b. Excluding gestating females					
Climatic models	Model with averaged temperatures (N = 1,074)	Sex	F: 0.06	1.24	.27
		Age	0.26	4.99	.026
		Temperature	0	0.01	.92
		Age*Temperature	−0.01	4.64	.032
	Model with averaged rainfalls (N = 851)	Sex	F: 0.05	0.69	.40
		Age	0.03	8.31	.004
		Rainfall	0.64	0.73	.39
		Age*Rainfall	−0.13	3.84	.051
Demographic model	(N = 1,074)	Sex	F: 0.09	2.15	.14
		Age	−0.01	0.88	.35
		N° adult males	−0.02	0.59	.44
		N° adult males*Age	0.01	3.92	.048
Physiological model	(N = 419)	Age	0.01	0.59	.44
		Dominance rank	HR: −0.35 LR: −0.36	3.62	.028
		Reproductive status	L: 0.07 NC: 0.03	0.20	.82

Estimates, *F* and *p* values are displayed as well as the sample size of each model. Note that the estimates associated to sex (F: females) was set to males; the estimates associated to dominance rank (HR: high-ranking, LR: low-ranking) was set to middle-ranking individuals; the estimates associated to female's reproductive status (P: pregnant females, L: lactating females, NC: noncycling females) was set to cycling females. Significant effects are shown in bold and trends in italics.

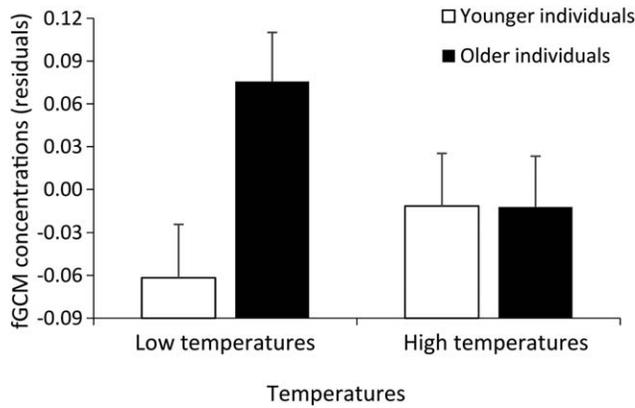


FIGURE 4 Interaction effect between age and temperature on fGCM concentrations. For the sake of clarity, we represented averaged residuals (\pm SEM) for two classes of age (younger than average individuals: $N = 585$; older than average individuals: $N = 648$) and two classes of temperatures (lower vs. higher than average) while these variables were considered as continuous in the associated statistical model (i.e., no associated p -values). Residuals were obtained from the full model excluding the two variables of interest (individual age and temperature)

both sexes (excluding pregnant females; long dry: $N = 465$, $F = 12.60$, $p < .001$; short rainy: $N = 105$, $F = 3.25$, $p = .081$). Note that during these two seasons, the climatic variables and the number of males were not correlated (e.g., with temperatures, long dry: $r = -0.13$; short rainy: $r = -0.15$), suggesting that both effects were independent and additive. Finally, because the long dry season is probably the ecologically most challenging season in mandrills, we further studied the effect of temperature variation on fGCM concentrations during that season only (there was no variation in rainfall patterns). We found that the lower the temperatures, the higher the fGCM concentrations (excluding pregnant females; $N = 465$, $F = 57.36$, $p < .0001$).

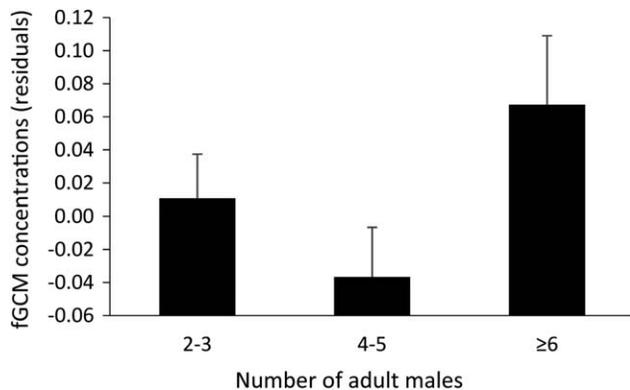


FIGURE 5 Number of adult males and fGCM concentrations. For the sake of clarity, we depicted three classes of the number of males (2 and 3 males, $N = 597$; 4 and 5 males, $N = 473$; ≥ 6 males, $N = 163$) while this variable was considered as continuous in the associated statistical model (i.e., no associated p -values). Residuals (mean \pm SEM) are displayed (residuals were obtained from the full model excluding the variable of interest, here the number of adult males)

