

## RESEARCH ARTICLE

# Dominance Rank and Fecal Testosterone Levels in Adult Male Chimpanzees (*Pan troglodytes schweinfurthii*) at Ngogo, Kibale National Park, Uganda

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In an attempt to describe hormone–behavior interactions in a sample of wild male chimpanzees, we quantified testosterone in 67 fecal samples obtained from 22 adult male chimpanzees at Ngogo, Kibale National Park, Uganda. A mixed-model methodology that controlled for age-class identified a significant positive association between testosterone levels and dominance rank. The results are consistent with those reported from a separate, smaller chimpanzee community in the same population in a study that analyzed testosterone levels in urine [Muller & Wrangham, 2004]. As in that earlier study, our results held during a period of social stability, which is not consistent with predictions of the “challenge hypothesis.” We concur with Muller and Wrangham [2004] that the challenge hypothesis requires modification to explain the chimpanzee data, because fission–fusion sociality in chimpanzees makes challenges unpredictable. We also discuss the utility of fecal samples and a mixed-model statistical method for behavioral endocrinology studies. *Am. J. Primatol.* 64:71–82, 2004. © 2004 Wiley-Liss, Inc.

**Key words:** dominance rank; chimpanzee; testosterone; fecal hormones; challenge hypothesis

## INTRODUCTION

Males in nonhuman primate species that typically form multimale groups often form dominance hierarchies and put considerable effort into attaining and maintaining high rank. Many behavioral data [reviewed in Cowlshaw & Dunbar,

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1991] and a growing body of data on genetics [e.g., Altmann et al., 1996; de Ruiter et al., 1994] support the argument that this effort, if successful, can bring reproductive payoffs.

Testosterone is a good candidate for a physiological factor that links male dominance rank and potential reproductive success. Testosterone is an anabolic steroid that maintains musculoskeletal performance [Bribiescas, 2001], stimulates muscle metabolism in vitro [Tsai & Sapolsky, 1996], and is associated with muscle mass gain in male vertebrates [Kemnitz et al., 1988; Welle et al., 1992]. Because it acts in such ways, and may mediate among dominance status, reproductive maturation, and aggressive behavior, one might expect to find a positive association between dominance rank and testosterone levels in male primates. Such correlations have been found in captive all-male groups of rhesus macaques (*Macaca mulatta*) [Bercovitch, 1993; Rose et al., 1971] and squirrel monkeys (*Saimiri sciureus*) [Coe et al., 1983]. Notably, Muller and Wrangham [2004] found a positive correlation between rank and testosterone in wild chimpanzees at Kanyawara (but see below). Further evidence from these studies supports the hypothesis that testosterone levels and aggression are causally linked. For example, Rose and colleagues [1971] also found highly significant correlations between testosterone levels and both the frequency with which individual rhesus macaques threatened and chased others, and the frequency with which they received submissive signals. Furthermore, Coe et al. [1983] found that testosterone production and aggression frequency increased abruptly during the breeding season in adult male squirrel monkeys.

Conversely, several studies have failed to demonstrate significant positive correlations between dominance rank and testosterone levels (e.g., rhesus macaques [Gordon et al., 1976], vervet monkeys [Steklis et al., 1985], chimpanzees [Seraphin, 2000], Japanese macaques [Barrett et al., 2002], and tufted capuchin monkeys [Lynch et al., 2002]). One major reason for the absence of such correlations may be that elevating testosterone imposes costs, and males only do so when the potential benefits exceed those costs. Two such costs include compromised survivability due to increased metabolic expenditures [Bribiescas, 2001; Ketterson & Nolan, 1992; Marler & Moore, 1988; Marler et al., 1995; Welle et al., 1992] and immunosuppression [Daynes & Araneo, 1991; Grossman, 1995; Grossman et al., 1991; Folstad & Karter, 1992; Olsen & Kovacs, 1996]. That is, while high testosterone levels may facilitate reproductive effort by promoting dominant/competitive behaviors and muscle anabolism (to aid in mate attraction and competition with conspecifics for access to mates), testosterone may also inhibit immune function in the face of pathogenic challenge or injury, thus increasing morbidity and mortality. [Muehlenbein 2004a, b].

