Plasma Testosterone Concentrations in Adult Tree Swallows During the Breeding Season

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ABSTRACT.—We studied seasonal profiles of circulating testosterone concentrations among male and female adult Tree Swallows (*Tachycineta bicolor*) breeding in nest-box colonies near Ames, Iowa, USA. Mean plasma testosterone in males was elevated during nest establishment (0.63 ± 0.86 ng/ml) and incubation stages (0.28 ± 0.26 ng/ml), and was significantly lower after hatching (0.03 ± 0.05 ng/ml) when males began provisioning nestlings. Male swallows do not incubate and high testosterone during the incubation stage may facilitate pursuit of extra-pair matings. Female testosterone concentrations were an order of magnitude lower than those of males (nest establishment, mean = 0.06 ± 0.09 ng/ml) and did not change significantly over the breeding season. These testosterone profiles support the hypothesis that elevated testosterone in males is associated with defense behaviors and obtaining additional mating opportunities during the first part of the breeding season, but is incompatible with parental care once the eggs have hatched. *Received 8 September 2010. Accepted 19 March 2011.*

Androgens such as testosterone promote secondary sex characteristics, sperm production, and aggressive behavior in male birds. Elevated circulating testosterone increases song production, mate guarding, and pursuit of extra-pair mating opportunities by males while also decreasing parental behavior (reviewed in Lynn 2008). Testosterone concentrations in temperate zone-breeding passerines generally are highest at the start of the breeding season when males compete for territories and mates, but this pattern varies with mating system and whether or not the male participates in parental care (Wingfield et al. 1990). For example, hormonal correlates of parental and sexual behavior have been studied in two closely related species: Blue-headed Vireo

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(*Vireo solitarius*) and Red-eyed Vireo (*V. oliva-ceous*). Both are socially monogamous, but differ in their relative contribution to care of offspring. Male Blue-headed Vireos participate in nest construction, incubation, and provisioning young and do not have a pre-nesting peak in testosterone. Male Red-eyed Vireos, however, exhibit parental care only after nestlings are present, and circulating testosterone is elevated early in the nesting season, but declines by the nestling stage (Van Roo et al. 2003).

We examined temporal profiles of plasma testosterone in male and female Tree Swallows (*Tachycineta bicolor*) during the breeding season. Tree Swallows are socially monogamous and secondary cavity nesters. There is competition for typically scarce natural nesting sites, which are defended by both males and females (Holroyd 1975, Winkler 1992). Males contribute to nest building, do not incubate, but contribute about equally to provisioning nestlings (Quinney 1986). If either the male or female parent is lost, the remaining parent will increase their nestling food provisioning rate. This increase neither fully compensates for loss of the other parent nor can it be sustained for an extended period of time, resulting in reduced reproductive success (Leffe-laar and Robertson 1986). Tree Swallows also have unusually high rates of extra-pair fertilizations for a socially monogamous species. Whereas extra-pair paternity rates for socially monogamous species in general average 18.7% of broods and 11.1% of offspring (reviewed in Griffith et al. 2002); 50–87% of broods in nest box populations of Tree Swallows contain extra-pair young and extra-pair males father 38–53% of the offspring in those broods (Barber et al. 1996). Testosterone is thought to influence the tradeoff between behaviors that facilitate obtaining additional mating opportunities versus investing in offspring care (Raouf et al. 1997). We predicted testosterone concentrations would be highest in males during nest establishment and would remain elevated during incubation when males can obtain extra-pair matings. Their testosterone concentrations should decrease when eggs hatch and males begin provisioning nestlings.

Both male and female Tree Swallows defend the nest, although males have a greater maximal alarm call-rate and longer call duration compared to females. Several other defense measures including number of dives towards predators and time defending the nest do not differ between males and females (Winkler 1992). Elevated female aggression has been associated with peaks in female testosterone concentrations in other species (Cristol and Johnsen 1994, Woodley and Moore 1999). However, females displaying aggressive behavior do not always have a corresponding increase in testosterone (Elekonich and Wingfield 2000, Jawor et al. 2006). We predicted testosterone concentrations in female Tree Swallows would be lower than those of males, but would be elevated during the territory and nest establishment phase and decline once incubation began.

METHODS

Field and Laboratory Procedures.—We collected blood samples to assay testosterone concentrations from Tree Swallows using nest boxes near Ames, Iowa, USA, (42° 01′ N, 93° 37′ W) in May–June 2009. Boxes were spaced ~20 m apart, and there was considerable interaction (nest box and mate guarding) among breeding birds.

We captured 25 male and 30 female adult Tree Swallows during three time periods: pair bond and nest-box establishment (hereafter nest establishment), incubation, and the nestling stage. We caught individuals during nest establishment either with mist nets or upon entrance into a nest box during nest building. Not all females had brood patches at this stage, and we classified individuals based on the presence of a cloacal protuberance in males or the brown plumage of second-year females. We confirmed our classification for seven of 10 females and three of seven males through later recapture at a nest, data from previous years, or wing length in the predominately female (≤113 mm) or male (≥122 mm) ranges (Stutchbury and Robinson 1987). We caught all birds during the incubation and nestling stages using nest-box traps. Males and females were easily distinguished during these two stages based on presence of a brood patch in females. Each bird was sampled only once.

