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Effects of forest fragmentation on the physiological stress response of black howler monkeys

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Abstract

Habitat loss and fragmentation can create severe environmental problems for forest species, and may ultimately lead to local population extinction. One mechanism that might play a role in this process is the physiological stress response of individuals, because animals that experience chronic stress might show reduced rates of reproduction and survival. In this study, we investigated whether black howler monkeys *Alouatta pigra* inhabiting forest fragments experienced greater physiological stress than those in continuous forest. Faecal cortisol metabolite levels were compared between monkeys inhabiting fragmented and continuous forests in Southern Mexico. The sex of the animals was taken into consideration, while activity patterns and aggressive events were also recorded to examine differences in behaviour and sociality. A chemiluminescent enzyme immunoassay was utilized to determine faecal cortisol metabolite levels. Our results showed that cortisol metabolite levels were higher in howler monkeys from forest fragments, and that they vary through time. Patterns of locomotion also differed between forest types, as monkeys from fragments travelled at higher frequencies, while monkeys from continuous forests performed more of their movements within tree crowns. There was no difference in the frequency of feeding, resting and social interactions between fragmented and continuous forest monkeys. Higher faecal cortisol metabolite levels in fragmented areas are likely the result of suboptimal environments and could have long-term adverse effects on monkey health, fertility and survival. We propose that faecal cortisol metabolite measurements can be a useful conservation tool for monitoring populations of long-lived species subsisting under suboptimal conditions.

Introduction

A main concern for conservation biologists is the decline of biodiversity as a consequence of habitat loss and fragmentation (Fahrig, 2003). This is particularly true for primates, which are highly vulnerable to forest fragmentation (Cowlishaw & Dunbar, 2000; Chapman & Peres, 2001). The survival and persistence of primates in forest patches often depend on their ability to utilize the surrounding vegetation matrix, together with their home range size, their behavioural and dietary plasticity and the nature and intensity of fragmentation (Marsh, 2003). However, fragmentation effects on primate physiology have rarely been considered, even though in recent years habitat degradation has been repeatedly proposed to act as a stressor for wild fauna (Romero, 2004; Wikelski & Cooke, 2006), potentially having long-term negative consequence onto individuals and hence onto populations.

Individual physiology is naturally regulated by periodical environmental cues (e.g. photoperiod and seasonal temperature change). However, environmental perturbations such as ecological events (e.g. storms, predator attacks) or social events (e.g. changes in the social hierarchy) disrupt homeostasis (Reeder & Kramer, 2005), promoting immediate physiological or behavioural adjustments to cope with the problem (Wingfield, 2005). When an organism encounters such environmental stressors, the adrenal gland increases glucocorticoid production (either cortisol or corticosterone), thus increasing gluconeogenesis and energy availability, until the stressor passes (Sapolsky, 2002). This reaction helps the individual to overcome emergency situations and to re-establish homeostasis (Sapolsky, Romero & Munck, 2000). However, if the perturbations persist over a long time, the sustained high levels of circulating glucocorticoid hormones can have negative effects on the health of an organism, suppressing proper functioning of the immune system, promoting severe protein loss (muscle wasting), inhibiting growth, producing neurone cell death and inhibiting reproductive functions (Sapolsky & Pulsinelli, 1985; McEwen, 2000). As a result, the stress response can be used to monitor potential threatened animal populations (Homan *et al.*, 2003; Romero, 2004).

Table 1 Group compositions, total hours of observation and faecal cortisol metabolite levels (ng g^{-1}; mean ± SE) of black howler monkeys *Alouatta pigra* from Forest Fragments and Continuous Forest

