

Testosterone and mate choice in the dark-eyed junco

DAVID A. ENSTROM, ELLEN D. KETTERSON & VAL NOLAN, JR

Department of Biology and Center for the Integrative Study of Animal Behaviour, Indiana University

*(Received 17 May 1996; initial acceptance 21 August 1996;
final acceptance 13 February 1997; MS. number: A7608)*

Abstract. Investigations of the effects of testosterone on male mating effort have focused on behaviours associated with intra-sexual interactions. We tested whether testosterone may also affect male reproductive behaviour by mediating the intensity and frequency of male courtship. In two simultaneous choice experiments, we assessed the relative attractiveness of testosterone-implanted males, whose testosterone levels were manipulated to match naturally occurring spring peaks, and empty-implanted control males, whose testosterone levels were lower and resembled those of males caring for young. In both experiments, testosterone-males outperformed control-males, giving both more frequent and more exaggerated displays. Females showed a significant preference for the peak-testosterone males as measured by both female attendance and female courtship displays. We concluded that variation in testosterone can affect male attractiveness to females and thus male copulatory success. Thus, testosterone has the potential to mediate all aspects of male reproductive behaviour and may provide a mechanism through which males adjust their full range of breeding behaviours (i.e. inter-sexual as well as intra-sexual and parental behaviour) to fit their current status. In a second experiment, we tested the effect of oestradiol treatment on female mating preferences. No evidence was found that oestradiol changed the preferences of females.

© 1997 The Association for the Study of Animal Behaviour

The reproductive success of males is often determined primarily by the number of matings they obtain (Bateman 1948; Trivers 1972). In many species, however, males may also enhance their reproductive success by providing care for developing young (Trivers 1972). These two components of male reproductive success tend to involve mutually exclusive activities, and the pattern of allocation to mating effort versus parental effort is predicted to reflect the relative costs and benefits associated with this reproductive trade-off (Stearns 1989).

Because birds are oviparous and males have the capacity to provide most forms of parental care, birds have been a focus for the investigation of male reproductive trade-offs (e.g. Trivers 1972; McKittrick 1993; Ketterson & Nolan 1994; Smith 1995). Socially monogamous birds (Gowaty 1985) in particular are intriguing subjects for

investigation because they form pair bonds and generally show some form of paternal care, but they also may increase their fitness by allocating time and effort to the pursuit of extra-pair fertilizations (e.g. Gowaty & Karlin 1984; Gowaty 1985; Westneat et al. 1990; Birkhead & Møller 1992).

In birds, the hormone testosterone plays a critical role in mediating male reproductive trade-offs (Wingfield et al. 1987, 1990; Ketterson & Nolan 1992, 1994), and seasonal patterns of testosterone secretion vary interspecifically with the mating system. In polygynous species with little or no paternal care, male testosterone levels tend to remain high throughout the breeding season. In contrast, testosterone in monogamous species tends to be high during territory establishment and pair formation, but then falls as the season progresses except for temporary increases in response to challenges from other males (Wingfield et al. 1990). Thus testosterone fluctuates with the stage of the nest cycle and is often high during periods of female fertility and low during incubation and care of young. This variation is unlike what occurs in males of

Correspondence and present address: D. A. Enstrom, Center for Biodiversity, Illinois Natural History Survey, Champaign IL 61820, U.S.A. (e-mail: denstrom@uiuc.edu). E. D. Ketterson & V. Nolan, Jr, are at the Department of Biology, Indiana University, Bloomington, IN 47405, U.S.A.

polygynous species in which there is little response to challenge, apparently because testosterone levels are maximal throughout the breeding season (Wingfield et al. 1990).

Experimental field studies have addressed the role of testosterone in the reproductive allocation of monogamous male birds (e.g. Silverin 1980; Wingfield 1984; Saino & Møller 1995; Ketterson et al. 1996). In socially monogamous males, experimentally prolonging natural peak levels of testosterone throughout the breeding season enhances male behaviours associated with intra-sexual competition for mates and inhibits male parental behaviour, producing male investment patterns that resemble those found in polygynous species. These studies have focused on testosterone's effect on the intra-sexual aspects of male mating effort (i.e. the effect of testosterone on territorial behaviour, aggression and mate guarding). It remains unclear, however, whether male courtship behaviour is responsive to within breeding-season variation in circulating testosterone. Some investigators have suggested that basal breeding-season levels of testosterone are adequate to stimulate courtship behaviour and that male courtship performance will not be enhanced by increasing testosterone beyond this level (Wingfield et al. 1990). Others assume a link between testosterone, secondary sexual characters (including behavioural traits) and female mating preference, suggesting that courtship performance will reflect individual variation in testosterone and male condition (e.g. Folstad & Karter 1992; Zuk et al. 1995). Socially monogamous males augment their mating effort largely by investing in the pursuit of extra-pair fertilizations, and it is becoming increasingly apparent that inter-sexual dynamics (i.e. male attractiveness and female choice) strongly influence male success in obtaining extra-pair fertilizations (e.g. Lifjeld & Robertson 1992; Burley et al. 1994; Kempenaers et al. 1995; Gray 1996). Therefore, to assess fully the nature of testosterone-mediated reproductive trade-offs, we must examine the effect of testosterone on male attractiveness and female mate choice.

