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Nonbreeding season pairing behavior and the annual cycle of testosterone in male and female downy woodpeckers, *Picoides pubescens*

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Abstract

Studies in birds show that testosterone (T) concentrations vary over the annual cycle depending on mating system and life history traits. Socially monogamous species show pairing behavior throughout the year and low levels of male–male aggression and are underrepresented in these studies, yet the function of testosterone could be particularly important for sexual and social interactions occurring outside the breeding season. We measured fecal T concentrations over the annual cycle and the frequency of interactions between male and female downy woodpeckers (*Picoides pubescens*) from late fall through early spring. We validated the fecal assay by collecting blood in conjunction with a subsample of our fecal samples: fecal T correlated with circulating levels in the blood. The annual peak level of T in males was relatively low and short-lived, similar to that of other bird species with low levels of male–male aggression and high paternal care. The annual cycle of female T resembled the male pattern, and the ratio of male T to female T was close to 1.0. Likewise, the frequency of aggression among females was similar to the frequency among males. Overall, testosterone levels in both sexes were variable, even in winter. In other bird species, sexual behavior during nonbreeding periods correlates with circulating levels of T in males. Based on this observation, we tested the hypothesis that T in winter was positively related to the frequency of interaction between mated downy woodpeckers. The results showed no such relationship. We discuss this finding and further relate the annual cycle of T in both males and females to behaviors that appear to facilitate mate choice and retention of the pair bond during conspecific challenge.

Keywords: Testosterone; Downy woodpecker; *Picoides pubescens*; Pair bond; Annual cycle; Courtship; Sex roles; Aggression; Reproductive behavior; Mate choice

Introduction

A large field of study in avian behavior is centered on the evolution and maintenance of different mating systems (Andersson, 1994). Although the distinction between monogamy and other mating systems in birds has blurred due to the discovery of extra-pair fertilizations found in presumed monogamous species (Møller and Birkhead, 1992), general trends between mating pattern and certain behavioral repertoires remain. For example, polygynous systems often feature high levels of male–male aggression, and strongly monogamous species have high levels of paternal care (reviews by Ketterson and Nolan, 1994; Møller and Birkhead, 1993; Schwagmeyer et al., 1999). Often, monogamous species with high levels of paternal care also maintain pair bonds in which mates may interact year-round (e.g., Potter and Cockrem, 1992; Rees et al., 1996).

Behavioral differences among species may correspond to differences in seasonal hormone profiles. For example, in avian species where males invest little in parental care, males have extended periods of high circulating levels of testosterone (T) during the reproductive season, whereas males in more parental species have only short-lived elevations in T (Wingfield et al., 1990). Wingfield et al. (1990) also showed that the amplitude of the seasonal T

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peak was related to the degree of male–male interaction taking place in a breeding population. They considered the seasonal peak in T as the highest average concentration measured in a population during the reproductive cycle. Males from populations with moderate to high levels of male–male interaction, in which males aggressively defended territories and guarded mates, showed seasonal peaks in T from 5 to 10 times higher than concentrations observed later in the breeding cycle during the parental phase [e.g., pied flycatchers, *Ficedula hypoleuca* (Silverin and Wingfield, 1982); song sparrows, *Melospiza melodia* (Wingfield, 1984 and Wingfield et al., 1989); starlings, *Sturnus vulgaris* (Ball and Wingfield, 1987)]. In contrast, males with low levels of male–male interaction, which showed very short periods of male–male aggression or no aggression at all, exhibited smaller T peaks at the start of the breeding period [e.g., western gull, *Larus occidentalis* (Wingfield et al., 1982); emperor penguin while courting, *Aptenodytes forsteri* (Groscolas et al., 1986)].

The studies cited above focused primarily on behavior and physiology during the breeding season. Patterns of T secretion in winter are not as well studied. Circulating levels of T are relatively low in most bird species during the nonbreeding season (Dawson, 1983; Hirschenhauser et al., 1999; Potter and Cockrem, 1992; Wingfield, 1984). Despite this, individuals may interact with others of the opposite sex, sometimes expressing behaviors with great similarity to courtship (Hall et al., 1987; Hegner and Wingfield, 1986; Kellam, 2003a; Lincoln et al., 1980; Murton and Westwood, 1977; Sharp, 1996). In some cases, these nonbreeding season courtship-like behaviors appear to correlate with T levels (or T precursors, Sharp, 1996; Wingfield et al., 1997).

Although little work has been done on how T might facilitate sexual behaviors in a nonbreeding context, Wingfield et al. (1990) did provide a theoretical framework for this phenomenon. They described two thresholds of T concentration. The first one, a nonbreeding baseline, was represented by the level of T found in male birds during the nonbreeding season before the expression of any sexual behavior. In most birds, this level is close to zero. Increases in T over the nonbreeding baseline up to a second threshold, the breeding season baseline, were thought to facilitate sexual behaviors such as courtship (Wingfield et al., 1990).

While courtship-like behaviors may not be expressed at all in some species during the nonbreeding season, males in some monogamous species interact with a former or future mate at this time of year. We collectively refer to these interactions as pair bond maintenance (cf. Kellam, 2003a). Interaction between mates, whether characterized by elaborate displays or simple spatiotemporal association, could be advantageous to either or both partners. Maintaining a cohesive relationship or bond between pair members may be a way for individuals to (1) protect and nurture the mate (Hogstad, 1995; Koivula et al., 1994), (2) aid in hormonal or behavioral synchrony in preparation for breeding (Fowler, 1995; Hirschenhauser et al., 1999; Rowley, 1983), or (3) regularly assess the quality or commitment of the mate (Black, 1996; Kellam, 2003b; Wagner, 1996; Zahavi, 1977). Although T in males is unequivocally involved with courtship in spring, whether T facilitates the subtle behaviors associated with pair bond maintenance outside the breeding season is not known.

