Correlates of stress in free-ranging male chacma baboons, *Papio hamadryas ursinus*

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In male baboons, social instability is known to increase concentrations of glucocorticoids (GCs), a class of steroid hormones associated with the vertebrate stress response. The stress response may be particularly marked among male chacma baboons, whose social groups often demonstrate a strong mating skew and high rates of infanticide. Here we test this prediction and explore additional correlates of variation in concentrations of GCs measured in faeces. We collected 482 faecal samples from 13 males over 14 months. Changes in the male hierarchy were the major source of GC variation. Such instability occurred as the result of a new immigrant male's take-over of the alpha position, a resident male's rise to the alpha position, and the rise in rank of a young natal male. All three events were correlated with elevated GCs among all males and higher rates of male–male aggression than were found during periods of stability. The interaction between rank and instability had a significant effect on GC concentrations; low-ranking males had higher GCs during stable periods, whereas high-ranking males had higher GCs during unstable periods. Males that dispersed had elevated GCs in the month following immigration but not at other times, including the month preceding emigration. Finally, entering into a sexual consortship led to a temporary increase in a male's GC measures. By contrast, seasonal changes and a male's age were unrelated to GCs. GC variation in chacma males was primarily explained by temporary elevations following events that relate to reproductive fitness.

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There has been a recent explosion of interest in stress physiology as technical advances have allowed researchers to measure stress hormones, or glucocorticoids, noninvasively from free-ranging animals (Whitten et al. 1998; Wasser et al. 2000; Khan et al. 2002; Lynch et al. 2003; Beehner & Whitten 2004). Glucocorticoids are a class of steroid hormones in vertebrates that are secreted by the adrenal cortex in response to a wide variety of stressful stimuli and conditions (Nelson 2000). An individual showing a stress response generally has elevated concentrations of circulating glucocorticoids, which serve to mobilize energy reserves and curtail nonessential metabolic processes (Sapolsky 2002). While the stress response is an adaptive response to acute stressors over short periods of time, it is also physiologically costly and can be detrimental to essential catabolic bodily processes

when sustained over extended periods. Some hallmarks of prolonged activation of the stress response are gastric ulcers, atherosclerosis, stunted growth, and suppression of the immune system (Sapolsky 2002).

The pioneering work by Sapolsky (1983) on anubis baboons (*Papio hamadryas anubis*) demonstrated that socially dominant males show relatively low circulating concentrations of glucocorticoids, whereas subordinate males typically show relatively high concentrations. Sapolsky (1992, 1993) suggested that the elevated stress levels among low-ranking males may have resulted from the unpredictability and loss of social control that accompanies low status in a baboon troop. Subsequent work demonstrated that the predictability and social control that high-ranking males enjoyed when the dominance hierarchy was stable disappeared when male rank positions were disrupted and there was instability in the hierarchy. During such periods of instability, the stress profiles of high-ranking males were higher than those of low-ranking males (Sapolsky 1993). Sapolsky (1992) hypothesized

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that, while high-ranking male baboons had the most control over their daily feeding, mating, or social activities during periods of stability, the same individuals had the most to 'lose' when the status quo was upset.

Since Sapolsky's work, a multitude of studies have examined the relationship between stress physiology and dominance rank in vertebrates, but no consistent relationship has emerged (reviewed in Creel 2001). While dominance rank can be a useful 'summary' of many behavioural events within a group (e.g. the direction of aggression), it may not be that informative in cross-species comparisons (Creel 2001; Sands & Creel 2004). Rather, to understand the physiological costs of different social ranks, it may be more useful to evaluate the way in which dominance is achieved and maintained in a species (Goymann & Wingfield 2004). For example, in some cooperatively breeding species, dominance is associated with both high rates of aggression and high GCs (e.g. wild dogs, *Lycaon pictus*: Creel et al. 1997), while in species with strong kin relationships, subordinance is associated with elevated GCs, possibly due to a lack of social support (Abbott et al. 2003). It has become increasingly apparent that there is no simple relationship between dominance and GCs across taxa. Therefore, our approach was to identify the behaviours and events that are associated with elevated GCs in a highly social primate, the chacma baboon.

Behavioural events such as changes in dominance rank, the acquisition of mates, and dispersal can have significant reproductive consequences for the individuals involved. The fitness benefits of these behaviours have been well studied, but the costs have received little attention. While researchers could previously only quantify the physical costs (e.g. injuries, decreased feeding time, or even death) of behavioural events, we can now quantify the physiological costs as well. Sapolsky (1992, 1993) found that changes in the male dominance hierarchy in anubis baboons are associated with elevated glucocorticoids for all group members. Mating behaviours are also associated with elevated GCs in seasonally breeding primates (Schiml et al. 1996; Strier et al. 1999; Barrett et al. 2002; Lynch et al. 2002). Although baboons (*Papio* spp.) mate throughout the year, the temporary consortships that males form with oestrous females may be an additional stimulus for GC elevation. Finally, in addition to causing stress for residents, immigration into a new group can be associated with elevated GC concentrations for the immigrant himself (van Schaik et al. 1991; Alberts et al. 1992).

Here we evaluate the relationship between faecal glucocorticoids (fGCs) and several ecological, demographic and behavioural variables in a population of free-ranging chacma baboons. We consider male glucocorticoid variation in relation to the following variables: dominance rank, instability in the dominance hierarchy, consort status, infanticide risk, dispersal, aggression, grooming, age and seasonality. We hypothesize that variation in fGC concentrations will be better explained by variables related to reproductive fitness, such as rank changes, mating behaviour and dispersal, than by ecological and demographic variables such as seasonality and age. We predicted that rank changes at the top of the male dominance hierarchy would have a stronger effect on fGC measures than other rank changes because the former have a greater potential for reproductive consequences. This holds particularly true for chacma males, because mating skew is high, rank changes are common, and infanticide occurs at high rates (Cheney et al. 2004).

