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EXOGENOUS TESTOSTERONE IN MALE DOWNY WOODPECKERS LEADS TO REDUCED CALLING BEHAVIOR OF BOTH MALES AND THEIR FEMALE PARTNERS DURING THE NON-BREEDING PERIOD

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ABSTRACT.—Numerous studies have shown that testosterone (T) increases singing rates of passerine birds, but much less is known about the influence of T on non-song vocalizations, particularly in non-passerine species. Woodpeckers (order Piciformes) give several non-song vocalizations in a variety of social contexts throughout the year, including whinny and pik calls. We gave free-living male Downy Woodpeckers (*Picoides pubescens*) subcutaneous T implants during the non-breeding period to determine whether the incidences of these calls were greater among experimental males and their female partners compared to control males and females. We also tested whether calls were given more or less often when pair members were spatially proximate, and whether T treatment affected this pattern. Both call types were more likely given when pair members were nearby (<40 m), and this was true regardless of T treatment. Surprisingly, both males with T-implants and their female partners showed significantly lower incidences of whinny calls than control birds, and the female partners of T-implanted males also gave fewer pik calls. We attributed these patterns to the possible effect of T on non-vocal behaviors that influenced the social relationship between members of a pair. *Received 15 May 2013. Accepted 31 December 2013.*

Key words: bird calls, Downy Woodpecker, non-breeding season, pair bond maintenance, *Picoides pubescens*, testosterone.

Acoustic communication in birds can include a variety of signal types, many with multiple, overlapping functions. Birds use acoustic signals to attract mates, defend territories, warn against predators, and maintain social relationships such as the pair bond (Smith 1994, Nowicki and Searcy 2005). Because these activities are so important for reproductive success, there is an abundance of research on proximal mechanisms influencing the expression of acoustic signals. However, nearly all investigations of bird acoustic signals focus on songs, which are typically the most complex signals of a species' vocal repertoire and are most frequently given during the breeding season when reproductive hormones are at their highest concentrations. The effect of testosterone on birdsong is particularly strong: testosterone increases the propensity of males (and even females) to sing, the complexity of songs, and the duration of certain structural components of songs (Galeotti et al. 1997, Brenowitz et al. 1998, De Ridder et al. 2002, Van Roo 2004, Kurvers et al. 2008). These effects can be linked to testosterone-mediated increases in the size or activity of song control nuclei and the medial preoptic nucleus of the brain (Tramontin et al. 2000, Dawson et al. 2001, Ball et al. 2002, Brenowitz and Lent 2002). The relationship between testosterone and bird song is consistent with the widely supported hypothesis that elevated testosterone levels in birds and other animals facilitate male aggressive behaviors that relate to the defense of breeding territories and acquisition of mates (Wingfield et al. 1990).

Less is known about the effects of testosterone (hereafter T) on non-song forms of acoustic communication in birds, even though such signals may be expressed in a reproductive context (Marler 2004). Data suggest an activational role for T for some of these signals. For example, the advertisement calls of House Sparrows (*Passer domesticus*; Schwagmeyer et al. 2005) and Rufous Whistlers (*Pachycephala rufiventris*; McDonald et al. 2001), territorial calls of Red Grouse (*Lagopus lagopus scotica*; Mougeot et al. 2005), aerial alarm calls of domestic chickens (*Gallus gallus*; Gyger et al. 1988), and the sexually selected 'rusty gate' call of male and female Grey Partridges (*Perdix perdix*; Fusani et al. 1997) have all been shown to increase in frequency with supplemental T. The T-mediated call in the grouse was related to male territorial defense; in all other cases the non-song vocalizations were directed at females and could be used by females to assess male quality.

The role of T in facilitating behaviors during the non-breeding period has been investigated

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much less than during springtime. However, pair bond relationships and courtship behaviors are present in some species at this time, and the expression of these behaviors has been found to positively correlate with T or T precursors (Sharp 1996, Wingfield et al. 1997, Frigerio et al. 2004). Testosterone concentrations in many temperate birds are near zero in fall and winter (i.e., ''non-breeding baseline,'' Wingfield et al. 1990), but birds of some species become photostimulatory several months before breeding (Nicholls et al. 1988, Dawson 2008). This allows for small rises in testicular volume if not suppressed by environmental variables such as poor food supply, weather conditions, or lack of social stimulation (Dawson 2008). Plasma concentrations of T in birds between the non-breeding baseline and the breeding season levels that support male aggression are thought to facilitate sexual behavior including courtship (Wingfield et al. 1990).

The Downy Woodpecker (*Picoides pubescens*) is one species in which courtship behavior including copulation solicitations can be observed in winter (Kellam et al. 2004). Downy Woodpeckers are socially monogamous and year-round residents. They seem to defend small territories while nesting, but territorial defense away from a nest is poorly documented in the literature (Jackson and Ouellet 2002; but see Gordon and Confer 1996). Aggressive interactions appear to be more focused on mate selection than on spatial resources, since most (78%) same-sex aggressive displays observed from November through April took place within 40 m of a bird of the opposite sex (Kellam et al. 2004). Testosterone is found in males and females at low concentrations throughout the year (~5 ng/g feces or ~3 ng/ml plasma androgen equivalent), with population averages showing a small peak around 10 ng/g in April prior to egg laying (Kellam et al. 2004). Despite low population averages, some individuals during the nonbreeding period have levels of T equal to or exceeding those of breeding birds (Kellam et al. 2004).

A study using T implants in males from January through March was conducted to help determine the function of these elevated T levels. T-implanted males showed closer intrapair spatial association when experiencing a high degree of intrusions onto their home range by neighboring males (Kellam et al. 2006). T-males also showed reduced frequencies of male-male aggressive visual display and spatial association with same-sexed neighbors (Kellam et al. 2006).