In this study, we documented the annual cycle of T in male and female downy woodpeckers (*Picoides pubescens*) and the cohesiveness of pair bonds during the nonbreeding season. Temperate-zone woodpeckers including *P. pubescens* are an excellent group to use for studying the hormonal correlates of sexual behavior both in breeding and nonbreeding contexts because most species are strongly monogamous (few extra-pair fertilizations; Haig et al., 1994; Michalek, 1998), exhibit high levels of paternal care (Winkler et al., 1995), and have some degree of association with their mate throughout the year (Kellam, 2003a,b; Kilham, 1962, 1970; Lawrence, 1967). To date, the hormonal patterns in bird species with these characteristics have not been well studied, even in the breeding season. *P. pubescens* is of particular interest because it has an unusual social system during the winter (Matthysen, 1993). Unlike most other woodpeckers, territorial defense by downy woodpeckers is absent most of the year (Kellam, 2003b; Matthysen et al., 1993). Even during breeding, territoriality is restricted to a very small area around the nest (Lawrence, 1967). This suggests that male T likely has behavioral functions other than facilitating male–male aggression via territorial defense.

We took advantage of the unique social behavior of *P. pubescens* to test two predictions based on the hypothesized relationships in other birds among T level, male parental care, and the frequency of male–male interactions. First, the patterns of circulating male T during the breeding season should be similar to those in other species with low male–male interaction and high paternal care (e.g., western gull; Wingfield et al., 1982). Second, T levels in males during the nonbreeding season should correlate positively with the frequency of male–female interactions. In addition to evaluating these predictions, we documented the timing and context of reproductive behavior in downy woodpeckers to relate the annual cycle of T to the reproductive cycle. We also compared the frequency of interactions among individuals of the same sex with male and female T profiles.

Methods

All procedures relating to the observation, handling, and housing of downy woodpeckers were approved by the Purdue University Animal Care and Use Committee (protocols #97-084-2, #99-064, and #00-032).

Field methods

We studied the behavior and hormone levels of color-banded, free-living downy woodpeckers at the Ross Biological Reserve near West Lafayette, IN, USA. We captured woodpeckers as they entered tree trunk-mounted traps baited with a commercial suet product between March 1998 and May 1999 and between November and April 2000–2002. Each trap was closed manually from a blind 15 m away, and woodpeckers were removed immediately upon capture.

We collected fecal samples to assess circulating levels of testosterone (T) in the woodpeckers. Samples were collected after placing woodpeckers in wire-mesh holding cages lined with aluminum foil. Feces and urea were well mixed in the samples (mean dry mass = 33.2 ± 2.0 SE mg; n = 157), and few had any significant fluid component. Samples deposited on the aluminum foil adhered easily to a cotton applicator, which we used in transferring samples to microcentrifuge tubes. All samples were kept cool in the field until frozen to −20°C for long-term storage.

During November and December 1998–1999 and January to April 1999–2002, we attached a 1.1-g radio transmitter (one of two models: SOPB 2011, Wildlife Materials, Carbondale, IL, USA; BD-2, Holohil Systems, Carp, ON, Canada) to both males and females using an adjustable leg-loop harness. Transmitter batteries lasted approximately 32 days. Individuals wearing radio transmitters were located once per 1.5 days to determine spatial proximity of males and females and the frequency of conspecific interactions. These social data were recorded during 30-min focal observations with an audiocassette recorder. We documented aggressive interactions between individuals whenever (or if) they occurred during an observation. Although there were several kinds of displays exhibited by individuals, we treated all aggressive displays the same in most analyses by simply recording whether they were given by individuals during any particular 30-min focal observation. Every 10 min, we conducted an instantaneous scan sample to determine spatial proximity of conspecifics. If woodpeckers came within 40 m of one another during one or more of the scans, we considered them to be in association; otherwise, we scored the tracked individual as having no association on that day. In another study, Matthysen et al. (1993) used a 50-m threshold to describe downy woodpecker social association. We used a 40-m threshold because our study site was previously marked with a 40 × 40 m grid, and this improved the distance estimates. Spatial association is not the only way to quantify the strength or cohesiveness of a pair bond. However, we rely on mate association frequency in this study as a robust measurement of pair bond maintenance.

Males and females were sometimes spotted near one of several individuals of the opposite sex over the course of a fall or winter season; however, they almost always associated predominantly with one particular individual of the opposite sex, which we assumed to be the mate (but see Mate switching). We confirmed mated partners by finding nests during the breeding period. Without exception (n = 19), presumed mates during the nonbreeding season tended to the same nest in the following breeding season. We report the time of observed copulations and nesting to show how the seasonal peak in T corresponded with reproduction in our population.

