

Life History Trade-Offs and Behavioral Sensitivity to Testosterone: An Experimental Test When Female Aggression and Maternal Care Co-Occur

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Abstract

Research on male animals suggests that the hormone testosterone plays a central role in mediating the trade-off between mating effort and parental effort. However, the direct links between testosterone, intrasexual aggression and parental care are remarkably mixed across species. Previous attempts to reconcile these patterns suggest that selection favors behavioral insensitivity to testosterone when paternal care is essential to reproductive success and when breeding seasons are especially short. Females also secrete testosterone, though the degree to which similar testosterone-mediated trade-offs occur in females is much less clear. Here, I ask whether testosterone mediates trade-offs between aggression and incubation in females, and whether patterns of female sensitivity to testosterone relate to female life history, as is often the case in males. I experimentally elevated testosterone in free-living, incubating female tree swallows (*Tachycineta bicolor*), a songbird with a short breeding season during which female incubation and intrasexual aggression are both essential to female reproductive success. Testosterone-treated females showed significantly elevated aggression, reduced incubation temperatures, and reduced hatching success, relative to controls. Thus, prolonged testosterone elevation during incubation was detrimental to reproductive success, but females nonetheless showed behavioral sensitivity to testosterone. These findings suggest that the relative importance of both mating effort and parental effort may be central to understanding patterns of behavioral sensitivity in both sexes.

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Introduction

Among the most widespread behavioral trade-offs are those between mating effort and parental effort, where individuals divide limited resources between attracting mates or repelling rivals on the one hand, and caring for offspring on the other [1–3]. Naturally or experimentally elevated testosterone is often associated with greater investment in mating effort via ornaments, armaments, or aggressive behavior (i.e. traits and behaviors that attract a potential mate or deter same-sex competitors), whereas lower androgens are often associated with greater investment in parental care [4–12]. Thus, the androgen testosterone (T) is thought to be one of the proximate mediators of this trade-off, as T can either directly (e.g. by binding androgen receptors) or indirectly (e.g. by interfering with prolactin signaling) affect the expression of aggressive and parental behaviors [13,14].

The vast majority of research on the role of T in the trade-off between parental care and intrasexual aggression has been undertaken in males. Males with experimentally elevated T often exhibit more frequent or intense aggression towards rivals, while provisioning and/or incubating relatively less than control males [15–25]. However, there are many studies that do not support this pattern [26–29]. Attempts to reconcile these mixed results in males have focused on two main hypotheses, both of which relate to

paternal care and the fact that shifts from high to low testosterone are somewhat limited in their temporal flexibility [4,29,30]. The ‘essential paternal care’ hypothesis suggests that if male care is especially important for reproductive success, then selection may favor behavioral insensitivity to T [29,31,32], and so, T should be less likely to affect behavior in species with significant paternal care. The ‘short season’ hypothesis posits that behavioral insensitivity to T should evolve when breeding seasons are especially short (e.g. in single brooded or arctic dwelling species) because selection ought to favor a rapid transition from mate attraction to parental care [27,30,33–35]. Each of these hypotheses attempts to explain cases of behavioral insensitivity to T, with the assumption that sensitivity to T should be favored when T-mediated mating effort (e.g. intrasexual aggression, mate guarding, etc.) is particularly important for reproductive success [4,10,27,36] because T may enhance the ability to obtain a mate or keep away rivals (e.g. [37]). Conflict arises, of course, when components of mating effort and parental effort overlap in time, with the prediction that one or another behavior might evolve behavioral insensitivity to T.

Historically, the sexes were placed at opposite ends of the spectrum for this trade-off, with males investing primarily in mating effort and females investing primarily in parental effort [38,39]. More recent research shows that females also display

exaggerated traits and behaviors that may be important for mating effort (i.e. in attracting or acquiring mates or territories [40–43]), but we know much less about trade-offs between aggression and parental care and the degree to which these trade-offs are also mediated by T in females. Many of the phenotypic effects of androgens are not sex-specific (e.g. muscle development, immune function), and organizational effects of T often masculinize female phenotype [5,44]. Furthermore, females secrete physiologically and behaviorally relevant levels of T [31,32], and they express androgen receptors in a variety of neural and peripheral tissues [44], indicating that activational effects of T on female phenotype may extend beyond this hormone's biochemical role in estrogen synthesis.

