THE ROYAL SOCIETY

*Received* 23 January 2004
*Accepted* 3 March 2004
*Published online* 5 May 2004

# Elevated testosterone reduces choosiness in female dark-eyed juncos (*Junco hyemalis*): evidence for a hormonal constraint on sexual selection?

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Because testosterone (T) often mediates the expression of attractive displays and ornaments, in the absence of constraints sexual selection should lead to an evolutionary increase in male T levels. One candidate constraint would be a genetic correlation between the sexes that leads to a correlated response in females. If increased T in females were to have deleterious effects on mate choice, the effect of sexual selection on male T would be weakened. Using female dark-eyed juncos (*Junco hyemalis*), we tested whether experimentally enhancing female T would lead to a decrease in discrimination between two classes of males, one treated with T (T-males) and one control (C-males). The two female treatments (T-implanted and C-females) spent equal amounts of time with both classes of males, but T-treated females failed to show a preference for either male treatment, whereas C-females showed a significant preference, albeit in an unexpected direction (for C-males). T-females were less discriminating than C-females, irrespective of the direction of their preference. To our knowledge, this is the first study to show that circulating hormones can alter female choosiness without reducing sexual motivation. Our results suggest that hormonal correlations between the sexes have the potential to constrain sexual selection on males.

**Keywords:** testosterone; mate choice; sexual selection; constraints

## 1. INTRODUCTION

Testosterone (T) is an important mediator of the expression of traits and behaviours used by male vertebrates during courtship (Ligon *et al.* 1990; Saino & Møller 1995; Zuk *et al.* 1995). Several studies, both correlative and experimental, have shown that females prefer males with higher T levels (Wingfield 1984; Zuk *et al.* 1995; Alatalo *et al.* 1996; Enstrom *et al.* 1997; Fusani *et al.* 1997; Hill *et al.* 1999). If males with higher T consistently obtain more mates, then an evolutionary response to sexual selection is expected, assuming that opposing selective pressures are not too strong and that sufficient additive genetic variance for T exists. Although T is typically thought of as a 'male' hormone, most female vertebrates have at least some circulating T (Wingfield 1994; Staub & De Beer 1997; Wingfield *et al.* 2000; Ketterson *et al.* 2001) and T rises in response to a social challenge in females of some species, just as it does in males (Langmore *et al.* 2002; but see Elekonich & Wingfield 2000). If male and female T levels are controlled by common genetic factors, female T levels should show a correlated response when sexual selection favours an increase in male T levels (Lande & Arnold 1985).

Because hormones mediate suites of traits (Ketterson & Nolan 1999), such an evolutionary increase in female T concentrations could potentially affect several aspects of female sexual behaviour, including mate choice. It is known that hormones have both organizational and activational effects on the development of heterosexual versus homosexual partner preferences (reviewed in Adkins-Regan 1998), but the effect of T on female choice among males has not been explored. Treatment of females with T has been shown to facilitate male-typical behaviours, such as aggression, and even behaviours that tend to be limited to males, such as song (Kern & King 1972; Searcy 1988; Hausberger *et al.* 1995; Nespor *et al.* 1996; Adkins-Regan 1999). If T affects mate choice in an analogous way, females with increased T might be expected to become more 'male-like' (that is, less choosy) in their mate-choice behaviour (Owens & Thompson 1994; Johnstone *et al.* 1996). Such an effect could oppose a build-up of genetic covariance between trait and preference (Fisher 1930; Lande 1981; Kirkpatrick 1982), potentially constraining sexual selection for increased T concentrations in males (Lande & Arnold 1985; Lande 1987; Price *et al.* 1987).

We examined the effect of artificially increased T on female mate-choice behaviour in a common songbird, the dark-eyed junco (*Junco hyemalis*). Juncos form socially monogamous pairs and exhibit biparental care; extra-pair fertilizations are common and increase the opportunity for sexual selection (Ketterson *et al.* 1998; Nolan *et al.* 2002). In both male and female juncos, T levels decrease following a peak early in the breeding season (Ketterson & Nolan 1992; Ketterson *et al.* 2001). When males are given

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subcutaneous T implants that maintain this peak throughout the entire breeding season, they are more attractive (Enstrom *et al.* 1997; Hill *et al.* 1999) and obtain more extra-pair fertilizations than controls (C) (Raouf *et al.* 1997), through either mate choice or male–male competition. Such extra-pair fertilization success indicates that higher levels of T are favoured by sexual selection and must be countered by correlated effects in males or in females.

