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# Testosterone is associated with harem maintenance ability in free-ranging grey-headed flying-foxes, *Pteropus poliocephalus*

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Males of many vertebrate species aggressively defend their reproductive interests by monopolizing females, and the ‘challenge hypothesis’ predicts that testosterone levels in reproductive contexts rise to facilitate males’ competitive behaviours necessary for meeting social challenges. The hypothesis is successful in explaining patterns of testosterone secretion in many avian species, but remains comparatively unexplored in mammals. ‘Circulating plasma testosterone levels (T)’ were studied in relation to harem maintenance in grey-headed flying-foxes, *Pteropus poliocephalus*. In this species, harems provide mating opportunities and so a male’s ability to maintain a harem is likely to correlate with his fitness. We hypothesized that if T reflect a male’s ability to withstand challenges from competitors, then T should be linked to successful harem maintenance. To test this, we temporarily removed males from their territories prior to and during the short mating period, recording their harem sizes both before removal and after reintroduction. Most males successfully reclaimed their territory and a harem, but during the mating period, males with higher T had harems closer to their original size, and males with lower T suffered reduction in harem size. Our findings highlight the role of T in harem maintenance in a major mammalian taxon with complex forms of social organization.

**Keywords:** territoriality; androgens; fruit bat; palaeotropical

## 1. INTRODUCTION

Social organization is the outcome of complex trade-offs between individual foraging needs, defence against predation and mating (Stearns 1992). ‘Circulating plasma testosterone levels (T)’ mediate social interactions through persistence-promoting effects on aggressive behaviour during the formation of territories, dominance disputes and mate-guarding (Adkins-Regan 2005; Archer 2006). Higher T have

fitness costs as well as benefits: high T may promote mating effort through social dominance, territoriality and access to mates (Rohwer & Rohwer 1978; Alatalo *et al.* 1996), but it may also suppress immunity (Malo *et al.* 2009; but see Boonekamp *et al.* 2008) and parental effort (Hegner & Wingfield 1987). According to the ‘challenge hypothesis’ (Wingfield *et al.* 1990), T are highest during periods of social instability, for example, when territories are established or defended, when aggression between males is common and during peak female receptivity. T may rise further if challenges to a male’s social status occur, facilitating competitive behaviours that are necessary for meeting the challenge. The hypothesis has found support in a wide range of vertebrates, mainly birds, yet it remains relatively unexplored in mammals (review by Archer 2006; Moore 2007).

Here, we examine the role of T in harem maintenance of grey-headed flying-foxes, *Pteropus poliocephalus*, in the wild. This endangered temperate to subtropical fruit bat (Hall & Richards 2000) forms seasonal breeding colonies of thousands of individuals. Males attract and defend (‘maintain’) groups of up to six females (‘harem’) in mating territories. Centrally located males are larger and have more mating opportunities than peripheral ones, and territorial aggression, associated with frequent injury, intensifies towards the mating period (March–May; the most likely time of conception is April, when there is a concomitant peak in mating activity (see Nelson 1965; Martin 1995; Welbergen 2005)). T peak around this time (McGuckin & Blackshaw 1991). As T are higher in droppings of dominant harem males than in subordinates of the tropical bat *Saccopteryx bilineata* (Voigt *et al.* 2007), we hypothesized that if T reflects a male’s ability to withstand social challenges from competitors for mates, then T should be linked to harem maintenance success. To test this, we temporarily removed *P. poliocephalus* males from their mating territories and examined the relationship between T and the ability of individual flying-fox males to reclaim a harem from conspecific competitors.

## 2. MATERIAL AND METHODS

### (a) *Bats and experimental protocol*

We conducted our study at Dallis Park colony near Murwillumbah, New South Wales, Australia (see Welbergen *et al.* 2008). At the beginning of the reproductive season, from January to April 2006 (‘pre-mating period’, late January to mid February; ‘mating period’, late March to mid April; Klose *et al.* 2009), 36 roosting adult males ( $\geq 2$  years old) actively defending mating territories were randomly selected from the central colony area, assigned to one of the two study periods and sampled independently. This was because residents were known to be centrally located (Welbergen 2005). The number of adult females (‘harem size’; easily recognized by the formation of relatively tight clusters) in each male’s territory,  $3.3 \pm 1.6$  body lengths along tree branches (Welbergen 2005), was recorded daily between 12.00 and 16.00 AEST (Australian Eastern Standard Time), at least twice over 72 h. Males were subsequently caught between 13.00 and 17.00 AEST using a capture noose device (Welbergen 2005). A wing vein blood sample ( $\geq 200$   $\mu$ l) was obtained using an insulin syringe within 180 s of capture. Plasma was separated and stored as described previously (Klose *et al.* 2006). Males were marked with colour steel bands and individually housed nearby for 72 h, with ad libitum access to a standardized fruit diet and water. From a platform overlooking the colony, bats were released in the late afternoon. We followed each bat using binoculars as it returned to the colony, identifying the exact tree where it landed. Twenty-four hours post-release, we again recorded the presence of the respective males and harem size in their original territory,