More generally, testosterone may only be associated with the frequency or intensity of male aggression and/or with dominance rank in the presence of receptive females, such as during mating seasons for seasonally breeding species, or in situations of social instability, such as during the formation of dominance relationships, the establishment of territorial boundaries, or challenges by a conspecific male for territory or access to mates (the “challenge hypothesis” [Wingfield et al., 1990]). A number of studies utilizing nonhuman primates have supported the challenge hypothesis. For example, Sapolsky [1983] found that increased testosterone levels were associated with increased dominance status only during periods of instability in the male dominance hierarchy in a group of wild olive baboons (*Papio anubis*). During periods of relative social stability, high testosterone levels and aggressive behavior were unrelated to social status. Likewise, Cavigelli and Pereira [2000] found that in a population of free-ranging

ring-tailed lemurs, male aggression rates and fecal testosterone levels were not significantly correlated during the premating period. However, during the mating period, the correlation between fecal testosterone and aggression was significantly positive and peaked on days of female estrus. Muller and Wrangham [2004] found that testosterone levels in wild male chimpanzees at Kanyawara, Kibale National Park, were elevated when parous females with full sexual swellings were present, but not when fully swollen nulliparous females were present. Aggression rates and intensity also increased in the presence of fully swollen parous females, but not in the presence of fully swollen nulliparous females. In apparent contrast to predictions of the challenge hypothesis, however, the positive relationship between male rank and testosterone levels at Kanyawara held during a period of stability in the male dominance hierarchy.

We sought to identify any potential relationships between testosterone and dominance rank in wild male chimpanzees at Ngogo, Kibale National Park. The Ngogo chimpanzee community is the largest documented population in the wild, and has the largest number of males of any known community. Although there are a large number of males, researchers have found that they can be ranked in a linear hierarchy (see below). Thus, Ngogo is an excellent place to test the hypothesis that testosterone is correlated with dominance rank. Furthermore, this is the first study to utilize a mixed-model methodology to evaluate the behavioral endocrinology of wild primates.

## **MATERIALS AND METHODS**

### **Study Site**

Ngogo is in the Kibale National Park in western Uganda, and is maintained by the Makerere University Biological Field Station. The park is located between 0°41'N, 30°19'E, and 0°13'N, 30°32'E, and has a total area of approximately 750 km<sup>2</sup>. The Ngogo study area is approximately 25 km<sup>2</sup> and contains a mix of mature, regenerating, and swamp forest; *Acanthus* scrub; and other vegetation types [Ghiglieri, 1984; Struhsaker, 1997].

### **Subjects**

The Ngogo chimpanzee community was originally studied by Ghiglieri in the late 1970s and early 1980s [Ghiglieri, 1984]. Research and habituation efforts resumed at Ngogo in 1991, and have been continuous since 1995. All adult and adolescent males are well habituated and are observable within 5–10 m on the ground. At the time of this study, the Ngogo community had a total of about 150 members, including 24 adult males and 14 adolescent males.

### **Assessment of Male Dominance Ranks and Age Classes**

The behavioral data used here were collected during 1,700 hr of observations of the Ngogo chimpanzees from June through December 2002 (D. Watts, unpublished data). Most of the observation time (1,263 hr) was devoted to focal samples of adult males, during which all pant-grunts to or by focal individuals, all acts of aggression that they gave or received, the identities of the aggressors and their targets, and the outcomes of aggression were recorded. Ad lib data on pant-grunts and charging displays were also collected. Data were entered into actor-receiver matrixes, and the Linear Hierarchy procedure in MatMan (Noldus Information Technology Leesburg, VA) was used to examine for evidence of

linearity and to rank-order the males. With MatMan, one can calculate Landau's linearity index, with corrections for unknown relationships ( $h'$ ), and Kendall's coefficient of linearity ( $K$ ). Separate analyses were run on pant-grunts alone and on all agonistic interactions; accordingly, alpha was set at 0.025. Both tests involved 10,000 matrix permutations.