When applied to females, T implants have, in some cases, lead to an increase in female aggression at the expense of maternal care [45–48], but once again, there are exceptions to this pattern [31,47,49–51]. A full investigation of how T mediates behavioral trade-offs must also ask whether the T-mediated trade-offs seen in males operate similarly in females. This knowledge will provide insight into whether the hypotheses posed to explain male patterns of behavioral sensitivity/insensitivity can be extended to females. Furthermore, any comprehensive theory of the evolution of behavioral mechanisms must recognize that interspecific patterns of behavioral sensitivity to hormones may not simply reflect *male* life history. These patterns also may relate to trade-offs between parental effort and mating effort *in females*, as well as the degree to which there is sexual conflict in the resolution of these trade-offs in the two sexes.

As a first step towards this more comprehensive view, I measured the behavioral effects of experimentally elevated T in female tree swallows (*Tachycineta bicolor*). Specifically, I examined the hormonal underpinnings of a trade-off between female aggression and incubation behavior to ask whether hypotheses developed for explaining patterns of behavioral sensitivity to T in males also may apply to females. Tree swallows are ideal for examining this question because previous work suggests that females are faced with trade-offs between mating effort and parental effort during their relatively short breeding season. For example, focal observations suggest that free-living females trade-off investment in aggression and parental care (provisioning), and cross-fostering demonstrates that aggressive females tend to have poor quality offspring, likely due to a combination of reduced pre- and post-hatch maternal care, i.e. provisioning and incubation [52,53]. Tree swallows are single-brooded and the time from clutch initiation to completion of breeding is ~1.5 months [54]. Thus, while not as short as arctic breeding seasons for which the short season hypothesis was developed in male birds (e.g. ~1 mo. [55,56]), the tree swallow breeding season is shorter than other songbirds breeding in the same area [57]. Female care of offspring is essential to reproductive success in this species because a reduction in provisioning, incubating or brooding significantly decreases the quality or quantity of offspring produced [53,58–60]. Whereas both sexes provision offspring, females alone incubate eggs [54], and thus, studying the effects of T on incubation avoids the potential confounds of male care.

Aggressive behavior in the context of competition for nesting cavities is also an essential component of reproductive success for female tree swallows, as females aggressively defend a nesting territory that includes the cavity and the several meters surrounding it. Further, evidence to date suggests that female aggressiveness indeed functions in the context of maintaining access to these territories or mates (i.e. mating effort), much like it does in males of many species. For example, female intruders are a common challenge, with most populations having an excess of

one year-old female floaters that are capable of breeding but do not breed because they lack a nesting cavity [61]. Throughout the breeding season, these female floaters engage in frequent intrusions with cavity holders, and these interactions can escalate to overt aggression, with the possibility of eviction or even death [62,63]. Females that are more aggressive are more likely to obtain a nesting cavity when cavities are limited, suggesting that selection favors females that are more aggressive during female-female competition [64]. Because females cannot mate or breed without a nesting cavity [54] and males do not respond aggressively to young female intruders [65], it may be especially important that females aggressively defend their nesting cavities against rival females in order to have reproductive success, much like male-male aggressiveness is a key component of male reproductive success by facilitating access to territories and mates.

If the short breeding season or importance of parental care shape female responsiveness to testosterone, then incubating female tree swallows should not alter their incubation behavior or their aggressive behavior in response to a T-implant. On the other hand, if the relative importance of aggression favors behavioral sensitivity to T in spite of its potential negative effects on maternal care, then T-implanted females should be significantly more aggressive and incubate significantly less than their control counterparts. Finally, sensitivity to T in one behavior but not another would instead suggest some degree of modularity of behavior, where selection can decouple one or another behavior from circulating T levels, according to the most adaptive combination of phenotypes [11,66].

Materials and Methods

Ethics Statement

All research was approved by IACUC at both Duke University (A050-07-02; author's affiliation at the time of field work) and University of Pittsburgh (0704588A) in accordance with Federal banding and collecting permit (21523-H), PA State Banding permit (BBN00227), and PA State Game Commission Special Permit for Scientific Study.

General Methods

This study was conducted using free-living tree swallows breeding near the University of Pittsburgh's Pymatuning Lab of Ecology in Linesville, Pennsylvania, USA (41°40' N, 80°26' W) in spring 2008. All swallows were breeding at nestboxes located at the Linesville State Fish Hatchery or the nearby Pennsylvania State Gamelands, where average distance (\pm se) between nestboxes was 114 ± 8 m. Beginning early in the breeding season, nests were checked at least every third day to determine clutch initiation date, clutch size, and the onset of incubation. I followed each nest until hatching or failure. Females were banded with one U.S. Fish and Wildlife metal band and one color band. In addition, each female was marked with dabs of non-toxic acrylic paint on the wings and rump, to facilitate individual identification during behavioral trials [67]. This study focused on adult females (≥ 2 years old) to minimize potential age-related variation in female reproductive behavior because adult and subadult females may differ in many aspects of condition and reproductive behavior, including incubation [54,68–70], though not aggressiveness [64]. Subadult and adult females are readily distinguished based on plumage coloration, with adult females displaying primarily greenish-blue plumage and subadults displaying brown plumage [71].