Testosterone and Reproductive Trade-offs in the Junco

In the dark-eyed junco, *Junco hyemalis*, a socially monogamous North American bunting (Emberizinae), testosterone influences patterns of

male allocation to parental effort versus mating effort. In a long-term study, free-living males received implants of testosterone to prolong naturally occurring early-season peak levels of testosterone throughout the breeding season (Ketterson & Nolan 1992). Compared to control-males, testosterone-treated males (testosterone-males) (1) sang at higher rates, (2) occupied expanded home ranges, (3) fed their young less frequently and (4) defended their nests and offspring less effectively (Ketterson & Nolan 1992; Chandler et al. 1994; Ketterson et al. 1996; M. Cawthorn, D. Morris, E. D. Ketterson & V. Nolan, Jr, unpublished data). Thus, by experimentally maintaining normal peak levels of testosterone beyond the period in which those levels occur in nature, males can be created that reduce their parental effort, apparently in favour of increased extra-pair mating effort.

We conducted two experiments to test the effect of elevated testosterone on male courtship and attractiveness and on female mate choice. In the first experiment, we examined the relative preference of females for testosterone- versus control-males. In the second experiment, we treated half of the test females from the first experiment with oestradiol and again measured their relative preference for testosterone- versus control-males. Oestradiol treatment has been shown to enhance female sexual behaviour and has been used to promote pre-copulatory displays during avian choice experiments (e.g. Searcy 1992). Thus, our second experiment allowed us to assess whether the female mating preferences revealed by the first experiment persisted even when female levels of oestradiol were elevated.

METHODS

Study Species

We studied the Appalachian subspecies of the dark-eyed junco, *J. h. carolinensis*, at Mountain Lake Biological Station (MLBS) of the University of Virginia, Giles County, Virginia, where juncos breed in abundance above ~1000 m elevation (see Chandler et al. 1994 for a description of the study site). During the breeding season, males hold all-purpose territories and assist females in defending and feeding offspring. Females build the nest and incubate. Juncos are weakly dimorphic and dichromatic, with females slightly

smaller and duller in colour than males (Miller 1941; Pyle et al. 1987). The social mating system is monogamy, but in our study population, extra-pair fertilizations are common and appear to influence male reproductive success (Ketterson et al. 1996; Raouf et al., in press).

Juncos engage in a variety of courtship displays during the breeding season. Males produce two distinct song types, 'long range song', primarily used during intra-sexual territorial interactions, and 'short range song', primarily associated with courtship (Titus, in press). Males also engage in a number of non-vocal displays during courtship. Courting males erect the contour feathers of the body (ptilorection); during intense ptilorection, which precedes copulation, males appear to double in size. Courting males also fan their tails, sometimes rapidly and repeatedly, showing their white outer rectrices, and during intense courtship they often pick up and carry nesting material in front of the female. Female sexual display is restricted to a pre-copulatory display similar to those described for other passerine species (e.g. Nolan 1978).

Capture and Housing

In April and early May 1993, we captured male and female juncos in mist nets and seed-baited potter traps. Most were captured prior to the onset of breeding; only two of 35 females showed any brood patch development at the time of capture. We housed males singly and females in groups of two or three in 32 separate cages ($1.22 \times 0.61 \times 2.44$ m high) in an outdoor aviary constructed for the purpose. All birds were visually isolated from members of the opposite sex. We supplied water, seed (primarily white and red millet and cracked corn), and Purina turkey starter ad libitum. Vitamins and meal worms (Tenebrionidae) were also provided approximately every other day. We weighed the birds upon capture and measured their wing (flattened), tail and tarsus length. We also measured the colour of the crown and breast feathers of all males, using the Munsell neutral value scale, and also scored the percentage of white in their three outer tail feathers. Plumage colour and size were used to sex and age the birds.

Mate Choice Experiments

Male attractiveness to females was assessed in two experiments, one on 9–24 May 1993, and

the other on 14–29 June 1993. Each experiment consisted of a series of trials in which a single female was allowed to choose between two simultaneously presented males.

General Methods

Male stimulus pairs were matched for age (yearling or older), size (≤ 3 mm wing length and ≤ 1.5 g mass) and plumage colour (tail, breast and crown). After administering general anaesthesia (Metophane), we implanted all males with two Silastic tubes (Dow Corning 1.47 mm i.d., 1.96 mm o.d.) subcutaneously above the left thigh. One member of each pair, the testosterone-male (selected by coin toss), received implants packed with 10 mm of crystalline testosterone (Sigma Chemical Company). Implants of this size maintain the level of plasma testosterone within the range of the birds' normal early spring maximum (Ketterson & Nolan 1992). The other member of each stimulus pair (the control-male) received empty implants.

We conducted the trials in a Y-shaped outdoor aviary (Fig. 1). Each pair of males was used to assess the preference of two, three or four females. The afternoon before males of a stimulus set were to be used in trials, they were placed in separate chambers on either side of the test aviary, where they were visually isolated from one another (Fig. 1). The following morning we observed the stimulus males for 30 min prior to the start of the first trial in order to quantify behavioural differences between testosterone- and control-males in the absence of females. Female preference trials, conducted between 0630 and 1200 hours, consisted of two parts, a period in which she could observe and assess the males followed by a period in which she could actively choose a male. During the assessment period, the female was confined for 20 min in a small cage ($0.31 \times 0.31 \times 0.62$ m) placed 2.1 m from the front of both male cages; she was in full view of both males (Fig. 1) and could watch and hear them. After 20 min the female was released (by a remotely controlled trigger) from the small cage into the larger aviary for a 40-min period, during which we monitored her behaviour to determine which male she preferred. Males courted females in all trials, both during the period of assessment and during the period of active choice.