We took a blood sample of ~100 μl within 5 min of capture from a wing vein, collecting blood into heparinized capillary tubes that were immediately cooled on ice. We separated plasma from cells by centrifugation within 4 hrs and stored the plasma at −80° C. We measured plasma testosterone in duplicate using a radioimmunoassay kit (DSL-4100) from Diagnostics Systems Laboratories (Brea, CA, USA) in September 2009. This RIA kit measures testosterone
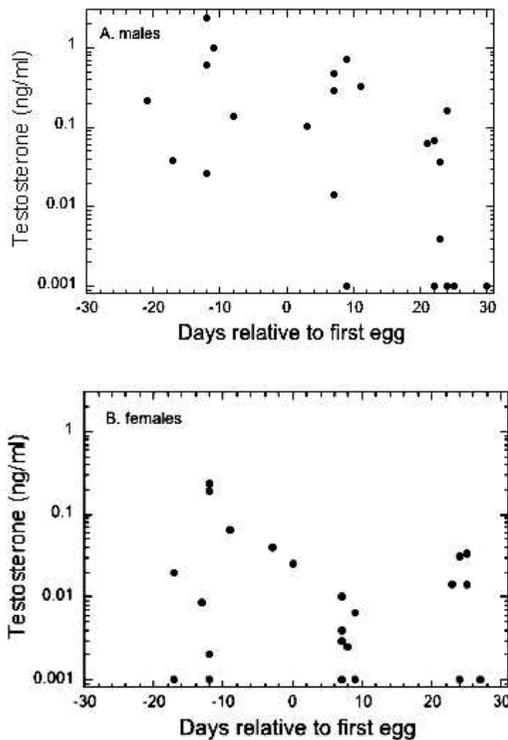
FIG. 1. Plasma testosterone concentrations in adult Tree Swallows relative to the date when the female of the pair initiated egg laying. (A) Data from males. (B) Data from females. Incubation begins ~4 days after the first egg is laid and eggs hatch ~14 days later. Testosterone concentrations are displayed on a logarithmic scale.

concentration in plasma directly using I^{125} labeled testosterone. The kit was modified by running all volumes at 25% of the suggested kit volume. Samples were run in two separate assays. The intra-assay and inter-assay coefficients of variation were 0.11 and 0.07, respectively.

We calculated the time in days relative to the initiation of laying in that bird's nest for each sample. We assumed egg-laying began on the mean date of initiation of laying for the colony (18 May) for individuals sampled prior to egg-laying that did not remain to nest in our colony ($n = 11$). Varying this estimated egg lay date by ±7 days did not affect our results significantly. Incubation in Tree Swallows typically begins with the penultimate egg (~4–5 days after laying initiation) and hatching occurs ~14–15 days after the onset of incubation (Zach 1982).

Statistical Analyses.—Plasma testosterone concentrations were not normally distributed and we log-transformed testosterone concentrations for all

TABLE 1. Mean testosterone (T) concentrations in adult male and female Tree Swallows during three stages of the breeding season in nesting colonies near Ames, Iowa, USA.

Breeding stage	Mean ± SD T (ng/ml)	n
Males		
Nest establishment	0.63 ± 0.86[a]	7
Incubation	0.28 ± 0.26[a]	7
Nestling care	0.03 ± 0.05[b]	11
Females		
Nest establishment	0.06 ± 0.09[a]	10
Incubation	0.01 ± 0.01[a]	11
Nestling care	0.02 ± 0.02[a]	9

[a,b] Breeding stages with different superscripts have significantly different mean testosterone (T) concentrations in Tree Swallows (Tukey-Kramer HSD $P < 0.05$).

statistical analyses. We performed an analysis of variance with plasma testosterone as the dependent variable using a model that incorporated time in days relative to the first egg, males or females, and the interaction between the two variables. There was a significant interaction between males or females and time relative to egg lay; thus, we used separate Tukey-Kramer honestly significant difference (HSD) tests to compare mean testosterone concentrations among the different breeding stages for males and females. We used JMP statistical software for all statistical analyses (SAS Institute Inc. 2009).

RESULTS

Circulating testosterone concentrations in Tree Swallows ranged from 0.01 to 2.4 ng/ml in males and 0.01 to 0.23 ng/ml in females during the breeding season (Fig. 1). Gender ($F_{1,53} = 12.7$, $P = 0.0008$), days relative to the first egg ($F_{1,53} = 5.7$, $P = 0.006$), and the interaction between the two variables ($F_{1,53} = 4.9$, $P = 0.01$) significantly affected testosterone concentration. Testosterone concentrations over the breeding season were about an order of magnitude higher in males than in females (male mean = 0.27 ng/ml; female mean = 0.03 ng/ml).