One of the objectives of our study was to determine whether T level correlated with nonbreeding season mate association frequency. Ideally, we might have captured each individual to obtain a fecal sample on a weekly basis throughout the period that we monitored behavior. However, some woodpeckers were trap-shy and could not be captured so frequently. In addition, regular access to baited traps could have changed the pair bond relationship, as data show that pair interactions in downy woodpeckers differ when food is plentiful versus when it is scarce (Kilham, 1970; Lawrence, 1967; Matthysen et al., 1991). Consequently, we tested for hormone-behavior correlations by comparing the concentration of T in each fecal sample with the frequency of mate association calculated over the 3 weeks following the date when the fecal sample was collected (in the case of radio transmitter attachment) or the 3 weeks previous to the date when the fecal sample was collected (in the case of radio transmitter removal).

Laboratory methods

Frozen fecal samples were dried overnight in a laboratory drying oven. The dry samples were then weighed to the nearest 0.1 mg. We extracted T by mixing feces with 2 ml 90% methanol in test tubes overnight. Then we centrifuged the samples and evaporated a 500-μl aliquot. We added 500 μl 10% ethyl acetate in *iso*-octane and approximately 2000 cpm ^{3}H-labeled testosterone (to measure recovery following extraction) to each dried extract. Testosterone was separated from other steroids using column chromatography (see Wingfield and Farner, 1975). Purified fractions were evaporated, and 550 μl of phosphate-buffered saline with 0.1% sodium azide and gelatin (PBSG) was added to each tube. Tubes were mixed for 1 h, and two 200-μl aliquots were used for radioimmunoassay (RIA) and one 50-μl aliquot was used to calculate recovery (Wingfield and Farner, 1975). After assembling a standard curve using nine serially diluted concentrations of T (range: 1.95–500 pg/100 μl distilled H_2O), we added 10,000 cpm of tritiated T (NET-553; New England Nuclear Research Products, Boston, MA) and antitestosterone antibody (T-3003; Wien Laboratories, Succasunna, NJ) to each sample and standard tube. These tubes were incubated overnight, and the unbound steroid was separated from bound steroid by adding 0.5 ml dextran-coated charcoal solution. The tubes were then centrifuged, and the supernatant decanted into scintillation vials. We added Ultima Gold scintillation fluid (PerkinElmer Life and Analytical Sciences, Boston, MA), and the vials were counted for radioactivity. Values were corrected for percent recovery of the original tracer label, aliquoting, and initial fecal sample dry mass. Mean recovery value was 38.6%, and concentration limits for reliable measurement averaged 0.85 ± 0.07 SE ng/g feces (range = 0.16 to 7.40). The coefficient of interassay variation across four assays was 8.6%, and intraassay variation was 1.6%, based on a comparison of concentrations of T measured from standard tubes.

Validation study

Testosterone acts on target tissues via the bloodstream, and fecal T concentrations have been shown to correlate with circulating levels of T in the blood in other species (Bishop and Hall, 1991; Cockrem and Rounce, 1994; Hirschenhauser et al., 1999). However, our study species and the T extraction method we used differed from other studies. Therefore, we validated our procedure for analyzing fecal T by implanting three captive male woodpeckers with two T-filled Silastic tube implants (tubing: Dow Corning, Midland, MI; 1.47 mm i.d., 1.96 mm o.d.). Implants were sealed at each end with Silastic medical adhesive (Dow Corning) and had an effective length of 10 mm. The males were housed individually in an outdoor aviary for 14 days in October 2000. We collected feces and blood (from wing vein) periodically throughout captivity, both before and after insertion of the implants.

Blood plasma collected from three captive males as well as from eight free-living males without implants was analyzed for androgen using a direct RIA without chromatography. Cross-reaction of the anti-T antibody with dihydrotestosterone is approximately 70%. We extracted androgen from plasma by adding dichloromethane and prepared samples for RIA according to the procedures described above. Mean recovery value across two assays was 75.9%. The coefficient of interassay variation was 10.6%, and intraassay variation was 2.5%, based on a comparison of androgen concentrations measured from standard tubes.

Statistical analysis

We analyzed our data in two primary ways. First, we looked for general trends among various hormonal and behavioral variables within the population of woodpeckers as a whole. These analyses usually involved calculating mean values for each variable according to month, with separate analyses for males and females. Specifically, we used a paired *t* test to compare male and female monthly display rates. Chi-square (χ^2) tests were used to determine whether the frequency of certain behaviors differed among months. Pearson correlation tests were used to determine whether the monthly mean fecal T in males and females varied with the monthly frequencies of aggressive interaction observed within the population.

Second, we analyzed our data by employing repeated-measures analysis of covariance models (PROC MIXED; Littell et al., 1996) that used measurements from the same individuals in different months to examine monthly trends in hormone data found within individuals rather than the population as a whole. We also used repeated-measures models to test whether individual males' or individual females' average mate association frequency was explained by mean fecal T concentration. We calculated statistical power for these tests following Cohen (1988). Repeated-measures analyses often assume that within-subject residuals are uncorrelated, but this assumption can be violated in nonexperimental data when behavioral and hormonal variables correlate with time. To correct for this temporal correlation, we employed a first-order autoregressive covariance structure that allowed residuals calculated for consecutive observations on the same individual to be more correlated than residuals calculated for nonconsecutive observations (Littell et al., 1996). For our validation study, we used a repeated-measures model, a paired *t* test assuming unequal variances and a Pearson correlation test to determine the relationship between plasma androgens and fecal T. For selected repeated measures models, we generated multiple comparisons using the '/DIFF' option in LSMEANS.

In a specific test to determine whether fecal samples from aggressive females had T concentrations consistently above the monthly means, we classified each sample as having above- or below-average T concentration and then, using a binomial sign test, compared the frequencies of the two categories in a preselected group of aggressive females versus the entire population. Male data were not analyzed in this way because fewer males were classified as aggressive and we lacked fecal samples from them.

