

# Experimentally Elevated Plasma Testosterone Levels Do Not Influence Singing Behaviour of Male Blue Tits (*Parus caeruleus*) During the Early Breeding Season

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## Abstract

Sexual selection theory suggests that females might prefer males on the basis of testosterone (T)-dependent secondary sexual traits such as song. Correlational studies have linked high plasma T-levels to high diurnal song output. This has been confirmed in experiments where T-levels were kept high at times when natural T-levels have decreased. However, surprisingly little is known about the relation between T-levels during the early breeding season and song. In many passerine birds males sing at a high rate at dawn early in the breeding season, referred to as the dawn chorus. In blue tits (*Parus caeruleus*), the dawn chorus coincides with the fertile period of the female, whereas diurnal song occurs throughout the breeding season. Previous studies on blue tits showed that characteristics of the dawn chorus correlate with male reproductive success. We experimentally elevated plasma T-levels in male blue tits during the pre-fertile and fertile period. Our aim was to test whether increased plasma T-levels affect dawn song characteristics and increase the amount of diurnal song. Although T-implants successfully raised circulating T-levels, we did not find any difference between T- and control males in temporal performance measures of dawn song or in diurnal song output. Our results suggest that either there is no direct causal link between song output or quality and individual T-levels, or experimental manipulations of T-levels using implants do not permit detection of such effects during the early breeding season. Although we cannot exclude that individual T-levels are causally linked to other (e.g. structural) song parameters, our results cast doubt on T-dependence as the mechanisms that enforces honesty on song as a sexually selected trait.

## Introduction

The role of testosterone (T) in the expression and regulation of avian reproductive behaviour is widely accepted (Balthazart 1983). In many temperate-zone birds, reproductive behaviour is highly seasonal, and this coincides with changes in plasma

T-levels in males (Wingfield & Farner 1980). T-levels peak during territory acquisition and courtship in spring, and usually decline to near non-breeding baseline levels when males provide parental care (Wingfield et al. 1990). There is good evidence for a link between T and singing behaviour of temperate-zone songbirds (Arnold 1975;

Heid et al. 1985; Marler et al. 1988; Rost 1990), in that high singing activity during spring coincides with the period of high plasma T-levels (e.g. Nottebohm et al. 1987; Johnsen 1998).

Correlational studies have shown that high T-levels are linked to high diurnal song activity (e.g. Hunt et al. 1995; Johnsen 1998; but see Saino & Møller 1995; Galeotti et al. 1997). This is confirmed by studies that experimentally altered the time profile of high T: high exogenous T-levels, at the time when natural T-levels dropped below the breeding baseline, caused an increase in song rate in several species [Silverin 1980; Nowicki & Ball 1989; Ketterson et al. 1992; Beletsky et al. 1995 (but see their Table V on red-winged blackbirds *Agelaius phoeniceus*); Enstrom et al. 1997; Hunt et al. 1997; De Ridder et al. 2000; Van Duyse et al. 2002]. T might not only influence song output, but also the quality or complexity of the song. For example, seasonal changes in repertoire size may be linked to changes in T-levels (Nottebohm et al. 1986; Eens 1997).

Clearly, high exogenous T triggers or enhances the expression of singing behaviour during the later stages of the breeding season, when natural T-levels are low. However, does variation in T-levels above the breeding baseline relate to variation in song output or song characteristics during the pre-fertile and fertile period? This is a crucial point in our understanding of the role of T in sexual selection. If females select males on the basis of T-dependent secondary sexual traits (e.g. song characteristics, see Andersson 1994), then males with higher T-levels will have a selective advantage. However, high T-levels also carry costs. Elevated T-levels may reduce survival (Nolan & Ketterson 1992), or reduce investment in parental care (Silverin 1980; Ketterson et al. 1992), and they may have an immunosuppressive effect (e.g. Duffy et al. 2000; Peters 2000). It is therefore likely that the optimal T-level depends on the quality or condition of each male, and thus acts as a link relating the expression of secondary sex traits to male condition.