In this study, we allowed captive female juncos to choose between males with enhanced T (T-males) and control males (C-males). Individual females were tested twice: once with normal T levels (as a C-female) and once with enhanced T (as a T-female). Male juncos tend to be less choosy than females, at least with respect to a particular plumage trait (Hill *et al.* 1999; Wolf *et al.* 2004). Therefore, we predicted that T-females would be more 'male-like', showing a decrease in choosiness between males relative to C-females.

## 2. MATERIAL AND METHODS

### (a) *Study area and species*

We conducted our research at Mountain Lake Biological Station in Giles County, VA, USA (37°22′ N, 80°32′ W) on *J. h. carolinensis*, the subspecies of the dark-eyed junco that breeds at high altitudes in the Appalachian Mountains (Nolan *et al.* 2002). Females build nests, incubate eggs and brood nestlings; both sexes feed nestlings and fledglings. Although female plasma T levels are almost always lower than those of males, both sexes exhibit a similar seasonal profile (Ketterson *et al.* 2001). Female T levels are particularly low during nest building and egg laying, when choosing among potential mates should be most important. All procedures used in this study were approved by the Bloomington Institutional Animal Care and Use Committee.

### (b) *Capture and housing*

In spring 2000, 21 male and 25 female juncos were captured in baited mist nets and potter traps primarily before the onset of breeding (four females and two males had begun to nest). Birds were housed in compartments of an outdoor aviary; males were housed singly and females were housed in groups of two or three. Prior to the mate-choice trials, birds were visually isolated from individuals of the opposite sex; male song was audible throughout the aviary. All birds had *ad libitum* access to food and water.

### (c) *Hormone manipulation*

We classified males as yearlings or older and measured their wing length, tarsus length, mass, amount of white in the outer rectrices (tail white; Hill *et al.* 1999) and cloacal protuberance size (Nolan *et al.* 2002). We divided 20 males into 10 age-matched dyads (six of yearlings, four of older males), matching as closely as possible for tail-white score (maximum difference = 0.5) and wing length (maximum difference = 3 mm). One male from each dyad was randomly selected to receive increased T. Males were anaesthetized with methoxyflurane (Metofane, Pitman-Moore Inc.). T-males received two 12 mm implants of Silastic tubing (Dow Corning, 1.47 mm inside diameter, 1.96 mm outside diameter), which were inserted under the skin of the flank and packed with 10 mm of crystalline T (Sigma Chemical Co.); C-males received two empty implants. Females were measured and randomly assigned to the T or C group in the same way as males, except that they were implanted with one 7 mm long tube (either empty or packed with 5 mm of T). This hormone treatment is designed to produce levels of T similar to those found at the breeding season peak in both sexes (Ketterson *et al.* 2001) and does not affect circulating levels of oestradiol in females (Clotfelter *et al.* 2004). Both sexes were given at least one week to acclimatize before we began mate-choice trials.

### (d) *Mate-choice trials*

We measured female preference using previously established protocols (Enstrom *et al.* 1997; Hill *et al.* 1999), which we describe briefly below. We performed two sets of preference trials in which each of 25 females was given the opportunity to choose between the T-male and the C-male of a dyad. In the first set of trials (3–26 June), 12 T-females and 13 C-females were presented with a dyad randomly assigned from a pool of 10 dyads. Each female was then switched from one treatment group to the other by substituting the original implant for one of the opposite treatment; females were allowed at least one week to acclimatize to their new treatment before being used in the second set of trials (23 June–12 July).