For all procedures described above, we employed only two-tailed tests and assumed $\alpha = 0.05$. Both dependent and independent variables were log-transformed as needed to ensure that assumptions for normality were not violated. Interactions between independent variables were excluded from models when not significant.

Results

Validation study

Log-transformed plasma androgen concentration accounted for a significant amount of variance in log-transformed fecal T for three captive males given T-filled implants ($F_{1,2} = 104.2$, $P = 0.010$). Fecal samples taken the day following implant insertion contained significantly higher amounts of T than samples collected immediately before implantation (paired *t* test: $t = 8.35$, $n = 3$, $P = 0.014$). In fact, postimplant levels of T were extremely high (up to 34.4 ng/ml) and were unlikely to be found in unmanipulated woodpeckers. These results were unexpected, since implants were similar in construction but half the size of those used in dark-eyed juncos (*Junco hyemalis*), a slightly smaller bird (Ketterson et al., 1992). Because of the unusually high T concentrations produced by the implants, we augmented the validation study by collecting concurrent plasma and fecal samples from eight free-living, unmanipulated males during the spring. In these birds, plasma androgen and fecal T concentrations were also significantly correlated ($r = 0.87$, $n = 8$, $P = 0.004$; Fig. 1).

Annual cycle of testosterone

Six (of 157) fecal samples had T concentrations below the detection limit of our assay. In these cases, we used the detection limit calculated for each sample (mean = 0.80 ± 0.2 SE ng/g, range = 0.29–1.70 ng/g) to represent fecal T concentration in our analyses (cf. Wikelski et al., 2000).

Fecal T concentrations in male woodpeckers varied considerably among individuals (Fig. 2a). After statistically accounting for this variation, we found that mean T levels within individuals were low in late summer and fall and began to rise during the winter with separate peaks in December and April. A multiple comparisons analysis within the repeated-measures model indicated that these peaks were significantly greater than the T levels in all other months except March. In the study by Wingfield et al. (1990), the seasonal peak in plasma T was considered the highest average concentration in the population during the reproductive cycle. Thus in our data set, we consider the seasonal peak in male downy woodpeckers to be 10.14 ng/g, despite there being considerable individual variation.

Mean fecal T in females was similar to the levels measured in males (Fig. 2b). The ratios of male to female T each month averaged 1.2 ± 0.2 SE and never exceeded 2.0 for any month. Female T rose through the fall and then remained relatively constant until April, when T peaked at 12.31 ng/g. A multiple comparisons analysis of the female T data showed that summer and early fall levels were significantly less than the spring and December values.

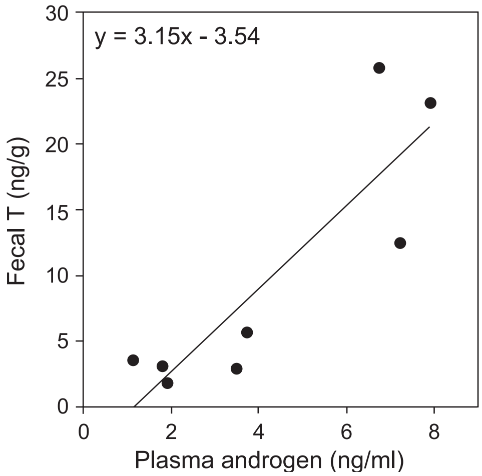

Fig. 1. Results from validation of fecal testosterone (T) levels in free-living male downy woodpeckers. Concurrent fecal and plasma samples were analyzed for androgen as described in Methods. The positive relationship is significant ($P = 0.004$).

Timing of pair displays and reproduction in relation to testosterone

Direct interaction between pair members was uncommon (Table 1). When interactions did occur, most were displacements, which involved one individual flying to the perch position of another, forcing it to move. Two interactions included rhythmic swinging of the head (bill-waving) and repeated spreading of the wings (wing-flicking) previously described by Jackson and Ouellet (2002). Focal observations that included displacements occurred with similar frequency across months except in February, when they were significantly more frequent than expected by chance ($\chi^2 = 14.16$, $df = 4$, $P = 0.007$; January excluded due to insufficient sampling). Increased male–female conflict in any month was not reflected in the male or female T cycles (compare Table 1 with Fig. 2).

We also observed a few nonaggressive interactions in all months of study during which mates came within 1 m of each other (Table 1). For example, during 10 focal observations, we observed males flying to and sitting on a branch next to a female. In seven of these cases (two in December), females gave a solicitation pose, a display frequently followed by copulation when given in the spring. However, males flew away without copulating. Average T in males and females was low during November and most other months when these and other close male–female interactions were observed.

Males did mount soliciting females between April 19 and May 15 when we witnessed seven copulations during our nest searches. Nests of 17 pairs were initiated during the first week of May, and young fledged during the first 2 weeks of June with little difference among years (mean fledge date, 1997–2001: 8-Jun ± SE 1.1 days). Male testosterone peaked in April (Fig. 2a), which corresponds with the date of most copulations.

Given that the months of May and June represent the period when males are incubating and feeding young, we used the procedure described in Wingfield et al. (1990: 840) to calculate the breeding season baseline for T in downy woodpeckers as 4.26 ng/g.