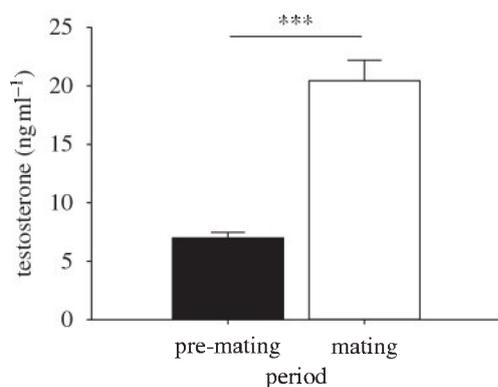


Figure 1. T (mean  $\pm$  s.e.m.) in male *Pteropus poliocephalus* in pre-mating ( $n = 16$ ) and mating ( $n = 20$ ) periods. \*\*\* $p < 0.001$ .

at least twice within 72 h. Harem size counts were used to calculate 'harem size change' as the percentage difference between the median number of females in the male's territory before and after experimental removal. Failure of a male to return to his territory was counted as a complete loss of the harem.

#### (b) Hormone analyses

T were measured by direct duplicate <sup>3</sup>H radioimmunoassay (RIA; Wingfield & Farner 1975). Briefly, steroids were extracted twice with redistilled dichloromethane. Testosterone was isolated by stepwise polarity-based celite column chromatography using a solvent gradient of ethyl acetate (EA) in iso-octane (IO). Testosterone was eluted at 20 per cent EA in IO, dried under nitrogen and resuspended in phosphate-buffered saline. Tracer (NET-553 [1,2,6,7,16,17-<sup>3</sup>H(N)], Perkin Elmer, USA) added to the initial sample allowed for correction of extraction and separation losses. The RIA was performed using highly specific antibodies (T-3003, Fitzgerald Industries, USA), activated dextran-coated charcoal count separation and an assay-specific standard curve. Each RIA contained two zero standards (doubly distilled water, 'blanks'), three-pooled bat plasma and three buffer standards, each containing a known amount of steroid (T-1500, Sigma, Germany). Recovery was  $75.01 \pm 5.46$  per cent ( $n = 36$ ), the lower limit of detection (assay sensitivity)  $0.03 \text{ ng ml}^{-1}$  and accuracy  $95.04 \pm 0.34$  per cent by recovery of known lower standards plus a pool of chiropteran plasma (approx. 0.2 ng). The inter-assay coefficient of variation (CV) was 6.51 per cent and the intra-assay CV 4.25 per cent. Blanks were below assay sensitivity. T did not differ between the beginning and end of captivity in a separate sample of bats (paired samples *t*-test,  $t = -0.72$ ,  $p = 0.496$ ,  $n = 7$ ), so it is unlikely that androgen levels were influenced by captivity. Unlike glucocorticoids, T did not increase in flying-foxes in response to restraint stress (Reeder *et al.* 2006).

Statistical tests were carried out in PASW (Windows v. 17.0). Data were assessed for normality and constant error terms, and transformed if necessary. An independent samples *t*-test compared T between reproductive periods. General linear mixed modelling (GLMM) procedures were used to determine the effects of T on the harem variables (harem size and change in size were independent,  $r_p = 0.238$ ,  $p = 0.162$ ,  $n = 36$ ), with random factor 'individual bat', fixed factor 'period' and covariate 'T'. Average values are reported as the mean  $\pm$  s.e.m.