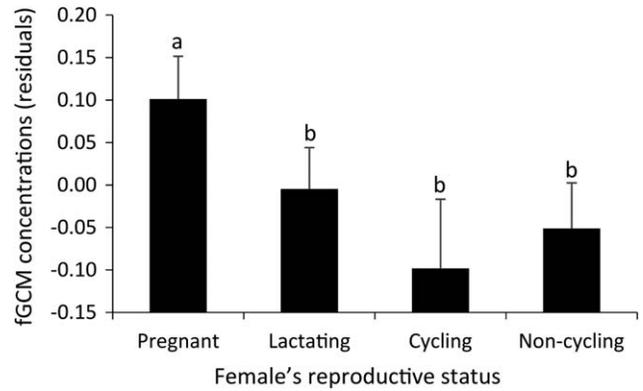


FIGURE 6 Female reproductive status rank and fGCM concentrations. Averaged residuals (\pm SEM) are displayed per different reproductive status. Residuals were obtained from the full model excluding the variable of interest (female reproductive status). Significant differences ($p < .023$ in all instances, except between pregnant and lactating females where $p = .095$) across classes are indicated using different letters

4 | DISCUSSION

Using fecal samples collected over a 5-year period on 99 mandrills of both sexes and all ages, we found substantial seasonal variation in cortisol production. In particular, we found that fGCM concentrations peaked during the short dry season (Dec-Jan), with a 1.34-fold increase compared to the short rainy season, characterized by the lowest fGCM values recorded. This effect appeared to be mainly driven by samples collected from pregnant females that are generally numerous at this time of the year (Figure 1). However, this seasonal peak during the short dry season remained detectable after pregnant females were excluded. We propose that this observed elevation during the short dry season resulted from other cumulative, small-magnitude effects, including a combination of climatic events (rainfall and temperature) and demographic changes (number of adult males), although the largest variation of these two variables was recorded during the long dry season.

When comparing the raw values of fGCM concentrations across the three seasonal predictors tested, we found that gestation, as expected, showed the strongest effect size, with a 1.27-fold increase in pregnant females compared to any other mature females. This is followed by climatic events, where we noted a 1.18 and 1.11-fold increase for temperatures and rainfall, respectively, recorded below the average values calculated from all data points. Changes in the number of adult males impacted fGCM concentrations the least, with only a 1.05-fold increase recorded in social settings where more males were present in the group than on average.

4.1 | Climate

Animals living in harsh or/and unpredictable environments generally show modified physiological responses, such as an increase in glucocorticoids during challenging periods of the year (Wingfield, 2013). For example, yellow baboons living in the semi-arid Amboseli basin experience strong seasonal and annual variation in rainfall and temperatures

(Altmann, Alberts, Altmann, & Roy, 2002). These unpredictable climatic events result in variation in the production of glucocorticoids with the most elevated concentrations found during hotter months (Gesquiere et al., 2008). Similarly, geladas live in quite inhospitable environments characterized by high altitudes and nighttime temperatures often below freezing, both predicting elevated glucocorticoids in this species (Beehner & McCann, 2008). These climatic effects that we also observed in mandrills may be explained by either the metabolic demands related to harsh environments such as cold temperatures or the nutritional challenges experienced by animals during constraining periods (as per: Beehner & McCann, 2008) or a combination of both.

In contrast to geladas, where fGCM concentrations were not higher during the season characterized by the highest food scarcity (Beehner & McCann, 2008), we propose that the climatic effects we found reflect the impact of food shortage rather than inclement weather on fGCM concentrations. Indeed, in Gabon, temperatures remain relatively buffered and quite elevated throughout the year (across the study period: mean \pm SD and min-max monthly temperatures, daytime: 24 ± 2.6 and $15.7\text{--}32.3^\circ\text{C}$; nighttime: 20.8 ± 1.5 and $15.4\text{--}31.3^\circ\text{C}$), suggesting that the metabolic challenges possibly undergone by the study mandrills are probably relatively small compared to other primates experiencing dramatic climatic changes (and that all show an important environmental signature on glucocorticoid concentrations: e.g., Beehner & McCann, 2008; Gesquiere et al., 2008; Sapolsky, 1986; Wingfield, 2005). Rainfall, in contrast, largely vary throughout the year and are virtually absent during the long dry season (mean \pm SD and min-max monthly rainfall: 0.02 ± 0.02 and $0\text{--}0.07$ mm) possibly impacting food availability. While an equatorial environment may not entail drastic food shortages, mandrills' nutritional needs may be not totally met during the dry seasons, explaining associated rises in fGCM concentrations (although the highest values were found during the short dry season and not the long dry season). Additional analyses of time budgets, nutritional values of food items and markers of metabolic status (Emery Thompson 2017; Higham, Girard-Buttoz, Engelhardt, & Heistermann, 2011) are now necessary to fully elucidate this notion.