Mate association and testosterone

In addition to specific male–female interactions, T may correlate with the frequency at which male and female are seen in proximity (within 40 m). The mean monthly frequency of mate association among downy woodpecker pairs was highly variable (Fig. 3). The highest mean frequency of association occurred in March, and the lowest frequency of association occurred in December, but there were pairs in each month that were frequently together

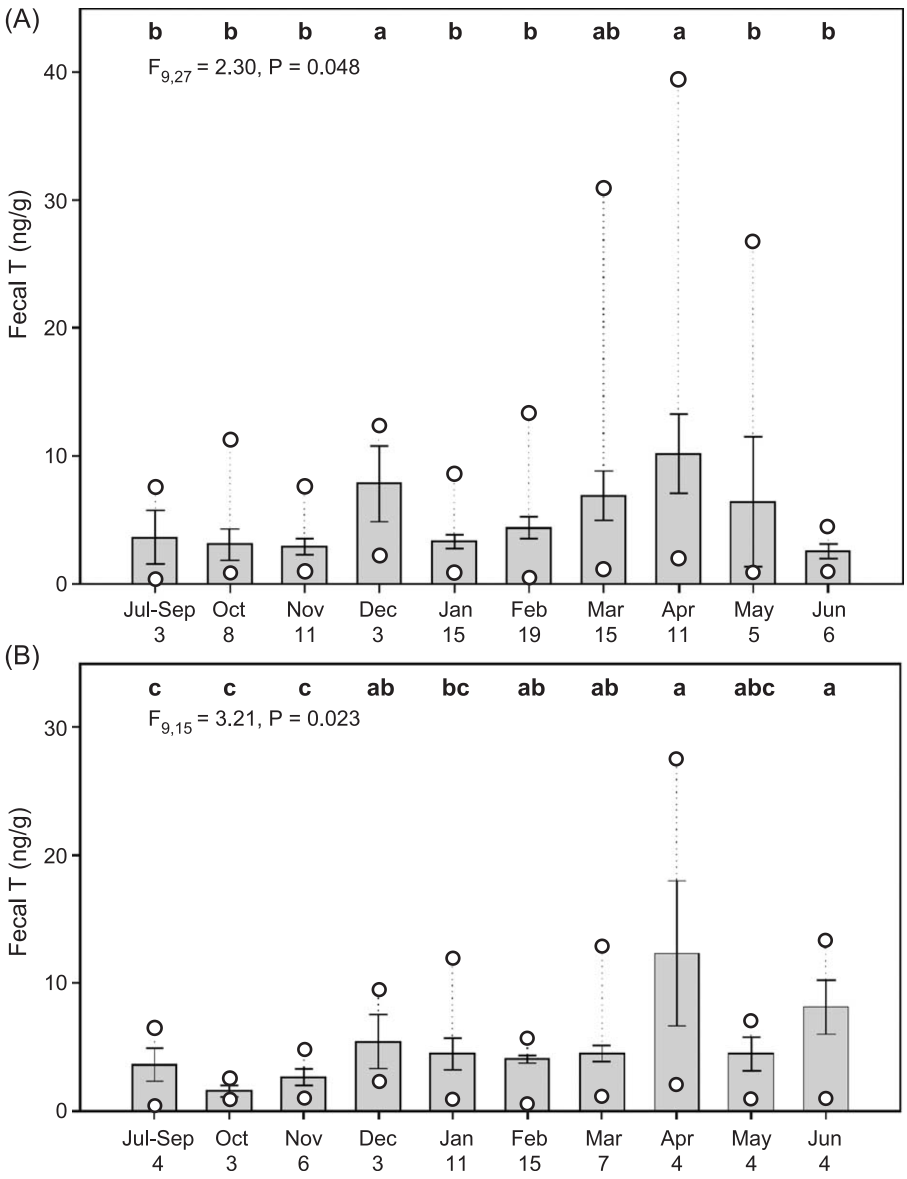

Fig. 2. Fecal testosterone (T) concentrations of male (A) and female (B) downy woodpeckers over the annual cycle. Gray bars show population means (± SE), and open circles indicate extreme individual values. Sample sizes are given below each month. Some of the same individuals were sampled in multiple months, so we tested for differences in T between months by conducting a multiple-comparisons analysis within a repeated measures model. T concentrations measured in months sharing the same letter (a, b, or c) were not significantly different. Summary statistics for each repeated measures analysis are listed for both sexes.

(>50% of observations) and pair members that were never seen in proximity.

To determine if T facilitated nonbreeding season pair bond maintenance, we tested whether fecal T concentration correlated with month (nested within year), age (<2 or >2 years), and mate association score (mate within 40 m or not, based on whether the individual had been seen near its mate within 3 weeks of the date of the fecal sample). We included age in the model because older individuals with more pairing and reproductive experience may differ from inexperienced birds in level of mate interaction and T concentration (Hall et al., 1987; Wingfield, 1990). A repeated-measures analysis indicated that male T and mate association frequency were not significantly related (Table 2). However, statistical power of this test was low (0.34; power analysis assumed an effect size of 2.13 ng/g, or 50% of the breeding season baseline). There was no significant age effect in males.

We also found no significant relationship between mate association and T levels in females (Table 2). Power of this test was also low (0.55; assumed effect size of 2.13 ng/g). Contrary to what was found for males, female fecal T was significantly related to female age: older females had lower T concentrations than younger ones. T levels did not change significantly across months. However, this analysis only used a subset of the data previously used to test for monthly changes in T within individuals, and the larger analysis did show monthly differences (Fig. 2b).