We had two a priori criteria for female preference. First was the amount of time the female

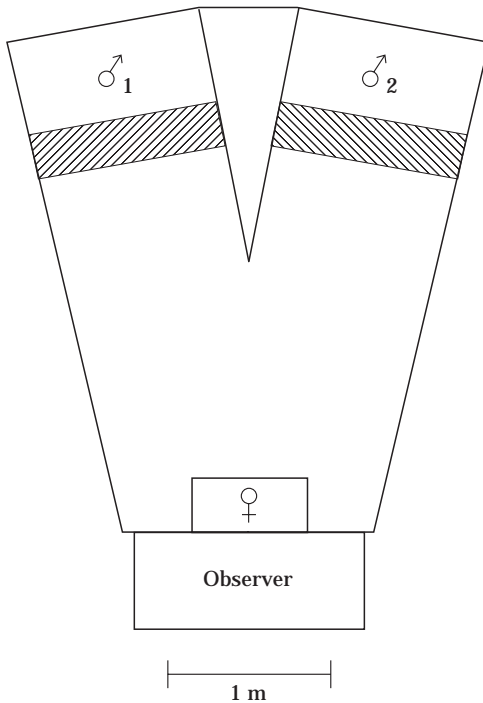


Figure 1. Schematic overview of the test aviary. Hatched areas represent the 'choice areas'.

spent adjacent to one male or the other (hereafter 'attendance time') in the designated 'choice areas'. The choice area for each male included the hardware-cloth front wall of his chamber, a perch 0.6 m above the floor of the cage on the hardware-cloth wall, and a plywood platform that extended outward 0.6 m from the hardware-cloth wall at ground level. The second criterion of preference was the number of pre-copulatory displays the female directed toward a male while she perched in his area. All parts of the cage not included in one of the choice areas were considered neutral, and the time spent by females in these areas was not scored as indicating a preference. We considered a trial to be successful if the test female spent at least one quarter of the choice period (≥ 10 min) in either or both (combined) of the designated choice areas. Females that failed to meet this criterion were re-tested. Six females, two in the first experiment and four in the second, were re-tested once; in their second trial, four of these met our criterion, and the other two were removed from the experiment (after failing to meet our criterion for a second time). We also

classified males as 'winners' and 'losers' on the basis of female attendance time. Trials in which the female did not spend at least 20% more time with one of the males (i.e. time with the more attended male/time with less attended male > 1.2) were considered ties.

Experiment 1

In experiment 1, each of 10 male stimulus sets was used to test two or three females, and 27 females met the standard of at least 10 min in the choice areas. We recorded male behaviour on videotape and later quantified male activity level (number of perch changes), song rate (long range song and short range song), ptiloerection and the incidence of tail fanning and nest-material displays. Ptילוerection was scored on a scale from 1 to 3 depending on its intensity (1=feathers noticeably erect; 2=feathers at least half erect; 3=feathers fully erect). For each minute of the period, males received a score equal to the most exaggerated ptילוerection achieved. We then added these scores and divided by the number of minutes in the period to get a mean ptילוerection score. Each male's courtship performance was averaged over all the trials in which he participated.

Experiment 2

In experiment 2, we re-tested 24 of the females used in experiment 1. Half of these (chosen at random) received one Silastic implant packed with 8 mm of crystalline β -17-oestradiol (1.47 mm i.d., 1.96 mm o.d.). The other 12 females were given empty implants of the same size. Trials began 2 weeks after the females were implanted. We used behavioural (pre-copulatory display rate) and physical (the development of a brood patch) indicators to confirm the effectiveness of our oestradiol treatment.

In experiment 1, females favoured the more shaded side of the test aviary. To combat this bias, we attempted to shade the entire aviary for the second experiment. We also used a balanced design with regard to male position and female treatment to allow us to account for these factors in our analysis. Males of each stimulus set were presented in both positions (i.e. with the testosterone-male on both the right and the left sides of the Y-aviary) to both oestradiol-treated and control females. Hence, we tested four

females with each of six of the male stimulus sets that had been used in experiment 1. In experiment 2, no female was tested with a stimulus set that she had seen in experiment 1.

Plasma Sampling and Hormone Assays

An initial blood sample was taken from all males just prior to implantation, at least 10 days after capture and 2 weeks before the start of experiment 1. Birds were removed from the aviary one at a time and taken to a bleeding station (~100 m from the aviary). From the brachial (wing) vein we drew 100–300 μ l of blood, yielding 50–150 μ l plasma. Bleeding was completed within 15 min of capture, and we waited 20 min before returning to the aviary for the next bird. All initial samples were taken between 0900 and 1200 hours. A second blood sample was taken 3–4 h after the males were used in experiment 1 (between 1400 and 1800 hours). Again, males were removed and bled one at a time, and we waited at least 20 min before returning to the choice aviary for the second male. Plasma was separated and frozen and then stored until we completed the hormone assays. We used radioimmunoassay (Wingfield & Farner 1975) to determine the testosterone concentration in these plasma samples (protocol in Ketterson et al. 1991).