4.2 | Reproductive seasonality: group composition and female reproductive status

Mandrills reproduce seasonally, a pattern that may further explain the observed seasonal variation in fGCM concentrations. Indeed, seasonality probably evokes a multitude of social and physiological changes in both sexes, including changes in group composition and individual reproductive status. In several seasonal mammalian species, the mating season is often characterized by an overall rise in glucocorticoid production (e.g., sifakas *Propithecus verreauxi*: Fichtel, Kraus, Ganswindt, & Heistermann, 2007; redfronted lemurs *Eulemur rufifrons*: Ostner, Kappler, & Heistermann, 2008b; muriquis *Brachyteles arachnoides*: Strier, Ziegler, & Wittwer, 1999; Japanese macaques *Macaca fuscata*: Barrett, Shimizu, Bardi, Asaba, & Mori, 2002; and in other mammals: Pavitt, Walling, Mostl, Pemberton, & Kruuk, 2015; Quispe, Villavicencio, Addis, Wingfield, & Vasquez, 2014). In mandrills, most females are cycling during the long dry season (Figure 1), and while the number of adult males

varies throughout the year, larger numbers of males are recorded from the long dry to the short dry seasons (Brockmeyer et al., 2015). Intense male-male competition therefore probably increases when males are more numerous, which may further contribute to increases in fGCM levels observed in all individuals (particularly in old animals). In yellow and chacma baboons, the immigration of new adult males and its association with elevated glucocorticoid levels in both sexes are probably related to a combination of infanticide risk, male-male competition and more generally to a rise in the number of aggression events following immigrations (Alberts et al., 1992; Engh et al., 2006a; Ostner et al., 2008b). We suggest that the same determinants may apply here.

The highest peaks of fGCMs were observed in pregnant females, a finding previously reported for semi-free ranging mandrills, based on a smaller sample size (19 females, 340 fecal samples: Setchell et al., 2008). Similar results were found in several other primates (e.g., yellow baboons: Gesquiere et al., 2008; tamarins *Saguinus oedipus*: Ziegler, Scheffler, and Snowdon, 1995 and in other mammals, e.g., Barrette, Monfort, Festa-Bianchet, Clutton-Brock, & Russell, 2012; Pavitt et al., 2016). These high glucocorticoid levels during gestation partly result from interactions between the HPA axis and the different hormones released during gestation (estrogen, corticotrophin-releasing hormone; McLean & Smith, 1999). Glucocorticoid production during this key period allows the fetus to grow appropriately while excess or deficiency in production result in disorders during fetal development (Keller-Wood & Wood, 2001). Additionally, in mammals, elevated concentrations of glucocorticoids, often observed at the end of the gestating period (Keller-Wood & Wood, 2001), induce labor (Goldkrand, Schulte, & Messer, 1976; Smith, Chan, Bowman, Harewood, & Nd Phippard, 1993), although we did not observe such an increase at the end of the gestation in the studied females (results not shown; but see: Setchell et al., 2008). Following gestation, we observed a gradual decrease in fGCM production. Concentrations remained high in lactating females (although not significantly different from cycling or noncycling females), however, as also reported for other primates (e.g., Weingrill et al., 2004; but see: Setchell et al., 2008) and probably reflecting a combination of increases in energetic demands, metabolic burden and infanticide risk suffered by lactating females (e.g., Emery Thompson, Muller, Kahlenberg, & Wangham, 2010; Engh et al., 2006a). Overall, cycling females exhibited the lowest fGCM values, which is in contrast with reports on chacma baboons where cycling females receive the most aggression (Huchard & Cowlshaw, 2011), possibly translating into higher fGCM concentrations. A study combining hormonal analyses and fine-grained monitoring of mandrills' social behavior is now indicated to elucidate whether patterns of aggression, coalition, or affiliation impact fGCM production.