Table 1
The number of behavioral observations ("focals") during which interactions between members of the opposite sex were observed in downy woodpeckers (1998–2000)

Month	Focals	Nonagonistic interactions		Heightened conflicts[a]	Displacements initiated by[b]		Proportion focals with displacements[c]
		Copulation solicitations[d]	Other[e]		Male	Female	
NOV	108	0	1	0	3	1	0.04
DEC	95	2	1	0	3	1	0.04
JAN	12	0	0	0	1	0	0.08
FEB	132	3	0	1	18	3	0.17
MAR	229	1	4	1	9	4	0.06
APR	71	1	1	0	6	1	0.10
Total	647	7	7	2	40	10	

[a] Involved aggressive bill-waving and wing-flicking displays plus one or more displacements during a single daily observation period.

[b] The individual initiating the displacement was the male or female that forced its partner to move from a perch.

[c] Includes observation periods with heightened conflicts.

[d] Involved a female bowing low as is done during copulation; however, the behaviors documented here did not result in copulation.

[e] All other interactions resulting in a male or female coming within 1 m of their partner, excluding aggressive encounters such as displacements or triangle conflicts.

Same-sex conflicts and testosterone

Woodpeckers performed agonistic displays to same-sex conspecifics during all months of observation (Table 3). The frequency of focal observations including these interactions tended to be highest in winter among males and lowest in winter among females (males: $\chi^2 = 7.29$, $df = 4$, $P = 0.12$; females: $\chi^2 = 10.39$, $df = 4$, $P = 0.03$; January data were excluded due to insufficient sampling). Overall, however, males displayed to other males no more frequently than females displayed to other females (Table 3). The sexes exhibited the same kinds of displays during same-sex interactions too, consisting of bill-waving and wing-flicking interspersed with chases and displacements. During 78% (38 out of 49) of the observation periods during which we documented these displays, the displaying birds of the same sex faced each other, while an individual of the opposite sex was found 15–40 m away. Sometimes the displaying birds would fly toward the opposite-sex individual and perch within 1 m of its position, but this was usually temporary, as the approaching individual was displaced by its same-sex opponent. These interactions are often referred to as "triangle conflicts" in the literature (Lawrence, 1967; Short, 1971), but 2 of these 38 conflicts involved five to six individuals rather than just three. In both of these larger conflicts, four females chased each other through the forest, all of them pausing to bill-wave and wing-flick while facing one of the others. One to two males followed the groups of females but never displayed to any of the other birds.

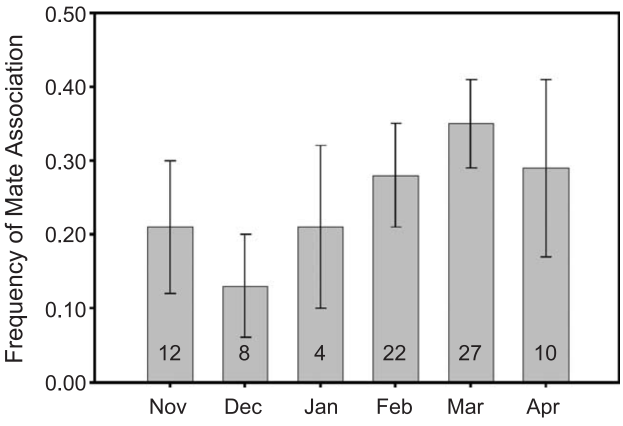

Fig. 3. Mean (± SE) frequency of mate association in the study population of downy woodpeckers by month, 1998–2000. Frequency of mate association is the fraction of daily observations during which a male was within 40 m of its mate. The number of individuals used to estimate the monthly mean is listed within each bar.

Over 10% (5 of 49) of same-sex conflicts were prolonged. With only 30 min of observation per day, we could not determine the proportion of time that an individual spent displaying on any given day. However, displays observed among the same individuals on successive days were considered part of the same overall conflict. One 3-day conflict was between displaying males; the other four multiday conflicts were among displaying females and lasted 3–6 days. All other same-sex conflicts were not continued from one daily focal observation to another.

Correlation analysis (excluding January data due to insufficient behavioral sampling) showed that there was no significant relationship between the monthly frequency of male–male interactions and monthly mean male T concentration. We also found no significant relationship among females. Several days or weeks usually separated behavioral observations from fecal sampling. Therefore, within-individual comparisons between T and the occurrence of specific aggressive interactions were not possible. However, from the 10 females participating in at least one of the multiday conflicts described above, we collected six fecal samples between February and April. All the samples had T concentrations above their respective monthly population means. This frequency of above-average samples was significantly different from random (binomial sign test:

Table 2
Results from repeated measures models showing the relationship between mate association, month, age of an individual at the time of sampling, and fecal testosterone concentrations in male and female downy woodpeckers

Independent variable	Least-squares mean log fecal T ± SE		*F* (*df*)	P
Males				
Mate association during fecal sampling period[a]	With mate	0.74 ± 0.06	0.10 (1,11)	0.753
	Without mate	0.74 ± 0.06		
Month[b]	Nov	0.75 ± 0.10	3.19 (4,22)	0.033
	Dec	0.89 ± 0.12		
	Feb	0.48 ± 0.09		
	Mar	0.73 ± 0.08		
	Apr	0.86 ± 0.13		
Age	<2 years old	0.72 ± 0.07	0.09 (1,11)	0.776
	>2 years old	0.76 ± 0.10		
Females				
Mate association during fecal sampling period[a]	With mate	0.64 ± 0.04	1.26 (1,9)	0.290
	Without mate	0.65 ± 0.04		
Month[b]	Nov	0.57 ± 0.06	1.57 (5,19)	0.216
	Dec	0.58 ± 0.05		
	Jan	0.67 ± 0.06		
	Feb	0.67 ± 0.04		
	Mar	0.66 ± 0.04		
	Apr	0.72 ± 0.05		
Age	<2 years old	0.75 ± 0.03	6.68 (1,9)	0.030
	>2 years old	0.54 ± 0.07		

[a] Statistical power of these tests was low (<0.56).