The exact ages of the adult males at Ngogo are unknown. Males were assigned to the following age categories, based on physical characteristics and the history of observations: 1 = old, 2 = old prime, 3 = prime, 4 = young prime, and 5 = young. The "young" and "young prime" categories included males that were first recognized as adolescents and ranked in order of relative age according to the date at which they appeared to have attained full adult body mass. Following Goodall [1986], the males with noticeably worn teeth and/or some thinning of hair and loss of muscle mass, and that were noticeably less vigorous than they were in earlier studies at Ngogo, were categorized as "old." It can be difficult to distinguish prime from old males (or "prime" and "middle-aged" males in Goodall's [1986] scheme) by physical characteristics alone [Boesch & Boesch-Achermann, 2000; Mitani et al., 2002]. All males in these categories were adult when first recognized, and old prime males were those that appeared to become less vigorous during the history of study.

### **Fecal Sample Collection and Hormone Analyses**

A total of 67 fecal samples were collected opportunistically from 22 adult male chimpanzees from July to September 2002. One to five samples were obtained per individual (Table I). All serial samples were collected on nonconsecutive days. A portion of each fresh sample was dried on an aluminum dish for approximately 2 hr at 100°C [Seraphin, 2000] inside a portable Coleman oven placed atop a kerosene stove. After the samples were desiccated, they were individually packaged with silica gel and transported to the Laboratory of Reproductive Ecology and Environmental Toxicology at Emory University, Atlanta, where extractions and radioimmunoassays for testosterone were performed. For each extraction, a 0.3-gm sample of feces was homogenized in 4 ml of methanol: acetone (8:2, v/v) and filtered with a 0.2 µm nylon centrifuge filter (Centrex MF; Scheicher & Schuell, Keene, NH). The filtrate was extracted on Sep-Pak VAC C18 columns (500 mg) (Water Corp., Milford, MA). An equal volume of water was added to dilute the sample, which was then layered onto a column that was primed according to the manufacturer's instructions. The column was washed with 5 ml of water, and the steroid fraction was eluted with 3 ml of methanol. Extraction recovery, measured by the addition of  $I^{125}$  labeled steroid to fecal samples prior to extraction, averaged 65% for testosterone.

The assay used reagents from the Equate Testosterone RIA kit (Binx, South Portland, ME). The testosterone RIA used an antibody raised in rabbit against testosterone. The second antibody was a PEG goat anti-rabbit antibody solution. An aliquot of each extract was reconstituted in working buffer (0.1% gelatin phosphate buffered saline) at a 1:5 dilution.  $I^{125}$  testosterone tracer (50 µl) and 100 µl antiserum (diluted 1:2) were added to 100 µl aliquots of standards (diluted 1:10 to give concentrations of 1–100 ng/dL), samples, and controls (diluted 1:10). After vortexing and incubation overnight at room temperature, 500 µl second antibody (diluted 1:2) was added. After a 20-min incubation at room temperature, the incubates were centrifuged at 1500 rpm × gm for 60 min at 4°C. The supernatant was decanted, and the radioactivity in the precipitant was

TABLE I. Sampling Description

Subject	Number of samples	Age class <sup>a</sup>	Dominance rank	Mean testosterone (ng/gm) <sup>b</sup>
AY	4	2	18	2.66 (2.61)
BT	4	3	1	7.20 (4.63)
BA	4	4	7	11.83 (6.92)
BE	4	4	13	5.76 (3.04)
BF	4	2	8	6.65 (4.22)
BRU	2	5	20	10.38 (8.79)
DZ	1	4	22	2.23
DO	4	3	14	9.03 (4.00)
EL	3	2	4	7.10 (5.43)
GRA	4	5	15	8.88 (6.05)
HA	4	3	5	6.49 (2.86)
HO	4	3	2	11.56 (6.17)
LO	3	3	3	13.55 (3.00)
MG	4	3	10	9.19 (7.89)
MI	2	4	6	10.21 (10.39)
MO	5	3	11	7.56 (2.34)
OR	3	5	15	12.74 (10.43)
PA	3	3	9	4.60 (4.73)
PI	4	3	19	8.01 (4.81)
RU	4	1	17	7.35 (3.91)
ST	4	4	21	14.52 (6.45)
TY	3	3	12	3.29 (1.70)

<sup>a</sup>1, old; 2, old prime; 3, prime; 4, young prime; 5, young 2.