METHODS

Study Site and Subjects

Research was conducted in the Moremi Game Reserve in the Okavango Delta of Botswana. The habitat consists of grasslands that flood annually and elevated woodlands that range in size from one to hundreds of hectares (Hamilton et al. 1976; Bulger & Hamilton 1987; Ross 1987; Cheney et al. 2004). During the annual flood (between June and October), the baboons ford the submerged floodplains and move between the woodland 'islands' throughout an approximately 5-km² range. The density of baboons in this area is one of the highest reported for chacma baboons (Cheney 1987).

Chacma males form strict, linear dominance hierarchies in which high-ranking individuals monopolize access to fertile females (Bulger 1993). Perhaps because this population lives at very high densities (Hamilton & Bulger 1992), changes in rank are frequent, occurring on average 1.5 times per month in a group containing 10–15 males, with a change in the alpha male on average every 6–7 months (Cheney et al. 2004). Males that acquire alpha rank are usually young adults that have recently immigrated into the group, although young adult natal males also occasionally rise to the alpha position (Hamilton & Bulger 1990; Kitchen et al. 2003). Immigrant males that attain alpha status frequently commit infanticide (Palombit et al. 2000). Recently deposed alpha males typically remain in the group, often forming close behavioural 'friendships' with lactating females. These friendships may protect the males' offspring from potential infanticide (Hamilton & Bulger 1990; Palombit 2000).

As they mature, natal males begin to rise in dominance rank. First, they rise above all adult females. Then, as they approach fully adult size (at 8–9 years of age), they begin to rise in rank above the older low- and middle-ranking adult males. Natal males typically emigrate at some point during this rise in dominance rank (8–10 years of age), although some males remain in their natal groups (Hamilton & Bulger 1990).

The study group, C, has been observed since 1978 (e.g. Bulger & Hamilton 1987). Since mid-1992 the group has been under continuous observation on an almost daily basis (Cheney et al. 2004). The ages and matrilineal relationships of all natal individuals are known, as are the origins of many immigrants and destinations of many emigrants. The group is fully habituated to humans on foot. During the 14 months of this study, the group contained 82–91 individuals, including 9–11 adult males (>8.5 years of age), 29–31 adult females (>6 years of age),

Table 1. Characteristics of adult males and number of faecal samples collected

ID	Age	Rank	Dispersal status*	Tenure	N faecal samples
BG	Young	High	Immigrant	April 2002–End	42
GM	Young	High	Immigrant/emigrant	April 2002–June 2002	11
BJ	Young	Low	Resident	Entire study	56
MG	Young	High	Natal	Entire study	50
PO	Young	High/Low	Natal	Entire study	55
NK	Young	Low/High	Natal	October 2002–End†	19
RY	Old	High	Resident	Entire study	53
TH	Old	High	Resident	Entire study	53
FG	Old	High/Low	Resident/emigrant	Start–September 2002	26
AP	Old	Low	Resident	Entire study	56
AU	Old	Low	Resident	Entire study‡	46
SO	Old	Low	Immigrant/emigrant	May 2002–July 2002, September 2002	7
WA	Old	Low	Resident	Start–March 2002	8

*Immigrant and emigrant refer to males that dispersed during the study period. Resident refers to non-natal males that entered the group prior to the study period.
†Natal male that reached maturity during the study and was considered an adult as of October 2002.
‡Old male that was occasionally not seen.

and their immature offspring. A total of 13 adult males were used as subjects for this study (Table 1).

Behavioural Data Collection

We used 10-min focal animal samples (Altmann 1974) to collect behavioural data on all 13 adult males in the group between January 2002 and February 2003. Males were sampled at the same rate (approximately 2.5 times per week) for a total of 1201 focal animal samples (14–140 samples/male) and 23.3 h of observation for each male present for the entire study.

Hormone Collection and Analysis

We collected 482 faecal samples from the 13 males over the same 14-month period (Table 1). A sample was collected in the morning from each male approximately once every 8 days. Using a combination of opportunistic and targeted sampling, we were usually able to obtain a faecal sample from each male before any male was sampled twice. Because males were in the group for different lengths of time, the total number of samples from each male varied. For males that remained in the group throughout the study (N = 6), this variation was minimal (range 50–56 samples/male). For males that arrived and/or disappeared during the study (N = 7) the number of samples was proportional to residency time (range 7–46 samples/male). In both cases, however, faecal sampling was evenly distributed across males for any given time period.

We extracted hormones from faeces in the field using methods described by Beehner & Whitten (2004). All samples were collected in the morning, placed in a methanol/acetone solution, homogenized, filtered and loaded onto a reverse-phase C18 cartridge (Sep-Pak Plus, Waters Associates, Milford, Massachusetts, U.S.A.). Following solid-phase extraction, all samples were immediately frozen (−10°C) until transported to Emory University

for analysis. There, steroids were eluted from cartridges with methanol and subsequently frozen (−80°C) until the time of radioimmunoassay (RIA). Immediately prior to RIA, aliquots of samples were evaporated under nitrogen and reconstituted 1:1 in working buffer (phosphosaline gelatin). All samples were assayed for glucocorticoids (GC) using a corticosterone RIA kit (ICN Diagnostics Division, Costa Mesa, California, U.S.A.) previously validated for use in baboons (Beehner & Whitten 2004). Interassay coefficients of variation were $9.81 \pm 3.29\%$ (high control, $N = 14$) and $15.37 \pm 1.99\%$ (low control, $N = 14$). The intra-assay coefficient of variation was $8.98 \pm 5.19\%$ (faecal extract pool, $N = 6$). All samples were run in duplicate and mean concentrations are expressed in pg/g.