All trials were conducted in a single Y-shaped outdoor aviary in which each dyad member was caged at the end of one of the arms of the Y (see Enstrom *et al.* (1997) for description). When a female entered an arm of the Y she could not see the male in the other arm; dyad members could not see one another. Dyad members were placed in the Y aviary at *ca*. 16.00 on the day preceding a trial and allowed to acclimatize until the trial, which took place between 06.00 and 12.00 the next morning. To control for any possible effect of position of the different arms of the Y (Enstrom *et al.* 1997), we alternated male treatment groups between the left and right arms on each day of the experiment. We conducted two or three 50 min trials each morning unless it rained. For 20 min prior to the first trial each morning, we observed the behaviour of the male dyads in the absence of a female (the pre-assessment period). We then introduced the female confined to a small cage (0.31 m × 0.31 m × 0.62 m) at the base of the Y (i.e. distant from the males), and allowed her to see and be seen by both males; during this 20 min assessment period we observed male behaviour. At the end of the assessment period, we opened the female's cage by remote control and observed her behaviour for 30 min (the choice period), during which she could move about and approach the males. The observer (J.W.M.) was blind to both male and female treatment.

During each 30 s interval of the pre-assessment and assessment periods we noted the presence or absence of five male behaviours: movement (changing perch), long-range song (Titus 1998), short-range song, erection of the body feathers (ptiloerection) and tail spreading. These songs and displays are thought to be important in mate attraction and courtship (Nolan *et al.* 2002). During each 15 s interval of the choice period, we noted only female behaviour, and noted the presence or absence of hopping, still perching, flying, preening, bill scraping and engaging in precopulatory display (Enstrom *et al.* 1997).

We defined attendance time as the number of 15 s intervals a female spent in the space (an elevated platform and a perch) immediately next to either of the males, the choice areas (Enstrom *et al.* 1997). Attendance time was scored by recording the female's location (in one of the two active choice areas or in a neutral area of the aviary) at the end of each 15 s interval. A similar method of scoring choice has been found to correlate

highly with actual time spent near the stimulus (Wolf *et al.* 2004). Total attendance time may be interpreted as a measure of the motivation to associate with males, or of the readiness to choose. We considered a female to have been responsive if she spent more than 20% of her time in the choice areas. Non-responsive females were retested with a different dyad within two weeks of the original trial.

The second set of trials tested the same females with the opposite hormone treatment (12 C-females and 13 T-females). Females were tested in roughly the same order as in the first set of trials and using the same protocol. All females were tested with a dyad of males that they had never seen before in captivity.

Female preference was measured as the proportion of total attendance time (number of 15 s intervals spent in either of the choice areas) that was spent with the T-male. In other words, a preference score above 0.5 represents a preference for T-males, while a preference below 0.5 represents a preference for C-males. We were also interested in the degree of discrimination between males, irrespective of which male was preferred. To this end, we quantified choosiness as the absolute deviation of a female's preference score from 0.5. Choosiness scores thus ranged from 0 (equal time spent with each male) to 0.50 (all attendance time spent with one male).

### (e) *Hormone assays*

To measure hormone concentrations in males and females, we obtained two blood samples (each of 100–300 µl, yielding 65–185 µl of plasma) from the alar vein. Birds were bled once after the first set of trials and again after the second. All samples were collected within one week of a bird being used in a mate-choice trial. Implant switching and removal were performed just after bleeding. Plasma T and corticosterone were separated from other steroid hormones using column chromatography and measured using radioimmunoassay as in Casto *et al.* (2001); for detailed procedures see Wingfield & Farner (1975), Ball & Wingfield (1987) and Ketterson *et al.* (1991). Intra-assay coefficients of variation ranged between 5.8% and 14.6% for the T assay, and between 9.1% and 14.6% for the corticosterone assay. Inter-assay coefficients of variation were 3.5% and 7.1% for the T and corticosterone assays, respectively.

### (f) *Statistical analyses*

Because each female was tested twice, once in each treatment group, we used two-factor ANOVAs without replication (the equivalent of a paired *t*-test) to assess differences in preference and choosiness (Sokal & Rohlf 1995, pp. 342–352). This analysis included female treatment and individual female as effects, which allowed us to test for differences as a result of T treatment while correcting for differences among individual females. Despite its statistical power, this paired model was unable to control for other factors that might have affected preference or choosiness, so we also used a larger ANOVA model, which included treatment, treatment order (T first or C first) and position of the T-male (in left arm or in right arm) as fixed effects, Julian date as a covariate, and all possible interactions among fixed effects. To determine whether T affected the overall motivation to associate with a male, we also examined possible effects on total attendance time using the latter ANOVA model.