<sup>b</sup>Standard deviation in parentheses.

determined by 5-min counts in a gamma counter. Sensitivity was 6 ng/dL. Cross-reactivity was 1.7% for dihydrotestosterone, and <0.1% for all other steroids.

Accuracy was tested by the addition of steroid standards to a chimpanzee extract. The mean percentage of observed concentration to expected values in the Equate testosterone assay was  $91.4 \pm 5.0\%$  ( $n=6$ ). Serial dilutions of a pooled chimpanzee male fecal extract produced displacement curves that paralleled the standard curve (standard curve:  $y = -12.188 \log x + 35.032$ ,  $n=6$ ,  $P < 0.001$ ; dilution sequence:  $y = -13.880 \log x + 37.883$ ,  $n=5$ ,  $P < 0.001$ ).

Internal controls were run in every assay and consisted of human serum controls (male and female) provided with the kit [Equate] along with clinical serum standards (BioRad 1,2) and chimpanzee fecal extracts. Intra-assay coefficients of variation averaged  $2.7 \pm 1.1\%$  for the male serum control ( $n=5$ ) and  $4.4 \pm 1.4\%$  ( $n=19$ ) for duplicates of chimpanzee fecal extracts. Inter-assay coefficients of variation were 4.2% for the Equate female serum control (4.8 ng/dL), 4.6% for the Equate male serum control (55.0 ng/dL), 4.2% and 7.2% for the BioRad controls 1 (4.7 ng/dL), and 2 (58.2 ng/dL) and 10.1% for three chimpanzee samples assayed in two separate assays.

### Statistical Analyses

The data were entered into an Access database that was then imported into SAS for analysis with SAS/STAT software (SAS System for Windows, version 8.2; SAS Institute Inc., Cary, NC). We used PROC MIXED was used to perform a mixed-model, repeated-measures analysis of associations between male domi-

nance rank and testosterone levels. Mixed modeling allowed us to use all data points, and hence to include individuals with missing observations. It also allowed us to examine within-subject effects of continuous variables and to control for age as a fixed within-subject covariate. Age was used as a covariate in the model because we expected the hormone levels to vary by age (i.e., testosterone levels would differ between young and old adults).

The mixed model had to take into account the fact that the hormonal data consisted of repeated measures at up to five unequally spaced time intervals. We accomplished this by using a time-series covariance structure that did not assume equal spacing. Potential correlations between the variables of interest (dominance rank and testosterone) should remain stable during the 3-month data collection period. Therefore, the data were fit with a compound symmetry covariance structure that assumes that correlations remain constant. By using such a time-series covariance structure, we accounted for differences in the number of samples collected for each animal, and for the unequal time periods between sequential samples. Furthermore, this methodology allowed us to avoid averaging hormone levels for individuals and sampling intervals. Pearson correlations controlling for age were also used to confirm the results of the mixed models.

## RESULTS

The Ngogo community had a clear alpha male (BT) throughout the study period (all other males pant-grunted to BT, but he pant-grunted to none). Data on pant-grunts alone produced a highly significant linear hierarchy ( $h' = 0.94$ ,  $K = 0.92$ ,  $P = 0.0001$ ; 14.8% of dyadic relationships unknown). Data on all agonistic interactions gave similar results ( $h' = 0.97$ ,  $K = 0.97$ ,  $P = 0.0001$ ), with fewer unknown relationships (4.3%) and one tied relationship. We used the reordered linear hierarchy based on all agonistic interactions to assign ranks to individual males. In addition, a Spearman rank-order correlation of male display rate on dominance rank revealed a highly significant correlation ( $R^2 = 0.95$ ,  $n = 27$ ,  $P < 0.001$ ) between dominance rank and the rate at which males gave charging displays. This analysis included not only the 22 adult males sampled in this study, but also several adolescent males that ranked above the lowest-ranking adults. No major rank challenges occurred between male dyads during the period of sample collection, and no samples were collected from individuals during or just after active hunts or boundary patrols.

