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High faecal glucocorticoid levels predict mortality in ring-tailed lemurs (*Lemur catta*)

R. Ethan Pride[†]

Department of Ecology and Evolutionary Biology, Princeton University, Princeton, NJ 08525, USA

Glucocorticoid levels are commonly used as measures of stress in wild animal populations, but their relevance to individual fitness in a wild population has not been demonstrated. In this study I followed 93 ring-tailed lemurs (*Lemur catta*) at Berenty Reserve in Madagascar, collecting 1089 faecal samples from individually recognized animals, and recording their survival over a 2 year period. I evaluated faecal glucocorticoid levels as predictors of individual survival to the end of the study. Animals with high glucocorticoid levels had a significantly higher mortality rate. This result suggests that glucocorticoid measures can be useful predictors of individual survival probabilities in wild populations. The 'stress landscape' indicated by glucocorticoid patterns may approximate the fitness landscape to which animals adapt.

Keywords: glucocorticoid; lemur; mortality; survival; cortisol; faecal steroid

1. INTRODUCTION

Glucocorticoids, steroid hormones secreted in parallel with the 'fight-or-flight' stress response, are commonly measured indicators of stress in vertebrates (Wingfield & Romero 2001). Glucocorticoids divert energy from long-term storage to cope with immediate crises (Sapolsky *et al.* 2000), and elevated glucocorticoid levels have been observed in animals facing both natural challenges (Alberts *et al.* 1992; Foley *et al.* 2001; Goymann *et al.* 2001) and anthropogenic environmental disturbances (Creel 1997; Wasser *et al.* 1997; Wingfield & Romero 2001). Because high glucocorticoid levels suggest the presence of environmental threats, and chronically high glucocorticoid levels are themselves associated with health risks (Dhabhar & McEwen 2000), it is believed that high glucocorticoid levels indicate lower individual fitness or population viability. Population-level research supports this proposition (Romero & Wikelski 2001), but there has not yet been an empirical test of glucocorticoids as predictors of individual fitness in a wild population.

Since glucocorticoid levels fluctuate in response to environmental challenges, they provide a spatio-temporally fine-grained measure of the stress landscape animals' experience. If glucocorticoid differences indicate individual differences in survival, then glucocorticoid patterns could reveal even short-term or subtle selection pressures that would otherwise be difficult to detect. Because they can be measured in faeces, glucocorticoids provide a powerful non-invasive tool for evolutionary ecologists and wildlife managers (Hofer & East 1998). However, if glucocorticoid levels do not predict individual survival, then it is inappropriate to use glucocorticoid analysis to infer fitness costs or demographic probabilities, greatly limiting their utility.

In this study I present the first test of faecal glucocorticoid levels as predictors of individual mortality in a wild animal population.

2. METHODS

(a) *Study site and system*

I conducted this study on a free-ranging population of ring-tailed lemurs (*Lemur catta*) at Berenty Reserve in southern Madagascar. Berenty's population of ~350 ring-tailed lemurs has been studied for four decades and is habituated to humans (Jolly & Pride 1999). Ring-tailed lemurs are prosimian primates that live in social groups of up to 25 animals; they are semi-terrestrial, diurnal, site-faithful, and typically range less than 1000 m per day (Sauther *et al.* 1999). *L. catta* defecate regularly after dormant periods, facilitating faecal sample collection.

(b) *Survival*

I observed six *L. catta* groups for 16 months between July 1998 and July 2000 (2–5 days per group per month), distinguishing all non-infant individuals by facial characteristics. At the end of the study period, I characterized all observed animals by current survival status (living, dead). Individual females that had disappeared during the study were considered dead as there are no known cases of individual female group transfer in four decades of study. Males that disappeared during the study ($n=18$) were excluded from the analysis because *L. catta* males emigrate between troops every 3–5 years, and survival status of missing males was therefore unknown. A total of 93 non-infant animals (52 females, 41 males) were included in the analysis.

(c) *Faecal sample collection and preparation*

I collected 1089 faecal samples opportunistically from known individuals during the study (11 ± 6 samples per individual). Each sample was collected immediately after an animal's defecation; date, time and identity of the donor animal were recorded. Samples were stored in 95% ethanol at room temperature for 2–7 months, then frozen (−86 °C). To dry samples, I evaporated the excess ethanol by leaving samples overnight under a fume hood and then freeze-dried the samples. I shifted each sample manually through a fine wire mesh, and extracted steroids from the faecal powder using published methanol vortex methods (Wasser *et al.* 2001). Powder and extract were stored at −20 °C.

(d) *Glucocorticoid concentration*

To quantify the concentration of glucocorticoids in each faecal sample, I used an ^{125}I cortisol radioimmunoassay kit (Pantex, Santa Monica, CA), as modified for prior *L. catta* faecal glucocorticoid studies (Cavigelli 1999). Since no chromatographic purification or HPLC analysis of faecal extract was performed, glucocorticoid concentrations reported as 'cortisol' comprise cortisol as well as other faecal steroid metabolites detected by the Pantex cortisol antibody. These concentrations have been shown to correlate with baseline plasma glucocorticoid concentrations in *L. catta* (Cavigelli 1999). Assay sensitivity was 1 ng ml^{-1}.