Study Design

To determine which characteristics were most important for determining faecal glucocorticoid concentrations (fGCs), we used five predictor variables in a stepwise regression (definitions found in subsequent section): dispersal status, rank, age, consort status and stability of the hierarchy. We then analysed significant predictor variables individually. To determine whether chacma males show a similar pattern to anubis males, we examined the interaction between rank and stability. Next, we examined the relationship between several behavioural characteristics and fGCs, mainly rates of aggression and grooming and the formation of friendships. Finally, we examined seasonality, a potentially confounding variable.

Predictor Variables

Dispersal status

Dispersal status is a categorical variable that separates males that immigrated or emigrated during the study period from males that remained resident throughout the study (Table 1). Two new males immigrated into the group (one subsequently emigrated), one non-natal male

immigrated twice and emigrated twice, and one non-natal male emigrated from the group. The latter two males had each entered and left the group several times prior to this study.

Rank

Rank is a categorical variable that divides the male hierarchy in half (at the median), resulting in two rank categories: 'high' and 'low'. We included males at or below the median rank in the 'low' rank category. We recalculated this variable monthly or whenever we observed a change in the hierarchy (three males switched between high and low categories during the study, see Table 1).

Age

Age is a categorical variable that divides males (at the median age) into two age categories, 'young' and 'old' (Table 1). We included males at or above the median age in the 'old' age category. We knew all ages for natal males ($N = 3$) and we assigned ages to immigrant males ($N = 10$) based on dental characteristics (Kitchen et al. 2003).

Consort status

Consort status is a categorical variable that indicates whether a male was 'in consort' or 'not in consort' at the time of the faecal sample. We considered a male as 'in consort' with a female under two conditions: (1) the female had to have visible signs of swelling of the perineal skin, and (2) the male had to show signs of mating (i.e. mounting and copulating) and mate guarding (i.e. grunting towards the female, following the female, threatening other males). Because there is approximately a 1.5-day lagtime between hormone secretion and faecal steroid excretion in baboons (Wasser et al. 1994), we classified a faecal sample as an 'in consort' sample if the male had been observed with an oestrous female on either of the 2 days prior to collection. Males that were not observed for more than 5 h on either of the 2 days prior to sample collection were not scored for this variable ($N = 138$ faecal samples).

Stability

Stability is a categorical variable that indicates whether the top of the male dominance hierarchy was stable (i.e. no rank changes) or unstable (i.e. recent rank changes) at the time of each faecal sample. We determined male dominance ranks through daily observations of approach–retreat interactions (supplants), using both focal animal and ad libitum sampling (Altmann 1974). Because the frequent rank changes that occur among low-ranking individuals do not appear disruptive to the rest of the group, stability refers only to the stability of the first and second rank positions.

Our definition of stability differs from that of Sapolsky (1983), who used 'instability' to refer to ambiguity in dominance relationships. There was never a case where rank ambiguity existed in the study population; rather, all rank changes were conspicuous, abrupt, and permanent. Therefore, we primarily define 'instability' as approximately 1-month periods following changes in the first

two rank positions, with one exception. The 'natal male rise' periods refer to two longer periods encompassing the rise of two different natal males to the beta (second) rank and their challenge for the alpha position (a position each male subsequently achieved). Because the time between these two events was short, we considered the entire period unstable. Unless superceded by other events, we defined the male dominance hierarchy as returning to stability 1 month after a take-over, because this time period roughly coincided with the behavioural return to baseline. In the multivariate analyses, we classified all periods of time as either 'stable' or 'unstable'.

Stability Categories

To examine periods of instability in more detail, we divided the study period into eight periods (Fig. 1) based on the specific changes at the top of the male hierarchy. For subsequent analyses, we grouped these eight periods into four categories, the same 'stable' category as used in the multivariate analysis, and three 'unstable' categories as follows.

Stable

Two periods when no changes occurred in the top two positions of the male hierarchy (17 May 2002–30 July 2002; 1 September 2002–16 December 2002).

Unstable

Natal male rise. Two periods when two different natal males rose rapidly through the hierarchy, reached the second-ranking position, and had frequent aggressive interactions with the alpha male. These periods terminated on the day that each male became alpha (3 January
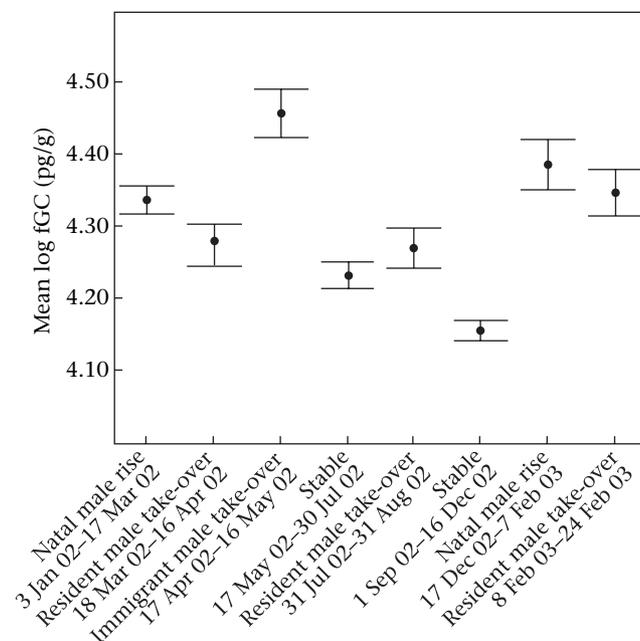

Figure 1. Variation (mean ± SE, pg/g) in faecal glucocorticoid concentrations (fGCs) during the study. The study was divided into eight periods based on changes at the top of the male hierarchy.