[b] Month data reported here differ from those given in Fig. 2 because in this analysis, only individuals for whom both fecal and behavioral data were available were used, and least-squares means remove interindividual variation.

Table 3
Frequency of behavioral observations ("focals") per month based on recording agonistic displays and conflicts between members of the same sex (1998–2000)

	Focals per month	Birds observed per month	Focals per bird (mean ± SE)	Focals with displays	Focals with repeat display participants[a]	Multiday conflicts[b]	Frequency of focals with displays
Male and male–male interactions							
Nov	69	13	5.2 ± 1.6	4	0	0	0.06
Dec	67	13	5.2 ± 0.9	1	0	0	0.01
Jan	8	4	1.8 ± 0.5	2	0	0	0.25
Feb	55	17	3.1 ± 0.5	6	1	1	0.11
Mar	111	24	4.5 ± 0.9	8	2	0	0.07
Apr	34	13	2.6 ± 0.6	2	0	0	0.06
Total	344			23		1	
							Average[c] 0.06 ± 0.02 SE
Female and female–female interactions							
Nov	39	13	2.9 ± 0.9	5	2	0	0.13
Dec	28	9	3.1 ± 0.9	2	0	0	0.07
Jan	4	3	1.3 ± 0.3	0	0	0	0.00
Feb	77	14	5.5 ± 1.6	4	1	1	0.05
Mar	118	24	4.9 ± 0.7	16	2	3	0.14
Apr	37	16	2.3 ± 0.5	7	1	0	0.19
Total	303			34		4	
							Average[c] 0.12 ± 0.03 SE

[a] Only a small number of the focal observations with displays each month involved the same displaying individuals.

[b] Multiday conflicts lasted 3–6 days and involved the same individuals displaying to one another during sequential daily focal observations.

[c] Frequency of focals with displays in January was excluded from analysis due to small sample size. There was no significant difference between male–male and female–female display frequencies.

n = 6, P = 0.031), suggesting that the subset of females participating in multiday conflicts had significantly higher fecal T than average in the population during the months when the samples were collected. No fecal samples were collected from the two males participating in a multiday conflict.

Mate switching

At least two female "floaters" were observed to enter into the prolonged and multiple-female interactions discussed above, yet these females appeared to have no mate and remained solitary for at least 3 weeks after the conflicts. Indeed, no observed conflict resulted in changes in pair membership. However, changes in pair membership did occur and same-sex conspecific interactions may play a role in pair formation and maintenance.

We radio-tracked 27 different male–female pairs involving a total of 45 individuals. Nine individuals associated first with one, and then another member of the opposite sex in the same fall–spring study period. In six cases, the switch occurred after the death of the first mate (usually depredation); in the other three cases (occurring in February and March), switches occurred while the former partner was still alive. One male initiated a mate switch, with his former partner remaining unpaired through April when her transmitter battery died and we lost track of her. Females initiated the other two mate switches, each leaving their former mate without a partner for at least several weeks.

Discussion

Duration of seasonal testosterone peak

Prior studies have shown that paternity rates (proportion of young fathered by the male parent) are relatively high in species where males make relatively large contributions to incubation and nestling care (Ketterson and Nolan, 1994; Møller and Birkhead, 1993; Schwagmeyer et al., 1999). This correlation between mating system and male parental care can be further expanded by adding a third dimension, seasonal male testosterone (T) profile. For example, Wingfield et al. (1990) found that annual T peaks are short-lived in males of strongly monogamous and paternal species compared to the peaks in males of polygynous species. However, hormone data are rare for species that exhibit high levels of both paternity and male parental care. Species in the family Picidae may prove useful as models in this area of research.

Male woodpeckers both incubate and feed nestlings at similar rates as females (Winkler et al., 1995). Few data are available on extra-pair fertilizations (EPFs) in woodpecker species; however, existing studies have found very low (<4%) frequencies of EPFs, even among the cooperatively breeding red-cockaded woodpecker (*Picoides borealis*; Haig et al., 1994; Michalek, 1998). Acorn woodpeckers (*Melanerpes formicivorous*) also maintain genetic monogamy when nests are cared for by a single male–female pair (Dickinson et al., 1995). In accord with these behavioral patterns, limited hormone data show that male woodpeckers have low circulating levels of T during breeding periods relative to species with greater extra-pair paternity and less male parental care (Khan et al., 2001; M.S. Stanback, J.C. Wingfield, and W.D. Koenig, unpublished data). Here, we show, as predicted, that male downy woodpeckers exhibit a short-lived peak in T in early spring. Mean T levels in our population peaked in April when copulations occurred and were significantly lower by May when nests were initiated.