Hormone Manipulation and Analyses

I implanted 14 control females and 14 testosterone-treated females. All females were captured using nestbox traps during mid to late incubation (mean \pm se = 8.1 ± 0.1 days after clutch completion, range: 6 to 10 days). Upon capture, a blood sample (approximately 50 to 100 μ L) was collected from the alar vein into heparinized capillary tubes to assess pre-implant levels of circulating T for control and experimental females. All blood samples were collected within 5–11 min (mean \pm se = 8.0 ± 0.4 min) of capture of the bird. There was no relationship between collection time and T levels in either treatment group for pre- and post-implant samples (Pearson's $|r| < 0.4$, $p > 0.17$), and so I did not control for time in further analyses. After brief treatment with a topical anesthetic, all females were given a subcutaneous implant (8 mm long) made of silastic tubing (I.D. = 1.47 mm, O.D. = 1.96 mm) and sealed at both ends with silastic glue. The implant was inserted under the skin along the flank, using a trocar needle. Experimental females' implants were previously packed with 5 mm of crystalline testosterone, whereas control females received empty implants. Handling times during the implantation procedure were short and did not differ between treatment groups (control: 12.9 ± 0.4 min; experimental: 12.7 ± 0.4 min; unpaired t-test, $t = -0.31$, $p = 0.76$). Most females resumed normal breeding activities within a few hours (2.54 ± 0.37 h, range: 0.45 to 8.75 h, as indicated by nest temperature data), though two females did not return after implantation (one control, one experimental), and these females were eliminated from further analyses, leaving $n = 13$ per treatment group. The latency to first incubation did not differ between the two groups (control: 2.5 ± 0.7 hours; experimental: 2.4 ± 0.5 hours; unpaired t-test: $t = 0.16$, $p = 0.88$). At the end of the study, females were re-captured using nestbox traps and mist-nets to remove implants (13.6 ± 1.3 days after implantation, range: 5 to 31 d). At this time, a second blood sample was collected to confirm that the circulating T levels were significantly higher after implantation in the T-treated group than in the control or pre-implant samples, though I was unable to capture one female from each group. In addition, sufficient plasma volume was not collected from all females, thus sample sizes for hormone analyses are $n = 4$ for pre-implant T-females, $n = 9$ for pre-implant control females, $n = 10$ for post-implant T-females, and $n = 8$ for post-implant control females.

Blood samples were stored on ice in the field and centrifuged the same day. Plasma was drawn off the top with a Hamilton syringe and then stored at -20°C until hormone analyses. I quantified plasma T using a commercially available enzyme immunoassay (Assay Designs, Inc. 901-065) that has already been validated for use in female songbirds with comparable circulating levels of testosterone [49]. This assay reports high linearity (slope = 0.975, $R^2 = 0.999$) and low cross-reactivity with androstenedione (7.2%) and other steroid hormones (all $< 1\%$). The assay sensitivity is 0.00567 ng/mL. Briefly, a trace amount of radio-labeled T was added to each sample, and plasma was extracted twice with diethyl ether. Each sample was run in duplicate on the plate, and T concentrations were based upon a 9-point logistic standard curve, with the help of a curve-fitting program (Microplate Manager, Bio-Rad Laboratories). To obtain final T concentrations, I corrected for sample volume and used extraction efficiencies to account for incomplete recoveries (efficiency: $93.7 \pm 0.6\%$). All samples were run on one plate, with intra-assay variability of 9.78%. Three samples were undetectable by the assay ($n = 2$ from the pre-testosterone group, $n = 1$ from the post-control group), and so I used the assay sensitivity (0.00567 ng/mL) to calculate a maximum possible T value for these samples, corrected for

plasma volume (i.e. assay sensitivity divided by the plasma volume).