The cause for a reduction in male-male interaction remains unclear. One possibility is that a T-male's dominance status or degree of readiness to respond to social challenge could have been maintained through other means and at a greater average distance from neighboring males, such as by increased vocalization rates (Boseret et al. 2006; also see De Kort et al. 2009). If T implants increased the calling rates of woodpeckers, this may have led to reduced interactions with other males.

Here, we used data collected during Kellam et al.'s (2006) study to examine the effect of T implants on the expression of two vocal signals given by free-living male Downy Woodpeckers and their female associates: 'whinny' calls and 'pik' calls. Whinny calls are loud and of relatively long duration (1.5 secs; Mahan 1996). They may be used in territorial defense, to assert dominance over neighbors, and to initiate contact with a mate (Kilham 1962, Short 1982, Ritchison 1999). There is little indication that males and females use the call in different contexts, but much of our understanding of this call comes from anecdotal reports and small observational studies. Mahan (1996) found that use of whinny calls by males varied across months, with most whinnies given in April. However, use of whinny calls by females did not vary across months (Mahan 1996). Pik calls are short notes averaging 5 msecs in duration (cf., 'teak' call of Mahan 1996) that may be given singly or repeated for several minutes at a time. Pik calls may serve as short-distance contact calls between mates, and may also indicate a state of mild alarm (Sullivan 1984, Ritchison 1999, Jackson and Ouellet 2002). Mahan (1996) found that pik calls were given year-round, but most frequently in April.

We predicted that whinny calls, because of their reported role in asserting dominance and territorial defense, would be given at greater rates by T-males than controls. No *a priori* prediction was made regarding the effect of T on the rate of pik calls, but the signal is included here because of its function in pair interactions, which could be altered by testosterone treatment. We also report on acoustic signals given by females because any change in male vocal behavior might affect the rate at which a female responds to the male signals. Lastly, we document whether whinny and pik calls are given more often when pair members are spatially proximate or more distant from one another, and whether T treatment alters this effect.

If testosterone has an effect on calls related to courtship or other pair interactions, the effect may be most evident when mates are close by.

METHODS

Our study was conducted at the Ross Biological Reserve, Tippecanoe County, Indiana from 5 January to 31 March 2001–2002. Details of the field methods were described in Kellam et al. (2006). In brief, 21 male and 24 female Downy Woodpeckers were captured over 2 years using manually operated tree-trunk mounted traps baited with suet. The population was studied previously (Kellam 2003a), so most individuals were marked with a unique combination of colored leg bands. For this study, woodpeckers were equipped with a 1.1-g radio transmitter (BD-2, Holohil Systems, Carp, ON, Canada; transmitter: body mass ratio ≤4%) using a leg-loop harness. The transmitters facilitated collection of behavioral data after each bird's release.

Testosterone Manipulation.—Male woodpeckers were transported a short distance from site of capture to a field laboratory where they were placed under anesthesia by intramuscular injection of saline containing a mixture of ketamine and xylazine (62.5 μg ketamine and 12.5 μg xylazine administered per gram of body mass). Ten males were randomly assigned to the experimental set (T-males) and given a subcutaneous Silastic tube implant (Dow Corning, Midland, Michigan; effective length: 7 mm, 1.47 mm i.d., 1.96 mm o.d.) packed with crystalline testosterone (T; Sigma-Aldrich, St. Louis, Missouri). The remaining 11 males (C-males) were given empty implants as a control. Radio transmitters were attached to all males before they were released at the site of capture after a 3-hr recovery period. Woodpeckers were recaptured after 5 weeks to remove spent radio transmitters and assess implant efficacy. T-males had significantly greater cloacal protuberance lengths and significantly higher concentrations of T in their feces than C-males (Kellam et al. 2006). The average T levels of T-males (15.2 ng/g feces; range 5.6–25.8 ng/g) were within the range shown by an unmanipulated population during April (range 1.9–39.4 ng/g; Kellam et al. 2004) and significantly higher than the average levels in C-males (5.5 ng/g; range 2.8–10.2 ng/g; Kellam et al. 2006).

Focal Observations.—We tracked each radio-marked woodpecker about once every 2 days for the ~30-day life of the transmitter. Data collection began 3 days following capture to minimize any residual effect of transmitter attachment and anesthesia (males only) on the woodpeckers' behavior. Focal observations were conducted during all daylight hours but ceased during periods of precipitation or high winds. The home ranges of experimental and control birds were intermingled throughout the study site as a result of random group assignments. Therefore, the mix of focal observations in the morning and afternoon always included each group.

Instantaneous scans were conducted every 10 mins during a 30-min focal observation that began as soon as a woodpecker was in view. These scans were used to document the identity and distance of conspecific associates, which were also wearing radio transmitters. Scans revealed that all woodpeckers tended to associate with a particular member of the opposite sex during focal observations, and these were considered to be mates. Such associations were observed in an earlier study of the same population as early as November (Kellam et al. 2004). The majority (67%) of these non-breeding partnerships persist into the breeding season, with much of the remainder (22%) breaking up because of death of a mate (Kellam et al. 2004).

We conducted time budget analyses during the same 30-min focal observations described above to measure the rate of acoustic signaling given by each woodpecker. Pik and whinny calls were recorded along with the behaviors of pecking, moving, looking and other activities as reported in Kellam et al. (2006). Birds rarely stayed in one place for more than a few secs at a time so we used a lapel microphone attached to an audiocassette recorder for hands-free recording of behavioral data. These audiocassettes were later transcribed in real time using event recorder software (a C++ program written by JRL) to count the number of calls given during each 30-min observation. Analyses were based on 7.2 ± 5.4 SD focal observations per bird. This represents a subset of a greater number of observations conducted on each bird for the purpose of delineating home range use (reported in Kellam et al. 2006) and male-female partnerships. Home range observations did not include time budget data, so they could not be used to quantify vocalization frequencies. Overall, the number of focal observations per bird varied because of differences in transmitter battery life and by some

TABLE 1. Number of 30-min focal observations conducted on Downy Woodpeckers used to analyze the effect of testosterone (T) implants on vocalization rates.

