Understanding testosterone variation in a tropical lek-breeding bird

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Male reproductive coalitions, in which males cooperate to attract females, are a rare strategy among vertebrates. While some studies have investigated ultimate aspects of these relationships, little is known about the mechanistic role that hormones play in modulating cooperative behaviours. Here, we examined male testosterone variation in a tropical lekking bird, the wire-tailed manakin (*Pipra filicauda*), which exhibits cooperative male–male display coalitions. We found that testosterone levels in territorial males were comparable to those of temperate breeding birds, a surprising result given their environmental, social and reproductive dynamics. In addition, social status rather than plumage was a strong predictor of testosterone variation. Territorial males had significantly higher testosterone levels than did two other plumage classes of floater males, who do not hold territories. We hypothesize that testosterone variation plays an important role in the establishment of male dominance hierarchies (competition), while concurrently facilitating stable display partnerships (cooperation).

Keywords: cooperation; competition; lekking; reproductive coalitions; testosterone; wire-tailed manakin

1. INTRODUCTION

Comparative avian studies have been, and continue to be, integral to our understanding of how testosterone underlies male life-history variation [1]. Yet, despite the great diversity of reproductive strategies exhibited by tropical birds, most endocrine work has focused on temperate breeders. In general, tropical males exhibit lower testosterone levels during breeding than do temperate males; lower testosterone is hypothesized to minimize testosterone-mediated survival costs over prolonged breeding seasons [2,3]. However, tropical males of some species can exhibit peak testosterone levels comparable to those of northern males [2–5]. Further study across a broader spectrum of species

with diverse reproductive strategies is needed to elucidate the social and ecological factors underlying this variation [4].

Several ecological factors are hypothesized to influence variation in testosterone within and among tropical species [2,4]. Elevated breeding testosterone levels are expected in species with relatively high degrees of seasonality [2,5], during periods of social instability [6], and in species where males compete each season for new mates [7]. The degree of sociality may also influence testosterone patterns because cooperatively breeding species tend to exhibit lower testosterone than do those with monogamous or polygamous mating systems [2,4]. However, endocrine studies of cooperation in tropical species have focused primarily on cooperative breeders (i.e. where helpers contribute to offspring care), largely ignoring lek systems where males form coordinated display coalitions (but see [8]).

Here, we examined plasma testosterone variation in the wire-tailed manakin (*Pipra filicauda*), a lekking species where territorial males form male–male display coalitions with non-territorial partners and neighbouring territory holders in order to attract mates. Cooperation in this system is characterized by coordinated behaviours that yield mutual benefits for each display partner. Specifically, male partners coordinate their display elements, and these partnerships provide benefits to both non-territorial males (increased probability of territorial inheritance [9]) and territorial males (increased reproductive success [10]). While the frequency of cooperative partnerships varies both within and among males of different social status [11], all males engage in these partnerships, as they are an essential component of male fitness [9–11]. However, despite the cooperative benefits of display coalitions, these partnerships are also thought to play an important role in the establishment of dominance hierarchies as males compete for social status. As such, male–male display coalitions represent a rare mix of cooperation and competition and thus provide a unique social context in which to examine testosterone's role in mediating opposing behaviours.

2. MATERIAL AND METHODS

We studied wire-tailed manakins (*P. filicauda*) at Tiputini Biological Station in the Orellana province of eastern Ecuador (0°38' S, 76°08' W). Birds were captured on leks during a two week period in late December 2008 and in early January 2010. The breeding season in this population lasts from November to March, and our sampling coincided with peak reproductive activity. Following Ryder *et al.* [9], we categorized males based on a combination of plumage characteristics and social status (territorial versus floater). Males exhibit delayed plumage maturation where adult coloration (definitive plumage) is not obtained until their third year of life (figure 1a–c). Categories were: (i) *formative floaters* with all-green plumage (first year; figure 1a) and no territories, (ii) *pre-definitive floaters* with mixed plumage (second year; figure 1b) and no territories, (iii) *definitive floaters* with adult plumage (third year and beyond; figure 1c) but no territories, and (iv) *definitive territory holders* with adult plumage (third year and beyond; figure 1c) and established territories. Birds were marked with aluminium bands and unique colour combinations. Male social interactions and territorial status were determined using focal behavioural observations and scan sampling on each lek (see the electronic supplementary material for details).

Captured birds were blood sampled and testosterone levels were determined by direct radioimmunoassay (RIA) (see the electronic supplementary material for detailed blood sampling and RIA methods). For each capture, we recorded maximum possible time

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Figure 1. Plumage of wire-tailed manakins is a good proxy for age in both (a) formative and (b) pre-definitive males, but lacks resolution once a male obtains (c) definitive plumage.

in the net (net time), time from net removal to blood sampling (bleed time) and time of day because these factors could influence hormone levels. However, net time ($r^2 = 0.001$, $F_{1,68} = 0.09$, $p = 0.77$), bleed time ($r^2 = 0.001$, $F_{1,68} = 0.07$, $p = 0.78$) and time of day ($r^2 = 0.003$, $F_{1,68} = 0.25$, $p = 0.62$) were not related to individual variation in testosterone levels; thus, these factors were removed from subsequent analyses. We used a general linear model to determine whether testosterone levels varied with social status (three levels). Formative floaters were excluded from statistical analyses because of small sample sizes ($n = 4$). Social status in this case incorporates both plumage variation and dominance (territorial versus floater). We conducted post hoc comparisons using Tukey's HSD test. Hormone data were log transformed, and analyses were conducted using JMP v. 8.