We used the blue tit, *Parus caeruleus*, a small passerine, as a model to study the relationship between T and singing behaviour during dawn and during the day. The dawn chorus is likely to play a role in sexual selection (Staicer et al. 1996; Gil & Gahr 2002). In Parids, the dawn chorus – in contrast to song during the day – is closely linked to female fertility: males start singing at dawn a few days before the first egg is laid and stop soon after the female lays the last egg (Mace 1987; Poesel et al. 2001). In blue tits, song output during the dawn chorus is cor-

related with aspects of female fecundity (Poesel et al. 2001), and strophe length at dawn might function as an indicator of male quality (Kempenaers et al. 1997). Further, Foerster et al. (2002) found that natural plasma T-levels during the fertile period tended to correlate with song output.

The aim of this study was to investigate whether increasing plasma T-levels in reproductively active male blue tits affected their singing behaviour. The natural profile of circulating plasma T in male blue tits shows a single peak during nest building (pre-fertile period) and egg laying (fertile period; Foerster et al. 2002), following the typical pattern of socially monogamous temperate-zone birds with a single clutch (Wingfield et al. 1990). We experimentally elevated plasma T-levels of males towards the high end of the natural range during nest building and egg laying, but not during chick feeding (in contrast to other studies investigating the effect of T on song, see Fig. 1 and Discussion). To investigate the influence of plasma T on dawn song characteristics and diurnal singing activity during the early breeding season, we compared singing behaviour of males with experimentally elevated plasma T-levels (T-males) to that of control males (C-males).

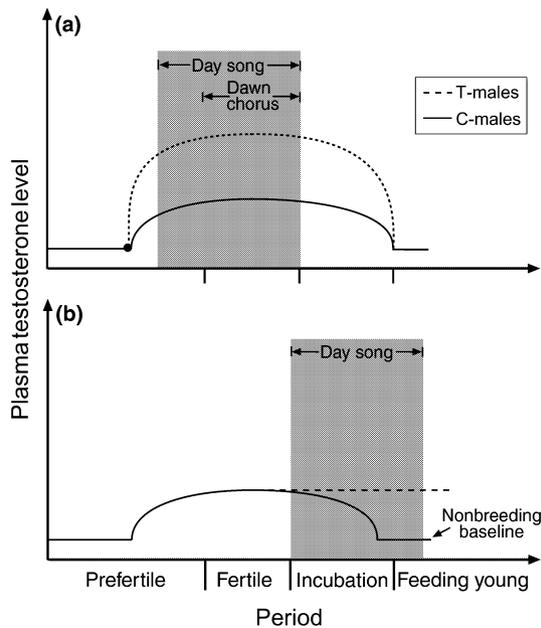
## Materials and Methods

### Study Site and Population

Our experiment was conducted in 1999 and 2000 in a population of blue tits breeding in a mixed deciduous woodland at Kolbeterberg (48°13'N, 16°20'E) in Vienna, Austria. Breeding birds in the study area have been colour-banded since 1998. From early Mar. onwards, we checked all nestboxes at least every second day to detect breeding activity. Breeding pairs were identified when they started to build a nest. In 1999 and 2000, the first egg was laid on 5 and 7 Apr., respectively, and the median laying date was 9 and 12 Apr., respectively.

### Experimental Procedure

During the last week of Feb. and the first week of Mar. in 1999 and 2000, we caught males at night when they slept in a nestbox. We determined the age of all birds (yearling or older, following Svensson 1992) and assigned the first individual of each age class randomly either to the testosterone (T-males) or control group (C-males). We then alternated the treatment for all further males of the same age class. In total, we implanted 35 males with T-pellets and