Group	*n*	Focal observations	Mean (and range of) observations/bird	Observations with whinny calls	Observations with pik calls
Control males	11	76	6.9 (1–16)	18	15
Control females	16	85	5.3 (2–12)	22	24
T-males	10	103	10.3 (7–18)	7	13
T-females	8	62	7.8 (4–19)	8	10

birds flying across a river. No direct hormone manipulations were conducted on females, but females were classified as T-females if they were the mate of a T-implanted male. Otherwise, females were classified as C-females. By chance, T-males and T-females had a greater average number of focal observations per bird than the control groups (Table 1). We included sampling rate as a variable in our statistical analyses to reduce any bias this may have caused (see below).

Social Context of Vocalizations.—It was beyond the design of our experiment to record the non-vocal behavioral responses of conspecifics receiving the vocal signals of the woodpecker under focal observation. However, if a non-focal individual gave a call during our observations, we were able to document it. Of particular interest was whether the calls of the woodpecker being observed were reciprocated by the mate. We noted the identity of the responding conspecific (when known), the latency of response, and the call type used (whinny or pik). Information on call reciprocation in this species has not been published elsewhere and it may clarify the function of whinny and pik calls with regards to pair bond maintenance. We assumed calls were reciprocated by the mate if the mate gave a vocal signal within 9 secs of a call made by the bird under focal observation. This 9-sec window was determined by looking at the distribution of latency values. There was a natural gap between 9 secs and 15 secs and another between 22 secs and 31 secs. To be conservative in our assumption of reciprocation, calls given by mates more than 9 secs after a call by the focal individual were assumed to be coincidental rather than responses to the initial call.

Statistical Analyses.—We hypothesized that T-treated males would utter whinny calls at a higher rate than control males, and that T treatment might also affect the rate of pik calls. Two repeated-measures statistical tests were carried out for males, one for each vocal signal. Two other models were run to analyze frequencies of female pik and whinny calls. We used SAS software (Version 9.3, SAS Institute, Cary, NC, USA) to construct generalized linear mixed models (PROC GLIMMIX; Littell et al. 2006) that tested whether the response variable, calls per hour, was dependent on T-treatment. Each bird was observed multiple times, so bird identity was used as a random, repeated factor. Goodness of fit for each model was confirmed following Littell et al. (2006) using the variable χ^2/df, which was near 1.00 in all cases. Significance was assumed at $\alpha = 0.05$.

There were some focal observations in which a woodpecker gave a series of repeated piks for several minutes, and pikking rates calculated for these observations were much higher than the 0–2/hr range typical of most observations. It is unclear whether a long series of piks has a different biological meaning than just a few piks scattered throughout a focal observation. If single piks and a series of piks are given in response to different stimuli, the pikking rate as calculated by piks per hr might be biased in a way that was independent of testosterone treatment since not all woodpeckers would be exposed to these different stimuli during our observations. Therefore, to minimize confounding effects, in a second analysis the rate of each vocalization was converted to a binomial distribution in which each focal observation was rated as to whether the vocalization was given or not given. We then constructed generalized linear mixed models that tested whether the binomial response variable (e.g., presence or absence of pik calls; hereafter 'binomial model') was affected by T treatment (T-implant or C-implant).

All models included a second independent variable and interaction term relating to whether the focal observation was being conducted on a woodpecker spatially proximate to its mate. Proximity to the mate was defined as a categorical variable indicating whether the woodpecker being

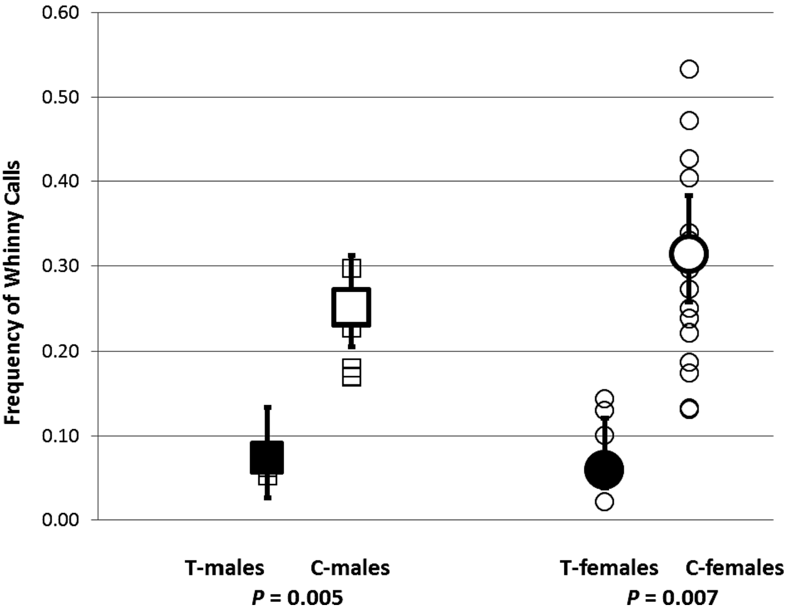

FIG. 1. Proportion of 30-min focal observations during which male and female Downy Woodpeckers gave at least one whinny call. Presented are least-squares means (± SE) output from repeated-measures mixed models run for each sex. Calling incidence of both males (large squares) and their female social partners (large circles) was significantly dependent on whether males had been given subcutaneous testosterone (T) implants or control (C) implants. Smaller squares and circles are the predicted values for individuals within each group as output from models that included implant type, proximity to the mate (>40 m or ≤40 m), sampling rate, and an interaction term.

observed was within 40 m of its mate during any one of the three scans conducted during a focal observation. A distance of 40 m was used because it ensured the two individuals were out of visual range. The function of whinny calls may be different depending on whether mates are in visual contact (i.e., pair interaction versus territorial functions). Since we did not specifically note whether male and female were in visual contact when giving whinny calls within 40 m of one another, we also ran the statistical models described above using shorter distances of association (10 m, 20 m, and 30 m). Results from these tests were not qualitatively different to the results reported below using the 40 m distance.

