

The Influence of Testosterone on Territorial Defence and Parental Behavior in Male Free-Living Rufous Whistlers, *Pachycephala rufiventris*

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We studied a population of rufous whistlers, *Pachycephala rufiventris*, throughout a single breeding season in central New South Wales, Australia. We evaluated the relation between plasma testosterone (T) and reproductive behaviors using both simulated territorial intrusions (STIs) and subcutaneous T implants. We compared circulating T values to aggression levels of males (using STI) during pair bond and territory establishment and again during incubation. Although plasma T levels were significantly lower in the latter period, male responsiveness to STI, in terms of proximity to decoy, call rate, and number of attacks on the decoy, was indistinguishable between the two breeding stages. T levels of males exposed to STI were not different from the levels of unexposed free-living males at the same breeding stage. The effect of exogenous T on parental behavior was examined by comparing duration of incubation bouts of males and their mates prior to and after T treatment. T males significantly reduced the amount of time they incubated following implantation, whereas Control males maintained their incubation effort. After cessation of breeding activities, T males displayed significantly higher call rates due to increased use of the primary intersexual advertisement call in this species. The reduction of incubation behavior following T implantation emphasises the functional significance of the rapid decline in T in free-living males during incubation. The results from both experiments suggest that intersexual advertisement, rather than territorial aggression, may be dependent on high T levels in this species. © 2001

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Prior to the onset of breeding, male birds experience an increase in circulating testosterone (T) above levels maintained in nonbreeding periods, facilitating gonadal recrudescence, sperm production, and the expression of male secondary sexual characteristics (see Wingfield and Moore, 1987; Wingfield, Hegner, Dufty, and Ball, 1990, for reviews). However, in many species, free-living males have notably higher seasonal excursions of T than captive conspecifics (Wingfield, 1980; Wingfield and Moore, 1987; Wingfield *et al.*, 1990). This difference is thought to arise from frequent conspecific agonistic interactions experienced while establishing territories and/or defending a mate (Wingfield *et al.*, 1990). Because the intensity of aggressive behavior is often facilitated by elevated plasma T (e.g., Arnold, 1975; Harding, 1981; Nowicki and Ball, 1989; Wingfield and Farner, 1993), attainment of high circulating T during periods of frequent agonistic interaction may be advantageous. These behavioral influences on T secretion in breeding males were formalized in a paradigm by Wingfield (1985), Wingfield, Ball, Dufty, Hegner, and Ramenofsky (1987), and Wingfield *et al.* (1990) called the “Challenge Hypothesis.” This model predicts that T levels are elevated above a breeding season baseline (a level adequate for gonadal recrudescence and expression of male secondary sex characteristics) only when males are “challenged” by conspecifics or during periods of frequent male–male agonistic interactions, thereby increasing the intensity (and presumably improving the outcome) of aggressive encounters.

Testosterone influences the intensity of aggression

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in many species, but such aggression may be counterproductive for males engaged in parental care. In species with biparental care, males typically show a rapid reduction in circulating T levels with the onset of parental duties (Wingfield and Moore, 1987; Wingfield *et al.*, 1990). Experimental elevation of T levels in males of such species decreases their level of parental care in favor of persisting with more aggressive activities characteristic of the early breeding period (e.g., Silverin, 1980; Wingfield, 1984, 1985; Hegner and Wingfield, 1987; Vleck and Dobrott, 1993; Raouf, Parker, Ketterson, Nolan, and Zeigenfus, 1997). Wingfield and collaborators (1990) also argue that the pattern of T secretion is influenced by a species' life history traits. For example, males from polygynous, promiscuous, or lekking species, which do not provide any parental care to eggs or chicks, maintain elevated T levels throughout most of the breeding season (see Wingfield *et al.*, 1990, for review).

Theoretically then, plasma T levels reflect both a species' mating system as well as an individual male's recent social history. To date, the majority of studies examining the effect of T on parental care have focused on migratory, socially monogamous north temperate passerines. In these species territoriality is usually abandoned after breeding events cease, with many species then typically forming large migratory flocks which move away from the breeding grounds to overwintering sites. In contrast, Australian rufous whistlers *Pachycephala rufiventris* aggressively defend all-purpose territories for the entire time they remain on the breeding grounds before migration begins, including a period of several months after breeding activities have ceased (Bridges, 1994a, b; McDonald, 2001). In other respects, rufous whistlers have a similar life history to most north temperate passerine species, being socially monogamous, territorial, and displaying biparental care. However, the Whistler family (*Pachycephalidae*) are members of the corvine assemblage of Australia's old endemic passerines, believed to have evolved independently on this continent before the arrival of Eurasian lineages (Boles, 1988; Russell, 1989). Thus, by studying the influence of T on the breeding behavior of the rufous whistler we can see if the pattern identified in north temperate birds is shared by other phyla. Moreover, the few endocrine studies of Australian old endemics suggest that T excursions may be reduced in amplitude (Borgia and Wingfield, 1991; Poiani and Fletcher, 1994) and there may be less dependence on T to mediate reproductive activities (Astheimer and Buttemer, 1999).