2002–16 March 2002; 17 December 2002–7 February 2003).

Resident male take-over. Three 1-month periods after three resident males separately assumed the alpha position; these were the two natal males described above and one other male that had been resident in the group for 3 months at the second rank prior to attaining the alpha position (17 March 2002–16 April 2002; 31 July 2002–31 August 2002; 8 February 2003–24 February 2003). Here we use the term 'take-over' to refer only to a change in the alpha rank position. In contrast to fallen alpha males in some other primate species, alpha male baboons that have fallen in rank usually remain in the group.

Immigrant male take-over. A single 1-month period beginning with the arrival of a male that rapidly (within days) rose to the alpha position (17 April 2002–16 May 2002). A second male, who later became the alpha (see resident male take-over), also immigrated during this period.

Behavioural Correlates of fGC Variation

We evaluated two behaviours in relation to fGC variation: male–male aggression and male–female grooming. Male–male aggression included all agonistic interactions between adult males (i.e. fights, chases, and vocal or visual threats). Male–female grooming included all grooming interactions (initiated or received) between a focal male and an adult female. We predicted that male–male aggression (initiated or received) would be positively correlated with fGCs, whereas male–female grooming would be negatively correlated with fGCs.

Male–Female Friendships

During times of infanticide risk (i.e. following a take-over by a potentially infanticidal male, Palombit et al. 2000), lactating females with a male friend have lower fGCs than those without (Beehner et al. 2005). To analyse the fGC concentrations of male friends, we divided males into those with a female friend (always a lactating female) and those without a female friend during the same period. We identified a male friend as any male with which a lactating female had significantly more 'friendship' interactions (i.e. grooming, approaches, tolerance for infant handling) than with any other male (Palombit et al. 1997; Beehner et al. 2005). Of the seven males during this period that were not themselves involved in rank changes, three had friends and four did not.

Seasonality

To measure the possible confounding effects of seasonal variation in productivity on fGC concentrations, we combined information on mean monthly temperature and total monthly precipitation (UNESCO 1963) using the Gaussen method (Gaussen 1954 cited in Dufour-Dror & Aytekin 2004), which calculates the number of months in which twice the mean maximum temperature ($2T$, in °C) is greater than the precipitation (P, in mm). When examining seasonal effects on fGC concentrations, we modified this technique and calculated the difference, $2T - P$, for each month to produce a measure of productivity that could be compared to mean monthly fGCs. For P we used the total precipitation, measured locally at our research site, from the current and the previous month, to account for the delay between accrued precipitation and environmental productivity.

Data Analysis

We performed all statistical analyses using SPSS version 11 (Chicago, Illinois) for Macintosh. All hormone data were logarithmically transformed to meet the assumptions of normality; logarithmically transformed fGC values did not deviate from normal either overall or for any male (Kolmogorov–Smirnov tests: $P > 0.05$). Because we were interested in addressing the relative contribution of factors that were aspects of individuals (rank, age and dispersal status) as well as of time (stability and consort status), we entered all fGC values (rather than means for each male) into the multivariate analyses. Faecal GC values did not differ significantly across individuals overall (ANOVA: $F_{12,469} = 1.49$, $P = 0.12$) or within any of the stability categories described above (ANOVA: stable: $F_{11,235} = 1.19$, $P = 0.30$; natal male rise: $F_{10,108} = 0.66$, $P = 0.757$; resident male take-over: $F_{10,76} = 1.13$, $P = 0.35$; immigrant male take-over: $F_{9,19} = 1.47$, $P = 0.23$). These findings, in combination with our regular sampling of males, indicate that no single male affected our results disproportionately. We performed a stepwise regression on fGC values using a P value to enter of less than 0.05.

Following the multivariate analysis, we analysed all independent variables that were significantly associated with fGC concentrations in further detail. When an ANOVA indicated a significant difference across factors, we used a Tukey's multiple comparisons test to determine which groups were significantly different from others.

In our analyses of behavioural data, we used nonparametric statistics. We used correlation analysis to relate individual behavioural and fGC means. For male–male aggression, we used a chi-square test to determine whether observed frequencies were significantly different from expected (based on all observations) as well as binomial tests to compare pairs of time periods. Statistical tests are two-tailed, and the statistical threshold for all analyses was set at $P = 0.05$.

RESULTS

Multivariate Analysis

We entered fGC values into a stepwise multiple regression with the following predictor variables: dispersal status, rank, age, consort status and stability. A stepwise multiple regression entered stability and dispersal status (in that order) as significant predictors of fGC measures,

Table 2. Final model of multivariate stepwise regression with log faecal glucocorticoid (pg/g) as the dependent variable ($r^2 = 0.18$, $P < 0.001$)

Predictor	Included	Standardized β	P
Stability	Yes	−0.405	<0.001
Dispersal status	Yes	0.160	0.001
Consort status	No	0.098	0.053
Age	No	−0.026	0.61
Rank	No	0.019	0.71

while age and rank were not entered (Table 2). Although consort status approached significance ($P = 0.053$), it was not included in the model. Faecal GCs were higher during periods of instability and among males that dispersed during the study (Table 2). Including all variables in the regression resulted in the same significant variables (stability and dispersal), and the order in which the variables were entered did not affect the results (data not shown). To minimize confounding effects, in the ensuing analyses we excluded consort samples where appropriate as well as samples from the first month following a male's immigration (see below), resulting in a sample size of 267 faecal samples.