RESULTS

Testosterone Assay

Prior to implantation, males in both treatments had similar circulating levels of testosterone, approximating levels of free-living males caught while feeding nestlings (Ketterson et al. 1991). Circulating testosterone measured 3–4 h after males were used in trials differed according to treatment: plasma levels of testosterone-males were high and resembled those seen in free-living males during the spring peak of testosterone (Ketterson & Nolan 1992), but levels in the control-males were lower and resembled those of males caring for young (Fig. 2).

Experiment 1

Male behaviour

During the 30-min period prior to the introduction of the first test female, testosterone-males sang long range song at a significantly higher rate

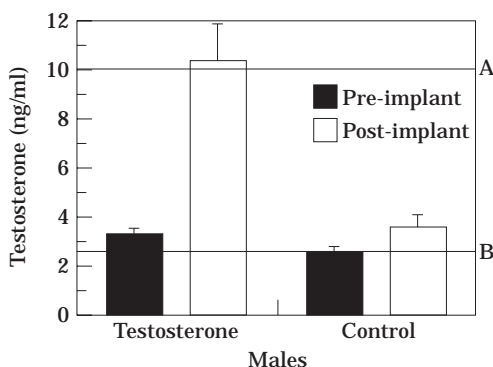


Figure 2. Mean \pm SE plasma testosterone concentration (ng/ml) of testosterone-treated and control-males. Line A indicates the mean testosterone titre (10.2 ± 0.5) for free-living adult males at the time of territory formation and pairing in spring ($N=13$). Line B indicates the mean testosterone titre (2.3 ± 3.2) for males when caring for young ($N=4$; Ketterson & Nolan 1992).

($\bar{X}=1.3$ songs/min) than did control-males ($\bar{X}=0.22$ songs/min; Wilcoxon signed-ranks test: $N=11$, $z=-2.8$, $P=0.01$). Testosterone-males were also significantly more active during this period, changing position more often ($\bar{X}=18.8$ perch changes/min) than did control-males ($\bar{X}=13.6$; Wilcoxon signed-ranks test: $N=10$; $z=-2.7$; $P<0.01$).

Male behaviour also differed according to treatment during the period of female assessment. Testosterone-males sang significantly more bouts of short range song and were again more active than controls (Table I). Tail-fanning and nest-material displays did not occur during this period.

During the period of active choice, male behaviour was largely determined by the position of the female (Tables II, III). Males of both treatment groups displayed vigorously when approached by the female. When the female was present in the choice area, males had higher ptileorection scores, gave more nest material displays and sang more short range song. In contrast, males sang significantly less long range song when the female was present than when she was absent. Testosterone-males engaged in more behaviours associated with intense courtship than did control-males (i.e. tail-fanning and nest-material displays, Table II).

Female behaviour

In experiment 1, females spent significantly more time with testosterone-males ($\bar{X} \pm SE =$

Table I. Comparison of male behaviour (means) during the 20-min period of female assessment (experiment 1)

Behaviour	Testosterone males	Control male	<i>P</i>
Long range song (songs/min)	1.09	0.70	0.24
Short range song (bouts/period)	1.59	0.26	0.03
Perch (changes/min)	19.14	12.79	0.03
Mean ptiloerection score	2.00	0.38	0.11

Wilcoxon signed-ranks tests: $N=10$; all P -values are two-tailed.

Table II. MANOVA of male behaviour during the period of active female choice

Factor		<i>F</i>	<i>df</i>	<i>P</i>
Male treatment	Wilks' $\lambda=0.75$	2.18	5,32	0.08
	Ptiloerection	0.54	1,36	0.65
	Tail display	4.07	1,36	0.02
	Nest material display	6.33	1,36	0.003
	Long range song	1.16	1,36	0.93
	Short range song	0.32	1,36	0.53
Female presence	Wilks' $\lambda=0.48$	6.85	5,32	<0.001
	Ptiloerection	17.65	1,36	<0.001
	Tail display	0.81	1,36	0.14
	Nest material display	8.81	1,36	0.001
	Long range song	4.33	1,36	0.001
	Short range song	3.30	1,36	0.06

Table III. Comparison of male behaviour (means) in the presence and absence of the female during the 40-min period of active female choice (experiment 1)

Behaviour	Female present	Female absent
Long range song (songs/min)	0.02	0.70
Short range song (bouts/period)	0.14	0.05
Nest (displays/min)	0.08	0.006
Mean ptiloerection score	2.3	1.2

16.8 \pm 2.0 min) than with controls ($\bar{X} \pm \text{SE} = 9.8 \pm 1.7$ min; Wilcoxon signed-ranks test: $z = -2.01$, $P = 0.04$; Fig. 3). We used a repeated measures ANOVA (Table IV) to test the effect of male stimulus set on female preference (e.g. Kroodsmas 1989), which revealed a significant effect of treatment, but not of stimulus set. Seventeen of 24 females (there were three ties) spent more time with the testosterone-male than with the control-male (binomial one-tailed test: $N=24$, $P=0.03$).