**Fig. 1:** Schematic overview of natural seasonal fluctuations and experimental manipulations of plasma levels of testosterone (T) in male temperate-zone songbirds. (a) Following our experimental procedure, T-males were implanted around the start of nest building and levels of T were kept at a constant high level until the nestling feeding period. Control males followed a similar seasonal pattern, but average T-levels were lower. ‘•’ Indicates the time of implantation. The shaded area indicates the period when we recorded the dawn chorus and scored the amount of spontaneous singing during the day. (b) In all other experimental studies (except Van Duyse et al. 2002; see text for details), T-males received implants that resulted in higher T-levels during the incubation and/or nestling feeding period, when T-levels in control males have declined to non-breeding baseline levels. The shaded area indicates the period when spontaneous singing activity was recorded in these studies

33 with C-pellets. Of these, 27 T-males and 25 C-males bred in the study area. Song data were only recorded for a subset of the breeding males (see below). We implanted round pellets (diameter: 3 mm, height: 1 mm; supplied by Innovative Research of America Inc., Sarasota, FL, USA), through a small dorsal incision in the skin. The cut was sealed with tissue adhesive (Histoacryl, Braun, Melsungen, Germany). The birds were kept in separate wooden containers until dawn and then released near their territories. T-implants contained 7.5 mg crystalline T in binding material; control pellets consisted of pure binding material. The pellets were designed to release T during a period of 60 d and to elevate the plasma level of circulating T to about 5 ng/ml. This level was similar to the highest natural plasma T-level previously measured in a male blue tit in this population (Foerster et al. 2002). The implants

dissolved and were absorbed completely after this period (see Foerster & Kempenaers 2004, 2005).

**Hormone Analysis**

To check whether the implants worked properly, we caught eight T- and six C-implanted birds during the nest building or egg laying stage (between 17 Mar. and 24 Apr.). There were no differences in the average capture date between T- and C-males ( $t = -0.186$ ,  $df = 12$ ,  $p = 0.86$ ). We confirmed that the implant was still present for all 14 birds. We were not able to catch more males during this period, because they were not easily attracted to food baits. We avoided using song playback to attract birds, because this could influence both T-levels and their subsequent singing behaviour. From all recaptured implanted birds, we took a blood sample of approx. 100  $\mu$ l within 30 min of capture. We immediately centrifuged the blood in the field, removed the plasma and put it on ice until storage at  $-70^{\circ}\text{C}$  in the laboratory. We determined circulating levels of plasma T using a radioimmunoassay, described in detail by Fusani et al. (2000). Average recovery was 90% and the sensitivity of the assay was 26.7 pg/ml for a plasma sample of 20  $\mu$ l. The intra-assay variation was 2.8%. All samples were measured in a single assay.

**Song Recording and Analysis of the Dawn Song**

We recorded the full dawn song of 31 male blue tits in 2 yr between 1 and 23 Apr. In 1999, we recorded six C-males (two yearlings and four older) and 11 T-males (nine yearlings and two older); in 2000, we recorded six C-males (four yearlings and two older) and eight T-males (six yearlings and two older). All singing males were identified by their colour rings either during or after the recording. We recorded dawn song using a Sony Stereo Cassette Recorder WM-D6C (Sony Electronics Inc.). A Telinga Stero-condenser microphone (Stero-Dat-Mic, Telinga Microphones, Tobo, Sweden), centred in the focus of a Telinga pro 5 parabolic sound reflector with a diameter of 57 cm, was connected to the recorder. We digitized recordings on a PC with Cool Edit 2000 (now called Adobe Audition, Adobe Systems Inc.) (sample frequency: 44.1 kHz, resolution: 16 bit) and analysed dawn song characteristics with the software package AVISOFT SASLAB PRO 3.5 (Specht 1993; time resolution: 5.8 ms, FFT: 256).