Stability of the Hierarchy

Instability at the top of the male hierarchy appeared to be the best predictor of fGC measures. To take a closer look at these periods of instability, we assessed fGC variation across four categories (stable, natal male rise, resident male take-over and immigrant male take-over; see Methods). Overall, fGCs varied significantly across these four categories (ANOVA: $F_{3,263} = 25.47$, $P < 0.001$) with the highest concentrations during the immigrant male take-over. In pairwise comparisons we found significant differences between all categories except the natal male rise and the immigrant male take-over (Fig. 2). Interestingly, fGC concentrations during the resident male take-over period were fairly low, despite the fact that this period included a take-over by a male that had only been in the group for 3 months and thus posed an infanticidal threat. When we repeated the analysis using a mean fGC value per male per time category, we also found significant differences across periods (ANOVA: $F_{3,40} = 25.79$, $P < 0.001$) with significant pairwise differences between all time categories, except between the natal male rise and the resident male take-overs.

The previous analysis incorporated all males, including those individuals whose arrival or change in rank status defined a given time period (e.g. the immigrant males during the immigrant male take-over period, the natal males during the natal male take-over period, and the deposed alphas in all cases, henceforth 'participants'). Participants had higher fGC concentrations overall than males that were not directly involved in the rank changes or immigration event ('nonparticipants', ANOVA: $F_{1,265} = 16.85$, $P < 0.001$). Even among nonparticipants, however, fGC concentrations differed significantly across
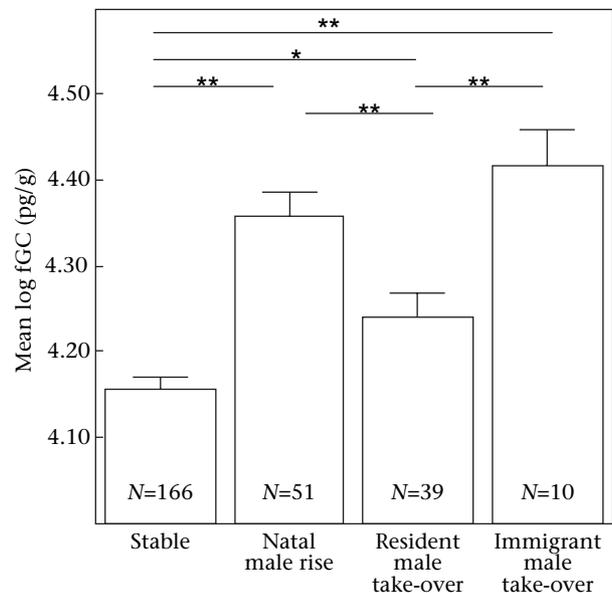

Figure 2. Comparison of faecal glucocorticoid concentrations (fGCs) (mean + SE, pg/g) for each stability category. Only nonconsort samples and samples not associated with a recent immigration were used in analysis. Pairwise differences were compared using Tukey's method. *$P < 0.05$; **$P < 0.01$.

stability categories (ANOVA: $F_{3,237} = 20.76$, $P < 0.001$) and differences between categories matched the results for all males.

Next, we tested whether the effect of rank instability was restricted to upheavals only among high-ranking males. We compared fGCs during a 1-month period in which there were no changes in the hierarchy followed by a 1-month period when several low-ranking males changed ranks. We were necessarily restricted to a short period of time for this analysis because changes in the lower ranks of the hierarchy were so frequent. There was no significant difference in fGC concentrations across these two periods (ANOVA: $F_{1,69} = 0.83$, $P = 0.37$).

To separate the possible confounding effects of stress associated with the arrival of a new male from that associated with the potential for infanticide, we made a three-way comparison. We compared fGCs during the immigrant male take-over period (involving two unfamiliar males and heightened infanticide risk), the period following the take-over by the short-term resident (involving heightened infanticide risk only), and all other unstable periods. These periods differed significantly (ANOVA: $F_{2,232} = 7.82$, $P = 0.001$). However, the short-term resident take-over was not significantly different from the other unstable periods ($P = 0.21$), whereas the immigrant male period differed significantly from both the short-term resident take-over ($P = 0.003$) and other unstable periods ($P < 0.001$). We repeated this three-way comparison using only nonparticipants, yielding significant differences (ANOVA: $F_{2,180} = 5.71$, $P = 0.004$). Specifically, mean fGCs were lower during the short-term resident male take-over than the immigrant male take-over ($P = 0.003$) and other unstable periods ($P = 0.046$). The immigrant male take-over period had higher fGCs

than other unstable periods, but the difference was not significant ($P = 0.11$). Thus, the resident period was characterized by lower fGCs than the immigrant male period, suggesting that the arrival of new males creates additional stress beyond the threat of infanticide.