Because preference scores were proportions, they were arcsine transformed before analysis (Sokal & Rohlf 1995, p. 421). To generate transformed values of choosiness scores for analysis, we took the absolute value of the difference between a female's transformed preference score and 45 (the arcsine-transformed value of 0.5, which represented equal time spent with each male). Total attendance time was ln transformed before analysis.

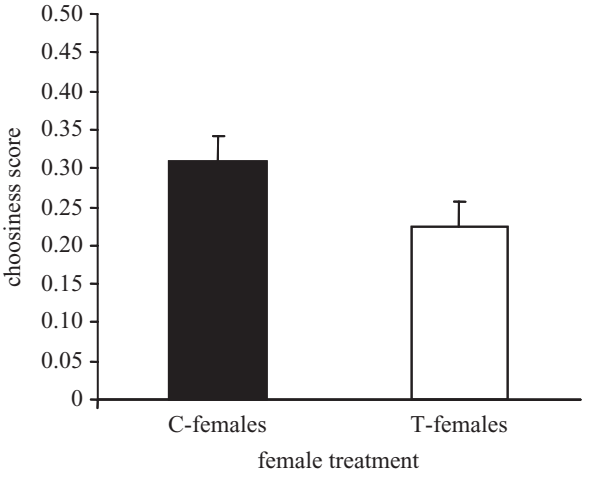


Figure 1. Female choosiness according to hormone treatment ($n = 25$, mean ± s.e.). A choosiness score of 0.50 represents all time spent with one of the two males in a dyad; a score of 0 represents equal time spent with each male.

To test whether differences in male appearance, despite our efforts to control for them, affected female preference and choosiness, we added covariates to our paired ANOVA model. For the analysis of preference, these covariates were the phenotypic values (wing length, tail white and mass) of the T-male minus those of the C-male. For the analysis of choosiness, we used the absolute values of the differences between the T-male and the C-male.

We used Wilcoxon paired signed-rank tests to analyse behavioural data. We measured the effects of T on female behaviour by comparing a female's behaviour as a C-female with her behaviour as a T-female. We tested for differences in male behaviour using three paired comparisons: (i) pre-assessment versus assessment periods, to measure the effect of female presence on male behaviour; (ii) T-males versus C-males in the pre-assessment period; and (iii) T-males versus C-males in the assessment period. Because dyads of males were used in multiple trials per day and the behaviour of a male could vary among days, we used averages of male behaviour on each day as independent observations.

The effect of hormone treatment on T and corticosterone concentrations in both sexes was analysed using separate ANOVAs. For females, treatment order and a treatment × treatment-order interaction were incorporated into the analyses. Because corticosterone concentrations may increase rapidly owing to stress, analyses of corticosterone concentrations for both sexes included handling time (measured in s elapsed between capture and completion of bleeding) as a covariate (Schoech *et al.* 1999). Correlations between T and corticosterone were also examined in both sexes. To normalize the data, all hormone concentrations were multiplied by $10^4$ and logarithmically transformed before analysis.

All analyses were performed with SPSS v. 11.0 for Windows.

## 3. RESULTS

### (a) *Female mate choice*

T treatment decreased the choosiness of females (figure 1). While this difference was marginally non-significant in

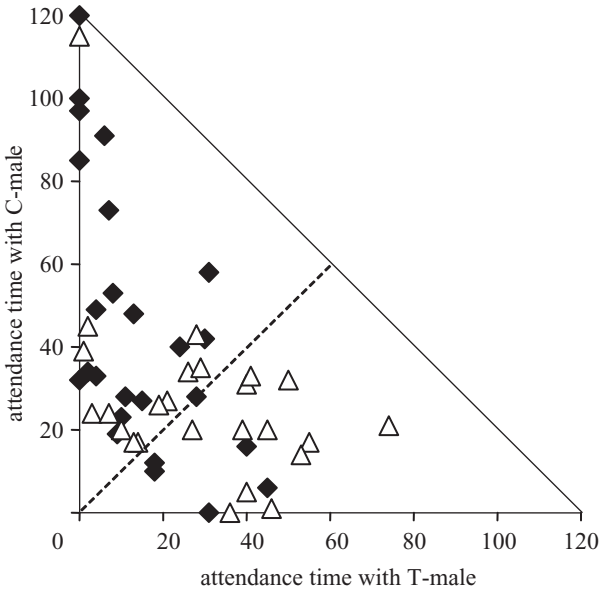


Figure 2. Female attendance time measured as the number of 15 s periods in which the female was in the male choice area. Points near the 45° indifference line represent females that were less choosy; i.e. they spent nearly equal time with each male. T-females (white triangles) were less choosy than C-females (black diamonds); that is, T-females tend to cluster around the 45° indifference line.