	Age class[a]	Group 1	Hours of observation	Mean (± SE)	Group 2	Hours of observation	Mean (± SE)
Forest Fragments	Adult	SA ♂	32.8	591 ± 161	CB ♂	19.0	421 ± 42
		CH ♂	34.3	328 ± 26	RA ♂	19.2	340 ± 47
		TO ♀	32.2	314 ± 32	FR ♂	18.6	358 ± 25
		MI ♀	29.8	438 ± 36	DE ♀	18.6	380 ± 31
					MA ♀	19.3	422 ± 83
	Juvenile	0			1♂		
	Infant	1♀			1♀		
	Newborn	2			2		
	Total	7			9		
Continuous Forest	Adult	BA ♂	34.9	299 ± 6	ME ♂	19.2	297 ± 35
		CI ♀	36.0	380 ± 58	CA ♂	17.3	320 ± 54
		XO ♀	33.9	290 ± 20	MO ♂	14.4	321 ± 63
					PE ♀	17.3	291 ± 19
					PA ♀	16.3	298 ± 17
					JO ♀	13.8	317 ± 31
	Juvenile	1 ♂			1♂, 1♀		
	Infant	0			1♀		
	Newborn	2			2		
	Total	6			11		

[a] Observations and faecal cortisol metabolite measurements were restricted to adult individuals. Number of individuals of other age classes and total number of individuals per group included.

Cortisol (the main glucocorticoid hormone in primates) has been used previously to determine primate physiological responses to a variety of different stressors, including psychological stress (e.g. Norcross & Newman, 1999), social stress (e.g. Muller & Wrangham, 2004) and physical stress (e.g. Muller & Wrangham, 2004). However, few attempts have been made to use cortisol to evaluate environmental stress following habitat fragmentation (Chapman *et al.*, 2006).

The aim of this study was to determine whether black howler monkeys *Alouatta pigra* experience higher levels of cortisol when inhabiting forest fragments than when in a continuous forest. Although *Alouatta* has previously been described as a primate genus that shows a relatively high tolerance to human disturbance (Silver & Marsh, 2003), the black howler monkey *A. pigra* is the most threatened species of the genus because of its restricted distribution and its exposure to high rates of deforestation (Horwich, 1998; Aguilar, Martínez & Arriaga, 2000). The effects of this disturbance on its physiology may be deleterious in the long term.

Methods

Study site and subjects

We studied four groups of black howler monkeys from October 2002 to May 2003, sampling each one of them in four different occasions. The first two groups inhabited small forest remnants (< 2 ha, hereafter 'Forest Fragments') at ejido Leona Vicario, Balancán (17°48′ N; 91°32′ W) in the State of Tabasco, Mexico. In this area, clearance for cattle ranching and agriculture has been common since 1972 and the annual deforestation rate is estimated at 2% (Aguilar *et al.*, 2000; Pozo-Montuy, 2006). Annual precipitation is 1850 mm, and the mean temperature is 26.0 °C (Moguel-Pérez, 2005). The two other groups were located in a large forested area (1400 ha, hereafter 'Continuous Forest') at Campo Experimental Forestal of Instituto de Investigaciones Forestales, Agrícolas y Pecuarias (18°16′ N; 90°43′ W) Escárcega in the State of Campeche, Mexico. Here, the annual precipitation is 1380 mm and the mean temperature is 24.1 °C (Barrueta Rath *et al.*, 2003).

Howler monkeys in both areas are accustomed to people and one of the groups in Balancán has been monitored previously (Pozo-Montuy & Serio-Silva, 2007). The individual identification of each animal was performed before the beginning of formal sampling. Our study focused on adult males and females (Table 1): in Forest Fragments, the female reproductive condition was variable [births were recorded in October (female TO) and December (MI and MA)], while in Continuous Forests all females were lactating and no additional births were registered.

Behavioural data collection

In order to obtain behavioural data and one faecal sample from each identified individual, every howler monkey group was followed for five consecutive days during each sampling period. By recording the behaviours during the days before faecal collection, we were able to get a 'picture' of the social environment of each group.

A 2 min instantaneous focal-animal sampling method was used to record daily activity patterns (Altmann, 1974). Each set of focal animal observations lasted 1 h, followed by a change of subject, allowing us to cover the entire group at least twice during each sampling day. When the animals were out of sight of the observer for 10 min, the focal sampling was stopped, and started again with another visible subject. Table 1 summarizes the total hours of observation per individual. Each group was observed on average for 106.7 ± 7.8 h (mean ± SE). We recorded the following activities: feeding, resting, travelling (locomotion of animals from one tree to another in order to start a new activity), moving (short locomotion bouts within the tree crown) and social interactions. Social interactions included both affiliative and agonistic behaviours, which encompass playing, embracing and sexual behaviour for the former, and branch breaking, howls and chases for the latter.