We investigated the following song characteristics: (1) the onset of dawn song relative to sunrise (min

before sunrise); (2) the duration of the dawn song, which is the duration of continuous singing (in min) including pauses of <5 min; (3) mean strophe length (in s), whereby we defined a strophe as a unit of song with pauses of <0.5 s between notes (see Fig. 1 in Poesel & Kempenaers 2000); (4) mean pause length, defined as the mean duration of the interval between two consecutive strophes (in s); (5) mean percentage performance time, whereby the percentage performance time is calculated as strophe length divided by the sum of strophe and pause length  $\times 100$ ; (6) song rate as the mean number of strophes per minute throughout the dawn chorus; (7) versatility, defined as repertoire size multiplied by the number of song type switches, which we used as a measure of song complexity. Because males differed in the duration of their dawn song, we divided the calculated versatility by the duration of the dawn chorus and used this measure in the analyses. For each strophe, we determined to which song type it belonged (based on the classification by Bijmens 1988; cf. Poesel et al. 2001) and we defined repertoire size as the number of different song types a male sang during a single dawn chorus. This underestimates true repertoire size (i.e. the total number of song types a male can produce), unless males sang all their song types during a single dawn chorus, which seems unlikely (Bijmens 1988). Sample sizes are less than 31 in some analyses because the recording date is missing for one male and the start of the dawn chorus was not noted for six males (two C- and four T-males).

Song characteristics might change over the breeding season (Poesel et al. 2001). However, T- and C-males did not differ significantly in absolute recording date (t-test,  $t = 0.154$ ,  $df = 28$ ,  $p = 0.88$ ), nor in recording date relative to the date on which their female laid the first egg (t-test,  $t = 0.145$ ,  $df = 28$ ,  $p = 0.89$ ). The T-implanted group contained 15 yearling and four older birds, whereas the control group consisted of six yearling and six older males. To control for potential age effects on song characteristics, we included age (first year vs. older) as a factor in the analyses.

### Measurement of Diurnal Song Activity

In our study area, blue tit males rarely sing during the day, and singing bouts contain few strophes. Diurnal song is therefore more sporadic and shorter event than the dawn chorus. Data on singing activity during the day were collected for 27 T- (17 yearling and 10 older males) and 20 C-males (12

yearling and eight older) between 07:00 and 12:00 hours. We followed each male for 30 min on 1–3 d (mode = 2) during nest building (pre-fertile period) and on 1–6 d (mode = 3) during the fertile period of the female (defined as starting 5 d before the first egg was laid until the day the penultimate egg was laid; cf. Birkhead & Møller 1992). At the end of every 2-min interval, we noted whether the male sang during this interval (yes/no). We calculated the number of intervals with song within a 30-min observation and averaged these values for each period separately. Eight T-males and five C-males were only observed during the pre-fertile period, and two T-males and one C-male were only observed during the fertile period of their female. Average diurnal singing activity did not differ between years (t-test,  $t = -0.44$ ,  $df = 29$ ,  $p > 0.65$ ), nor did it depend on age (t-test,  $t = -0.99$ ,  $df = 29$ ,  $p > 0.33$ ), so data were pooled across years and age classes.

### Data Analyses

Statistical analyses were performed with SPSS 9.0 for Windows (SPSS Inc., Chicago, IL, USA). Data shown are  $\bar{x} \pm SD$ . We tested whether the data were normally distributed and for homogeneity of variances. All tests are two-tailed.

### Ethical Note

Care was taken to minimize handling and disturbance of birds. After taking a bird out of the nestbox, it was transported in a dry, clean, cotton bag to our field laboratory and kept in the dark until processed. Implant insertion caused no more apparent distress than blood sampling. The total handling time of a bird never exceeded 20 min. After manipulation, birds were put singly in a dry, wooden container and kept in the dark until the following morning. Birds were released around dawn at the edge of the study area. All birds were observed on their former territory afterwards and they behaved normally. C-implanted and T-implanted males did not differ in the feeding rate of their young (Foerster & Kempenaers 2005). Furthermore, no males showed signs of illness or loss of body condition and no mortality resulted from this experiment. The small incision wounds healed entirely and we found no effect of T-implants on survival probability either within or between seasons (B. Kempenaers, unpubl. data). All procedures were approved by the Austrian Magistrate of Conservation (permit MA22-7332/97) and

the Austrian Ministry of Science and Transport (permit G\_Z 66015/10-Pr/4/99).