Results of the repeated-measures analysis showed that testosterone levels were positively and significantly associated with dominance rank, after age was adjusted for ( $F = 5.51$ ,  $df = 1$ ,  $P = 0.032$ ). With the repeated-measures analysis controlling for age group, testosterone was not significantly associated with the display rate at the 0.05 level ( $F = 3.74$ ,  $df = 1$ ,  $P = 0.071$ ). However, the power for this analysis was  $< 80\%$ , and thus the reader should not be convinced that the null hypothesis of a significant positive relationship between testosterone and display rate was not true.

Although the present study (which involved an admittedly small sample size) was not designed to assess differences in testosterone/dominance relationships in males in the presence vs. absence of swollen parous females, some post-hoc analyses were conducted. During the study period, males were associated with only two parous females in estrus. The first female (present with the adult males on 3–7 September) appeared to be infertile, and most males showed little interest in copulating with her. In contrast, the second female (present with the adult

males on 11–20 August) generated a great deal of excitement from the males. Of the 67 samples collected in this study, 19 samples (from 12 individuals) were collected during the presence of this swollen parous female. A Wilcoxon signed-rank test revealed that fecal testosterone levels were actually lower ( $t = -3.039$ ,  $P = 0.012$ ) during this period of swollen parous female exposure.

In samples from the 12 males that were obtained *during* swollen parous female exposure, a Pearson correlation (controlling for age) revealed no significant correlation ( $R = -0.42$ ,  $P = 0.20$ ) between dominance rank and mean testosterone value. In samples from those same 12 males that were *not* obtained during exposure to the swollen parous female, a Pearson correlation (controlling for age) revealed a significant correlation ( $R = -0.67$ ,  $P = 0.02$ ) between rank and testosterone. One of the alpha male's individual samples appeared to be a low outlier, and when it was removed from the dataset a Pearson correlation revealed a significant correlation ( $R = -0.74$ ,  $P = 0.01$ ) between rank and testosterone in samples obtained during exposure to the swollen parous female.

## DISCUSSION

### Testosterone and Dominance Rank in Nonhuman Primates

Muller and Wrangham [2004] found that urinary testosterone was positively associated with dominance rank in the Kanyawara chimpanzee community at Kibale, but only for afternoon (not morning) urine samples. Because the hierarchy at Kanyawara was stable during the study period, Muller and Wrangham [2004] interpreted their result as support for the hypothesis that male rank and testosterone levels are positively associated in chimpanzees, regardless of hierarchy stability. Using a mixed-model methodology that controlled for variation in age among individuals, we identified a significant positive association between testosterone and dominance rank in the much larger Ngogo chimpanzee community. We also identified a significant association during a period of relative social stability, which is not consistent with the challenge hypothesis in its original form [Wingfield et al., 1990]. However, our data do not necessarily contradict the challenge hypothesis, which requires modification for chimpanzees because challenges can occur unpredictably [Muller & Wrangham, 2004].

It often requires considerable effort for male chimpanzees to maintain their rank [Goodall, 1986; Nishida & Hosaka, 1996; Muller, 2002; Muller & Wrangham, 2004]. As Muller and Wrangham [2004] noted, the fission-fusion nature of chimpanzee society means that males repeatedly separate from and reunite with potential rivals, which can introduce uncertainty into their relationships. Also, males sometimes form alliances that they use to reverse ranks with dominant males, and a male's rivals might form an alliance against him while they are separated from him. Consequently, males need to assert themselves frequently against other males to maintain dominance over them. For example, although alpha male BT faced no challenges from subordinates during our study period, he displayed toward other males at the highest rate among the males. Beta male HO displayed at the second-highest rate, and DZ, a small male that was the lowest-ranking of the adults, displayed at the lowest rate. In contrast to Muller and Wrangham [2004], we did not identify a statistically significant association between testosterone and the display rate; however, the power of this analysis was <80%, and a small sample size may have accounted for this effect.