As stated earlier, females appeared to prefer to associate with males on the more shaded side of the aviary. When testosterone-males were in the

shaded position, females spent more time with them (10 of 10 trials); when testosterone-males were in the sun, females spent more time with them in seven of 17 cases (three ties). We had not expected male position to affect female behaviour and had randomly assigned male aviary position, and because this experiment was not balanced with respect to male position (the testosterone-males were more often in the sunny position), we could not take this variable into account in the ANOVA. Despite the apparent attraction of females to the shade, however, and the greater number of trials in which control-males were placed in the shaded position, the effect of

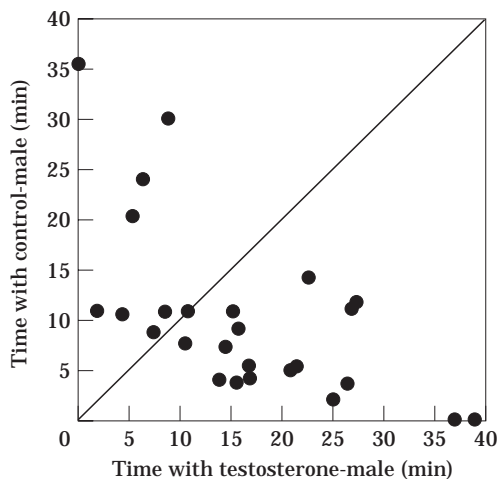


Figure 3. Female attendance time according to male treatment group (experiment 1).

testosterone on female attendance was significant.

Seven of the 27 females (26%) in experiment 1 performed 19 pre-copulatory displays. All of these displays were directed toward testosterone-males. Attendance correlated with their pre-copulatory display: females spent more time with the male they displayed to in six of the seven cases.

Experiment 2

Effects of oestradiol treatment on female condition

All 12 of the oestradiol-treated females developed brood patches after implant and prior to the start of experiment 2; none of the untreated females developed patches. There was no effect of oestradiol treatment on mass. Birds typically lost ~1 g (5% of their mass) in the first 10 days of captivity (at capture: $\bar{X}=21.1$ g; on ~day 10: $\bar{X}=20.1$ g) but then maintained that mass

throughout the rest of their captivity. The mass of both oestradiol-treated and control females did not differ at the time of implanting (oestradiol-treated females: $\bar{X}=20.2$ g; control females: $\bar{X}=19.9$ g; $T_{10} = -0.47, P>0.4$) or at the conclusion of experiment 2 (oestradiol-treated females: $\bar{X}=20.1$ g; control females: $\bar{X}=19.9$ g; $T_{10} = -0.49, P>0.4$). We sacrificed six oestradiol-treated and five control females at the end of the breeding season (12–20 July) to assess the physiological effects of oestradiol treatment. The oestradiol-treated females had significantly smaller ovaries (0.007 ± 0.001 g) than the controls (0.019 ± 0.005 g; $T_4 = -4.86, P<0.01$).

Female attendance

Females again spent significantly more time with the testosterone-males (20.5 ± 2.0 min) than with the control-males (8.9 ± 1.7 min; Wilcoxon signed-ranks test: $N=24, z = -2.8, P<0.01$; Fig. 4). We also analysed female attendance time using a repeated measures ANOVA to account for the potential effects of male position and stimulus set composition. As expected, we found a strong treatment effect. In spite of our efforts to shade the test aviary, we also found a significant position effect, but no effect of stimulus set (Table V). Significantly more females preferred the testosterone-male (18) to the control-male (five, with one tie; binomial test: $N=23, P<0.01$). There was no difference in the preferences of oestradiol-treated versus control females: 11 of 12 oestradiol-treated females, and nine of 11 controls, spent more time with the testosterone-males ($\chi^2_1=0.04, P>0.5$).

Female displays

In this experiment, 11 females gave 61 pre-copulatory displays, and nine of the 11 females

Table IV. Repeated measures ANOVA of female time spent in choice areas adjacent to testosterone- and control-males, with male stimulus set as a main effect (experiment 1)

Source	SS	df	MS	F	P
Between subjects					
Stimulus set	307.10	10	30.71	0.81	0.62
Error	606.37	16	37.90		
Within subjects					
Treatment	800.88	1	800.88	4.36	0.05
Treatment × stimulus set	1052.99	10	105.30	0.57	0.81
Error	2942.00	16	183.88		

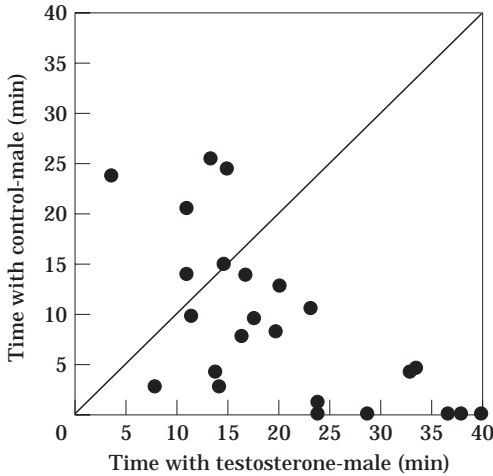


Figure 4. Female attendance time according to male treatment group (experiment 2).

spent more time with the male they displayed to. Females directed significantly more displays (57) toward testosterone-males ($\bar{X}=2.5$ displays/trial) than toward control-males ($\bar{X}=0.08$ displays/trial; Wilcoxon signed-ranks test: $N=24$, $z=-2.57$, $P=0.01$). Nine of 12 oestradiol-treated females performed pre-copulatory displays, but only two of 12 control females displayed ($\chi^2_1=8.23$, $P<0.01$). Oestradiol-treated females displayed at a significantly higher rate ($\bar{X}=4.5$ displays/trial) than did controls ($\bar{X}=0.33$ displays/trial; Mann-Whitney U -test: χ^2 approximation=4.8, $N=24$, $P=0.03$). Females that were used in both experiments and that were treated with oestradiol in experiment 2 significantly increased their pre-copulatory display rates in experiment 2 ($\bar{X}=4.7$

displays/trial) compared to experiment 1 ($\bar{X}=0.3$ displays/trial; Wilcoxon signed-ranks test: $N=12$, $z=1.96$, $P=0.05$). There was no significant change in the performance of untreated females between experiments (experiment 1: $\bar{X}=0.50$ displays/trial; experiment 2: $\bar{X}=0.47$ displays/trial; Wilcoxon signed-ranks test: $N=12$, $z=-0.3$, $P=0.8$).