## Results

### Testosterone Levels

The T-implants successfully increased plasma T-levels during the nest building and egg laying period (T-males:  $8.23 \pm 1.47$  ng/ml, range: 3.60–9.92 ng/ml, one outlier at 16.71 ng/ml is excluded,  $N = 8$ ; C-males:  $1.61 \pm 0.71$  ng/ml, range: 0.07–4.82 ng/ml,  $N = 6$ ;  $t = 3.64$ ,  $p < 0.005$ ). In the study population, natural T levels in untreated males ranged between 0.09 and 12.78 ng/ml during nest building and egg laying (Foerster et al. 2002; A. Peters, unpubl. data). Thus, the treatment produced plasma T-levels in the upper range of naturally occurring levels, with the exception of one T-implanted male with a T-level of 16.71 ng/ml. This male showed neither high, nor low expression of different aspects of behaviour (see Foerster & Kempenaers 2004, 2005), and was thus included in all analyses. Note that excluding this individual did not change the results.

### Dawn Chorus

T- and C-males did not differ in any of the seven evaluated characteristics of the dawn chorus (Table 1). Males recorded later in the breeding season started their dawn chorus later in the morning relative to sunrise (Pearson correlation,  $r = 0.41$ ,  $n = 25$ ,  $p = 0.04$ ). T-males started their dawn chorus slightly later than C-males, but this difference was not significant (Table 1). The dawn chorus lasted significantly longer in 2000 ( $25 \pm 8$  min) than in

1999 ( $17 \pm 8$  min), but T-males did not sing longer than C-males (Table 1). Percentage performance time declined over the breeding season ( $r = -0.50$ ,  $n = 31$ ,  $p = 0.005$ ), but there was no difference between T- and C-males (Table 1).

### Diurnal Song Activity

Diurnal song activity did not differ between T- and C-males, either during the pre-fertile period, or during the fertile period (Fig. 2). Song rates did not differ between the pre-fertile and the fertile period, either for T-males (pre-fertile:  $1.9 \pm 1.8$ , fertile:  $1.9 \pm 1.4$ , paired t-test:  $t_{16} = 0.02$ ,  $p = 0.98$ ) or for C-males (pre-fertile:  $1.8 \pm 2.0$ , fertile:  $1.9 \pm 1.5$ , paired t-test:  $t_{13} = 0.13$ ,  $p = 0.90$ ).

## Discussion

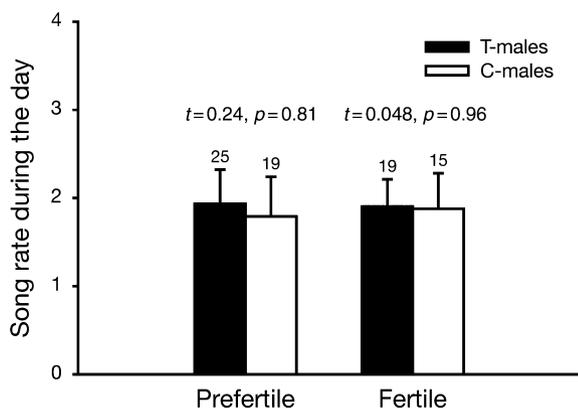
Male blue tits that received T-implants had significantly higher circulating levels of T during the pre-fertile and fertile period than males with control implants. However, we found no differences in dawn song characteristics, nor in diurnal singing activity, between males with experimentally elevated plasma T-levels and control males.

Most studies applying exogenous T-treatment in male birds found an increase in singing activity or song output during later stages of the breeding season (e.g. Silverin 1980; Ketterson et al. 1992; Beletsky et al. 1995; Hunt et al. 1997; De Ridder et al. 2000; Van Duyse et al. 2002). However, previous studies compared singing behaviour of males with experimentally elevated T-levels to that of control males with naturally low T-levels during incubation or chick feeding (but see Van Duyse et al. 2002;