3. RESULTS

Testosterone levels varied according to male social status ($F_{2,58} = 14.63$, $p < 0.0001$; figure 2). In particular, definitive territory holders had significantly higher testosterone levels than did non-territorial floater males (HSD, all $p < 0.05$; figure 2). Elevated testosterone was not associated with plumage maturation, as evidenced by the fact that testosterone did not differ between definitive and pre-definitive floaters (HSD, $p = 0.66$), but did differ between definitive plumage males of different social status (HSD, $p = 0.0002$; figure 2).

4. DISCUSSION

Territorial wire-tailed manakins males showed substantially higher testosterone levels than predicted based on cumulative evidence from tropical avian studies. Specifically, territorial males exhibited

Figure 2. Variation in male plasma testosterone concentration (ng ml^{-1}) was explained by social status: definitive territorial males had higher testosterone than all three classes of floater males.

testosterone levels ($2.9 \pm 0.5 \text{ ng ml}^{-1}$) comparable to those of temperate species ($2.8 \pm 0.4 \text{ ng ml}^{-1}$), and substantially higher than most tropical species studied to date ($1.3 \pm 0.2 \text{ ng ml}^{-1}$; data from [2]). In tropical birds, such higher concentrations are typically associated with short breeding seasons at high elevations [2,4,5], yet our study population breeds at low elevation over a prolonged breeding season (more than six months). Thus, elevated testosterone in territorial males was unexpected. However, our short sampling period precluded us from examining seasonal testosterone variation in this species.

Mating system dynamics may be another factor underlying high testosterone levels in territorial males [4]. For example, lekking species, which are characterized by strong reproductive skew and high degrees of male–male competition for mates, are expected to exhibit high testosterone levels relative to other mating systems. While elevated testosterone levels in territorial wire-tailed manakins are congruent with this prediction, males rarely exhibit overt aggression towards other males. Moreover, while male–male competition plays an important role in the establishment of the dominance hierarchy, and thus indirectly affects reproductive opportunities, female choice among male territories is the primary driver of reproductive variance [9,10]. Ultimately, understanding the mechanistic relationship between testosterone and reproductive success will require further study.

Elevated testosterone can also be associated with periods of social instability during territorial establishment [4,6]. In wire-tailed manakins, however, territories are maintained year round and display partnerships are stable through time [11]; thus, it is unlikely that elevated testosterone in territorial males during early breeding is the result of social instability. Given that territoriality is a prerequisite for breeding in wire-tailed manakins [10], elevated testosterone in territorial males may be indicative of physiological

readiness to breed. In addition, testosterone could activate complex courtship display [7], with subsequent androgen variation affecting display frequency among males of different status [11]. The link between testosterone and manakin display is further evidenced by increased androgen sensitivity in related muscle control centres [12]. While testosterone may promote display, these behaviours appear to be maintained in individuals with low circulating testosterone levels (i.e. floaters), possibly via androgen sensitivity and/or social facilitation [7].

Androgens have long been recognized as important modulators of behaviour, yet few studies have sought to establish the mechanistic link between hormones and cooperation [13]. Low testosterone levels are thought to facilitate cooperation because high testosterone might produce deterministic, inflexible responses (e.g. high male–male aggression) that are incompatible with the stable social bonds required of group members in cooperative systems [14]. Paradoxically, territorial wire-tailed manakins have high testosterone levels and they cooperate in long-term display partnerships. This pattern may be generalizable, as simultaneous work by DuVal & Goymann [8] found elevated testosterone levels in territorial lance-tailed manakin males (*Chrixophia lanceolata*), a species that represents an independent evolutionary origin of cooperative display. Although testosterone levels were substantially higher in wire-tailed manakins compared with lance-tailed manakins, territorial males of both species exhibit high testosterone and cooperation, which could occur if aggression is dissociated from testosterone control, as has been shown for other tropical species [15,16].

Ultimately, testosterone variation in wire-tailed manakins may facilitate both competition and cooperation among males of different social status. On the one hand, testosterone can promote competitive behaviours that influence status in a dominance hierarchy. On the other, testosterone can also dynamically respond to the social environment and therefore alter the expression of behaviours during subsequent social interactions (e.g. the reciprocal model [17]). For example, elevated testosterone can influence the probability of winning a dominance interaction, and this outcome can have down-stream effects on future hormone levels for both winners and losers [17]. While display coalition partnerships are stable, these social interactions are thought to be important in the establishment of dominance hierarchies whereby territorial males (high testosterone) play a dominant role in displays relative to floater males (low testosterone). Thus, relative testosterone levels may both predict the outcome of display interactions and serve to optimize display behaviours so as to maintain stable relationships. Such a pattern would facilitate cooperation among display partners by avoiding the social instability that might occur if both males in a coalition had high testosterone. This hypothesis is supported by testosterone patterns in both wire-tailed and lance-tailed manakins, where territorial (dominant) males have higher testosterone relative to helper (subordinate) males [8,14]. Male social interactions, however, are not limited to males of different status,

and the outcomes of dominance interactions among same status males could influence variation in testosterone levels within status groups and eventual social trajectory. Ultimately, testosterone may play an essential proximate role in both the competitive and cooperative function of wire-tailed manakin display coalitions.

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