Stability and Rank

Although rank was not a significant predictor of fGCs overall, we wanted to assess whether our population showed the same interaction between rank and stability as Sapolsky's (1993) anubis population. We found a significant interaction between rank and stability (ANOVA: $F_{1,266} = 4.166$, $P = 0.042$). Although differences across rank were not significant in either period (ANOVA: stable: $F_{1,165} = 1.96$, $P = 0.16$; unstable: $F_{1,98} = 1.99$, $P = 0.16$), fGCs were higher in low-ranking than high-ranking males during stable periods, whereas fGCs were higher in high-ranking than low-ranking males during unstable periods (Fig. 3). Both high- and low-ranking males had significantly higher fGCs during unstable periods than during stable periods (ANOVA: high-ranking: $F_{1,135} = 53.73$, $P < 0.001$; low ranking: $F_{1,128} = 13.10$, $P < 0.001$).

Consort Status

Consort status approached significance in the stepwise regression (Table 2), with males in consort having higher fGCs. There are two possible reasons why fGCs in consorting males may have been elevated. First, males with high fGC concentrations may be more successful at forming consortships with females. Alternatively, males in consortships may experience elevated fGC concentrations as a result of the consortship. To address the first hypothesis, we compared each male's consort frequency (proportion of time in consort) to his baseline (non-consort) fGC levels. Males that were in consort more

often did not have higher baseline fGCs than other males (regression: $r^2 = 0.002$, $P = 0.88$). To test the second hypothesis, we compared consort and nonconsort fGC means for each male. Consort fGC means were significantly higher than nonconsort means for all 12 males from which we had consort samples (Wilcoxon signed-ranks test: $T = 78.0$, $P = 0.001$). Finally, to control for the confounding effects of stability, we repeated the pairwise analysis for each male using faecal samples only from stable periods. In this limited sample of seven males, all had significantly higher fGCs when in consort (Wilcoxon signed-ranks test: $T = 28.0$, $P = 0.016$). Results suggest that for all males, consortships elevate fGC concentrations.

Dispersal Status

Males that immigrated or emigrated during the study had significantly higher fGC concentrations than other males (ANOVA: $F_{1,277} = 4.93$, $P = 0.03$). To consider the effects of immigration, we divided samples from males that dispersed into two categories: samples gathered in the month immediately following immigration, and samples gathered in subsequent months. Faecal GCs were significantly higher during the month following immigration than at other times (ANOVA: $F_{1,47} = 5.80$, $P = 0.02$). Faecal GC concentrations derived from samples collected outside the first month after immigration were not significantly different from samples for nondispersing males (ANOVA: $F_{1,265} = 0.52$, $P = 0.47$). Next, we considered the effects of emigration. In contrast to immigration, there was no difference between fGCs in the month preceding emigration and fGCs before this month (ANOVA: $F_{1,44} = 0.206$, $P = 0.652$). Therefore, it appears that dispersal is followed by a temporary rise in fGCs, but there is no evidence that high fGC males are more likely to emigrate.

Behavioural Correlates of GC Variation

We examined data from males that formed friendships in the period following the immigrant male take-over. Males with a lactating female friend had higher fGCs than males that did not, but the difference was not significant (ANOVA: $F_{1,20} = 2.85$, $P = 0.11$).

To examine the relationship between fGC concentrations and other aspects of behaviour, we first compared rates of aggression and grooming with individual fGC concentrations. This analysis included behavioural data only from the 10 males whose tenure in the group lasted more than 2 months. There was no correlation between mean male fGCs and rates of male–male aggression, either for aggression received or aggression initiated (Spearman's rank correlation: received: $r_S = 0.02$, $P = 0.96$, initiated: $r_S = -0.12$, $P = 0.75$). Similarly, there was no correlation between grooming received and mean male fGCs ($r_S = -0.06$, $P = 0.88$).

We then compared rates of male–male aggression across periods. First, we compared stable to unstable periods and found that aggression was more likely to occur during
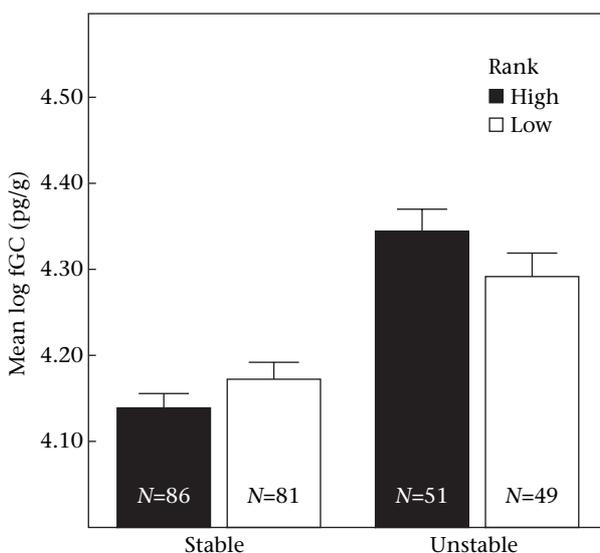

Figure 3. Comparison of faecal glucocorticoid concentrations (fGCs) (mean + SE, pg/g) for each rank and stability category.

unstable periods (binomial test: $P < 0.001$). Next, we separated the unstable periods into the three categories already described and compared rates in each stability category to the overall mean rate of aggression (0.24 incidents/h; Fig. 4). The stable category had lower than average rates of male–male aggression while all three unstable categories had higher than average rates, with the immigrant male category showing the highest rate (Fig. 4). Overall, these categories did not differ significantly in aggression rates (chi-square: $\chi^2_3 = 2.88$, $P = 0.41$). However, the resident male take-over and natal male rise categories both had significantly higher rates of aggression than the stable category (binomial test: $P = 0.05$ and $P = 0.02$, respectively). Despite having the highest rate of aggression, the immigrant male take-over category did not differ significantly from the stable category (binomial test: $P = 0.09$), a result that may have been due to small sample size.