To examine the importance of T in facilitating ap-

propriate reproductive behaviors in a migratory Australian old endemic, we asked whether (1) the intensity of STI responses correlates with plasma T levels in males and (2) experimentally elevated T levels in incubating males influence parental care.

METHODS

Simulated Territorial Intrusions

All field studies were carried out at Back Yamma State Forest in central western New South Wales, Australia (148°10'E 33°20'S). Animal ethics approval was obtained from the Animal Ethics Committees of the State Forestry Department and Wollongong University (Ethics Permit No. AE97/18). Methods used were equivalent to or more stringent than NIH standards. Although there is some controversy over terminology in this field, we have referred to all vocalizations made by whistlers as "calls" rather than "songs" and we have further categorized them as six phonetically discrete phrases which we termed "call types" (see McDonald, 2001, for details). Males and, to a lesser extent, females used these call types to formulate what others have termed songs (Bridges, 1992). All calls appear to be used for more than one function; however, the *extended song* and *short whistle* calls are the primary intersexual advertisement and territorial calls, respectively, of this species (McDonald, 2001).

Simulated territorial intrusions (STIs) were carried out between 0600 and 1330 h using procedures based on those described by Wingfield *et al.* (1987). Briefly, a furred 12 × 4 m mist net was placed within the territory of a focal male rufous whistler, with a covered cage containing an adult male rufous whistler (caught earlier that day) placed under the closed net. A new decoy male was used for each STI and in each case the bird was from a different area than the focal male. We observed the focal male for the next 10 min and recorded the type and frequency of calls and aggressive behaviors given. The caged decoy was then uncovered and a tape recording of adult male rufous whistler calls was played through a speaker next to the decoy at a natural volume. The continuous loop tape contained all six call types from a dawn chorus given by an adult male from a different area of the forest.

The tape was played for 10 min, during which the following behaviors were recorded: (1) *time within 1 m of decoy cage*, (2) *time perched on decoy cage*, (3) *fly to* (male flies aggressively toward and within 1 m of decoy cage), (4) *contact* (male contacts cage), (5) *bob*,

and (6) *tail flicks*. Both *bobs* and *tail flicks* are stereotypic aggressive displays of this species. *Bobs* involve a bow-like motion of the body with the bird's head held back and the tail lifted up, while *tail flicks* are simple up and down movements of the tail with the bird's body in a normal perching position (see McDonald, 2001, for details). After 10 min of STI with behavioral records, the net was lowered and the focal male captured at the earliest opportunity. The tape was left on the continuous loop and the decoy remained uncovered until the focal male was captured. These STIs were performed for eight males each during either the territory/pair establishment or incubation stages of the breeding season. Different focal males were used for each breeding stage, but the same tape was used for all STIs during the study. Territory/pair establishment (T/PE) males were captured from 10 to 13 October, while incubating (INC) males were captured from 8 November to 23 December 1997.

Immediately after the focal male was captured, a 300- μ l blood sample was collected by venipuncture of an alar vein, using heparinized capillary tubes, and stored on ice for up to 8 h. This blood collection protocol was repeated for Control males to facilitate hormonal profile comparison. Control males were captured without the aid of an STI during the same breeding stage. Plasma was harvested after centrifugation and stored at -20°C until T levels were evaluated for replicate samples using a double-antibody radioimmunoassay procedure and a Testosterone direct ^{125}I kit from Pantex (California, U.S.A., Cat. No. 135). This technique has a high sensitivity to T (0.1 ng/ml), an intraassay variation of less than 10%, and high specificity for T (relative specificity to T, 100%; to 5α -DHT, 6.9%; to androsterone, 0.5%; to other steroids that might be present $< 0.1\%$). All samples from this study were evaluated in a single assay, eliminating any interassay variation. Mean recovery of known amounts of T in samples is reported at 99.2% by the manufacturer.