Although the linear dominance hierarchy was relatively stable at Ngogo, contest competition over access to parous cycling females may have contributed to

the general rank-related elevation in testosterone levels, specifically because male mating success varies positively with dominance rank at Ngogo [Watts, 2003]. Two parous females (one infertile) were cycling during our study period, and samples collected during the fertile parous female's cycle were unexplainably lower than those collected at other times. Unfortunately, our project was not designed to specifically assess whether males experienced elevated testosterone levels on days when parous females were fully swollen, which explains the small sample size. However, Pearson correlations (controlling for age) did reveal significant relationships between rank and testosterone in 12 of the individual males that were sampled when exposed to the swollen parous female and when not exposed to her, and both sampling periods revealed significant associations. Interestingly, these associations were only significant when a single sample from the alpha male, considered to be a low outlier, was removed from analysis. Parasitological analyses of the same fecal samples utilized in the present study revealed that the sample from the alpha male that was excluded from analysis due to a very low testosterone value concomitantly had the heaviest infections (two intestinal parasites: *Strongyloides* sp. and *Entamoeba chattoni* (Muehlenbein, unpublished data)). Maintaining high testosterone levels may result in fitness costs to dominant animals by increasing susceptibility to infectious disease [Muehlenbein, 2004a,b]. Lowering testosterone levels during infection may represent an adaptive mechanism to prevent immunosuppression due to high testosterone levels, and to curb energetic investment in metabolically-expensive anabolic functions [Muehlenbein, 2004a,b].

We contend that our testosterone/rank associations are not the result of the presence of sexually receptive females. Although male testosterone levels in the Kanyawara group were elevated in the presence of parous females, copulation rates were actually equal for males with parous and nulliparous females, suggesting that aggression (contest competition) is the main driving factor of the testosterone/rank relationship, not actual mating [Muller & Wrangham, 2004]. Although the presence of fully swollen parous females may present an additional challenge to which males may respond by elevating testosterone levels, both our study and Muller and Wrangham's [2004] work identified testosterone/rank correlations overall, not just when males were mating.

Our data and those of Muller and Wrangham [2004] show that male chimpanzees exemplify at least two of the multiple types of relationships between male dominance and testosterone levels that may occur in nonhuman primates. As predicted by the challenge hypothesis, testosterone levels are elevated during breeding seasons in some species [Cavigelli & Pereira, 2000; Lynch et al., 2002]. In seasonally-breeding species that form multimale groups, the relationship between dominance rank and rates of aggression may determine whether or not testosterone is positively associated with dominance rank. In non-seasonally-breeding species that form multimale groups, males may engage in frequent contest competition for mating opportunities; increase levels of aggression among males, and between males and females; and may elevate testosterone levels in the presence of fertile females. In each of these cases, dominance rank and testosterone may be positively associated, but may not be during other circumstances because of the costly immunosuppressive effects of testosterone.

There may be a number of exceptions to the above-mentioned relationships. For example, high-ranking males may also experience elevated testosterone levels when they are involved in rank challenges, as Sapolsky [1983] found in wild olive baboons. Alternatively, male chimpanzees may elevate testosterone levels primarily in the presence of parous (not nulliparous) females. Rates of male

aggression are higher in the presence of fully swollen parous females than in the presence of fully swollen nulliparous females [Muller & Wrangham 2004] (Watts, unpublished data). However, testosterone levels apparently remain positively associated with dominance rank in chimpanzees even in the absence of high contest mating competition or direct rank challenges because of unpredictability in male–male social relationships. Finally, testosterone levels may be entirely independent of male dominance rank in species in which contest mating competition among males is unimportant (e.g., muriquis [Strier, 1996]). Given the enormous range of variation in primate socioecology, it is not surprising to find variation in the relationships between dominance rank and testosterone levels.