the paired model ($F_{1,24} = 3.46$, $p = 0.075$), it was significant when controlling for other factors ($F_{1,41} = 4.94$, $p = 0.032$). Treatment order ($F_{1,41} = 2.58$, $p = 0.12$) and male position ($F_{1,41} = 0.15$, $p = 0.70$) did not affect choosiness, but there was a significant interaction between the two factors ($F_{1,41} = 4.20$, $p = 0.047$). There was also a nearly significant interaction between treatment and T-male position ($F_{1,41} = 3.54$, $p = 0.067$). These interactions occurred because C-females, particularly T-first C-females, were more choosy when the T-male was on the right-hand side of the aviary and are probably statistical artefacts caused by a bias for the left side of the aviary (see below). There was also a nearly significant positive covariance between choosiness and Julian date, suggesting that females tended to be more choosy later in the experiment ($\beta = 0.67$, $F_{1,41} = 3.65$, $p = 0.063$). Other interactions in the model were not significant ($p \geqslant 0.17$).

Female hormone treatment also significantly affected preference (paired model: $F_{1,24} = 4.63$, $p = 0.042$). C-females as a group preferred C-males (percentage attendance time with T-male ± s.e. = 0.30 ± 0.058), but T-females tended to spend half of their time with each male (0.50 ± 0.057; figure 2). This treatment difference remained significant (full-model: $F_{1,41} = 9.19$, $p = 0.004$) despite significant effects of male position ($F_{1,41} = 12.63$, $p = 0.001$) and the treatment order × male position interaction ($F_{1,41} = 6.01$, $p = 0.019$). The position effect arose from the females' unexplained tendency to spend more time in the left arm of the aviary, despite our efforts to equalize conditions (Enstrom *et al.* 1997). Male behaviour did not differ with respect to position (Wilcoxon signed-ranks tests: $n = 25$, $p \geqslant 0.28$). Neither treatment order nor Julian date had a significant effect, and there were no other significant interactions (all $p \geqslant 0.47$).

When we considered differences in male appearance, female treatment groups still showed significant differences in preference ($p = 0.015$) and choosiness ($p = 0.049$). Preference for T-males showed a nearly significant dependence on differences in tail white ($\beta = 38.6$, $F_{1,21} = 4.29$, $p = 0.051$). Differences in mass and wing length did not covary with preference ($p > 0.35$). None of the phenotypic differences covaried with choosiness ($p > 0.16$).

Total attendance time of females did not differ with respect to female treatment (mean number of intervals ± s.e.: C-females = 59.1 ± 5.36, T-females = 56.0 ± 4.41; $F_{1,41} = 0.076$, $p = 0.785$), T-male position ($F_{1,41} = 1.99$, $p = 0.166$) or treatment order ($F_{1,41} = 0.774$, $p = 0.384$) and did not vary significantly with Julian date ($F_{1,41} = 2.68$, $p = 0.109$). There were no significant interactions.

### (b) *Female behaviour*

T-females were more active, spending significantly more time in flight, than C-females, but did not differ from C-females in any other behavioural categories (table 1). Individuals in both treatment groups performed very few precopulatory displays, and females tended to produce fewer when treated with T, though the effect was not significant (table 1). This low frequency of precopulatory displays is expected when female oestradiol levels are not manipulated (Enstrom *et al.* 1997). T-females were never observed singing during mate-choice trials or in the aviary (cf. Kern & King 1972; Hausberger *et al.* 1995; Nespor *et al.* 1996).