Effects of Exogenous T on Incubating Males

This experiment was carried out between 26 November and 24 December 1997 during the incubation phase. Initial behavioral observations were made the morning (0800 to 1030 h) after clutches were complete (Day 1) to determine the relative contributions of each member of a pair to incubation. These observations were made at least 10 m from the nest using either a Panasonic NV-MS4A video camera or 7×50 binoculars. Following these observations, call rates and call

types were recorded for 10 min between 1030 and 1100. The focal male was then caught in a mist net placed within the bird's territory (*without* using a STI), and a 300- μ l blood sample collected for T determination and fitted with a unique combination of colored leg bands. Males were then lightly anesthetized with methoxyfluorane inhalation and fitted with bilateral subcutaneous Silastic (Dow Corning, Midland, Michigan) implants (length, 5.5 mm; i.d., 1.47 mm). Control implants ($N = 4$) were empty and T implants ($N = 5$) were filled with crystalline testosterone (Sigma). Both implant types were placed along the birds' flanks and were sealed at both ends with Silastic medical adhesive.

After implantation on Day 1, the nests of both treatment groups were observed again on Day 4 between 0800 and 1030 h to record the incubation contribution of both sexes. Call type and rate were again monitored between 1030 and 1100 h. On Day 50, a third observation on total and type of calls used was conducted when breeding activities of the study population had ceased. Due to extremely low call rates at this stage (McDonald, 2001), Day 50 observations were carried out between 600 and 630 h, when call rates were higher. Although it was not possible to recapture all of the free-living males used in the experiment on Day 50 to remove implants, three birds were recaptured and had a 300- μ l blood sample taken. Implants were removed under anesthetic and the incision was sealed using medical-grade cyanoacrylate adhesive (Vet-bond) before birds were released at the site of capture.

Playback of whistler vocalizations were sometimes used to facilitate capture of focal males on Day 50 only. However, it is unlikely that this procedure affected endogenous T levels, as T levels in this species are not affected by exposure to playback alone (McDonald, Astheimer, and Buttemer, unpublished data).

Testosterone Levels of Implanted Males

Due to the low recapture success of T-implanted birds, we used captive rufous whistlers to measure plasma levels of T resulting from T implants identical to those used for the STI experiment above. Sixteen, postbreeding adult males were caught between 26 January and 5 February 1998 and a 150- μ l blood sample was taken from each bird immediately upon capture to assess its T level. These males were then placed in calico bags and transported to aviaries located at our field base, approximately 20 km SE of the Back Yamma study site. Birds were implanted on the day of capture, with eight males receiving bilateral T-filled

implants and eight others receiving empty (Control) implants, with treatments assigned randomly. After recovery, birds were housed in individual outdoor cages (40 × 80 × 40 cm). Individual cages were visually isolated from each other by cardboard partitions, but auditory separation could not be achieved at this field site. Water and mealworms were supplied *ad libitum* and refreshed twice daily. Birds were exposed to ambient photoperiod (approx. 15L:9D) and temperatures (ranging between 17° and 35°C) throughout the experiment. Blood samples (150 µl) were taken from the captive males on Day 4 of the experiment, after which Controls were released. T males, however, were held until Day 7, when a final sample (150 µl) was taken. The time elapsed between coming in visual contact with the whistlers and completion of blood collection was noted for all birds sampled. Implants were removed as described previously before birds were released at their capture site.

Statistical Analyses

T levels of males subjected to STI were compared to those of Controls caught during the T/PE and INC stages using a two-way ANOVA, following square root transformation of T levels to normalize their distribution (Zar, 1996). *Posthoc* analyses for all ANOVAs were performed using Tukey–Kramer tests with Bonferroni corrections. Total call rates and total number of aggressive displays were logarithmically transformed to normalize distributions for these behavioral characteristics (Zar, 1996). Two-way ANOVAs were then used to test the difference in totals of these three categories, with both stage of the breeding cycle and before or during the STI treated as factors. Student's *t* tests were used to assess changes in call frequency and changes in proportion of total call rates for all six call types measured during STIs. The influence of time of exposure to an STI on the rate of both *bobs* and *short whistle* calls, following logarithmic transformation, and circulating plasma T concentrations were examined using linear regression for each of the breeding stages examined. The effect of time after capture on plasma T levels was determined using linear regression of plasma T versus time after capture for each breeding stage.

We examined the influence of T on incubation by comparing the number and duration of male and female incubation bouts and the time that the nest was unattended prior to and following implantation in T-treated and control birds using paired Student's *t* tests.

Comparison of T-treated and Control male log-transformed call rates were assessed using a nested ANOVA with the fixed factors of treatment (T-filled or empty implants) and time (Day 1, 4, or 50) and the random factor of bird number (1–9) nested within treatment. The six call types were not analyzed separately due to their lack of independence. However, the proportion of total calls given as either *extended song* call or *short whistle* call during each observation was analyzed separately using ANOVAs identical to that for total call rates, with the proportion of each call type replacing total call rates (these two functional call types are discussed in McDonald, 2001).