### Methodological Considerations

Comparisons among studies investigating hormone–behavior interactions in male nonhuman primates are difficult, in part, because of the variety of methodologies utilized. For example, in his ground-breaking study, Sapolsky [1983] divided wild male olive baboons into high (1–5), middle (6–10), and low (11–13) ranking groups and compared average testosterone levels among these groups with a repeated-measures, two-way ANOVA. However, his sample included adults, subadults, and adolescents, and he did not control for age differences among these animals. Barrett et al. [2002] also employed a repeated-measures ANOVA using average testosterone values for six male Japanese macaques of known rank, and concluded that rank and testosterone levels were independent of each other. Likewise, Lynch et al. [2002] employed a Friedman related-samples test to compare mean testosterone levels across six wild tufted capuchin monkeys by 15-day sampling intervals, and concluded that their data supported the challenge hypothesis.

The present study differs from previous ones in at least three important ways. First, our study investigated testosterone–dominance relationships by employing the largest single group of wild primate males of known dominance ranks used to date. Second, we included only adults in the analyses, and controlled for age-class. Third, this is the first study to employ a mixed-model methodology to identify associations between hormones and dominance rank. Similar methodologies could easily be employed in future analyses involving other species.

It is becoming increasingly common to use fecal samples from captive and wild primates to determine steroid levels [Khan et al., 2002; Barrett et al., 2002; Lynch et al., 2002; Whitten et al., 1998]. Fecal samples are particularly useful for studies assessing hormone–behavior interactions because they represent hormone values averaged over a lengthy period of time, and are not significantly influenced by minor rapid fluctuations in the hypothalamic–pituitary–gonadal axis. However, as with any steroid assay, there are always concerns about the effects of diurnal variation on hormone levels. At least two studies have noted significant diurnal variations in steroid levels in primate fecal samples. Sousa and Ziegler [1998] identified higher cortisol levels in afternoon samples compared to morning samples in four captive female common marmosets (*Callithrix jacchus*). In contrast, Lynch et al. [2002] found that mean morning cortisol levels were significantly higher than mean afternoon values in wild male tufted Capuchin monkeys (*Cebus apella nigrurus*). In addition, testosterone showed a nonsignificant trend toward lower afternoon values, and animal age had a greater effect on hormone levels than did sample collection time [Lynch et al., 2002]. Nevertheless,

in a study by Khan et al. [2002], error bars for fecal glucocorticoids were surprisingly low in captive baboons (*Papio cynocephalus*).

Variation in fecal steroid levels may be lower in larger-bodied animals, such as chimpanzees, due to longer gut retention time. Unlike urinary steroids, which are excreted relatively rapidly [Mohle et al., 2002; Ziegler et al., 1989], fecal steroids are excreted slowly, reflecting an approximate 48-hr gastrointestinal transit time in chimpanzees [Milton & Demment, 1988; Whitten et al., 1998]. Fecal steroids may therefore be more appropriate for behavioral endocrinology studies because they are representative of long-term baseline hormone levels, and they appear to be less susceptible to diurnal fluctuations than are urinary steroid levels. For example, Muller and Wrangham [2004] found that urinary testosterone was positively correlated with dominance rank in the Kanyawara chimpanzee community at Kibale, but only for afternoon (not morning) urine samples. That is, the correlation between rank and testosterone levels in morning urine samples was nonsignificant, and Muller and Wrangham [2004] argued that afternoon samples are better indicators of the outcome of social interactions during the day. There is little doubt that urinary measures are more suitable for measuring short-term responses, such as acute stressors. However, associations between long-term social factors, such as the stable linear dominance hierarchies of the Ngogo and Kanyawara chimpanzee groups, and physiological measures such as testosterone may be more efficiently elucidated with the use of fecal samples and a mixed-model methodology than by urine samples and Pearson correlations or repeated-measures ANOVAs.

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