All models included a covariate representing sampling effort. Woodpeckers in each gender and experimental group were not observed an equal number of times (Table 1), so this variable helped account for any difference in vocalization rates or frequencies as a result of unequal sampling.

RESULTS

Whinny Calls.—One or more whinny calls were given by males in either treatment group during 14% (25 of 179) of focal observations. These calls were given during seven observations of four individual T-males and 18 observations of five individual C-males. Results from the repeated-measures binomial model showed that the propensity for T-males to give whinny calls was significantly lower than that of C-males ($F_{1,19} = 9.86$, $P = 0.005$; Fig. 1). The effect of mate proximity on a male's likelihood to give whinny calls was not significant ($F_{1,12} = 0.69$, $P = 0.42$; Fig. 2), nor was the interaction between hormone treatment and mate proximity ($F_{1,12} = 2.12$, $P = 0.17$). Similarly, the average number of whinny calls per hour was significantly lower among T-males (1.1 ± 0.1 SE) compared to C-males (1.4 ± 0.1 SE; $F_{1,19} = 5.17$, $P = 0.035$), and no significant effects of mate proximity were found ($F_{1,12} < 0.01$, $P = 0.95$).

Overall, 20% (30 of 147) of focal observations conducted on females included one or more whinny calls; eight of the 30 calls were given by three individual T-females. The repeated-measures binomial model showed that T-females were significantly less likely to give a whinny call than C-females ($F_{1,22} = 8.85$, $P = 0.007$; Fig. 1).

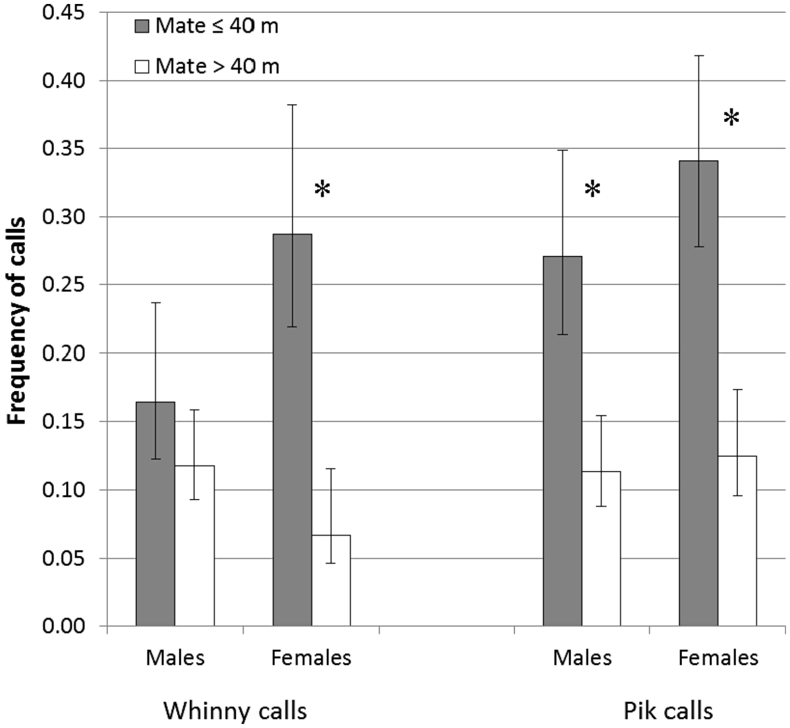

FIG. 2. Proportion of 30-min focal observations during which male and female Downy Woodpeckers gave at least one whinny or pik call. Presented are least-squares means (± SE) output from repeated-measures mixed models run for each sex and for each vocalization under the conditions of mate spatially proximate (≤40 m) or farther away. In the cases indicated by an asterisk, woodpeckers gave significantly fewer calls when the mates were separated by >40 m ($P < 0.05$).

Calling rate of T-females (1.1 ± 0.1 SE whinnies/hr) was significantly lower than C-females (1.5 ± 0.1 SE; $F_{1,22} = 12.94$, $P = 0.002$). Independent of T-treatment, females were significantly more likely to give whinnies when the mate was within 40 m ($F_{1,12} = 11.30$, $P = 0.005$; Fig. 1), and the number of whinnies per hr was greater ($F_{1,12} = 11.51$, $P = 0.006$). The interaction between T-treatment and distance from mate was not significant in either the binomial model ($F_{1,12} = 0.04$, $P = 0.85$) or the model testing whinnies per hr ($F_{1,12} = 1.89$, $P = 0.19$).

Pik Calls.—One or more pik calls were given by males in either treatment group during 16% (28 of 179) of focal observations. These calls were given during 13 observations of six individual T-males and 15 observations of six individual C-males. Results from the repeated-measures binomial model showed that there was no significant difference in the propensity to pik by T-males compared to C-males ($F_{1,19} = 1.49$, $P = 0.24$; Fig. 3). Likewise, male pik rate did not differ among T-males (1.5 ± 0.2 SE piks/hr) and C-males (1.4 ± 0.2 SE; $F_{1,19} = 0.09$, $P = 0.77$). The number of piks given per hour did not differ among males when the mate was nearby (≤40 m) versus far away ($F_{1,12} = 0.52$, $P = 0.49$). However in the binomial model, males were shown to be more likely to give pik calls when within 40 m of the mate ($F_{1,12} = 5.92$, $P = 0.030$; Fig. 2). There was no significant interaction between hormone treatment and mate proximity in either pik call model (binomial: $F_{1,12} = 0.27$, $P = 0.61$; rate: $F_{1,12} = 0.04$, $P = 0.85$).