Behavioral Measures

All 26 females were assayed for their level of aggressiveness using an established behavioral bioassay that is highly repeatable among females, independent of the female's mate [52] and independent of the identity of the decoy [64]. Importantly, aggression scores from this simulated territorial intrusion predict a female's ability to obtain a nesting cavity when cavities are limited [64], demonstrating that this assay maps onto an important component of reproductive competition. The assay measures the aggressive response of a focal female to a live, caged same-sex conspecific placed 1.5 m from the nestbox. For 5 min, I recorded aggressive behaviors directed at the decoy female: swoops and dives (to within 0.75 m), perching atop, hovering over or landing on the cage, and attempted pecking at the decoy. Swoops, dives, and close proximity are characteristic of natural female-female aggressive encounters, which involve aerial chases that can escalate to grappling and direct contact with one another [54]. Each female was assigned an aggression score, measured as the sum of all 5-sec intervals during which she responded aggressively to the simulated intrusion (ranging from 0 to 60 intervals). All decoy females were captured from at least 1 km away on the day of the trial and returned to their nesting site immediately afterwards, where they resumed normal breeding activities. Decoy females were not used in this study in any other way. The identity of the decoy did not affect the focal bird's aggressiveness ($n = 5$ decoys used in 26 aggression trials, $F = 0.66$, $R^2_{\text{adj}} = -0.05$, $p = 0.63$), and so I did not control for decoy identity in subsequent analyses. All aggression trials occurred between 0600 and 1100 Eastern Daylight Time at least two days after females were implanted (5.5 ± 0.5 days; range: 2 to 15 days), and all trials occurred during the incubation phase of the nesting cycle.

I quantified incubation behavior using small (17 mm diameter) battery-operated temperature sensors (iButton #1921G, Thermochron, Dallas, TX, USA). Before placing these sensors in the nest, each sensor was tested for accuracy. Only those sensors that measured within 1°C of each other were used in this study. Each iButton was then attached to a shirt button using Velcro and then wired securely into the nest cup, adjacent to the eggs. iButtons were placed in the nest on the day the female laid either the 4th or 5th egg of the clutch. Temperature logging began immediately after each focal bird was implanted, and the loggers recorded temperature to the nearest 0.5°C every 4th min. This technique provides a record of the temperature of the nest experienced by the eggs, and it accurately reflects the incubation behavior of females [69]. When females leave the nest, the temperature drops, and the temperature returns back to approximately 40°C when females return (**Figure 1**). In a few nests, temperature clearly tracked ambient temperature, suggesting that either the button was not placed close enough to the eggs to be incubated by the female, or that these females did not incubate at all ($n = 1$ control, 2 experimental). To conservatively measure temperature, I did not include these nests in future temperature analyses, although all findings are qualitatively similar if these nests are included.

I used two additional iButtons to record ambient temperature at 20 min intervals throughout the study. These loggers were placed in the shade at a central location at each of the two nearby field sites. Furthermore, treatment groups were counterbalanced by date and location to account for the possible effects of ambient temperature on nest temperatures (e.g. due to a cold snap). As

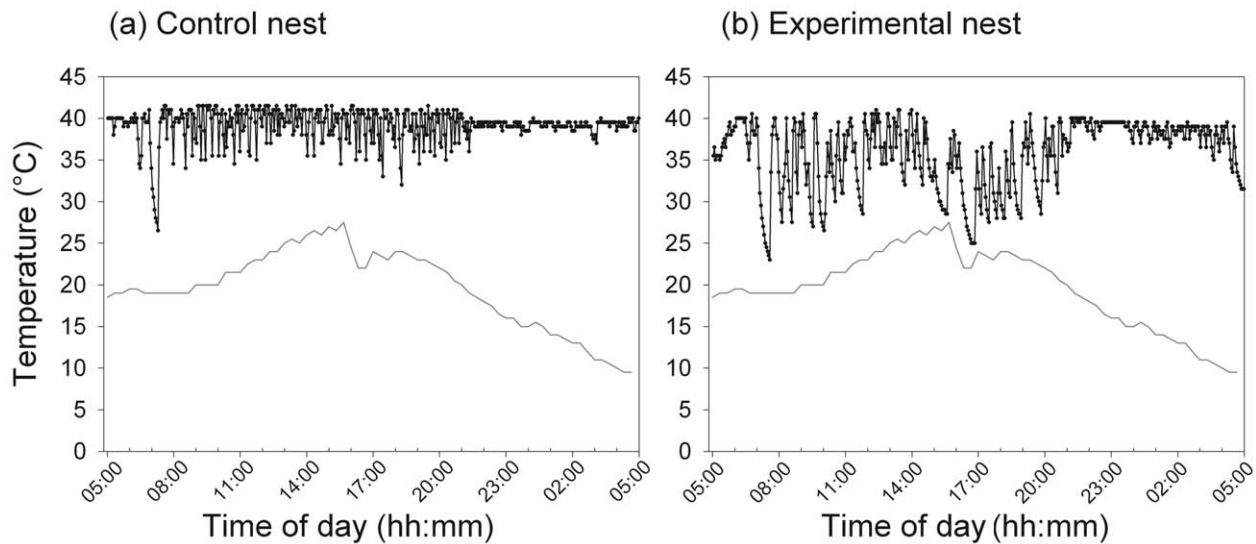


Figure 1. Daily temperature data. Exemplars of one full day (24 hours) of temperature data in (a) control and (b) experimental nests, beginning just before first light. Drops in temperature correspond to the female leaving the nest between bouts of incubation. Ambient temperature shown in gray line. Control and experimental nests were paired for location and date. doi:10.1371/journal.pone.0054120.g001

a result, I recorded nest temperatures in both experimental and control nests at the same exact times on the same days. Each day included either two or four focal females, half from each treatment group. Two exceptions arose, where an experimental female stopped incubating altogether, leaving 3 nests that were observed on those dates (i.e. 2 control, 1 experimental). Thus, sample sizes for incubation analyses are $n=9$ T-females and $n=12$ control females.