Evaluation of T levels of captive males used in the experiment were performed using a paired *t* test to analyze changes in T levels between Days 1 and 4 experienced by T males and Controls. As values were only available for T males on Day 7, an ANOVA was used to compare T levels in T males only between Days 1, 4, and 7. All variance values reported throughout are SEM.

RESULTS

Simulated Territorial Intrusions

Male rufous whistlers showed a robust response to STI, typically responding agonistically by cocking their tails and continually *bobbing*, with all males focusing their attention toward the decoy and not the speaker. Males in both breeding stages had significantly higher total call rates during STI than before STI ($F(1, 29) = 17.00, P = 0.0004$), with T/PE males increasing call rates from 56.5 ± 13.8 before STI to 105.3 ± 7.6 calls per 10 min during STI. Incubating males gave 62.4 ± 5.4 calls before and 91.8 ± 10.1 calls per 10 min during the STI. Total call rates did not differ significantly between the two breeding stages within treatments, nor was there a significant interaction between variables. Males subject to an STI also did not change the proportion of each of the six call types given from that they exhibited pre-STI observations.

Aggressive displays (*bobs* and *tail flicks*) increased dramatically during STIs (Fig. 1; $F(1, 29) = 193.61, P = 0.0003$), but again there was not a significant difference in the number of aggressive displays per STI between the two breeding stages or an interaction between treatment type, breeding stage, and the number of aggressive displays observed. Of the two aggressive behaviors, *bobs* was the main aggressive dis-

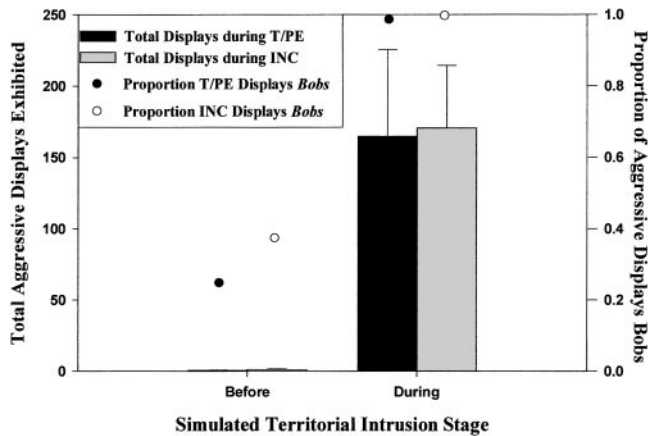


FIG. 1. Total number of aggressive behaviors per 10-min observation given by adult male rufous whistlers before and during exposure to a simulated territorial intrusion in the territory/pair establishment (T/PE; $n = 8$) and incubation (INC; $n = 8$) stages of the breeding cycle. Dots indicate proportion of total aggressive displays observed that were *bobs*. Error bars are SEM.

play used by male rufous whistlers, especially during STIs (Fig. 1).

Males in the T/PE stage spent significantly more time within 1 m of the decoy cage during STIs than did INC males (Fig. 2; $t = 3.79$, $df = 12$, $P = 0.0001$); however, the amount of time males from different breeding stages spent perched on the decoy cage was not significantly different. Similarly, there was no effect of breeding stage on the number of *fly to* (T/PE, 3.8 ± 1.8 ; INC, 3.9 ± 2.3) or *contact* behaviors (T/PE,

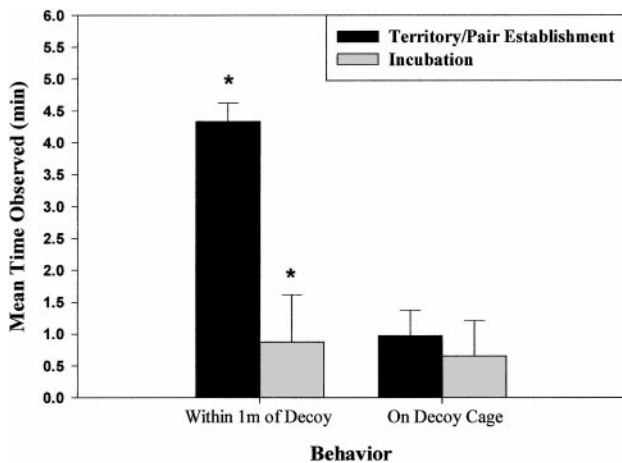


FIG. 2. Mean time adult male rufous whistlers spent both within 1 m of and actually perched on a decoy cage during a 10-min simulated territorial intrusion at the territory/pair establishment ($n = 8$) and incubation ($n = 8$) stages of the breeding cycle. Error bars are SEM, asterisks (*) indicate $P < 0.05$.