DISCUSSION

In both experiments, testosterone-males outperformed control-males in courtship behaviour, and females spent significantly more time associating with the testosterone-males and directed more pre-copulatory displays towards them. Thus, males whose testosterone levels were manipulated to match naturally occurring spring peaks were preferred over males whose testosterone levels resembled those of males caring for young. We conclude that variation in the level of circulating testosterone can affect male attractiveness to females during the breeding season.

Elevated Testosterone and Male Mating Effort

Testosterone is thought to mediate male allocation to mating effort and parental effort. Several lines of evidence suggest that testosterone enhances the ability of males to compete with each other for access to females. First, plasma testosterone is correlated with certain seasonal events. High testosterone is associated with aggression and territory formation and with periods of consorting (close following of females by males) and female fertility throughout the breeding season

Table V. Repeated measures ANOVA of female time spent in choice areas adjacent to testosterone- and control-males, with male stimulus set and male position in the aviary as a main effects (experiment 2)

Source	<i>SS</i>	<i>df</i>	<i>MS</i>	<i>F</i>	<i>P</i>
Between subjects					
Position	155.30	1	155.30	5.88	0.03
Stimulus set	91.40	5	18.28	0.69	0.64
Position \times stimulus set	184.04	5	36.81	1.34	0.30
Error	317.13	12	26.43		
Within subjects					
Treatment	1571.97	1	1571.97	10.26	<0.01
Treatment \times position	327.34	1	327.34	2.14	0.17
Treatment \times stimulus set	513.17	5	102.63	0.67	0.65
Treatment \times position \times stimulus set	429.65	5	85.93	0.56	0.73
Error	1836.90	12	153.08		

(Wingfield et al. 1990; Saino & Møller 1995). Second, plasma testosterone shown by males increases during staged territorial intrusions (Wingfield et al. 1990). During such encounters, testosterone levels rise, increasing male aggressiveness and their ability to repel rivals. Third, males respond to experimental manipulations of plasma testosterone in free-living populations of monogamous birds, including juncos. Males experimentally maintained at peak testosterone levels have larger territories (Wingfield 1984) and home ranges (Chandler et al. 1994) and spend more time consorting with their mates (Saino & Møller 1995). The present results suggest that testosterone also may affect male mating effort by increasing the frequency and intensity of male courtship behaviour, thus enhancing the relative attractiveness of males to females.

During the breeding season, females choose males in two contexts, as social mate and as copulatory partner, and the relationship between natural variation in testosterone and male attractiveness may be important in both. During the period of pair formation in early spring, males with relatively high testosterone may pair more rapidly or attract higher-quality females than males with lower levels. For example, testosterone in yearling males may peak at lower levels or reach peak levels later than in older males (Ketterson & Nolan 1992; see also Hill 1994). Yearlings of several passerine species also obtain mates later than adults (e.g. Flood 1984; Hill 1989) and may be less attractive to females than older males (Enstrom 1993). Differences in testosterone titre within male age classes may also affect relative male attractiveness and female choice of a social mate. Testosterone titre also varies between males later in the breeding season, affected by the stage of the nest cycle, mate fertility and by social interactions with mates and other conspecifics (e.g. Moore 1983; Wingfield et al. 1990). This persistent variation in testosterone may affect relative male attractiveness and fertilization patterns throughout the season. For example, evidence suggests that male attractiveness can play a key role in determining female choice of copulatory partner (e.g. Houtman 1992; Burley et al. 1994; Johnsen & Lifjeld 1995) and, ultimately, success in obtaining both within-pair and extra-pair fertilizations (Burley et al. 1996). Male birds can adjust their reproductive strategies depending upon their current circumstances (e.g.

Burley et al. 1994) and testosterone may mediate strategic differences in courtship behaviour related to age, mate fertility or relative attractiveness (Burley et al. 1994; Hill 1994; Saino & Møller 1995).

Although testosterone can influence behaviours associated with both intra-sexual and inter-sexual components of male reproductive effort, the relative importance of testosterone-mediated variation in each of these components to male reproductive success is less clear. The relationship between testosterone, aggressive behaviour and territory size seems to indicate an important role for testosterone in allowing males to compete with other males to acquire a territory and thus to be in a position to attract a social mate. High testosterone might also be expected to enhance the effectiveness of mate guarding by both enlarging the area defended and by enhancing male readiness to escalate aggressive encounters over females. Several studies, however, have called into question the effectiveness of both male territory defence and mate guarding in preventing their social mates from engaging in extra-pair copulations (Gowaty & Bridges 1991; Lifjeld & Robertson 1992; Kempenaers et al. 1995; Gray 1996). If mate guarding is ineffective, even high levels of testosterone might not enhance the intra-sexual component of male mating success in this context. In a number of species, the copulatory success of males appears to be determined largely by female choice of partner rather than by male competition (Lifjeld & Robertson 1992; Burley et al. 1994; Sheldon 1994; Kempenaers et al. 1995; Gray 1996). Thus, high testosterone levels after social pairing may benefit males primarily by enhancing their reproductive success through increased behavioural attractiveness.