Statistical Analyses

Statistical analyses were performed in JMP v. 10.0.0 (SAS Institute Inc. Cary, NC). All tests were two-tailed, and I report mean \pm se. Plasma testosterone levels were natural log transformed to achieve normality for statistical analyses, and back-transformed for visual representation in Figure 2. I analyzed the effects of hormone implants using a linear mixed model (LMM) with 'bird' as a random repeated factor and treatment (control vs. testosterone), time (pre- vs. post-implant), and a time*treatment interaction as fixed effects.

Aggression scores were not normally distributed, and so I compared aggressiveness of control and experimental females using a Mann-Whitney U test. I compared hatching success between control and T-implanted groups with a Fisher's exact test.

I used a general linear model (GLM) for temperature analyses, with average daytime nest temperature on the second day after implantation as the dependent variable and ambient daytime temperature and treatment as fixed effects. I selected day 2 post-implant because it allowed sufficient time for females to recover behaviorally and physiologically from capture, while still allowing all nests to be monitored a standardized number of days after implantation and before hatching (see also sensitivity analyses below).

Because hormonal and behavioral data were not collected on the same day, I performed three statistical analyses to test whether any effects of the hormone implants were likely to have been related to a short-term spike in T that had short-term effects on behavior (i.e. whether the day of sampling affected the results). Ideally, this sort of sensitivity analysis would include repeated sampling of hormones and behavior [72], though this is usually not

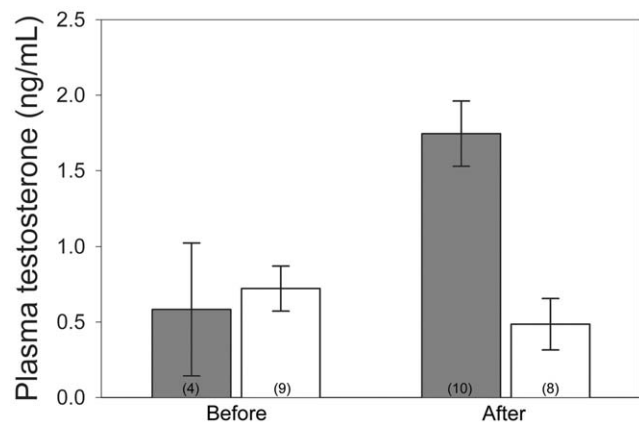


Figure 2. Plasma Testosterone. Experimental females (shaded bars) had significantly higher testosterone after implantation than before implantation. Experimental females also had significantly higher testosterone levels than control females (open bars). Sample sizes are shown in parentheses. These data are back-transformed from the natural log of plasma testosterone; error bars represent the standard error of these back-transformed means. doi:10.1371/journal.pone.0054120.g002

possible in free-living animals. This study has continuous sampling of incubation behavior, and so I first asked whether T-induced changes in incubation behavior changed over time (i.e. whether incubation behavior was less and less affected by the treatment on Day 1, Day 2, and Day 4 post-implantation) using a linear mixed model with day of sampling, treatment, and day*treatment interaction as fixed effects and 'bird' as a repeated random factor. I also used post-hoc Tukey's HSD tests to contrast successive days within each treatment group. Next, I used Pearson correlations to ask whether a given female's aggressiveness was negatively related to the time elapsed since implantation with T. Finally, I asked whether T titers in T-implanted females were correlated with the number of days elapsed since implantation.

Results

Linear mixed models on hormone data revealed a significant main effect of treatment ($F_{1,16.7} = 4.64$, $p = 0.046$) and a non-significant effect of time (pre- versus post-implant) in the whole dataset ($F_{1,20.0} = 1.78$, $p = 0.20$). However, there was a significant treatment*time interaction ($F_{1,20.0} = 6.51$, $p = 0.019$), demonstrating that T increased between pre- and post-implant samples in experimental females, but T slightly decreased over time in control females (Back-transformed means \pm se: Before, control: 0.72 ± 0.15 ng/mL, $n = 9$, Before, experimental: 0.58 ± 0.44 ng/mL, $n = 4$; After, control: 0.49 ± 0.17 ng/mL, $n = 8$, After, experimental: 1.75 ± 0.22 , $n = 10$, **Figure 2**). Thus, despite limited sample sizes at some time points, these data indicate the hormone implants increased plasma testosterone in the experimental group only. The mean elevated T levels in the experimental group lie at the high end of the natural range for incubating female tree swallows, reported by [73] as mean = 0.86 ng/mL, SD = 1.2 ng/mL.