Faecal collection and hormonal assay

We restricted the faecal collection to the morning of the last sampling day, usually collecting the first daily deposition from each adult. Faecal samples were labelled with the ID of each individual, kept in a cooler with frozen gel packs while in the field and in a domestic freezer once back in the camp. At the end of every field period, faecal samples were transported and stored in a freezer at −20 °C at the Instituto de Ecología, A.C., Xalapa, Mexico. Samples were stored for 3–10 months until the assay was conducted. We used a freezing procedure for storing the samples because it has been reported to have less time-storage effect on faecal glucocorticoid metabolites (Khan *et al.*, 2002). Overall, we obtained a total of 72 faecal samples.

Cortisol metabolites were extracted from faeces according to the procedure described by Valdespino, Asa & Bauman (2002). Briefly, 5.0 mL of phosphate/methanol buffer were added to vials that contained 0.5 g wet weight of each faecal sample and shaken overnight. Faecal suspensions were centrifuged at $800 \times \boldsymbol{g}$ for 60 min and the supernatants were decanted in cryotubes. The sediments of faecal material, together with the residuals from the vials, were dried in an oven overnight at 100 °C to obtain the dry weight of the samples.

Black howler monkeys are under protection in Mexican zoos and invasive practices other than anaesthesia with health-related purposes are proscribed. As a consequence, we were unable to apply an ACTH challenge, followed by HPLC to validate measuring native cortisol in faeces. However, there are studies on New World primates (i.e. *Callithrix jacchus*) that have found an increase in native cortisol (measured by commercial antibodies) after the application of a stressor (Heistermann, Palme & Ganswindt, 2006). Therefore, faecal cortisol metabolite levels were measured by a solid-phase chemiluminescent enzyme-immunoassay method (Wood *et al.*, 1984), using cortisol kits (Immulite Cortisol® Diagnostic Products Corporation, Los Angeles, CA, USA) and an automated analyser (Immulite®, Diagnostic Products Corporation, Los Angeles, CA, USA). The assay sensitivity was $0.2\,\mu g\,dL^{-1}$. The coefficient of variation for both intra-assay and inter-assay was 14.9 and 16.4%, respectively ($n = 6$, run in triplicate). We verified linearity assaying serial dilutions of faecal extracts, and we compared observed versus expected values with a *t*-test. We did not find any significant differences between observed and expected values of serial dilutions ($t = -0.89$, d.f. = 88, $P = 0.371$). Cross-reactivities with other compounds reported by kit manufacturers are: corticosterone 8.6%, fludrocortisone 0.20%, methylprednisolone 21%, prednisolone 49%, prednisone 5.9%, tetrahydrocortisol 0.90% and triamcinolone 0.022%. Values are reported in nanograms per gram of dry faeces weight ($ng\,g^{-1}$) to control for dietary effects on steroid excretion (Wasser *et al.*, 1993).

Data analysis

Faecal cortisol metabolite values were not normally distributed; therefore, they were log-transformed to fit parametric assumptions. They were then analysed with a general linear model repeated-measures ANOVA, with forest type and sex as independent variables, the group nested in the forest type and the sampling session as the repeated measure.

The time budgets of individuals in each group were determined by dividing the number of instantaneous scans in which an individual was recorded engaging in a particular behaviour by the total number of scans for that individual, and multiplied by 100. For each behaviour, we tested for differences in group time budgets using a Kruskal–Wallis test, with group as the independent variable. Affiliative and agonistic interactions were pooled as a single social interaction category and analysed using this test as well.

All statistical analyses were implemented on Statistica version 6 (StatSoft Inc., 2001), with a $P = 0.05$ level of significance. In all cases, two-tailed tests were used.