### 3. RESULTS

T in the mating period were significantly higher than in the pre-mating period ( $t = -6.70$ ,  $p < 0.001$ ,  $n = 36$ ; figure 1). As predicted, there was a significant effect of T on harem size both before removal of the male from the colony (GLMM;  $F_{1,36} = 23.008$ ,  $p < 0.001$ ) and after removal (GLMM;  $F_{1,36} = 18.743$ ,  $p < 0.001$ ). Males with higher T had larger harems in both cases. The change in harem size, however, varied with T differently depending on the mating

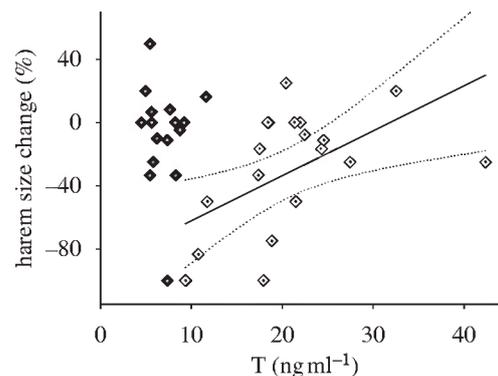


Figure 2. Experimentally induced short-term change in harem size in male *Pteropus poliocephalus* in relation to T during pre-mating ( $n = 16$ ; filled diamonds) and mating ( $n = 20$ ; open diamonds) periods.

period (GLMM;  $F_{1,36} = 4.041$ ,  $p < 0.046$ ; figure 2), with males with lower T experiencing a more pronounced harem size change in the mating period than the pre-mating period ( $-32.4 \pm 9.0\%$  versus  $-7.2 \pm 8.1\%$ , respectively).

Within 72 h of removal of the focal males, territories were either divided among neighbours ( $23/36 = 63\%$ ), occupied by a new male ( $12/36 = 34\%$ ) or remained unoccupied ( $1/36 = 3\%$ ). Following release, focal males headed straight back into the colony; many ( $24/36 = 67\%$ ) landed within 20 m of their original territory, mostly in their original tree ( $20/24 = 83\%$ ). The majority ( $32/36 = 89\%$ ) of focal males re-occupied their original territory within 24 h, three (8%) failed to return, and one returned without re-establishing a harem.

### 4. DISCUSSION

In accordance with our predictions, T in male *P. poliocephalus* was associated with harem maintenance ability. We observed a distinct seasonal variation in T. Levels were much lower during the pre-mating period than during the mating period, as would be expected under the 'challenge hypothesis'. T varied significantly with harem size, as described in feral horses (Khalil *et al.* 1998), but most intriguingly, mating period males with higher T performed better in re-acquiring harems after the experimental challenge than males with lower T. As in other species (Rohwer & Rohwer 1978; Li *et al.* 2004; Muller & Wrangham 2004; Voigt *et al.* 2007), T probably affects reproductive access through effects on territoriality and social dominance, and although we did not directly measure aggression, previous work has highlighted aggression peaks in the mating period (Welbergen 2005). Our findings provide a functional explanation for seasonal T variation in grey-headed flying-foxes (McGuckin & Blackshaw 1991) and highlight general relevance of T for harem maintenance.

From a conservation perspective, these findings are significant because they impinge on the current management practices for urban flying-foxes. Attempts to relocate colonies by ongoing disturbance

(Thiriet 2005) currently focus on the time of male competition to avoid disturbance of females with young. Here, we have experimentally confirmed high site fidelity (Welbergen 2005); males will return to their original territories even after days of captivity. Relocation attempts are thus unlikely to be successful. *Pteropus poliocephalus* is recognized as being in decline (Lunney *et al.* 2008; Welbergen *et al.* 2008), so alternative, suitable forest patches should be developed to attract bats upon return from annual migration.

## 5. CONCLUSION

Our study supports the 'challenge hypothesis' in Chiroptera, a mammalian key taxon in which it has not previously been tested (Archer 2006). This is, to our knowledge, the first study to demonstrate that a species with complex harem-type social organization shows an association between natural variation in T and a male's ability to maintain reproductive access. The generally rarely demonstrated relationship (Adkins-Regan 2005) supports the Wingfield *et al.* (1990) idea of a wider applicability of the hypothesis, which was built specifically upon evidence in birds. We have confirmed that it is also applicable to bats. T dynamics closely resembled those of previously studied temperate-zone vertebrates; however, we found that T and ability to maintain a harem were linked in the context of competition for mates. The long-term relevance of T for initial establishment of harems needs to be investigated, along with factors such as age and tenure of males. Research into costs and benefits of T in polygenous species may allow for exciting insights into the roles of T in the evolution of social organization. It may also allow for improved wildlife management decision-making through avoidance of anthropogenic interference during sensitive life cycle stages.

This research complied with Australian laws. It was conducted under ethics permits and licences issued by the New South Wales Director General of Agriculture Animal Ethics Committee, the New South Wales National Parks and Wildlife Service, the Australian Bird and Bat Banding Scheme of the Australian Department of the Environment and Water Resources, and the University of Queensland Animal Ethics Committee.

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