Experimental females were significantly more aggressive than control females (Mann-Whitney: $Z = 3.21$, experimental: 37.5 ± 3.0 , control: 18.6 ± 3.5 , $n = 13$ /group, $p = 0.0012$, **Figure 3**). The nests of experimental females were significantly cooler than controls, and this treatment effect was significant despite a significant effect of ambient temperature on nest temperature (GLM: $\chi^2_{2,18} = 34.4$, $p < 0.0001$, ambient temperature: $\chi^2 = 12.0$, $p = 0.0005$, treatment: $\chi^2 = 31.5$, $p < 0.0001$; **Figure 4**). Hatching success was dramatically different between control and experimental nests (Fisher's exact: $\chi^2 = 13.8$, $p = 0.0005$, with 0/13 experimental nests hatching and 9/13 control nests hatching).

Temporal variation in T levels, aggression scores, and incubation temperatures suggest that these results are unlikely to have been caused by a short-term spike in T that steadily declined throughout the duration of the experiment. Among T-implanted females, there was no detectable correlation between plasma T levels and the number of days elapsed since implantation (Pearson's $r = -0.27$, $n = 10$, $p = 0.44$), and there was a nearly significant positive correlation between female aggression score and the number of days elapsed since implantation (Pearson's $r = 0.55$, $n = 13$, $p = 0.052$), suggesting that, if anything, the behavioral effect of the T implant increased over time, rather than decreased. LMM revealed a significant treatment effect on nest temperature ($F_{1,48.4} = 13.6$, $p = 0.0006$), a significant effect of

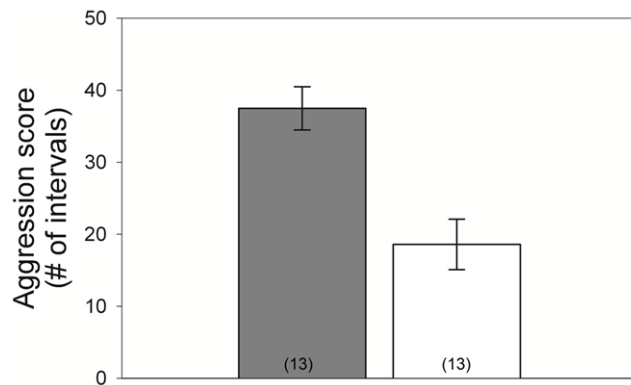


Figure 3. Aggression. Testosterone-implanted females (shaded bar) were significantly more aggressive than control females (open bar). Sample sizes are shown in parentheses; error bars represent the standard error of the mean.
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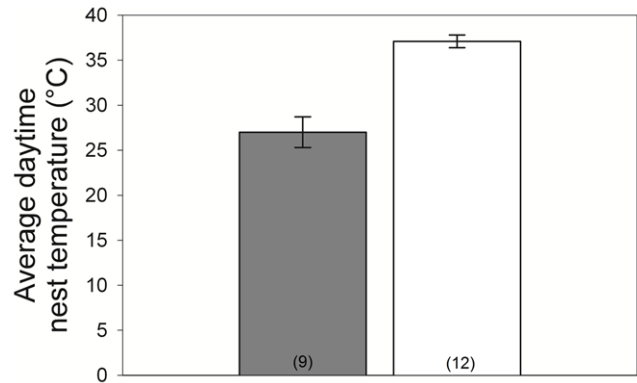


Figure 4. Incubation. Testosterone-implanted females' nests (shaded bar) were significantly cooler than control females' nests (open bar). Sample sizes are shown in parentheses; error bars represent the standard error of the mean.
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day ($F_{2,37.5} = 6.6$, $p = 0.0035$), but no significant interaction between treatment and day ($F_{2,37.5} = 1.8$, $p = 0.18$). Successive days did not significantly differ in temperature among control nests (Tukey's HSD: $p > 0.80$), but experimental nests steadily decreased in temperature over time, such that temperature on day 4 was significantly cooler than on day 1 after implantation (Tukey's HSD: $p = 0.0098$; average degrees above ambient on day 1 after implantation: control = $23.0 \pm 1.4^\circ\text{C}$, experimental = $15.6 \pm 1.9^\circ\text{C}$; on day 2: control = $23.1 \pm 2.4^\circ\text{C}$, experimental = $12.8 \pm 1.3^\circ\text{C}$; on day 4: control = $20.5 \pm 2.4^\circ\text{C}$, experimental = $8.6 \pm 1.5^\circ\text{C}$).