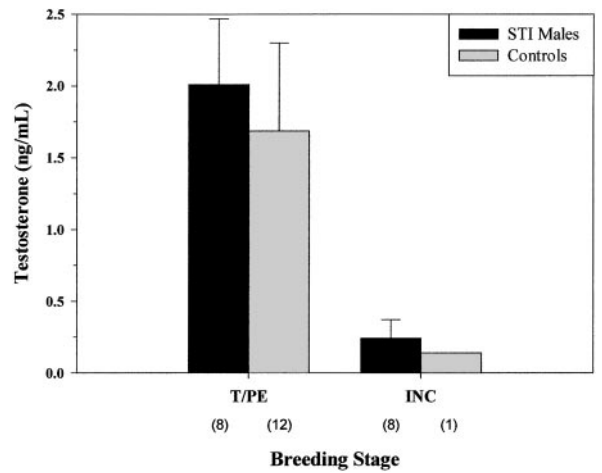


FIG. 3. Mean plasma concentration of testosterone (ng/ml) in adult males subjected to 10 min of simulated territorial intrusion (STI) males) or unmanipulated males (Controls) during the territory/pair establishment (T/PE) and incubation (INC) stages of the breeding cycle. Error bars are SEM. Numbers in parentheses indicate sample size.

11.5 ± 4.7 ; INC, 6.8 ± 4.9) performed by males during STI.

The mean time to capture focal males after 10 min of STI was 5.9 ± 1.9 min during T/PE and 6.5 ± 1.9 min during INC stages. T levels were not correlated with the time taken until capture or the time until blood collection was completed after capture in either breeding stage.

Both controls and males exposed to STI had significantly higher T levels during the T/PE than the INC stage (Fig. 3; $F(3, 29) = 3.64$, $P = 0.0465$). Males exposed to STI tended to have higher T levels than controls at the same breeding stage, but this difference was not significant for either of the stages examined. There was no relation between plasma T level and either the number of *bobs* males displayed or the change in proportion of total calls given that were *short whistles* (main territorial call) before and after STI.

Testosterone Implant Experiment

All nine pairs of birds used for the implant experiment had two-egg clutches, although modal clutch size for this species at a nearby site has been reported to be three (mean clutch size 2.63 ± 0.07 ; Bridges, 1994a). Air temperature was significantly higher on the day birds received implants (Day 1) than when they were observed 3 days later, averaging 35.1 ± 1.9

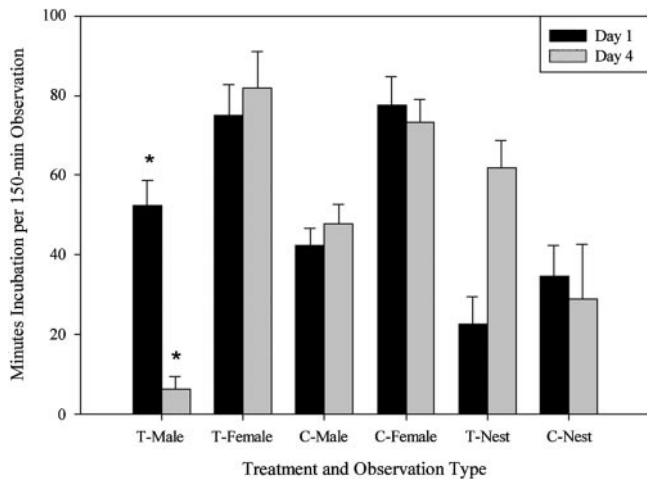


FIG. 4. Mean minutes of incubation per 2.5 h of observation provided by T males, Control males (C male), and their respective mates (T female or C female) and periods the nest was unattended for each treatment type (T nest or C nest) before (Day 1) and 72 h after (Day 4) receiving implants. Males were implanted with either testosterone-filled (T males, $n = 5$) or empty (Controls, $n = 4$) Silastic capsules. Error bars indicate SEM. An asterisk indicates significant difference ($P < 0.05$) between Days 1 and 4.

and $32.1 \pm 0.3^\circ\text{C}$ for T males and Controls, respectively, on Day 1 and dropping to $29 \pm 1.1^\circ\text{C}$ for T males and $28.5 \pm 1.2^\circ\text{C}$ for Controls on Day 4 ($F(1, 14) = 12.86$, $P = 0.003$). However, air temperature did not differ between treatments on either Day 1 or Day 4 and there was no interaction between treatment type and air temperature on the day of observation.