### (c) *Male behaviour*

During the pre-assessment period (female not present), T-males spread their tails more frequently than did C-males, but hormone treatment had no effect on any of the other four behavioural categories (table 2). After a female was introduced, males were less active (fewer perch changes), sang more long-range songs, and performed more ptiloerection (n.s.; table 2). During the assessment period (female present), T-males sang long-range songs more frequently than C-males, but did not significantly differ from C-males in any other behavioural category (table 2). T-males again tended to spread their tails more often, although the effect was not significant (table 2). Short-range song was recorded only occasionally in the assessment and pre-assessment periods, and no significant differences were found between treatment groups or between the assessment and pre-assessment periods. Short-range song tended to occur more often during the choice period when the female was directly in front of the male (J. W. McGlothlin, personal observation). Differences in male behaviour as a result of treatment observed in this study were similar to those seen by Enstrom *et al.* (1997).

### (d) *Hormone assays*

In both sexes, hormone treatment had the expected effect and T concentrations were within the physiological range (Ketterson *et al.* 2001). Plasma T was higher in T-males than C-males (mean T in ng ml$^{-1}$ ± s.e.: T-males = 12.58 ± 1.91, C-males = 1.13 ± 0.36; $F_{1,19} = 59.13$, $p < 0.0001$). T-females also had elevated T levels

Table 1. Female behaviour by hormone treatment during the active choice period.
(Reported values are medians followed by the interquartile range. Significance is tested by Wilcoxon matched-pairs signed-ranks tests, and tests with $p < 0.05$ are noted with an asterisk.)

| behaviour | C-females | T-females | *n* | *Z* | *p* |
|---|---|---|---|---|---|
| still perching | 89 (67.5–103.0) | 86 (71.5–95.0) | 25 | −0.31 | 0.76 |
| hopping | 76 (47.0–91.5) | 70 (59.0–80.0) | 25 | −0.20 | 0.84 |
| flying | 16 (4.5–33.0) | 32 (12.0–54.5) | 25 | −2.44 | 0.02* |
| preening | 1 (0.0–2.5) | 0 (0.0–2.0) | 25 | −1.16 | 0.25 |
| bill scraping | 3 (0.0–5.0) | 4 (1.0–7.0) | 25 | −1.66 | 0.10 |
| precopulatory display | 0 (0.0–2.0) | 0 (0.0–0.0) | 25 | −1.76 | 0.08 |
| ptiloerection | 3 (1.0–9.5) | 3 (0.0–17.5) | 25 | 0.00 | 1.00 |

(mean ± s.e.: T-females = 3.10 ± 1.66, C-females = 0.18 ± 0.076; $F_{1,44} = 237.71$, $p < 0.0001$) and treatment order did not significantly affect this difference ($F_{1,44} = 3.32$, $p = 0.08$). A slight residual effect of treatment order, however, was detected: T-first C-females had slightly higher T than C-first C-females (mean ± s.e.: T-first = 0.29 ± 0.16, C-first = 0.09 ± 0.021), while T-females were unaffected by order (mean ± s.e.: T-first = 3.09 ± 0.60, C-first = 3.12 ± 0.035). This manifested itself in a significant treatment × treatment-order interaction in the ANOVA ($F_{1,44} = 4.75$, $p = 0.035$). This small difference in plasma T levels as a result of treatment order is biologically negligible when compared with the large difference between treatment groups.

Corticosterone concentrations differed with respect to treatment for males, with T-males showing higher corticosterone (mean corticosterone in ng ml$^{-1}$ ± s.e.: T-males = 53.61 ± 10.58, C-males = 11.43 ± 1.80; $F_{1,18} = 30.65$, $p < 0.0001$), but concentrations did not vary significantly with handling time ($F_{1,18} = 0.15$, $p = 0.15$). Corticosterone also differed with respect to treatment in females (mean ± s.e.: T-females = 21.08 ± 2.43, C-females = 14.45 ± 1.99; $F_{1,42} = 10.33$, $p = 0.0025$) and varied positively with handling time ($F_{1,42} = 25.66$, $p < 0.0001$), but the effect of order and the treatment × treatment-order interaction were non-significant. When individuals were pooled with respect to hormone treatment, corticosterone and T concentrations were correlated in each sex (males: $r = 0.847$, d.f. = 19, $p < 0.01$; females: $r = 0.321$, d.f. = 46, $p < 0.05$).