Females gave pik calls during 23% (34 of 147) of focal observations. At least one pik call was given during observations of 14 females, including five T-females. Results from the repeated-measures binomial model showed that T-females were significantly less likely to call than C-females ($F_{1,22} = 4.40$, $P = 0.048$; Fig. 3). No difference was seen between T-females (1.8 ± 0.2 SE) and C-females (1.6 ± 0.2 SE) in the number of piks per hr ($F_{1,22} = 0.50$, $P = 0.49$). Female

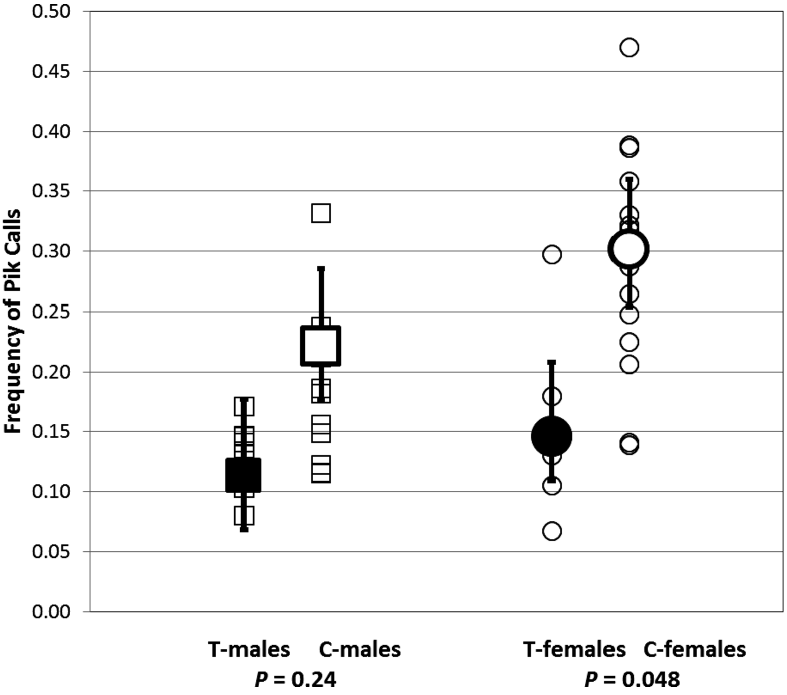

FIG. 3. Proportion of 30-min focal observations during which male and female Downy Woodpeckers gave at least one pik call. Presented are least-squares means (± SE) output from repeated-measures mixed models run for each sex. The incidence of calling in females was significantly dependent on whether their male social partner had been given a subcutaneous testosterone implant or control implant, but the males themselves showed no significant difference in calling related to their implant type. Smaller squares and circles are the predicted values for individuals within each group as output from models that included implant type, proximity to the mate (>40 m or ≤40 m), sampling rate, and an interaction term.

calling behavior was affected by mate proximity; females observed within 40 m of the mate were significantly more likely to give pik calls than females observed alone ($F_{1,12} = 9.37$, $P = 0.009$; Fig. 2), and they had a greater calling rate ($F_{1,12} = 10.08$, $P = 0.008$). There was no significant interaction found between T treatment and mate proximity using either model (binomial: $F_{1,12} = 1.63$, $P = 0.23$; rate: $F_{1,12} = 0.72$, $P = 0.41$).

Social Context.—Pik calls by both sexes and whinny calls by females were more likely to be given when a bird was spatially proximate to its mate, but sometimes the calls were given in other contexts (Table 2). There were 18 observations (of 117; 15%) when vocalizations were given but no conspecifics were detected within 40 m. Only 11of 35 (31%) observations on males associating within 40 m of another male documented a whinny call, and of those 11 observations, the mate was also present in five. Even at shorter inter-male distances, focal males only gave whinny calls half the time (9 of 18 observations when inter-male distance was ≤20 m, and 6 of 13 observations at <10 m).

Call reciprocation was fairly uncommon for both whinny and pik calls. There were 35 focal observations of males and females during which whinny calls were given. Six of these calls (17%) were reciprocated within 9 secs by the mate (average latency in response = 3.8 secs), and all were returned with a whinny call from the responding bird. In fact, extending the analysis to include six more observations beyond the 9-sec window, any call given by the mate up to 41 secs after a whinny call by the focal individual was always a whinny call rather than a pik call. Pik calls by focal individuals were reciprocated by the mate within 9 secs at a similar rate (6 of 44 focal observations; 14%). When pik calls were given by the focal woodpecker, there was an equal

TABLE 2. Number of focal observations of Downy Woodpeckers that included a vocalization when under different social contexts. Control and testosterone-treated woodpeckers are combined.

	Whinny calls		Pik calls	
Social context[a]	Male	Female	Male	Female
Association with mate[b]	11	24	18	27
Association with same-sex conspecific without mate	6	3	1	0
Other	4	1	2	2
No associations	4	2	7	5
Total	25	30	28	34

[a] Associations were defined as conspecifics observed within 40 m of one another on at least one occasion during a 30-min focal observation.

[b] With or without other conspecifics within 40 m.

likelihood that the mate would respond with a pik call or a whinny call (average latency = 6.2 secs).

DISCUSSION

Less than 22% of focal observations conducted on 35 individuals documented a vocalization, but this relatively low frequency was not unusual. Whinny call rates ranged from 1.1–1.5 per hour, which is greater than the 0.4 calls per hour documented by Mahan (1996) during non-breeding periods. Pik call rates ranged from 1.4–1.8, which is similar to the 1.5 calls per hour documented by Mahan (1996). Our results show that females gave both call types more frequently when located near the mate, and this was also true for males giving pik calls, independent of testosterone treatment. Males did not appear to give whinny calls any more frequently when near the mate than when away from the mate. However, the incidence of whinny calls given during close male-male interactions was not high, either, so it is unclear what circumstances most consistently lead to the production of whinny calls in males. What we do know is that the incidence and rate of whinny calls was reduced in the male and female experimental treatment groups. The incidence of pik calls was significantly lower in experimental females compared to controls, but no significant difference was found between male groups.