Testosterone, Mating Effort and Male Reproductive Success in the Junco

In the dark-eyed junco, elevated testosterone has the potential to enhance male mating effort through both intra-sexual and inter-sexual interactions. Free-living testosterone- and control-males do not differ in the amount of time they spend with their fertile mates (Chandler et al. 1997). Testosterone-males do sing more and have large home ranges than control-males, however, and testosterone-males therefore are more likely to encounter fertile extra-pair females (Chandler

et al. 1994). Since testosterone also increases the attractiveness of these males, as our data suggest, they are also more likely to be accepted by extra-pair females as copulatory partners. The increased attractiveness of testosterone-males should also increase their probability of siring the offspring of their social mate (e.g. Burley et al. 1994, 1996).

Since there is no difference in the consorting behaviour of testosterone- and control-males, we predict that if male-male competition were the only consideration, then they should be equally successful at siring offspring with their social mates. However, when we also consider the greater attractiveness of testosterone-males reported here, we predict that testosterone-males will be less likely than controls to lose paternity due to extra-pair fertilizations. Because of their larger home ranges, which make testosterone-males more likely to encounter fertilizable females mated to other males, and because of the greater attractiveness of testosterone-males, we predict that testosterone-males would also be more successful than control-males at gaining extra-pair fertilizations. DNA paternity analysis of the free-living experimental population is currently under way to address these predictions.

Oestradiol and Assessment of Female Preference

The results of experiment 2 suggest that oestradiol treatment does not alter the natural mating preferences of females in simultaneous choice experiments. Our results also suggest, however, that when experiments are designed to permit females to move between males, attendance time may provide an adequate index of preference. In our experiments, females displayed most often to the male they attended most. In other studies, males for which females have shown an association preference (sensu Hill 1990) also enjoyed a mating advantage (e.g. Hill 1990; Burley et al. 1994).

Although we did not find that oestradiol affected the preferences of females, we did find evidence of physiological changes brought on by oestradiol treatment. Only the oestradiol-treated females developed brood patches, and at the end of the breeding season oestradiol-treated females had significantly smaller ovaries than controls, suggesting that these females had accelerated rates of gonad regression.

Our results suggest that testosterone has the potential to mediate all aspects of male reproductive behaviour (i.e. inter-sexual as well as intra-sexual and parental behaviour) and may provide a mechanism through which males adjust all aspects of their reproductive behaviour to fit their current status. Future research should focus on how changes in social environment (both intra- and inter-sexual) and condition (e.g. health and age) affect testosterone-mediated shifts in male reproductive behaviour and on whether such shifts constitute adaptive shifts in strategic behaviour.

ACKNOWLEDGMENTS

We thank H. M. Wilbur and the Mountain Lake Biological Station (MLBS) of the University of Virginia and the Mountain Lake Resort Hotel for their generous support for this project, and our associates and collaborators, C. R. Chandler, M. Cawthorn, M. Chambers, L. Christensen, J. A. Hill, T. Kast, D. Morris, E. Snajdr, R. C. Titus and C. Ziegenfus, for their support and advice. J. Brawn, N. T. Burley, P. A. Gowaty, C. Phillips, S. K. Robinson, M. J. West and an anonymous referee provided critical comments on previous drafts. This research was supported by the National Science Foundation (IBN-9408061) to E. D. Ketterson and V. Nolan, Jr, by the Center for the Integrative Study of Animal Behaviour at Indiana University and by the University of Virginia through MLBS.

REFERENCES

- Bateman, A. J. 1948. Intrasexual selection in *Drosophila*. *Heredity*, **2**, 349-368.
- Birkhead, T. R. & Møller, A. P. 1992. *Sperm Competition in Birds: Evolutionary Causes and Consequences*. London: Academic Press.
- Burley, N. T., Enstrom, D. A. & Chitwood, L. 1994. Extra-pair relations in zebra finches: differential male success results from female tactics. *Anim. Behav.*, **48**, 1031-1041.
- Burley, N. T., Parker, P. G. & Lundy, K. 1996. Sexual selection and extra-pair fertilization in a socially monogamous passerine, the zebra finch (*Taeniopygia guttata*). *Behav. Ecol.*, **7**, 218-226.
- Chandler, C. R., Ketterson, E. D., Nolan, V., Jr & Ziegenfus, C. 1994. Effects of testosterone on spatial activity in free-ranging male dark-eyed juncos, *Junco hyemalis*. *Anim. Behav.*, **47**, 1445-1455.