**Table 1:** Effect of T on characteristics of the dawn song for 31 male blue tits recorded in 1999 and 2000

Song characteristic	T-males	C-males	Treatment	Year	Age	Recording date
Start of dawn chorus (min before sunrise)	$24.8 \pm 10.2$	$29.6 \pm 7.3$	$F_{1,22} = 1.91$ , $p = 0.18$	ns	ns	$F_{1,22} = 4.89$ , $p = 0.038$
Dawn chorus duration (min)	$20.7 \pm 6.7$	$19.4 \pm 11.7$	$F_{1,28} = 0.46$ , $p = 0.51$	$F_{1,28} = 7.94$ , $p = 0.009$	ns	ns
Song rate (strophes/min)	$12.9 \pm 3.3$	$13.4 \pm 3.3$	$F_{1,29} = 0.16$ , $p = 0.69$	ns	ns	ns
Mean strophe length (s)	$1.33 \pm 0.26$	$1.43 \pm 0.24$	$F_{1,29} = 1.08$ , $p = 0.31$	ns	ns	ns
Mean pause length (s)	$3.50 \pm 1.44$	$3.30 \pm 0.95$	$F_{1,29} = 0.17$ , $p = 0.68$	ns	ns	ns
Mean percentage performance time	$31.8 \pm 8.6$	$33.8 \pm 3.4$	$F_{1,27} = 0.63$ , $p = 0.43$	ns	ns	$F_{1,27} = 8.86$ , $p = 0.006$
Versatility	$1.0 \pm 1.5$	$1.3 \pm 1.0$	$F_{1,29} = 0.41$ , $p = 0.52$	ns	ns	ns

Data shown are  $\bar{x} \pm SD$ . Shown are the results of general linear models, with the different song parameters as dependent variable and experimental treatment (T or C implant) as a fixed factor. In the full model we controlled for recording date (covariate), male age (random factor) and year (random factor). For the confounding effects,  $F$ - and  $p$ -values are only shown if the variable was left in the final model (if  $p < 0.10$ ); otherwise ns (not significant) is indicated. None of the interaction terms were significant, so they were deleted from the models and are not shown



**Fig. 2:** Song rate during the day for T- and C-males in the pre-fertile and fertile period of their female. Song rate was estimated as the average number of 2-min intervals during which males produced song (see Materials and Methods for details). The numbers above the bars indicate sample sizes

Fig. 1b). Therefore, the observed differences in song activity are probably due to the fact that T-males continued singing at a high rate, whereas C-males decreased their song output following a natural decrease in T-levels at the end of the breeding season (when these males were engaged in parental duties). In line with this interpretation is the finding of Van Duyse et al. (2005) that blocking the pathways of T action (which should have the same effect as decreasing T-levels) early in the egg laying stage decreased the likelihood of showing dawn song in male great tits (*Parus major*). In contrast, we recorded singing behaviour during the pre-fertile and fertile period of the males' mates, and compared males with experimentally elevated T-levels to control males with naturally high T-levels (Fig. 1a). Thus, although exogenous T above a breeding baseline may allow high song activity later in the breeding season, our results provide no evidence for a causal link between plasma T-levels and song activity during the early breeding season. Similarly, Van Duyse et al. (2002) found that a significantly higher proportion of T-males than C-males showed diurnal song activity during the feeding stage, but not during egg laying (i.e. the fertile period) or incubation in great tits. In our study, experimentally elevated plasma T-levels did not influence singing behaviour, nor other male behaviours such as mate guarding and territory defence (Foerster & Kempenaers 2005). Furthermore, males with experimentally elevated plasma T-levels did not have a higher reproductive success (Foerster & Kempenaers 2004).

We found earlier that males with higher natural levels of plasma T sang with higher song output

(percentage performance time) during the dawn chorus (Foerster et al. 2002). However, our experimental results do not confirm that there is a direct causal relationship between T-levels and song output. We now discuss different explanations for the discrepancy between experimental and observational data.

#### Explanation 1

There exists no causal relationship between plasma T-levels above a certain threshold value and song output.