The role of testosterone is well known among vertebrate species in facilitating aggressive male-male interactions, especially when the interactions relate to defense of breeding territories or prospective reproductive partners (Wingfield et al. 1987). It thus seemed counterintuitive when Kellam et al. (2006) found that T-implanted male Downy Woodpeckers had significantly fewer close interactions with neighboring males than control males. Here we tested whether these same T-implanted males showed an increase in whinny vocalizations, which, like bird song (Catchpole and Slater 2008), could be used by neighboring males to assess a T-male's intentions or ability to defend the mate or territorial resources. Whinny calls have been described as signals used by males and females in territorial contexts, same-sex dominance interactions, and interactions with the mate (Kilham 1962, Short 1982, Ritchison 1999). Passerine song shares these same general functions (Nowicki and Searcy 2004), and supplementary T causes song rates to increase (Ball et al. 2002, Kurvers et al. 2008).

There were three possible outcomes of the experiment. First, our prediction could have been supported; T could have led to an increase in calling frequency. Second, T could have had no effect on calling frequency. Third, there could have been an unanticipated decrease in calling frequency related to the T treatment. This last outcome is best supported by our data. Failure to demonstrate the first outcome may be because of the time of year when our experiment took place and a reduced use of whinny calls to defend territory or advertise dominance status. Gordon and Confer (1996) reported that experimental playback of whinny calls and drums elicited whinny calls from resident Downy Woodpeckers. However, the woodpeckers ceased to respond to experimental playbacks after October (Gordon and Confer 1996). In winter, Downy Woodpeckers do not appear to defend exclusive territories since there is significant overlap in space use among individuals of the same sex (Matthysen et al. 1993, Kellam et al. 2006). Lack of territory defense could have prevented a T-mediated increase in vocal behavior. Males of other temperate-zone species that are implanted with T and exposed to simulated territorial intrusions react more aggressively than control males exposed to the same stimulus (Wingfield and Soma 2002). The same simulated intrusions conducted in winter do not necessarily elicit the same aggressive responses by T-implanted males as in the breeding season (Wingfield and Soma 2002). Future studies on Downy Woodpeckers might still document a relationship between circulating T and whinny calls, but the effect may only be present when birds are defending territories. Likewise, T may influence one or more of the other acoustical signals given by breeding Downy Woodpeckers that have aggressive or sexual functions, including ''kweek'' calls, ''tichrr'' calls, and drumming and tapping behavior (see Ritchison 1999 for descriptions).

Even if T has no activational role in producing whinny calls during the winter season, a second question remains. Why did the T implants lead to a decrease in the rate and incidence of whinny calls? A review of the literature generally failed to find any studies where application of T caused or was correlated with a decrease in avian vocal behavior. One exception was Titus et al. (1997), who reported that yearling male Dark-eyed Juncos (*Junco hyemalis*) implanted with T during the pre-breeding plastic song phase showed lower song rates than controls, and fewer song types. The authors concluded that T given to the juncos prior to song crystallization may have disrupted normal singing behavior at the neurological level. The influence of T on non-song vocalizations has not been widely investigated (Marler 2004), and this is particularly true for non-passerine species that lack song control nuclei (Ball et al. 2002). In the absence of neurophysiological evidence that would explain T-mediated inhibition of vocal behavior, a more likely explanation for the decrease in whinny calls among T-males is that T treatment did not directly cause this pattern; rather, the negative relationship between T-treatment and calling rate was simply correlative.

For example, there could be a correlation between a male's vocal behavior and the frequency of his interactions with other males. If whinny calls are given during close male-male interactions, then a reduction in one behavior could correspond to a reduction in the other. Kellam et al. (2006) showed that T-male spatiotemporal associations with other males were 62% lower in frequency than controls, and T-males spent about 73% less time engaged in aggressive behaviors such as displacements, chases, and displays. In the present study, the incidence of whinny calls in T-males was reduced by 72% compared to control males (Fig. 1). However, analysis of social context found that whinny calls were not routinely given by males during same-sex encounters. Only 32–50% of such encounters involved a whinny call, depending on the minimum distance between interacting males. Therefore, the reduction in male whinny calls may not be wholly explained by the lowered number of male-male encounters experienced by T-males.

The incidences of female whinny calls and male and female pik calls were shown to be highest when mates were spatially proximate (Fig. 2). Calls given by one member of a pair were uncommonly reciprocated by the other pair member, but this is not necessarily inconsistent with the ''contact calling'' function proposed by previous authors (Jackson and Ouellet 2002). Kondo and Watanabe (2009) indicate that the receiver of a contact call may respond to the call in a non-vocal manner including moving toward or away from the signaler or by simply looking in its direction. The present study was not designed to monitor the kind of responses given by a conspecific receiving vocal signals.

Pik calls may be of particular importance in pair bond interactions because woodpeckers benefit from the pik calls of their partner. Sullivan (1984) showed that the efficiency of solitarily foraging Downy Woodpeckers (males and females combined) improved when she played a recording of pik calls and woodpecker foraging sounds. Sullivan concluded that woodpeckers rely on audible cues from conspecific flockmates to modify the frequency of anti-predator vigilant scans they interject between foraging bouts. Sullivan's (1985) later work showed that both male and female Downy Woodpeckers give preferential alarm calls (''tichrr'' calls) when the mate is nearby. Combined, these results imply that mates can work together to protect one another from danger. There are numerous examples of this ''mate investment'' or ''mate protection'' behavior described in the avian literature (Hogstad 1995, Lemmon et al. 1997, Artiss et al. 1999, Kellam 2003b, Squires et al. 2007). This concept may help explain two results from our T-implant studies. We found T treatment in males led to a reduction in female pik calling (present study) as well as reduced foraging efficiency in females (Kellam et al. 2006). If T-females found the male partner less attractive because of the T-implant, or if T-females perceived that their male partner was less invested in pair interactions (e.g., fewer whinny calls), the females could have reduced their own investment in the pair bond. This would have resulted in reduced female calling behavior and lowered female foraging efficiency as a result of increased vigilance requirements relative to controls.