Exogenous T strongly affected the incubation behavior of male rufous whistlers (Fig. 4). Three days after implantation (Day 4), T males incubated significantly less than before implantation on Day 1 ($t = 6.54$, $df = 8$, $P = 0.0002$). However, neither the female partners of T males, control males, or the partners of controls significantly altered their level of incubation throughout the experiment (Fig. 4). Consequently, there was a tendency for T-male nests to be unattended for longer periods following implantation than those of controls, but this difference was not significant (Fig. 4).

The reduced incubation effort of T males was the result of a reduction in the number of incubation bouts observed per observation rather than the duration of each bout. Prior to implantation, T males averaged 3.8 ± 0.8 bouts per observation period, whereas 3 days postimplantation their bout frequency was reduced to 2.2 ± 0.7 bouts. In contrast, control males maintained the same incubation bout frequency, averaging 2.75 ± 0.5 and 2.75 ± 0.75 bouts per observation period,

respectively. The difference between T males and control birds after implantation was statistically significant ($t = 2.47$, $df = 7$, $P = 0.04$). In contrast, the incubation bout frequency of female partners was unchanged over this period.

Comparison of call rates of T males and Controls over the 3 sampling days (Days 1, 4, and 50) revealed a significant interaction between treatment and sample day ($F(2, 14) = 15.19$, $P < 0.05$). This is due to T males having a significantly higher call rate than Controls on Day 50, while the two treatments do not differ in observed call rate on either Day 1 or Day 4 (Fig. 5). The higher call rates of T-treated males on Day 50 is primarily due to a significant increase in the number of *extended song* calls (the main intersexual advertisement call) given by T males on Day 50 (20 ± 4.31 calls per 10 min) compared to Controls (1 ± 0.71 calls per 10 min; $F(2, 14) = 5.35$, $P < 0.05$). In contrast, only Controls (7 ± 3.35 calls per 10 min) were heard giving the *short whistle* call (the main territorial call) on Day 50; however, this difference was not statistically significant. Although the remaining four other call types were not assessed statistically, they tended to be given more often by T males on Day 50 than Controls.

Plasma T Levels of Implanted Birds

No implanted males were recaptured on Day 4; however, plasma T levels of three T males and two

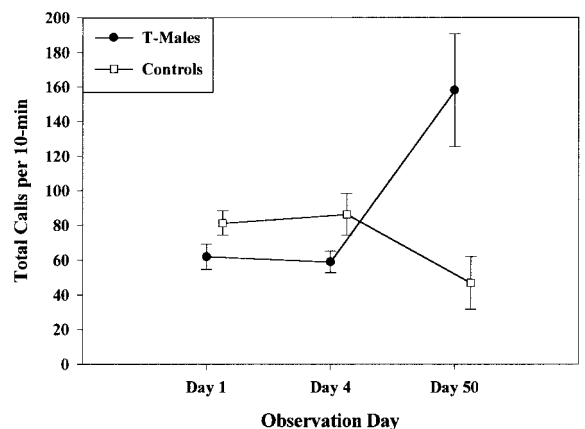


FIG. 5. The total number of calls given by adult male rufous whistlers in a 10-min observation before (Day 1), 72 h after (Day 4), and 7 weeks after (Day 50) implantation with either testosterone-filled (T males, $n = 5$) or empty (Controls, $n = 4$) implants. Day 1 and 4 call rate observations were carried out between 1030 and 1100 h, Day 50 between 600 and 630 h. Error bars indicate SEM. Control call rates are offset from the x axis to improve clarity.

Controls recaptured on Day 50 did not differ statistically, despite T only reaching detectable levels in two of the three T males (mean T, 3.1 ± 2.0 ng/ml). The T implants of these two males still contained a small amount of crystalline T, with the rest of the implant filled with yellowish fluid. In contrast, the T male lacking detectable levels of T had only one implant remaining and this was completely empty. Presumably the second implant had fallen out sometime during the experiment, although one implant should still have boosted this male's T levels higher than that of Controls during behavioral observations on Day 4.

Due to the difficulty of recapturing free-living implanted males, captive males were used to evaluate short-term plasma T concentrations following implantation. Captive T males had higher T concentrations in their plasma on Day 4 (averaging 28.6 ± 5.5 ng/ml; range, 13.2 to 62.5 ng/ml, $n = 8$) than captive Controls (averaging 3.3 ± 3.1 ng/ml; range, 0 to 23.2 ng/ml, $n = 8$). However, the one high value in Control birds (23.2 ng/ml) was entirely uncharacteristic, with six of the eight Controls having undetectable plasma T and one having 0.1 ng/ml of plasma T. If the outlier is excluded from the analysis, Control birds averaged just 0.01 ± 0.04 ng/ml of plasma T. Even with the inclusion of this outlying Control, the changes in plasma T concentrations from preimplant (Day 1) to postimplant (Day 4) in captive males was significantly greater in T males than Controls ($t = 4.11$, $df = 14$, $P = 0.0001$). By Day 7 T males had begun to experience a small decline in circulating T levels, averaging 26.7 ± 6.3 ng/ml, which was still significantly higher than T values on Day 1, but not Day 4.