Amplitude of seasonal testosterone peak

The amplitude of the seasonal T peak in a population of males may also vary with mating system and reproductive strategies. Wingfield et al. (1990) classified species into three groups according to whether males showed low, moderate, or high levels of aggression during the breeding season. Males in species with low levels of aggression rarely interacted and tended to have high levels of paternal care. They also had relatively low levels of T throughout the year. Males in species with moderate levels of aggression interacted frequently before the parental phase, but the frequency of interactions dropped after nestlings hatched. Those males, on average, showed seasonal T peaks approximately five times the breeding baseline levels. Males in species with high levels of aggression interacted regularly throughout the breeding season and exhibited little paternal care. They were often polygynous and had average seasonal T peaks 5–10 times the breeding baseline levels.

Downy woodpeckers' annual T cycle is comparable to that of the species that Wingfield et al. (1990) placed in the low aggression and high paternal care category. The seasonal peak in fecal T in our population of males was <2.5 times the breeding season baseline, lower than that found in more aggressive species.

The hormone data are consistent with several other patterns found in species with low male–male aggression and high levels of paternal care. For example, Wingfield et al. (1990) and Hirschenhauser et al. (2003) showed that males of these species had a greater hormonal response to social challenges than males with different combinations of life history traits. Therefore, downy woodpeckers may be expected to show a considerable increase in T over the population averages when participating in brief male–male competitive events. Unfortunately, we observed few male–male interactions and none of our fecal samples were from these aggressive males. However, the fecal T data do show that some individual males had extremely high fecal T concentrations, and it may be that those individuals had been recently engaged in a conflict. One sample had a T concentration nine times the population's breeding season baseline (or 5.5 times the baseline when fecal concentrations are converted to plasma concentrations using the regression equation in Fig. 1). This ratio of maximum concentration to breeding baseline concentration is consistent with the hormonal responsiveness of other monogamous species with high parental care (Wingfield et al., 1990).

Another interesting finding was that male and female downy woodpecker seasonal T profiles were quite similar. Wingfield (1994) showed that the sexes in sexually monomorphic species have similar T levels (see also Archawaranon et al., 1991; Hegner and Wingfield, 1987; Wikelski et al., 2000; Wingfield et al., 1982, 1989), and downy woodpeckers fit this basic relationship. In contrast, males in monogamous but sexually dimorphic species often have T concentrations three times higher than females (e.g., Hector et al., 1986; Schwabl and Sonnenschein, 1992). The reason for this pattern is not certain, but Wittingham and Schwabl (2002) recently showed that circulating T in females during the breeding season is correlated with the rate of competitive interactions among females. As we showed here, downy woodpecker females are at least equally aggressive to one another as males are to males, and the most aggressive females always had above-average fecal T concentrations respective to the month when samples were collected. We found that younger females had higher T levels than older ones as well, and this may result because older females might already have a high quality mate and may not initiate as many conflicts. Assuming that age is an important factor in mate choice, even if a younger female could win a conflict with an older female, the older female's mate might not benefit from such a mate switch and would be unlikely to accept the new partnership.

December testosterone peak

Average male T rose from November to December but dropped again in January. This early peak may not be unusual among birds. A number of species show small rises in T (at least in some individuals) during the late fall when most bird species become photosensitive (Dawson, 1983; Lincoln et al., 1980; Nicholls et al., 1988; Silverin, 1984; Temple, 1974; Wingfield et al., 1997). Assuming that downy woodpeckers in this study were photosensitive, males may have initiated T production in December, only to have it decreased again in midwinter when nonphotoperiodic cues such as lower air temperature could have slowed testicular development (Silverin and Viebke, 1994).

Pair interactions and testosterone

In addition to placing the downy woodpecker into the existing theoretical framework relating life history and hormonal profile, another goal of our study was to determine whether T levels influenced pair bond maintenance. Wingfield et al. (1990) hypothesized that increases in T level between the nonbreeding and breeding baselines facilitate sexual interactions. While individuals of many species of birds do not have the opportunity to interact sexually with a former or future mate during nonbreeding periods, individuals of other species do, including downy woodpeckers. Our observations indicate that explicitly sexual displays between mates were rare (Table 1). However, most downy woodpeckers did associate regularly with a particular individual of the opposite sex, increasingly so from December to April (Fig. 3). As downy woodpeckers do not form conspecific flocks at any time of year, we considered these spatial associations to be sexual in nature. With increased association frequency, other more courtship-like behaviors such as solicitation poses and the exchange of visual, vocal, and acoustic signals could occur.

Although we predicted that T levels during the nonbreeding season would be correlated with mate association frequency, our results revealed no significant relationship in either males or females. Previous studies have documented the role of T in increasing courtship behavior including song quality and frequency, display rates, and copulation (Balthazart, 1991; Brenowitz et al., 1998; Enstrom et al., 1997; Harding et al., 1983; Ketterson et al., 1992; Lincoln et al., 1980). Frequent mate association during the nonbreeding season may facilitate these behaviors, but perhaps the effects of T on mate interactions are limited to supporting particular displays and signals, and not spatial association itself. We encourage further study on how T might facilitate specific sexual displays related to pair bond maintenance in fall and winter.

Interactions between mates in a nonbreeding context are an important, yet frequently overlooked, mechanism for mate choice. Indeed, individuals may invest in their own reproductive success by defending, protecting, nurturing, or even divorcing their mate over winter (Black, 1996). Research on the proximate bases of these behaviors will not only allow a greater understanding of the costs and benefits of long-term pair bond maintenance, but it will also lead to more discovery in the field of reproductive behavior as nonbreeding season studies are compared with those conducted during the breeding season.

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