Most songbirds show a predictable pattern in the level of reproductive hormones with peaks during the breeding season and minima typically measured in winter (Wingfield et al. 1990). This pattern changes in species with long-term pair bonding, such as Downy Woodpeckers. In particular, hormone concentrations can be relatively high in late winter/early spring and quite variable between individuals (Kellam et al. 2004). Testosterone levels can in turn affect association patterns between mates at these times (Kellam et al. 2006). Here we showed that testosterone levels also affect vocal signaling rates. Our results indicate that T-implants can lead to a reduction in calling behavior, a finding that contrasts with most other avian studies examining the effect of T on song and non-song vocalizations. The fact that the birds we used were free-living and in a non-breeding condition could explain the discrepancy. The concentration of T used in the implants was within the species' natural range but was still artificially high in experimental males compared to controls. It is unknown whether or not the same neurophysiological mechanisms responding to T in a breeding bird are operative during mid to late winter. In addition, relatively little is known about the biology of pair associations in birds before the breeding season. Our results suggest that this is an important time of year for the maintenance and regulation of long-term pair associations.

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LITERATURE CITED

ARTISS, T., W. M. HOCHACHKA, AND K. MARTIN. 1999. Female foraging and male vigilance in White-tailed Ptarmigan (*Lagopus leucurus*): opportunism or behavioural coordination? Behavioural Ecology and Sociobiology 46:429–434.

BALL, G. F., L. V. RITERS, AND J. BALTHAZART. 2002. Neuroendocrinology of song behavior and avian plasticity: multiple sites of action of sex steroid hormones. Frontiers in Neuroendocrinology 23:137–178.

BOSERET, G., C. CARERE, G. F. BALL, AND J. BALTHAZART. 2006. Social context affects testosterone-induced singing and the volume of song control nuclei in male Canaries (*Serinus canaria*). Journal of Neurobiology 66:1044–1060.

BRENOWITZ, E. A., L. F. BAPTISTA, K. LENT, AND J. C. WINGFIELD. 1998. Seasonal plasticity of the song control system in wild Nuttall's White-crowned Sparrows. Journal of Neurobiology 34:69–82.

BRENOWITZ, E. A. AND K. LENT. 2002. Act locally and think globally: intracerebral testosterone implants induce seasonal-like growth of adult avian song control circuits. Proceedings of the National Academy of Sciences of the USA 99:12421–12426.

CATCHPOLE, C. K AND P. J. B. SLATER. 2008. Bird song: biological themes and variations. Second Edition. Cambridge University Press, Cambridge, UK.

DAWSON, A. 2008. Control of the annual cycle in birds: endocrine constraints and plasticity in response to ecological variability. Philosophical Transactions of the Royal Society of London, Series B 363:1621–1633.

DAWSON, A., V. M. KING, G. E. BENTLEY, AND G. F. BALL. 2001. Photoperiodic control of seasonality in birds. Journal of Biological Rhythms 16:365–380.

DE KORT, S. R., E. R. B. ELDERMIRE, E. R. A. CRAMER, AND S. L. VEHRENCAMP. 2009. The deterrent effect of bird song in territory defense. Behavioral Ecology 20:200–206.

DE RIDDER, E., R. PINXTEN, V. MEES, AND M. EENS. 2002. Short- and long-term effects of male-like concentrations of testosterone on female European Starlings (*Sturnus vulgaris*). Auk 119:487–497.

FRIGERIO, D., K. HIRSCHENHAUSER, E. MÖSTL, J. DITTAMI, AND K. KOTRSCHAL. 2004. Experimentally elevated testosterone increases status signaling in male Greylag Geese (*Anser anser*). Acta Ethologica 7:9–18.

FUSANI, L., L. BEANI, C. LUPO, AND F. DESSÌ-FULGHERI. 1997. Sexually selected vigilance behaviour of Grey Partridge is affected by plasma androgen levels. Animal Behaviour 54:1013–1018.

GALEOTTI, P., N. SAINO, R. SACCHI, AND A. P. MØLLER. 1997. Song correlates with social context, testosterone and body condition in male Barn Swallows. Animal Behaviour 53:687–700.

GORDON, A. L. AND J. L. CONFER. 1996. Do Downy Woodpeckers abandon their breeding territories in winter and relocate near a permanent food source? Kingbird 46:111–116.

GYGER, M., S. J. KARAKASHIAN, A. DUFTY, JR., AND P. MARLER. 1988. Alarm signals in birds: the role of testosterone. Hormones and Behavior 22:305–314.

HOGSTAD, O. 1995. Alarm calling by Willow Tits, *Parus montanus*, as mate investment. Animal Behaviour 49:221–225.

JACKSON, J. A., AND H. R. OUELLET. 2002. Downy Woodpecker (*Picoides pubescens*). The birds of North America. Number 613.

KELLAM, J. S. 2003a. Downy Woodpecker pair bond maintenance in winter: proximate and ultimate mechanisms. Dissertation. Purdue University, West Lafayette, Indiana, USA.

KELLAM, J. S. 2003b. Pair bond maintenance in Pileated Woodpeckers at roost sites during autumn. Wilson Bulletin 115:186–192.

KELLAM, J. S., J. R. LUCAS, AND J. C. WINGFIELD. 2006. The role of testosterone in male Downy Woodpeckers in winter home range use, mate interactions, and female foraging behaviour. Animal Behaviour 71:695–707.