DISCUSSION

Testosterone and Territorial Aggression in Male Whistlers

Male rufous whistlers display a pattern of T secretion like that documented for other biparental passerines, with T levels reaching a peak during territory/mate acquisition and mate guarding before declining dramatically with the onset of incubation (McDonald *et al.*, unpublished data). However, unlike many species studied previously, male territorial aggression persists throughout the breeding season in male rufous whistlers despite this precipitous fall in T level. Furthermore, the intensity of aggressive behaviors shown by males in response to STI were not correlated with their plasma T level nor associated with a rise in

T during STI, in contrast to expectations of the Challenge Hypothesis (Wingfield *et al.*, 1990).

There are several explanations that may account for the lack of correlation between T and aggression in this species. It is possible that the population density of rufous whistlers at Back Yamma was sufficiently high to provoke maximal secretion of T in breeding males, due to the resultant frequent intrusions by neighboring males, as noted in some species (Ball and Wingfield, 1987; Beletsky, Orians, and Wingfield, 1992). However, this explanation does not account for the lack of rise in T levels following STIs in males during the incubation stage.

Another possibility is that the STI was not perceived as an intrusion by male whistlers, perhaps because they were not receiving appropriate cues from the decoy or sufficient aural stimulation to increase T secretion. This seems unlikely as males dramatically increased their level of aggressive displays following the onset of STIs. Alternately, it is possible that STIs were perceived as intrusions, but were not maintained sufficiently long to stimulate T secretion. However, Wingfield and Wada (1989) found that T levels of male song sparrows began to rise within 1 to 4 min after exposure to STI. Because male whistlers in this study were exposed to a minimum of 10 min of STI and did not experience any significant rise in T levels, despite increased aggressive behavior, it is unlikely a longer period of STI would have yielded different results.

The most likely interpretation is that T and male territorial aggression are not coupled in this species, as evidenced by males exhibiting the same frequency and intensity of aggressive behaviors during STI in both T/PE and INC stages, despite large differences in T levels at these times. This suggests that maximum territory defence in male rufous whistlers is either independent of circulating T or influenced maximally by plasma T concentrations as low as those experienced by males during incubation. Current data do not allow us to distinguish between these possibilities, but existing evidence favors the former explanation. An uncoupling of T and territory defence has been demonstrated in other bird species. For example, breeding aggression in Lapland longspurs, *Calcarius lapponicus* (Hunt, Wingfield, Astheimer, Buttemer, and Hahn, 1995; Hunt, Hahn, and Wingfield, 1997), and snow buntings, *Plectrophenax nivalis* (Romero, Soma, O'Reilly, Suydam, and Wingfield, 1998), both breeding and autumnal territoriality in northern mockingbirds, *Mimus polyglottos* (Logan and Carlin, 1991; Justice and Logan, 1995), as well as autumnal territorial aggression in European robins, *Erithacus rubecula* (Kri-

ner and Schwabl, 1991), are not affected by circulating T. Further, males of the sedentary *morphna* race of the song sparrow *Melospiza melodia* show no seasonal difference in response to STI, nor does gonadectomy affect level of male aggression or the type of calls males give (Wingfield, 1994). Indeed field-based experimental research on this species by Soma, Tramontin, and Wingfield (2000) has determined that male aggression in the nonbreeding season is in fact regulated by estrogens at a time when T levels are near basal. This raises the intriguing possibility that territorial defence in other species, such as the rufous whistler, may still be dependent on sex steroids even when T levels are near basal. Rather than aggression being triggered maximally by near basal levels of T, the delivery pathway of sex steroids may instead be via a nonclassical mechanism such as plasma neurosteroids (Soma *et al.*, 2000). However additional data are needed to examine the extent to which such processes occur in whistlers.

Parental Behavior and Plasma T Levels of Male Whistlers

Although T did not influence the level of aggression in whistlers, T-implanted males greatly reduced the time they spent incubating and, because females did not compensate for this reduced effort, there was a tendency for T-male nests to be unattended for longer periods. Such an effect of elevated T demonstrates the functional importance of the substantial reduction in T levels of male rufous whistlers at the onset of incubation. Similar declines in T at the onset of parental duties has previously been documented in males of biparental north temperate species (e.g., house sparrows, *Passer domesticus* (Hegner and Wingfield, 1986), and pied flycatchers, *Ficedula hypoleuca* (Silverin and Wingfield, 1982)). Such declines in male T are believed to be important in reducing the tendency of T to interfere with male parental behavior (Silverin, 1980; Wingfield, 1984, 1985; Hegner and Wingfield, 1987; Oring, Fivizzani, and El Halawani, 1989; Vleck and Dobrott, 1993; Chandler, Ketterson, Nolan, and Zeigenfus, 1994; Raouf *et al.*, 1997; Cawthorn, Morris, Ketterson, and Nolan, 1998).