Circulating testosterone concentrations of males during the nest establishment stage did not differ from those during the incubation stage, but male testosterone concentration decreased significantly (Tukey-Kramer HSD, $q = 2.5$, $P < 0.05$) during the nestling stage (Table 1). Mean testosterone concentrations of females did

not differ significantly among breeding stages ($P > 0.05$).

DISCUSSION

Concentrations of circulating testosterone of male Tree Swallows were much higher than those of females, as expected, and particularly during the nest establishment and incubation stages. Testosterone did not decrease significantly in males until they began provisioning nestlings, when it was similar to that of females. Contrary to our predictions, we did not find evidence that females have elevated testosterone during nest establishment despite their active participation in nest defense (Winkler 1992). Rather, testosterone concentrations in females were generally low throughout the breeding season.

Bishop et al. (1998) measured testosterone in Tree Swallows in Ontario, Canada, during incubation (females only) or chick rearing (males only). They used a different assay and reported average values that were significantly higher than ours (~2.5 ng/ml in males and 0.9 ng/ml in females). Whether these differences are due to methodology (Bishop et al. 1998 measured testosterone after ether extraction whereas we measured it directly in plasma) or whether population differences exist is not known. Differences in seasonal testosterone patterns have been reported between populations of other species. Male Orange-crowned Warblers (*Oreothlypis celata*) in Alaska have a pre-incubation peak in testosterone and subsequent decline during incubation and nestling stages. In contrast, males on Catalina Island, California have elevated testosterone throughout the breeding season (Horton et al. 2010). Variation in testosterone profiles also occurs among Cliff Swallow (*Petrochelidon pyrrhonota*) populations, depending on breeding density (Smith et al. 2005).

We found testosterone was always low in female Tree Swallows. Elevated testosterone during nest stages that require parental behavior is frequently associated with negative consequences for reproductive success or other costs. For example, artificially elevating testosterone in female Dark-eyed Juncos (*Junco hyemalis*) resulted in delayed onset of egg laying after nest completion as well as lower body mass and delayed molt (Clotfelter et al. 2004, Zysling et al. 2006). Additionally, Zysling et al. (2006) found a negative correlation between cell-mediated immune function and total testosterone in female Dark-eyed Juncos. Veiga and Polo (2008) reported similar results in female Spotless Starlings (*Sturnus unicolor*), in which testosterone-treated females delayed egg laying, laid fewer eggs, and provisioned nestlings at a reduced rate in comparison to controls. Similarly, testosterone supplementation in males is known to disrupt or decrease parental behavior in many species (reviewed in Vleck and Vleck 2010). For example, male House Sparrows (*Passer domesticus*) supplemented with testosterone during chick rearing have reduced feeding rates and lower breeding success (Hegner and Wingfield 1987).

The low circulating testosterone concentrations we measured in both male and female Tree Swallows during the nestling stage likely reflects the importance of parental care by both members of the pair for optimizing reproductive success (Leffelaar and Robertson 1986). However, low testosterone is not an absolute requirement for the exhibition of parental care in some species, indicating some behavioral insensitivity to testosterone is possible (Van Duyse et al. 2000, Lynn et al. 2002). Hau (2007) suggested the negative effects often seen with elevated testosterone may be avoided if selection acts on the responsiveness of target tissues to testosterone. Additionally, McGlothlin et al. (2007) suggested the ability to produce short-term increases in testosterone may allow for temporary shifts in territorial and sexual behaviors without compromising overall parental care.

Decline in testosterone concentrations after egg laying may not be as critical for male Tree Swallows, which guard the nest but do not incubate, as for species in which the male incubates. There is a negative correlation between incubation behavior and testosterone concentrations in male European Starlings (*Sturnus vulgaris*) (Pinxten et al. 2007). Elevated circulating testosterone during the incubation stage may be beneficial to male Tree Swallows to facilitate extra-pair copulations (Raouf et al. 1997). The date of egg laying can vary by as much as 7 weeks in our colonies because females that have lost their nest often lay a second clutch. Consequently other receptive females may be present after a male's mate has begun incubation and may influence his testosterone level. A positive correlation between circulating testosterone in males and the presence of receptive females has been documented in other passerines such as European Starlings (Pinxten et al. 2003). Male

White-crowned Sparrows (*Zonotrichia leucophrys*) placed with sexually receptive females had higher plasma testosterone and exhibited greater copulatory behavior than those paired with non-receptive females (Moore 1983). The wide range of testosterone concentrations we found in male Tree Swallows (Fig. 1) may reflect variation in access to receptive females.

Our breeding season testosterone profile for male Tree Swallows supports the role of testosterone in behaviors associated with nest and mate defense, and obtaining additional mating opportunities through extra-pair copulations. The decrease in testosterone in males after eggs hatch, along with the continuously low testosterone profile in females, lends support to the predicted costs associated with possessing elevated testosterone, particularly those associated with simultaneously displaying parental behavior.

ACKNOWLEDGMENTS

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