Finally, we examined the behaviour of consorting males. Overall, consorting males had higher rates of aggression than other males (0.41 incidents/h versus 0.24 incidents/h) but these differences were not significant (binomial test: $P = 0.10$). However, consorting males generally tend to avoid other males (Smuts 1985), a pattern replicated in our data. Consorting males approached another male less than half as often as did nonconsorting males (0.47 and 1.12 approaches/h, respectively). Comparing occurrences of aggression based on the number of approach interactions, consorting males had a significantly higher rate of aggression per approach than other males (binomial test: $P < 0.001$). Thus, while consorting males rarely interacted with other males, any interactions they did have with other males tended to be aggressive.

Seasonality

Although mean monthly fGCs were lower in hot/dry months, the relationship was not significant (regression:
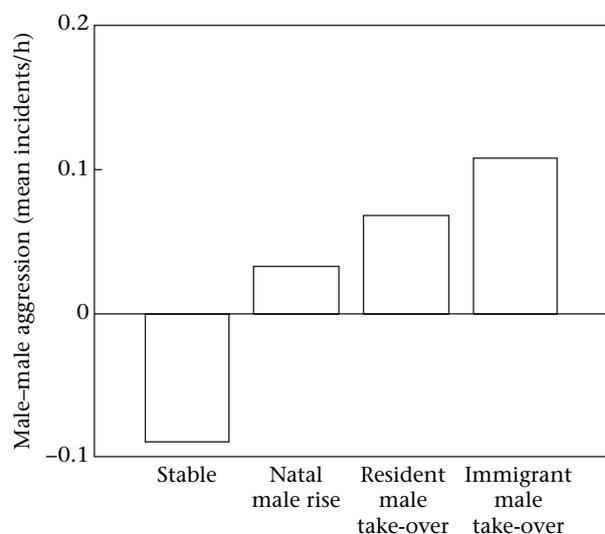

Figure 4. Deviations from the mean rate of male–male aggression (mean = 0.24 incidents/h) for each stability category.

$r^2 = 0.23$, $N = 14$, $P = 0.081$). However, it appears that rank stability may account for this tendency because all stable months were hot/dry months. Within stable and unstable periods, there was no relationship between fGC and the combined measure of temperature and precipitation (regression: stable: $r^2 = 0.10$, $N = 5$, $P = 0.60$; unstable: $r^2 = 0.03$, $N = 9$, $P = 0.68$). Because seasonal variation did not appear to contribute significantly to fGC concentrations independent of hierarchical stability, temperature and precipitation data were not included in any of the above analyses.

DISCUSSION

In this population of chacma baboons, behavioural events rather than demographic or ecological variables were the best predictors of variation in male glucocorticoid measures. Instability resulting from rank changes at the top of the hierarchy, recent dispersal events and consorting with females affected GC measures whereas age, rank and seasonal variables did not.

Stability in the male dominance hierarchy was the strongest predictor of male GC measures, replicating the results of Sapolsky (1986, 1993). In our study, instability in the male dominance hierarchy, with its attendant rise in GC concentrations, occurred following an immigrant male's take-over of the alpha position, a resident male's rise to the alpha position, and the rise in rank of a young natal male. As in anubis baboons (Sapolsky 1993), males in the upper half of the hierarchy showed the greatest increase in GC concentrations during periods of instability, having relatively low GCs during stable periods and relatively high GCs during unstable periods. However, GC concentrations increased for all males during periods of hierarchical instability, even among low-ranking males not directly involved in the rank changes. By contrast, the frequent rank changes among low-ranking males had no apparent effect on the GC concentrations of any individuals.

The period following an immigrant male's take-over of the alpha position was associated with the highest GC concentrations. Infanticide, the leading cause of infant mortality in this population (Cheney et al. 2004), is generally committed by recent immigrants that achieve alpha status. Although we have no evidence that this particular male committed infanticide, it was still not surprising to find that males showed physiological responses to this potential threat. Interestingly, the instability caused by this 'novel' immigrant male provoked a greater stress response for males than instability caused by a recent immigrant male that had been in the group for only 3 months (and therefore still presented an infanticidal threat). Consequently, it appears that the uncertainty surrounding the arrival of a novel male may represent an additional source of stress beyond the infanticidal threat.

Beehner et al. (2005) found that although all lactating females in this group showed elevated GC concentrations following the rise of an immigrant male to the alpha rank position, GC concentrations were lower among females with male 'friends'. By contrast, males showed a trend in the opposite direction: males that formed friendships

tended to show an increase in GC concentrations, whereas other males did not. These results suggest that, while friendships may alleviate stress among females, they may increase stress among males. This might occur because male friends are often the presumptive fathers of their friends' infants and, as such, actively protect these infants from infanticidal attacks (Palombit et al. 1997, 2000).

There was additional evidence that associating with females for reproductive gain was stressful. Males had higher GC concentrations when they were engaged in sexual consortships than when they were not. It was not simply the case that high GC males were more likely than other males to monopolize females but rather that entering into a sexual consortship was followed by a subsequent rise in GC concentrations for the consorting male. Mating behaviour has been associated with elevated GCs in seasonally breeding primates (Schiml et al. 1996; Strier et al. 1999; Barrett et al. 2002; Lynch et al. 2002), but little work has been done on nonseasonal breeders. Previous work on yellow baboons, *Papio hamadryas cynocephalus*, demonstrated that consorting males may experience some constraints on foraging activity due to decreased energy intake during mate guarding (Alberts et al. 1996). This suggests a metabolic source of stress for consorting males.