Discussion

Collectively, these results demonstrate that testosterone appears to mediate a behavioral trade-off between parental and aggressive behavior in incubating females: females with experimentally elevated T behaved more aggressively towards a same-sex competitor than did control females, and experimental nests had cooler temperatures, demonstrating that T-implanted females incubated less well than date-matched controls. Prolonged exposure to elevated T had clear fitness costs for females, as there was a robust negative effect of T-implantation on hatching success. These findings likewise demonstrate that females remain behaviorally sensitive to T during the incubation period, contrary to predictions based upon hypotheses used to explain interspecific patterns of behavioral sensitivity/insensitivity to T in males. Elevated T led to the enhancement of aggression at the expense of maternal care in a system where both female parental effort (incubation) and mating effort (defense of a breeding resource against same-sex rivals) are both important to reproductive success during a short breeding season.

The reduction of incubation and enhancement of aggression seen here in female tree swallows is similar to the T-mediated trade-off seen in many species of male birds (see references in Introduction). Because parental care is thought to be particularly important for female reproductive success, selection is thought to favor either lower levels of circulating T in females or reduced sensitivity to T [31,32]. Consistent with this view, females typically have relatively low levels of circulating T during parental phases of the breeding season [31,32], and some aspects of maternal care appear to be unresponsive to experimentally elevated T [47,49]. To my knowledge, however, the results I report here are the first to show that elevated T interferes with female incubation behavior, and this finding demonstrates that female insensitivity to T is not

necessarily the norm with respect to incubation behavior, despite the fact that female incubation is clearly essential to female fitness.

Prolonged exposure to T levels at the high end of the natural range of variation for incubating female tree swallows [73] resulted in a robust reduction in incubation and hatching success in this experiment. T-implanted females decreased incubation behavior sufficiently to decrease nest temperatures by approximately 10°C, ultimately leading to nest failure. Plasma T concentrations for female swallows reported here are at the high end of the normal range for tree swallows [73] and other temperate songbirds [31,32], suggesting that these values reflect natural levels of T (albeit high levels) that a female might experience during incubation. Repeated measurements of incubation temperatures reveal that the behavioral effect of the T-treatment increased over time (i.e. temperature in T-nests became closer and closer to ambient temperature over time), suggesting that the strong effect of T-implantation on incubation was unlikely to be driven by a short-term or suprphysiological surge in T that only affected incubation behavior during the first few days after implantation.

A logical next question is why are females behaviorally sensitive to T if sustained exposure to T levels at the high end of the normal range of variation can have such a detrimental effect on reproductive success. One possibility is that female sensitivity to T may be a byproduct of correlated evolution favoring sensitivity to T in males [74], much as T levels in females may reflect correlated evolution with male T levels [31,32,75]. However, previous studies that have compared behavioral sensitivity in males and females of the same species suggest the sexes may be able to evolve somewhat independently, with respect to behavioral sensitivity to T [20,47,49,76]. Another likely possibility is that exposure to the sustained high T levels seen in this experiment is a very rare occurrence, as female T titers typically decline during incubation in most temperate songbirds [31]. Indeed, control females saw a marginal decline in T levels during the study, and control females had significantly lower T levels than experimental females, suggesting that selection for or against sensitivity may not have a chance to operate under unmanipulated circumstances.

A third and not mutually exclusive possibility is that, like males, female patterns of behavioral sensitivity/insensitivity to T also may vary according to the ecology or life history of the species. While sustained and experimentally elevated T was clearly detrimental to hatching success, there are reasons to suspect that the behavioral sensitivity to T seen here may be adaptive under more natural conditions. For example, a short-term T surge may not necessarily have the same strong reduction in egg viability seen in this experimental setting because, like many songbirds, tree swallow eggs are hearty to incubation neglect on a short time scale, e.g. typically one day, but ranging up to several days due to adverse weather conditions [77,78]. In addition, this study coincided with a 4-day cold snap where ambient temperature = $8.5 \pm 0.1^\circ\text{C}$. Because experimental nests remained much closer to ambient temperatures than controls, it is feasible that this stochastic weather event may have exacerbated the observed negative effect on hatching success. Much more subtle effects on incubation and brooding are known to have deleterious effects on embryo development and ultimately chick condition [58,79–81], and chicks with reduced growth or smaller body mass may be less likely to survive to reproductive age [60,82]. Indeed, more aggressive female tree swallows tend to have chicks with reduced body mass [53], and the T-mediated trade-off between incubation and aggression observed here provides a mechanism that may partly account for this more subtle cost of aggression observed in nature.