Male incubation has been experimentally shown to be extremely important to reproductive success in biparental species (Smith, Yom-Tov, and Moses, 1982; Hegner and Wingfield, 1987; Bart and Tornes, 1989). It is possible that T-treated rufous whistler nests were more vulnerable to predators and nest parasites than those of control males; however, the low overall

breeding success of the population studied (13.3%) prevents reasonable speculation.

It is possible that the high T levels generated by the implants were at suprphysiological levels for this species. Although our implants were the size known to produce physiological T levels in the similar-sized house sparrow (Hegner and Wingfield, 1987), the much higher T levels produced in the rufous whistlers likely resulted from a substantially lower turnover rate of T in this species. Perhaps the maximal levels of T observed in the population during territory/mate acquisition (2–3 ng/ml) would not have resulted in the dramatic reduction of parental behavior that we induced with T implants. However, given that unmanipulated male whistlers experience nearly a 10-fold drop in T levels at the onset of incubation (McDonald, Astheimer, and Buttemer, unpublished data), we expect that even moderate elevations of T above incubation levels would still result in a reduction of male parental care.

Why Do Male Whistlers Exhibit a Peak in T Levels during Territory/Pair Establishment?

Given the absence of T-dependent aggressive behavior yet the apparent need to decrease T during incubation, why do male rufous whistlers experience high T levels during territory/mate acquisition? It is unlikely that successful spermatogenesis (e.g., Brown and Follett, 1977) or expression of secondary sexual characteristics require T levels of this magnitude (see Wingfield and Moore, 1987; Wingfield *et al.*, 1990, for reviews). The high levels of T prior to incubation may instead be important in stimulating particular mate-attracting behaviors in this species, in particular intersexual advertisement which, in turn, facilitates pair formation. Thus, while no significant behavioral differences were observed in male rufous whistlers on Day 4 of the T-implant experiment, T males gave significantly more *extended song* calls on Day 50. This call type is the primary intersexual attraction song of this species and was only extremely rarely given by either Controls or other unmanipulated males at this time. Thus, high T levels in this species may facilitate males in gaining mates and/or extrapair copulations.

The importance of T-mediated courtship behavior for successful breeding was demonstrated experimentally by Lehrman and colleagues in ring doves (see Lehrman, 1965, for summary). They found that female oviduct weight was significantly greater in birds exposed to intact males than in those exposed only to castrates. More recently, T has been shown to signifi-

cantly influence mate choice by female dark-eyed juncos, *Junco hyemalis* (Enstrom, Ketterson, and Nolan, 1997). Perhaps the T-stimulated *extended song* call type of male rufous whistlers has similar beneficial effects in increasing a male's attractiveness to females and/or stimulating her reproductive readiness. Such T-affected intersexual advertisement, but absence of T-affected territorial aggression, has been noted in Lapland longspurs (Hunt *et al.*, 1995, 1997), snow buntings (Romero *et al.*, 1998), and northern mockingbirds (Justice and Logan, 1995).

The correspondence of peak T levels with intersexual advertisement but not aggression is not predicted by the Challenge Hypothesis, which specifically states that the maintenance of T levels above those of the "breeding baseline" results from agonistic interactions alone (Wingfield *et al.*, 1990). There is, however, increasing evidence that sedentary species defending territories year round rely less on T to facilitate aggression during breeding than migratory species which maintain territories for a brief period. For example, some sedentary tropical passerines exhibit little variation in T annually and have very low peak levels of T when breeding (Levin and Wingfield, 1992; Wingfield and Lewis, 1993). Similarly, sedentary temperate Australian honeyeaters have low peak T levels and limited annual variation despite year-round territorial aggression (Astheimer and Buttemer, 1999). The rufous whistler population studied in this paper follows this pattern and, although it is migratory, males maintained territories outside the breeding season until migration. Interestingly, in populations of rufous whistlers where some males overwinter at breeding sites and in those known to be sedentary, males defend territories year round (Bridges, 1994a; personal observation). In light of the finding that alternate steroids influence territorial behavior in a species with year-round territoriality (Soma *et al.*, 2000), more attention must be paid to the influence of life history on the extent that species rely on T during aggressive interactions.

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