Another major predictor for male GC measures was dispersal status. Dispersing males had higher GC concentrations than resident males, primarily due to their extremely high GC concentrations in the month following their arrival. The majority of immigration events, however, occurred during periods of rank instability (in many cases causing rank instability). Thus, we cannot completely separate the effects of immigration from the effects of social instability. Both were significant predictors in our multivariate model. We found no evidence that a male's GC concentrations were elevated in the weeks before he emigrated from the group. Therefore, while changing groups appears to be a stressful event for male baboons, signs of physiological stress are detectable only after the event itself, possibly the result of the 'in-transit' period or the assimilation into a new group. Other primate studies have also found that recent immigrant males have elevated stress levels (van Schaik et al. 1991; Alberts et al. 1992) but this contrasts with evidence from spotted hyaenas, *Crocuta crocuta*, where males have low GCs following dispersal (Holekamp & Smale 1998). Differences between species in response to or in preparation of dispersal events deserves further study, but it may relate to differences in rank for dispersing males. Male hyaenas have very low rank following dispersal (Smale et al. 1997) whereas chacma males typically have high postdispersal rank (Hamilton & Bulger 1990).

Instability in the male dominance hierarchy, the best predictor of elevated GC concentrations, was consistently accompanied by higher rates of aggression when compared with stable periods. The energetic stress hypothesis suggests that aggression is associated with elevated GCs because of the metabolic demands of aggression (Muller & Wrangham 2004). The mechanism mediating the association between aggression and GCs in this population, however, remains unclear. While we lack the data to

properly test the energetic stress hypothesis, other aspects of our data do not support it. First, individual rates of aggression were not associated with individual mean GC concentrations. Second, even low-ranking males several ranks removed from the upheaval and unlikely to experience abrupt changes in metabolic demands had GC concentrations that corresponded with instability at the top of the hierarchy. Finally, while seasonal fluctuations in GC concentrations might indicate a primary role for energetic demands (Muller & Wrangham 2004), we found no seasonal variation among males in this population. In fact, Beehner et al. (2005) suggested that there may be comparatively little seasonal variation for baboons in this habitat due to the alternating rainy and flood seasons. Thus, although the association between stable periods and hot/dry months needs further exploration, the results of our study suggest that aggression is associated with elevated GCs for psychological reasons (Sapolsky 1983).

Although rank was not a significant predictor of GC concentrations, in at least two respects high-ranking males experienced greater stress than low-ranking males. First, during periods of instability, high-ranking males showed a greater increase in GC concentrations than did low-ranking males. This difference probably occurred because high-ranking males were more likely than low-ranking males to be directly involved in the rank changes that defined unstable periods. The heightened stress of high-ranking males during periods of instability takes on added importance when one considers that the male hierarchy was unstable for the majority of this study (9 of 14 months). Thus, instability may represent the normal condition in this population and chacma baboons may be comparable to other species in which high rank is associated with elevated GCs owing to the costs of acquiring and maintaining that rank (Goymann & Wingfield 2004). Second, high-ranking males were more likely than low-ranking males to engage in sexual consortships, and thus more likely than low-ranking males to experience the elevated GC concentrations associated with this behaviour.

Results from this study allow us to compare the causes of elevated GC concentrations in male and female baboons living in the same group. In one respect, the sexes certainly differ. Whereas elevated GC concentrations in males occurred during unstable periods marked by high rates of male–male aggression, Beehner et al. (2005) found that elevated GC concentrations among adult females in this population occurred only when their own infants were at risk from infanticide, and not in response to general male social instability. This is not to say, however, that the causes of physiological stress in females are exclusively psychological (based on events they observe involving others) whereas the causes of stress in males are behavioural (based on events involving themselves). In fact, we found no correlation between individual rates of aggression or grooming and GC concentrations in males. Furthermore, all males experienced higher GC concentrations when the hierarchy was unstable, even if their own rank was unaffected. Lastly, consorts were periods of elevated male GCs yet rates of aggression were not higher during these times, mainly because males in consort

generally avoided interactions with other males. However, when they did have an interaction with another male it was more likely to be an aggressive one. Thus, while the events themselves differ, GC measures for both males and females seem to reflect the anticipation of events that might occur. In males, the relevant event is male–male aggression, whereas in females the relevant event is infanticide.

The three events with the greatest impact on male GC measures (instability, consorting, and entering a new group) were all related to male reproductive fitness. In this population, changes at the top of the male hierarchy are important not only because the highest-ranking male limits the reproduction of other males by monopolizing access to fertile females, but also because a new alpha male can undo a rival's previous reproductive success through infanticide. Thus, for resident males, the arrival and subsequent alpha take-over of an immigrant male may be associated with both unpredictability and the perception that events are worsening, two major correlates of stress (Hofer et al. 1972; Hennessy et al. 1977; Dess-Beech et al. 1983; Sapolsky 1992; Wingfield & Ramenofsky 1997). At the same time, males that achieved high rank showed higher GCs than other males, suggesting that there is a physiological cost that partially offsets the substantial reproductive benefits of acquiring high rank. Similarly, immigration itself and consorting with females were also associated with elevated GC measures, countering some of the reproductive gains of these behaviours. While our data set does not allow us to compare the relative costs and benefits of these events quantitatively, this exploratory analysis has made a valuable step towards evaluating the physiological costs associated with several critical events in the lives of male chacma baboons.

Acknowledgments

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