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The hormonal and behavioral response to group formation, seasonal changes, and restraint stress in the highly social Malayan Flying Fox (*Pteropus vampyrus*) and the less social Little Golden-mantled Flying Fox (*Pteropus pumilus*) (Chiroptera: Pteropodidae)

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Abstract

This study examined behavioral and physiological responses (changes in inter-animal spacing, total glucocorticoids, testosterone, and body mass) to the formation of breeding and same-sex groups in two bat species, the socially gregarious Malayan Flying Fox (*Pteropus vampyrus*) and the less social Little Golden-mantled Flying Fox (*Pteropus pumilus*). We hypothesized that social instability, especially in the breeding groups and especially in *P. vampyrus*, would result in elevated glucocorticoids and that social facilitation of breeding and/or male–male competition would result in persistently higher levels of testosterone in breeding males. Seasonal rhythms in all measures were also predicted, and the glucocorticoid stress response was expected to vary by sex, season, and group type. Nearly all animals responded to group formation with elevated glucocorticoids, but, for breeding animals (especially aggressive male *P. vampyrus*), these responses persisted over time. In both species, breeding group formation resulted in elevated testosterone in males. Glucocorticoids, testosterone, testes volume, and body mass generally peaked in the breeding season in males (late summer and early autumn), but the seasonal glucocorticoid peak in females occurred in late winter and early spring. All animals responded to restraint stress with elevations in glucocorticoids that largely did not differ by sex, time of year, reproductive condition, group type, or, in lactating females, the presence of her pup. Changes in both behavior and physiology were more evident in *P. vampyrus* than in *P. pumilus*, and we believe that their underlying social differences influenced their responses to group formation and to the changing seasonal environment.

Keywords: Corticosterone; Cortisol; Group formation; Hypothalamic–pituitary–adrenal axis; Inter-animal spacing; *Pteropus*; Seasonality; Social influences; Stress; Testosterone

Successful reproduction involves a number of factors, including the regulation of various hormonal systems, which often must be accomplished within a changing physical and social environment. Although there are many different neuroendocrine components involved in regulating reproduction and the response to changing environments, the hypothalamic–pituitary–adrenal (HPA) axis and the hypothalamic–pituitary–gonadal (HPG) axis are particularly important (Reeder and Kramer, 2005; Romero, 2002; Wingfield and Sapolsky, 2003). Glucocorticoid hormones (cortisol and corticosterone, the endpoint of the HPA axis) are important regulators of energy balance, and increases in glucocorticoids are considered a hallmark of the stress response (Reeder and Kramer, 2005). The HPA and HPG axes interact with one another in complex ways and serve, in part, to regulate behavior (DeVries, 2002; Handa et al., 1994; Viau, 2002). In turn, behavioral processes can have profound influences on these and other physiological systems (Eherhart et al., 1983; Mendoza and Mason, 1989a). In many species, animals must navigate complex social environments in order to reproduce, often forging social relationships with both known and unknown individuals. Moreover, the natural social and/or mating system in which an animal typically finds itself has profound implications for how an individual approaches and responds

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to these social relationships (Mendoza and Mason, 1989b), both behaviorally and physiologically.

The current study evaluated the behavioral and physiological responses to breeding group formation and to the formation of same-sex groups. We also studied the basic character of the stress response and how this response varies by time of year and reproductive condition. These studies were performed comparatively, using two species of frugivorous flying foxes (Chiroptera: Pteropodidae: *Pteropus*) as model systems. The Malayan Flying Fox (*Pteropus vampyrus*) is a large pteropodid bat, weighing ~1.1 kg with a wingspan of ~1.5 m. *P. vampyrus* is socially gregarious and roosts in groups of tens to thousands throughout Southeast Asia. *P. vampyrus* is a highly seasonal and synchronous breeder, with most females giving birth to a single pup in the spring (Kunz and Jones, 2000). The second species, the Little Golden-mantled Flying Fox (*Pteropus pumilus*) is a relatively small pteropodid bat found in the Philippines, weighing ~200 g with a wingspan of ~0.3 m. Although many species of *Pteropus* are highly social and can be found in groups of up to several hundred or even thousand animals, *P. pumilus* roosts in small aggregations with infrequent social interactions (L.R. Heaney, personal communication; Mickleburgh et al., 1992). They display only mildly seasonal breeding and may give birth to a single pup once or possibly twice per year. Given their different social tendencies, these two species of pteropodid bats are excellent models for exploring social influences on mammalian physiology, as well as the general physiology of the HPA and HPG axes, including seasonal rhythms of these hormones and the response to stress. Both species breed relatively well in captivity, and they have some of the highest plasma glucocorticoid levels ever described in mammals (Reeder and Kramer, 2005; Widmaier and Kunz, 1993; Widmaier et al., 1994).