Results

Faecal cortisol metabolite levels from howler monkeys differed according to forest type (Table 2), with monkeys from Forest Fragments showing higher levels (mean ± SE = $399 \pm 23\,ng\,g^{-1}$) than those from Continuous Forest

Table 2 Results of the repeated measures ANOVA of faecal cortisol metabolite levels

Effect	d.f.	*F*	*P*-value
Forest type	1	8.57	0.015
Sex	1	0.01	0.942
Forest type × sex	1	0.18	0.677
Group (forest type)[a]	2	0.10	0.903
Group (forest type) × sex[a]	2	0.47	0.639
Residual	10		
Sampling	3	9.55	0.0001
Sampling × forest type	3	2.03	0.130
Sampling × sex	3	1.13	0.350
Sampling × forest type × sex	3	0.57	0.638
Sampling × group (forest type)[a]	6	0.12	0.993
Sampling × group (forest type) × sex[a]	6	1.40	0.247
Residual	30		

[a] Group nested in forest type.

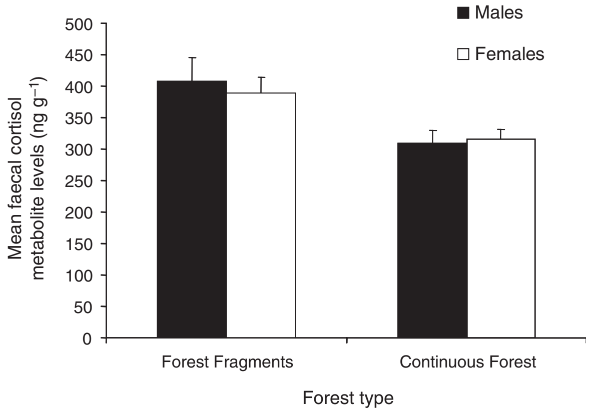

Figure 1 Differences in faecal cortisol metabolite levels (ng g^{-1}) between black howler monkeys *Alouatta pigra* from Forest Fragments and Continuous Forest. Means and standard errors for males and females in each forest type are shown.

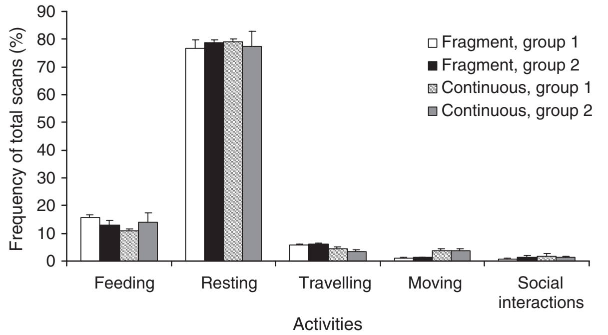

Figure 2 Relative frequencies of activity of four black howler monkey *Alouatta pigra* groups. Group means and standard errors are shown for each activity. White and black bars represent groups 1 and 2 of Forest Fragments, respectively; striped and grey bars correspond to groups 1 and 2 of Continuous Forest, respectively.

(313 ± 14 ng g^{-1}) (Fig. 1). Furthermore, there were differences in faecal cortisol metabolite levels through time (Table 2), with higher values for both monkeys from Forest Fragments and Continuous Forests during the first months of sampling (October–March) than in the last months (April–May). Cortisol metabolites did not differ between males and females, and the interactions among factors were also not significant (Table 2).

There were no differences in the frequencies of feeding, resting and social interactions among groups of howler monkeys from either forest type. However, monkeys in Forest Fragments spent more time travelling (Kruskal–Wallis test: $H = 11.41$, d.f. = 3, $n = 18$, $P = 0.009$), while monkeys in Continuous Forest spent more time moving within tree crowns (Kruskal–Wallis test: $H = 11.80$, d.f. = 3, $n = 18$, $P = 0.008$) (Fig. 2). Across the four groups, the most common activity was resting, followed by feeding and travelling (Fig. 2). Social interactions occurred at very low frequencies.

Discussion

Our results indicate that black howler monkeys living in Forest Fragments had higher faecal cortisol metabolite levels than those in Continuous Forest habitat. Similarly, in a variety of other vertebrate taxa, environmental disturbances, or poor-quality habitats, have been reported to influence the stress response and increase glucocorticoid hormone production (e.g. Marra & Holberton, 1998; Creel *et al.*, 2002; Homan *et al.*, 2003). The precise mechanism that is responsible for elevating cortisol metabolites in monkeys inhabiting Forest Fragments remains to be identified, but there are several possibilities.