Samples were assayed in duplicate over 20 assays; intra-assay variation was 6%; inter-assay variation from high, medium and low serum controls was 6, 7 and 18%. Faecal glucocorticoid concentrations are expressed in units of log(nanograms per gram dry faeces) as in prior studies (Cavigelli 1999).

I calculated a single cortisol level for each animal in the following way. To minimize bias due to unequal *ad libitum* sampling of individuals across months, I averaged each animal's mean cortisol level in each month over the study period, so animals were

[†]Present address: Department of Biology, The College of New Jersey, PO Box 7718, Ewing, NJ 08628, USA.

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Table 1. Mean cortisol and sample size in each sex and age class.

	cortisol (ng g^{-1} faeces) of animals that survived	cortisol (ng g^{-1} faeces) of animals that died
adult females:	26.9 ($n=36$, SD = 9.0)	60.1 ($n=6$, SD = 53.5)
dominant females (ranks 1 and 2)	26.0 ($n=13$, SD = 9.8)	77.6 ($n=1$)
subordinate females (all lower ranks)	26.4 ($n=23$, SD = 8.6)	44.8 ($n=5$, SD = 35.3)
adult males	29.2 ($n=26$, SD = 9.3)	41.9 ($n=2$, SD = 22.6)
subadult females	20.4 ($n=5$, SD = 5.9)	50.1 ($n=1$)
subadult males	31.0 ($n=6$, SD = 7.7)	13.2 ($n=1$)
juveniles	27.8 ($n=9$, SD = 18.4)	53.7 ($n=1$)
total	27.8 ($n=82$, SD = 10.3)	51.1 ($n=11$, SD = 41.1)

not over-represented by months in which they were more intensively sampled. To control for changes in cortisol across months caused by natural fluctuations or sample degradation during storage that could confound characterization of individual levels (Khan *et al.* 2002), I expressed animals' cortisol levels in each month as residuals from the population mean cortisol in that month (observed level minus population mean level), and averaged these to obtain a single value for each animal. Positive residual cortisol values indicate that an animal had higher cortisol levels than the rest of the population in the month it was sampled.

(e) *Analyses*

I compared cortisol of animals that survived and those that subsequently died by using a *t*-test, and evaluated cortisol as a predictor of survival using a logit regression. Because the regression would be heavily influenced by two outlier individuals, I performed a further analysis in which I divided the population into two discrete groups, high-cortisol (one standard deviation above the population average; >41 ng g^{-1} faeces) and low-cortisol (the rest of the population). Median cortisol in the high-cortisol group (52 ng g^{-1} faeces) was approximately twice the median level in the low-cortisol group (25 ng g^{-1} faeces). I compared survival of these two groups by using a χ^2 test.

I tested dominance rank as an alternative predictor of females' survival using a logit regression. Dominance ranks were determined by noting the direction of aggression among pairs of females during focal animal observations (three 10 min samples per animal per month). I expressed dominance both as an absolute rank, and as rank relative to the size of the group.

3. RESULTS

Of 93 animals whose survival status was known, 11 died and 82 survived to the end of the study period. All non-infant age and sex categories were represented in both groups (table 1).

Residual cortisol levels were significantly lower in surviving animals (mean ± s.e.m. -0.01 ± 0.02 log ng g^{-1} faeces) than in animals that subsequently died (0.17 ± 0.05 log ng g^{-1} faeces) (Student's *t*-test: $n=93$, $t=3.93$, $p=0.0002$). Probability of survival for non-infant animals decreased with higher residual cortisol (logit regression: $n=93$, $\chi^2=8.786$, $r^2=0.13$, $p=0.003$), although the effect is apparent only at high cortisol levels (figure 1).

Mortality during the 2 year study (figure 2) was significantly higher in the high-cortisol group (42%; 5/12 animals) than in the low-cortisol group (7%; 6/81 animals) (χ^2 test: $n=93$, $\chi^2=8.820$, $p=0.003$; odds ratio = 8.93 (95% confidence interval = 1.7–47.1)).

Female survival did not vary with absolute dominance rank (logit regression: $n=42$, $\chi^2=0.044$, $r^2=0.00$, $p=0.834$) or rank relative to the size of the group (logit regression: $n=42$, $\chi^2=0.794$, $r^2=0.02$, $p=0.373$).

4. DISCUSSION

This is the first study to show that variation in glucocorticoid levels among individuals in a wild population predicts their chances of survival. This result has several implications.

First, it suggests that individual variation in glucocorticoid levels, over the range observed within a small natural population, can be biologically meaningful. Plasma concentrations of circulating hormones can vary over orders of magnitude, so it is not necessarily self-evident that any statistically significant difference in concentration will be a biologically important difference. For example, the logistic regression here shows that faecal glucocorticoid variation at low to intermediate levels does not greatly affect chances of survival. However, these same data show that with a doubling in glucocorticoid concentration, as observed between the low-cortisol and high-cortisol groups, there is enough variation for a sixfold increase in mortality rate. Relatively small differences in individual glucocorticoid levels can be important, particularly at the high end of the naturally occurring range, and are therefore sensitive indicators of survival probability.