## 4. DISCUSSION

In this experiment, females with unaltered T levels preferred C-males over T-males. When females were implanted with T, their preference was significantly altered, and, on average, they spent equal time with each type of male. This difference in preference was primarily because of a decrease in choosiness. T-females discriminated between males to a lesser degree than did C-females, irrespective of the direction of their preference. Differences in preference and choosiness were not reflective of a difference in motivation to associate with males, as T-females spent as much time associating with males as did C-females. To our knowledge, this is the first study to demonstrate an effect of circulating hormone levels on female choice between males.

This result is consistent with the hypothesis that the effect of T on female mate choice might act as an evolutionary constraint, assuming that male and female T levels are genetically correlated. Normally, genetic covariance should build up between male trait and female preference owing to linkage disequilibrium generated by non-random mating (Lande 1981; Kirkpatrick 1982). It is this genetic covariance that leads to the runaway process that makes sexual selection a very strong evolutionary force. However, this process would be opposed by a pleiotropic effect of T on female preference. A decrease in female choosiness because of increased T would simultaneously decrease the mating advantage of males with higher T and oppose the generation of linkage disequilibrium between trait and preference, because males with higher T would tend to sire daughters who were less choosy. Without this genetic covariance, the runaway process could not occur, and sexual selection would be constrained in its ability to move male T concentration away from its natural-selection optimum. The results presented here, combined with the previously demonstrated advantage of T-males in attracting females (Enstrom *et al.* 1997; Hill *et al.* 1999) and obtaining extra-pair fertilizations (Raouf *et al.* 1997), suggest that such a constraint mechanism is plausible in dark-eyed juncos.

For this constraint to operate, T levels must be heritable and genetically correlated between the sexes. Although the heritability of T levels has not been measured in birds, evidence from domestic mammals suggests that T levels are heritable (Davis 1993; Robison *et al.* 1994). Additionally, T-mediated traits such as comb size in fowl (Johnson *et al.* 1993; Zuk *et al.* 1995; Tufvesson *et al.* 1999) and dominance in quail (Nol *et al.* 1996) have been shown to have a heritable basis. While the between-sex genetic correlation of T levels has not been directly measured, limited evidence supports covariation between male and female levels of T across sexually monomorphic bird species, which is consistent with genetic correlations within species (Wingfield 1994; Wingfield *et al.* 2000). In addition, bill colour in zebra finches (*Taeniopygia gutatta*), the expression of which is mediated by T (K. McGraw, personal communication), shows a positive between-sex genetic correlation (Price & Burley 1993).

Although a between-sex genetic correlation could potentially constrain the evolution of male T levels, this genetic correlation could be diminished if sexual selection for high T levels in males was very strong. This is most likely to occur in mating systems where the sex difference in the opportunity for selection ($\Delta I$) is large (Shuster & Wade 2003). Indeed, the best examples of sexual selection for T levels and T-mediated traits in birds come from

Table 2. Male behaviour by hormone treatment in the pre-assessment (no female present) and assessment (female present) periods.
(Reported values are medians followed by the interquartile range. Three comparisons were made using Wilcoxon matched-pairs signed-ranks tests: C-males versus T-males in the pre-assessment period; C-males versus T-males in the assessment period; and all males in the pre-assessment period versus all males in the assessment period. Sample sizes for assessment and pre-assessment periods differ because pre-assessment observations were not collected on each day of the experiment; see text for details. Asterisks indicate a difference at $p < 0.05$.)

| | pre-assessment period | | | | | assessment period | | | | | pre-assessment versus assement | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| behaviour | C | T | *n* | *Z* | *p* | C | T | *n* | *Z* | *p* | *n* | *Z* | *p* |
| perch changing | 38.5 (35.0–40.0) | 39 (35.8–40.0) | 20 | −1.16 | 0.25 | 34 (29.3–40.0) | 34.5 (32.5–40.0) | 23 | −1.27 | 0.21 | 40 | −2.55 | 0.01* |
| long-range song | 0 (0.0–2.8) | 0 (0.0–2.0) | 20 | −0.12 | 0.91 | 0 (0.0–4.83) | 1 (0.0–14.5) | 25 | −2.05 | 0.04* | 40 | −2.47 | 0.01* |
| short-range song | 0 (0.0–0.0) | 0 (0.0–0.0) | 20 | −1.0 | 0.32 | 0 (0.0–0.0) | 0 (0.0–0.0) | 25 | −1.34 | 0.18 | 40 | −1.34 | 0.18 |
| ptiloerection | 0 (0.0–1.0) | 0 (0.0–1.0) | 20 | 0.0 | 1.0 | 1 (0.0–6.0) | 0.5 (0.0–3.0) | 25 | −1.42 | 0.16 | 40 | −1.93 | 0.054 |
| tail spreading | 0 (0.0–1.0) | 1.5 (0.0–4.75) | 20 | −2.1 | 0.04* | 0.5 (0.0–2.83) | 2.5 (0.0–9.3) | 25 | −1.77 | 0.08 | 40 | −1.59 | 0.11 |