4.3 | Individual effects

Finally, we found that individual traits, considered here as confounding factors, also correlated with fGCM concentrations, with females exhibiting slightly higher concentrations than males, a common finding in mammals (e.g., Perret & Predine, 1984; Smith & French, 1997), probably resulting from differences in circulating steroid hormones between

the sexes and differences in hormone metabolism or, as in our case, due to the confounding physiological effects of including pregnant females. Additionally, and across models performed, older than average individuals (>10.0 years) generally exhibited increased fGCM levels, especially during ecologically challenging times of the year (i.e., low temperatures and low rainfall), resulting from possible physiological effects of aging. These physiological consequences may either result from a direct causation between aging and fGCMs involving reduced capacities of the endocrine system or from an indirect causation related to a reduced general condition in older animals, or a combination of both (see in red deer *Cervus elaphus*: Pavitt et al., 2016; and references therein). Contrary to our expectations, and while we observed possible effects of male-male competition in the form of the number of adult males present, we did not find any relationship between male dominance rank and fGCM production, which is in contrast with earlier findings on a semi-free ranging population of mandrills. In this captive population, the relationship between male dominance rank and fGCMs was condition-dependent: under unstable male dominance hierarchy, with numerous and frequent changes in the alpha position, as well as during mating periods, fGCM concentrations were found to be higher in dominant males, while under stable hierarchy, subordinates produced more glucocorticoids than dominant males (Setchell, Smith, Wickings, & Knapp, 2010). Similar condition-dependent effects were found in male chacma and olive (*P. anubis*) baboons (Bergman, Beehner, Cheney, Seyfarth, & Whitten, 2005; Sapolsky, 1992). In other species, however, contrasting results were obtained; ranging from no effect (e.g., Lynch, Ziegler, & Strier, 2002) to higher concentrations in either high-ranking males (e.g., Barrett et al., 2002) or subordinates (e.g., Ostner, Heistermann, & Schülke, 2008a; and see for review: Cavigelli & Caruso, 2015; Creel, 2001). These apparent multi-directional, complex relationships probably result from different social conditions and different temporal dynamics in the production of glucocorticoids, as recently proposed (Cavigelli & Caruso, 2015). While a more fine-grained study on rank-related effects of fGCMs is now required in male mandrills to fully elucidate possible multifactorial impacts on the production of glucocorticoids as a function of rank, we did not find any evidence that social stability (indirectly measured as the number of adult males present) or that seasonality (mating vs. other seasons) interact with males' dominance rank on fGCM production (results not shown).

Intriguingly, female's dominance rank, while less prone to reflect condition-dependent traits than male's dominance rank, was correlated with fGCM concentrations in our study population. Such an effect was absent in semi-free ranging female mandrills (Setchell et al., 2008), but similar results were also obtained in females belonging to three primate species with contrasting inheritance of female's rank. Indeed, in both ring-tailed lemurs (Starling, Charpentier, Fitzpatrick, Scordato, & Drea, 2010), where females do not inherit their mother's rank and Barbary (*M. sylvanus*: Edwards, Walker, Bodenham, Ritchie, & Shultz, 2013) and Japanese (MacIntosh et al., 2012) macaques, where female's rank is maternally inherited, females of intermediate dominance rank also show the highest levels of glucocorticoid concentrations. Fine-grained analyses on behavioral patterns (e.g., aggression, patterns of coalitions

and support) and matriline size may help illuminate whether or not these medium-ranking females undergo important social challenges.

To conclude, we found several small-magnitude seasonal signatures on fGCM concentrations in male and female mandrills reflecting the effects of predictable changes of the internal (pregnancy) and external (climatic, demographic) mandrills' environment. Altogether, these modest effects suggest that the production of glucocorticoids, as a metabolic hormone, allows animals to mobilize and redirect energetic resources toward appropriate, calibrated responses to changing climatic, demographic and physiological conditions. This study sets a baseline for future studies based on unpredictable events such as infanticide (Engh et al., 2006a) and the loss of close kin (Engh et al., 2006b), predation events (Engh et al., 2006a), or intense aggressive events (Wittig et al., 2008) and social instability (Bergman et al., 2005), which are all known to impact glucocorticoid production in primates, including humans (Flinn & England, 1997).

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SUPPORTING INFORMATION

Additional Supporting Information may be found online in the supporting information tab for this article.

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