Second, it suggests that the continued use of glucocorticoids as non-invasive indicators of population health is warranted. Populations exhibiting high glucocorticoid levels can be expected to have high mortality rates, and glucocorticoid measures may therefore be useful in refining population viability estimates. This finding reinforces prior work showing that differences in glucocorticoid levels among populations can predict their persistence or decline (Romero & Wikelski 2001).

Third, this result suggests that stress can be considered a mortality risk factor in wild populations as it is in humans. Causes of mortality are unknown for animals in this study, so it is impossible to assess whether high glucocorticoids themselves contributed to mortality. The detrimental physiological effects of chronic stress characterized in laboratory studies (Sapolsky *et al.* 2000) often occur at glucocorticoid concentrations much greater than those typically found in natural populations (Romero 2004). The data presented here do allow the possibility that

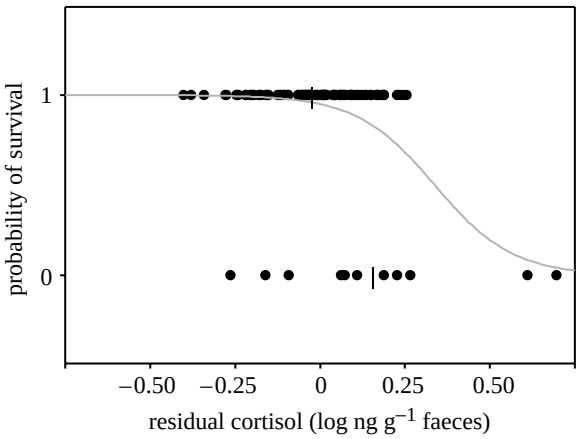

Figure 1. Survival probability decreased with higher cortisol. Vertical bars show median residual cortisol of animals that survived (-0.01 log ng g^{-1} faeces; top row) and those that died (0.11 log ng g^{-1} faeces; bottom row).

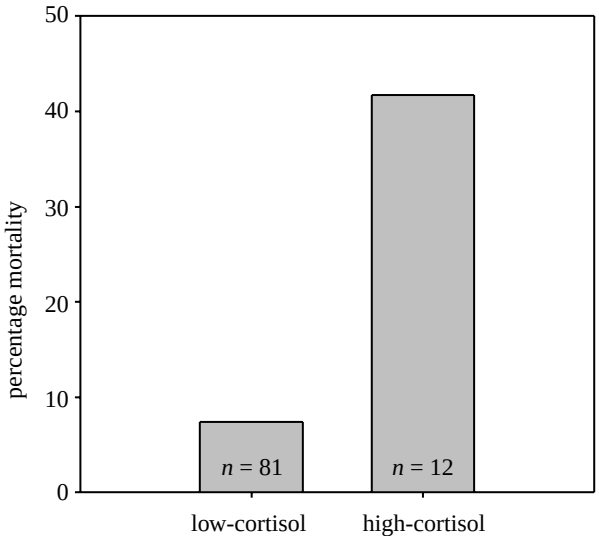

Figure 2. High-cortisol animals showed higher mortality than low-cortisol animals during the 2 year study.

stress-related disorders affected survival in the wild, but high baseline glucocorticoid levels could also simply reflect other survival threats, such as predation or conspecific agonism. In either case, it is clear that high glucocorticoid levels characterize animals with significantly higher chances of dying. Environmental pressures that raise glucocorticoid levels should be considered, directly or indirectly, as potential sources of mortality.

Conclusions regarding the generality of these results will depend on further studies, as many factors potentially confound faecal hormone analysis (Millspaugh & Washburn 2004). Mortality risks vary across species and environments, and some threats may correlate with glucocorticoid levels better than others. Causes of mortality that would correlate best with glucocorticoid levels include those that directly induce a stress response, such as predator attack or conspecific agonism (Goymann *et al.* 2001). Other causes of mortality will correlate to the extent that excessive stress increases the animal's vulnerability to them, such as susceptibility to infectious disease increasing due to glucocorticoid-suppressed immunity. In cases where glucocorticoids are not themselves influencing mortality, glucocorticoid responses to non-lethal stressors could confound correlations with other threats, resulting in a lack of overall predictive power.

In summary, these results provide the first validation of glucocorticoids as predictors of individual mortality in a wild population. Faecal glucocorticoid measurements provide a powerful tool for non-invasively monitoring wild animals. By discerning mortality risks at the level of the individual, wildlife managers may be able to identify 'at-risk' animals, diagnose threats according to which animals are affected, and ultimately design conservation efforts to benefit specifically these segments of the population. In addition, glucocorticoid analysis can help evolutionary ecologists assess the costs and benefits of behavioural, life history or social strategies in circumstances where measuring actual fitness parameters is impractical.

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