KELLAM, J. S., J. C. WINGFIELD, AND J. R. LUCAS. 2004. Nonbreeding season pairing behavior and the annual cycle of testosterone in male and female Downy Woodpeckers, *Picoides pubescens*. Hormones and Behavior 46:703–714.

KILHAM, L. 1962. Reproductive behavior of Downy Woodpeckers. Condor 64:126–133.

KONDO, N. AND S. WATANABE. 2009. Contact calls: information and social function. Japanese Psychological Research 51:197–208.

KURVERS, R. H. J. M., M. L. ROBERTS, S. R. MCWILLIAMS, AND A. PETERS. 2008. Experimental manipulation of testosterone and condition during molt affects activity and vocalizations of male Blue Tits. Hormones and Behavior 54:263–269.

LEMMON, D., M. L. WITHIAM, AND C. P. L. BARKAN. 1997. Mate protection and winter pair-bonds in Black-capped Chickadees. Condor 99:424–433.

LITTELL, R. C., G. A. MILLIKEN, W. W. STROUP, AND R. D. WOLFINGER. 2006. SAS system for mixed models. Second Edition. SAS Institute, Cary, North Carolina, USA.

MAHAN, T. A. 1996. Analysis of the acoustic signals of adult male and female Downy Woodpeckers. Thesis. Eastern Kentucky University, Richmond, USA.

MARLER, P. 2004. Bird calls: their potential for behavioral neurobiology. Annals of the New York Academy of Science 1016:31–44.

MATTHYSEN, E., D. CIMPRICH, AND T. C. GRUBB JR. 1993. Home ranges and social behaviour of the Downy Woodpecker *Picoides pubescens* in winter. Belgian Journal of Zoology 123:193–201.

MCDONALD, P. G., W. A. BUTTEMER, AND L. B. ASTHEIMER. 2001. The influence of testosterone on territorial defence and parental behavior in male free-living Rufous Whistlers, *Pachycephala rufiventris*. Hormones and Behavior 39:185–194.

MOUGEOT, F., A. DAWSON, S. M. REDPATH, AND F. LECKIE. 2005. Testosterone and autumn territorial behavior in male Red Grouse *Lagopus lagopus scoticus*. Hormones and Behavior 47:576–584.

NICHOLLS, T. J., A. R. GOLDSMITH, AND A. DAWSON. 1988. Photorefractoriness in birds and comparison with mammals. Physiological Reviews 68:133–176.

NOWICKI, S. AND W. A. SEARCY. 2004. Song function and the evolution of female preferences: why birds sing, why brains matter. Annals of the New York Academy of Science 1016:704–723.

NOWICKI, S. AND W. A. SEARCY. 2005. Song and mate choice in birds: how the development of behavior helps us understand function. Auk 122:1–14.

RITCHISON, G. 1999. Downy Woodpecker. Stackpole Books, Mechanicsburg, Pennsylvania, USA.

SCHWAGMEYER, P. L., H. G. SCHWABL, AND D. W. MOCK. 2005. Dynamics of biparental care in House Sparrows: hormonal manipulations of paternal contributions. Animal Behaviour 69:481–488.

SHARP, P. J. 1996. Strategies in avian breeding cycles. Animal Reproduction Science 42:505–513.

SHORT, L. L. 1982. Woodpeckers of the world. Monograph Series 4. Delaware Museum of Natural History Greenville, Delaware, USA.

SMITH, W. J. 1994. Animal duets: forcing a mate to be attentive. Journal of Theoretical Biology 166:221–223.

SQUIRES, K. A., K. MARTIN, AND R. I. GOUDIE. 2007. Vigilance behavior in the Harlequin Duck (*Histrionicus histrionicus*) during the preincubation period in Labrador: Are males vigilant for self or social partner? Auk 124:241–252.

SULLIVAN, K. A. 1984. Information exploitation by Downy Woodpeckers in mixed-species flocks. Behaviour 91:294–311.

SULLIVAN, K. A. 1985. Allarm calling by Downy Woodpeckers in mixed-species flocks. Auk 102:184–187.

TITUS, R. C., E. D. KETTERSON, AND V. NOLAN, JR. 1997. High testosterone prior to song crystallization inhibits signing behavior in captive yearling Dark-eyed Juncos (*Junco hyemalis*). Hormones and Behavior 32:133–140.

TRAMONTIN, A. D., V. N. HARTMAN, AND E. A. BRENOWITZ. 2000. Breeding conditions induce rapid and sequential growth in adult avian song circuits: a model of seasonal plasticity in the brain. Journal of Neuroscience 20:854–861.

VAN ROO, B. L. 2004. Exogenous testosterone inhibits several forms of male parental behavior and stimulates song in a monogamous songbird: the Blue-headed Vireo (*Vireo solitarius*). Hormones and Behavior 46:678–683.

WINGFIELD, J. C., G. F. BALL, A. M. DUFTY JR., R. E. HEGNER, AND M. RAMENOFSKY. 1987. Testosterone and aggression in birds. American Scientist 75:602–608.

WINGFIELD, J. C., R. E. HEGNER, A. M. DUFTY JR., AND G. F. BALL. 1990. The ''Challenge Hypothesis'':

theoretical implications for patterns of testosterone secretion, mating systems, and breeding strategies. American Naturalist 136:829–846.

WINGFIELD, J. C., J. JACOBS, AND N. HILLGARTH. 1997. Ecological constraints and the evolution of hormone-behavior interrelationships. Pages 22–41 *in* The integrative neurobiology of affiliation. Volume 807 (C. S. Carter, I. Lederhendler, and B. Kilpatrick, Editors). New York Academy of Sciences, New York, USA.

WINGFIELD, J. C. AND K. K. SOMA. 2002. Spring and autumn territoriality in Song Sparrows: same behavior, different mechanisms? Integrative and Comparative Biology 42:11–20.