On the whole, the observation that T implants significantly elevated aggression and reduced incubation behavior in female

tree swallows is not consistent with the two classic hypotheses for accounting for patterns of behavioral sensitivity/insensitivity in males because these hypotheses would predict that parental care should be unaffected by supplemental T [30,34]. While these hypotheses were developed to explain patterns of T insensitivity in males, a key question is whether this same logic should apply to a system where T-mediated trade-offs between parental care and aggression are seen in females.

Insights into this mismatch between hypotheses developed for males and the results seen here in females may lie in the assumption (for males) that behavioral sensitivity to T should be favored when T-mediated behaviors are particularly important for reproductive success (for parallelism, I refer to this as the ‘essential mating effort hypothesis’ [4,10,27,36]). Might it be that female tree swallows show behavioral sensitivity to T because this sensitivity is advantageous in the context of same-sex aggression and intrasexual competition? Behavioral sensitivity to T is thought to be beneficial for males in many species because it may enhance a male’s ability to defend a territory or obtain mates [37,83], though these benefits may very well apply to females as well [46,84,85], especially in light of growing evidence that female-female competition for mates and breeding resources may be an essential component of female fitness [41,43]. Thus, while the sustained elevation of T seen in this study was detrimental to female reproductive success, it may be useful to consider the potential benefits of a short-term, natural elevation in T. Aggressive interactions can lead to a short term rise in T that is thought to prepare individuals for success in future social instability, at least in males of many species [35,36]. Thus, if a brief spike in T permits an increased aggressive response to the female floaters that intrude at nesting cavities throughout the season, then behavioral sensitivity to T that enables aggressiveness may be adaptive, particularly if a fleeting socially induced elevation in T would have only a fleeting negative effect on incubation behavior. Further sampling of hormones and behavior is needed to address whether the effects of T-implants seen in this study can be extended to natural seasonal or socially-induced changes in T, aggression, or parental care in this system.

Moving forward, explicit consideration of the relative importance of parental care versus aggression will be an important comparison for reconciling patterns of behavioral sensitivity/insensitivity to T in males and in females in other species. For example, the T-induced suppression of paternal care in species with substantial male care (e.g. blue-headed vireo (*Vireo solitarius*) [17]) also does not match the predictions of the essential parental care hypothesis. The essential mating effort hypothesis, however, predicts behavioral sensitivity to T when aggression is particularly important to reproductive success, even if parental behaviors are also underway, as is often the case in males of socially monogamous but genetically promiscuous, biparental species. Shifting gears to females, the links between T and female aggression are rather variable across vertebrates [45,48,50,86–88]. In at least two cases, though, T increases female aggression in species where this behavior is associated with significant positive effects on female reproductive success [89,90], suggesting that the relative benefits of aggression should be contrasted with the importance of parental care to better predict patterns of behavioral sensitivity to T. Currently, a relative scarcity of data on the fitness benefits of female mating effort makes it difficult to test empirically how widely these hypotheses may apply to patterns of T-mediated behavior in females, or whether T-sensitivity would engender the same benefits in females that it would in males. Ultimately, a full understanding of these proximate mechanisms will require the integration of these experimental studies with

natural variation in behavioral and hormonal phenotypes in both sexes [6,87,90] as well as a metric that can directly compare the relative importance of parental care and aggression.

Many species appear to have resolved the trade-off between mating effort and parental effort with a decrease in T as they shift from mating to parental effort [4,31], and my finding that endogenous plasma T levels were low in control females is consistent with this view. When the competing demands of parental and mating effort overlap in time, the optimal strategy may also involve compartmentalization of each behavior, e.g. such that aggression depends upon T, but parental care can vary independently of T [11,66]. Because both parental and aggressive behaviors are often mediated by the same areas of the brain [91] and these nuclei are heavily enervated with sex steroid receptors [92], this potentially adaptive modularity of discrete behaviors may not be possible. Comparative neuroendocrinology demonstrates a range of proximate solutions to this evolutionary problem. Aggression may instead depend upon differences in neural expression of sex steroid receptors or steroidogenic enzymes [93–95] or aggression may be mediated by different signaling molecules altogether [13,96]. Environmentally or socially induced changes in the release of or sensitivity to T [35,36,97] or in the

amount of free versus bound hormone [29,98] may likewise permit the adaptive expression of parental and aggressive behaviors. The degree to which each of these proximate mechanisms may underlie the diversity of behavioral responses to T is as of yet unclear across species and sexes [29,99], and thus awaits further investigation.

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Author Contributions

Conceived and designed the experiments: KAR. Performed the experiments: KAR. Analyzed the data: KAR. Wrote the paper: KAR.

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