Given the small sample size ( $n = 7$ ), the correlation reported in Foerster et al. (2002) could have been a type I error ( $p = 0.03$ , not corrected for multiple tests). However, other studies showed that diurnal song rate correlated with natural levels of T (e.g. in Lapland longspur *Calcarius lapponicus*, Hunt et al. 1995; red-winged blackbird *A. phoeniceus*, Johnsen 1998), although this is not generally true (e.g. in the barn swallow *Hirundo rustica*; Saino & Møller 1995; Galeotti et al. 1997). The few studies that investigated the influence of natural T variation on variables other than song rate, found no effect on song complexity (Galeotti et al. 1997; Smith et al. 1997; This study). However, T could have affected (unmeasured) structural parameters of song such as the duration of specific song components (Galeotti et al. 1997), fundamental frequency (Cynx et al. 2005), stereotypy (Whaling et al. 1998) or tonality (Smith et al. 1997).

High T-levels and high song output during the early breeding season could have a common cause. For example, males that are subjected to high levels of aggressive interactions with neighbours or intruders might show both high T-levels and high song rate (Wingfield et al. 1990).

Alternatively, song may not only depend on high T-levels but also on other steroids (Balthazart 1983; Schlinger 1997). In the great tit and in the blue tit, plasma T-levels did not peak during the peak of dawn song activity (Van Duyse et al. 2003; Caro et al. 2005), suggesting that high T-levels alone are not enough to cause a peak in singing activity.

#### Explanation 2

There exists a causal relationship between T-levels and song output, but our experimental study did not allow us to detect it.

Our sample size might have been too small to detect an effect. However, our sample size is similar

to that used in comparable studies that found effects of T-treatment on song during later stages of the breeding season (e.g. Hunt et al. 1997; Van Duyse et al. 2002). Moreover, most differences between T- and C-males in this study are either very small, or in the opposite direction as predicted (i.e. control males started singing earlier, sang at a higher rate and with a higher percentage performance time and a larger versatility; Table 1).

Alternatively, we failed to detect an effect because of individual differences in the response to exogenous T. The number of androgen receptors and receptors for androgen metabolites probably varies between individuals (Nelson 2000), and thus the response to endocrine signals (Dufty et al. 2002). If the receptors were saturated by natural high T-levels early in the breeding season, we would not necessarily expect an effect of experimentally increasing T-levels (see also Van Duyse et al. 2005). In that case, natural variation in T-levels might still correlate with individual differences in singing activity, and females may still be able to discriminate against low quality males that do not produce enough T to saturate their receptors. Moreover, if the proportion of saturated receptors decreases during later stages of the breeding season as natural T-levels decrease, exogenous T might be able to affect behaviour (e.g. singing) at that time, which might explain the discrepancy between our results and those of previous studies. It also explains why Van Duyse et al. (2002) found an effect during late, but not during early stages of the breeding season. Note that we could not measure plasma T-levels in all individuals after the implantation (see Materials and Methods), so we compared T- vs. C-males, but did not correlate individual T-levels to song. However, assuming an individual-specific threshold of T needed for maximum song expression, such a correlation would not have been significant, because our treatment probably raised T-levels above this threshold.

So far, it has been assumed that the expression of a sexually selected trait (in this case song) is directly linked to the level of T. However, only few studies have experimentally tested this and the results (Van Duyse et al. 2002, This study) cast doubt on the validity of this assumption. The potential implications for our understanding of sexual selection should be considered. For example, if there is simply a (possibly individual specific) threshold T-level that must be exceeded for maximum song output or quality to be achieved, then the costs associated with maintaining higher T-levels cannot be the mechanism that keeps song an honest signal of male quality.

In conclusion, we were unable to detect an influence of experimentally elevated plasma T-levels on dawn song characteristics and diurnal singing activity. Our results suggest that either there is no causal link between song and plasma T-levels above a certain threshold during the early stages of the breeding season when females are fertile, or that experimentally increasing T-levels does not permit detection of such an effect. Although we cannot exclude that individual T-levels are causally linked to other (e.g. structural) song parameters, our results cast doubt on T-dependence as the mechanism that enforces honesty on song as a sexually selected trait.

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