In general, we hypothesized that the formation of breeding groups would transiently increase glucocorticoid levels in both sexes due to social instability and testosterone levels in males, due to social facilitation of breeding and/or male–male competition relative to the control groups (Capitanio et al., 1998; Schiml et al., 1996; Soto-Gamboa et al., 2005; Wingfield et al., 1990). Additionally, we hypothesized that, after an initial adjustment period, all animals would exhibit clear hormonal seasonal rhythms, as well as changes in body mass and changes in testes volume, that would vary by group type. These physiological changes would be accompanied by behavioral changes, here measured by changes in inter-animal spacing between different types of animals (e.g., between breeding males and females) over time. Changes were expected to be of greater magnitude in *P. vampyrus*, due to the predicted greater frequency of social interactions. Lastly, variability in the ability to mount a response to restraint stress by time of year and reproductive condition was assessed in all subjects. Because the glucocorticoid response to stress is superimposed upon baseline circadian and seasonal glucocorticoid rhythms (Reeder and Kramer, 2005; Romero, 2002), we hypothesized that a greater stress response would be evident in the fall during the mating season (when baseline glucocorticoids were also expected to be high and when groups were predicted to be less stable socially) than during the spring. We further hypothesized that pregnant and lactating females would show a blunted stress response that would be mediated by the presence of pups, as occurs in laboratory rats (Lightman et al., 2001; Stern et al., 1973).

Materials and methods

Animals and animal care

Subjects for this study included 48 *P. vampyrus* (22 males and 26 females) and 32 *P. pumilus* (16 males and 16 females). Animals were housed in captivity at the Lubee Bat Conservancy in Gainesville, Florida, USA. All of the research described in this paper was covered by an IACUC protocol from the Lubee Bat Conservancy (USDA Research Facility #58-R-0131; American Zoo and Aquarium Association Certified Related Facility #RF-4250000). Animals were housed in octagonal, double wire enclosures, measuring approximately 11 m in diameter and 2 m high. Enclosures contained an outdoor portion that encircled a smaller inside roost ("the nighthouse"; 3 m in diameter and temperature-controlled). Enclosures were designed to maximize the ability of bats to feed, rest, and fly freely. Animals were both wild-caught and laboratory-born and were all reproductively mature at the beginning of the study. Each bat was easily identified by a combination of at least three of the following markers: colored thumb bands, numbered thumb bands, ventral and dorsal fur bleaching of unique symbols, color-coded ball-chain necklaces enclosed in surgical tubing (LeBlanc et al., 2002), and uniquely coded transponders (Trovan, Santa Barbara, CA) implanted beneath the skin in the mid-scapular region. Animals were fed a mixture of fresh fruits, vegetables, and monkey chow (Purina) daily at 15:00 h. There was at least one food bowl per subject, and bowls were spaced sufficiently apart to minimize competition. Water was available ad libitum.

Group formation procedures

Subjects in each species were divided into 4 groups: 2 breeding groups (*P. vampyrus*: each had 5 males and 7 females; *P. pumilus*: each had 4 males and 4 females), and 2 same-sex control groups ($N = 12$ for *P. vampyrus* and 8 for *P. pumilus* for each sex). Based upon these groups, final samples sizes for subject type were: breeding females (*P. vampyrus*, $N = 14$; *P. pumilus*, $N = 8$), breeding males (*P. vampyrus*, $N = 10$; *P. pumilus*, $N = 8$), control females (in an all female group; *P. vampyrus*, $N = 12$; *P. pumilus*, $N = 8$), and control males (in an all male group; *P. vampyrus*, $N = 12$; *P. pumilus*, $N = 8$). The different sample size for breeding males and females in *P. vampyrus* was necessitated by the desire to reduce conflict between breeding males in this highly aggressive species.

Procedures for establishing groups varied slightly between the two species due to differences in colony management. In *P. vampyrus*, for the purposes of providing stability prior to the beginning of the study, pre-study same-sex groups containing all 48 subjects were formed in July of 2002 (2 male groups with 11 animals each and 2 female groups with 13 animals each). In mid-October, 2002, breeding and control study groups were created by rapidly moving animals from their pre-study groups into their assigned breeding and control study groups. Assignment of animals to study groups was randomized and balanced for age and size to the best of our ability, but management decisions necessitated the placement of some older (and larger) males into the control group in order to allow some younger, wild-caught males to breed. In *P. vampyrus*, the two breeding pens were adjacent (shared an opaque wire-mesh wall on one side), but the male and female control pens were as far apart as possible and not in visual contact (~40 m apart; on opposite ends of the complex).

In *P. pumilus*, same-sex pre-study groups were formed in early October, 2001, which was approximately 3.5 weeks prior to the formation of experimental groups. Animals were randomly assigned to each group. In this species, the two breeding groups were formed in late October by moving half of the animals from the pre-study same-sex groups into new enclosures. For this smaller species, the large octagonal pens were divided in half by the inclusion of a wire wall with an opaque shade-cloth covering, and the two breeding groups were adjacent to one another, on opposite sides of the wall. The two same-sex control groups were comprised of the animals remaining in the pre-study, same-sex groups (so 'group formation' for control animals in this species was not the formation of new social groups, but rather a social manipulation (the removal of some animals), which