species that have mating systems with very high mating skew, such as lekking species (Johnson *et al.* 1993; Zuk *et al.* 1995; Alatalo *et al.* 1996). Additionally, polygamous and socially monogamous species with high levels of overall dimorphism have been able to evolve high ratios of male to female T levels, suggesting that the genetic correlation between male and female T levels may have been broken in such species (Wingfield *et al.* 2000).

T may affect female choosiness either directly or indirectly by way of its effects on another physiological mechanism. For instance, T-females (like T-males (Ketterson *et al.* 1991) showed an increase in levels of corticosterone. Females with elevated corticosterone levels may be less discriminating because the effective cost of mate choice may be higher during periods of stress (Jennions & Petrie 1997; Widemo & Sæther 1999).

Our finding that C-females prefer C-males differs from the results of previous studies (Enstrom *et al.* 1997; Hill *et al.* 1999). This difference cannot be attributed to a failure of the T treatment to alter male behaviour, as T-males behaved similarly to T-males in previous studies (e.g. increased long-range song). The preference for C-males is also not likely to be a result of choice based on another trait. Despite our efforts to control for appearance, female preference showed a marginally significant dependence on differences in male tail white (females spent more time with males with whiter tails). However, T-males tended to have slightly more tail white than C-males (mean difference = 0.13). The preference of C-females for C-males in this experiment, then, may simply represent natural variation in mate-choice behaviour (Jennions & Petrie 1997; Widemo & Sæther 1999).

Regardless of the reasons for the unexpected preference of C-females for C-males, this preference did not extend to T-females, which were less choosy regardless of the direction of their preference. Moreover, T-males seem to have an advantage in obtaining mates most of the time. Not only do most of our mate-choice experiments show that T increases male attractiveness (Enstrom *et al.* 1997; Hill *et al.* 1999; D. A. Enstrom, M. Soensken, C. Ziegenfus, V. Nolan Jr and E. D. Ketterson, unpublished data), but also T-males obtained more extra-pair fertilizations in nature (Raouf *et al.* 1997).

Although we have suggested that the effect of T on choosiness may act as an evolutionary constraint, T may have a more general importance in mediating plasticity in choosiness. It has recently been suggested that plasticity in female mating preferences may often be adaptive (Jennions & Petrie 1997; Widemo & Sæther 1999). For example, only late-breeding female collared flycatchers (*Ficedula albicollis*) prefer to mate with males with large forehead patches, a strategy that increases reproductive success (Qvarnström *et al.* 2000). If T also decreases female choosiness in other species, patterns such as this could be explained by high T levels in the early breeding season that fall off as the season progresses. Also, in species where females compete for access to males, an increase in T as a result of female–female aggression (Langmore *et al.* 2002) may cause unmated females to be less discriminating about who they will mate with. Future studies should examine whether variation in levels of T and other hormones leads to variation in mating patterns in natural populations.

We thank M. Wade, E. Adkins-Regan and two anonymous reviewers for comments that greatly improved this manuscript. This experiment was conducted as part of the Research Experience for Undergraduates programme at Mountain Lake Biological Station (MLBS) of the University of Virginia, and we thank director H. Wilbur and associate director E. Nagy for providing facilities and support. E. Snajdr, I. Parker-Renga and K. Schubert assisted in bird care and experimental logistics, and C. Ziegenfus captured most of the birds used in this study. We also thank E. Clotfelter, J. Grindstaff, B. Heidinger and B. Van Roo for helpful discussions and suggestions. This research was funded by NSF/REU-sites award DBI-9732155 to the MLBS and NSF grant IBN 97-28384 to E.D.K. and V.N.

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