First, the process of forest fragmentation may have reduced food quality and/or availability. Forest fragmentation considerably alters the arboreal structure, floristic composition and diversity of plant species in the habitat, and has been shown to reduce the most important food resources for mantled howler monkeys *Alouatta palliata* (Arroyo-Rodríguez & Mandujano, 2006*a*). In addition, low fruit intake has been associated with higher cortisol levels in ring-tailed lemurs *Lemur catta* (Pride, 2005). Although feeding time did not differ between monkeys in Forest Fragments and monkeys in Continuous Forest, it remains possible that the howler monkeys were exploiting lower quality food resources. Indeed, the monkeys in Forest Fragments appeared visibly smaller than those in Continuous Forest, which may be indicative of nutritional stress. Second, Forest Fragments tend to be associated with canopy discontinuity, forcing monkeys to descend to the ground or use barbwire fences in order to reach other trees. One of the Forest Fragment groups in this study spent 11% of their locomotion time walking on the ground (Pozo-Montuy & Serio-Silva, 2007), which is likely to make them more susceptible both to infection from anthropogenic diseases (Gillespie, Chapman & Greiner, 2005), and to predation by domestic dogs and coyotes (G. Pozo-Montuy, pers. obs.). Finally, it is possible that monkeys inhabiting Forest Fragments exhibit higher faecal cortisol metabolite levels from a combination of local disturbance effects, including grazing, logging and human presence. Because reduction in forested areas in Balancán due to anthropogenic activities has been continuous over the last three decades, the likelihood that faecal cortisol metabolite levels in these monkeys are the result of just a transient stress response is low.

Faecal cortisol metabolite levels were also found to vary significantly through time. Glucocorticoids in animals may naturally vary with seasons (Romero, 2002), and our result may reflect such a natural change. For example, in this study, faecal cortisol metabolite levels were higher during the cooler months. The need for increased energy availability for thermoregulation could promote this seasonal rise. Further studies should address this seasonal variation in greater detail.

We did not find any significant differences in faecal cortisol metabolite levels between males and females, despite the fact that changes in the ovarian steroids or reproductive status of females are known to influence cortisol levels (e.g. Cynomolgus macaques *Macaca fascicularis*, Stavisky *et al.*, 2003; golden-lion tamarins *Leontopithecus rosalia*, Bales *et al.*, 2005). Our failure to detect any differences between males and females may be due to small sample sizes and the relatively short interval between sampling sessions.

No differences were found in the daily activity patterns for feeding, resting and social interactions. However, a difference was found in locomotion, with monkeys in Forest Fragments spending more time travelling but less time moving within tree crowns. These monkeys probably spend more time travelling because they must change trees more often in order to satisfy daily feeding or resting requirements; however, they may spend less time moving within tree crowns because these are smaller in Fragmented Forests, limiting flexibility in their use. It is also worth noting that social interactions among howler monkey individuals were performed at a low rate. This suggests that faecal cortisol metabolites levels were not influenced by overt aggressive events among individuals.

Although black howler monkeys have the ability to survive in degraded environments like the Forest Fragments in this study, the physiological effects that a chronic stressful situation may have on them could affect their health and survival, and, in the long term, may have a repercussion on population viability. Conservation actions that could be taken to ameliorate these effects, both in the forest fragments of Balancán, State of Tabasco, and elsewhere, could include an increase in connectivity among forest patches (in order to minimize the effect of canopy discontinuity and to increase the availability of resources) and the active conservation of larger patches (in which a higher number of large trees and tree species similar to those in undisturbed forests are likely to be found: Arroyo-Rodríguez & Mandujano, 2006*b*). Finally, the way in which anthropogenic disturbance is affecting the health of primates and other tropical taxa is a topic deserving a great deal more attention. Our study indicates that faecal cortisol metabolite measurements can be a powerful tool for monitoring populations of species with slow life histories that may be subsisting under conditions that will not ensure their long-term survival.

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