- Chandler, C. R., Ketterson, E. D. & Nolan, V., Jr. 1997. Effects of testosterone on the use of space by male dark-eyed juncos (*Junco hyemalis*) when their mates are fertile. *Anim. Behav.*, **54**, 543–549.
- Enstrom, D. A. 1993. Female choice for age-specific plumage in the orchard oriole: implications for delayed plumage maturation. *Anim. Behav.*, **45**, 435–442.
- Flood, N. J. 1984. Adaptive significance of delayed plumage maturation in male northern orioles. *Evolution*, **38**, 267–279.
- Folstad, I. & Karter, A. J. 1992. Parasites, bright males, and the immunocompetence handicap. *Am. Nat.*, **139**, 603–622.
- Gowaty, P. A. 1985. Multiple parentage and apparent monogamy in birds. *Ornithol. Monogr.*, **37**, 11–17.
- Gowaty, P. A. & Bridges, W. C. 1991. Behavioral, demographic, and environmental correlates of extra-pair fertilizations in eastern bluebirds, *Sialia sialis*. *Behav. Ecol.*, **2**, 339–350.
- Gowaty, P. A. & Karlin, A. A. 1984. Multiple parentage in single broods of apparently monogamous eastern bluebirds (*Sialia sialis*). *Behav. Ecol. Sociobiol.*, **15**, 91–95.
- Gray, E. M. 1996. Female control of offspring paternity in a western population of red-winged blackbirds (*Agelaius phoeniceus*). *Behav. Ecol. Sociobiol.*, **38**, 267–278.
- Hill, G. E. 1989. Late spring arrival and dull nuptial plumage: aggression avoidance by yearling males? *Anim. Behav.*, **37**, 665–673.
- Hill, G. E. 1990. Female house finches prefer colourful males: sexual selection for a condition-dependent trait. *Anim. Behav.*, **40**, 563–572.
- Hill, G. E. 1994. Testis mass and subadult plumage in black-headed grosbeaks. *Condor*, **96**, 626–630.
- Houtman, A. M. 1992. Female zebra finches choose extra-pair copulations with genetically attractive males. *Proc. R. Soc. Lond., Ser. B*, **249**, 3–6.
- Johnsen, A. & Lifjeld, J. T. 1995. Unattractive males guard their mates more closely: an experiment with bluethroats (Aves, Turdidae: *Luscinia s. svecica*). *Ethology*, **101**, 200–212.
- Kempnaers, B., Verheyen, G. R. & Dhondt, A. A. 1995. Mate guarding and copulation behaviour in monogamous and polygynous blue tits: do males follow a best-of-bad-job strategy? *Behav. Ecol. Sociobiol.*, **36**, 33–42.
- Ketterson, E. D. & Nolan, V., Jr. 1992. Hormones and life histories: an integrative approach. *Am. Nat.*, **140**, 33–62.
- Ketterson, E. D. & Nolan, V., Jr. 1994. Male parental behaviour in birds. *A. Rev. Ecol. Syst.*, **25**, 601–628.
- Ketterson, E. D., Nolan, V., Jr, Wolf, L., Ziegenfus, C., Dufty, A. M., Jr, Ball, G. F. & Johnsen, T. S. 1991. Testosterone and avian life histories: the effect of experimentally elevated testosterone on corticosterone and body mass in dark-eyed juncos. *Horm. Behav.*, **25**, 489–503.
- Ketterson, E. D., Nolan, V., Jr, Cawthorn, M., Parker, P. & Ziegenfus, C. 1996. Phenotypic engineering: using hormones to explore the mechanistic and functional bases of phenotypic variation in nature. *Ibis*, **138**, 70–86.
- Kroodsma, D. E. 1989. Suggested experimental designs for song playbacks. *Anim. Behav.*, **37**, 600–609.
- Lifjeld, J. T. & Robertson, R. J. 1992. Female control of extra-pair fertilization in tree swallows. *Behav. Ecol. Sociobiol.*, **31**, 89–96.
- McKittrick, M. 1993. Phylogenetic analysis of avian parental care. *Auk*, **109**, 828–846.
- Miller, A. H. 1941. Speciation in the avian genus Junco. *Univ. Calif. Publ. Zool.*, **44**, 169–440.
- Moore, M. C. 1983. Effect of female sexual displays on the endocrine physiology and behaviour of male white-crowned sparrows, *Zonotrichia leucophrys*. *J. Zool., Lond.*, **199**, 137–148.
- Nolan, V., Jr. 1978. *The Ecology and Behaviour of the Prairie Warbler, Dendroica discolor*. Ornithology Monograph no. 26. Washington, D.C.: American Ornithologist's Union.
- Pyle, P., Howell, S. N. G., Yunick, R. P. & DeSante, D. F. 1987. *Identification Guide to North American Passerines*. Bolinas, California: Slate Creek Press.
- Raouf, S. A., Parker, P. G., Ketterson, E. D., Nolan, V., Jr & Ziegenfus, C. In press. Testosterone affects reproductive success by influencing extra-pair fertilizations in dark-eyed juncos (Aves: *Junco hyemalis*). *Proc. R. Soc. Lond., Ser. B*.
- Saino, N. & Møller, A. P. 1995. Testosterone correlates of mate guarding, singing and aggressive behaviour in male barn swallows, *Hirundo rustica*. *Anim. Behav.*, **49**, 465–472.
- Searcy, W. A. 1992. Measuring responses of female birds to male song. In: *Playback and Studies of Animal Communication* (Ed. by P. K. McGregor), pp. 175–189. New York: Plenum.
- Sheldon, B. C. 1994. Sperm competition in the chaffinch: the role of the female. *Anim. Behav.*, **47**, 163–173.
- Silverin, B. 1980. Effects of long-acting testosterone treatment on free-living pied flycatchers, *Ficedula hypoleuca*, during the breeding period. *Anim. Behav.*, **28**, 906–912.
- Smith, H. G. 1995. Experimental demonstration of a trade-off between mate attraction and paternal care. *Proc. R. Soc. Lond., Ser. B*, **260**, 45–51.
- Stearns, S. C. 1989. Trade-offs in life-history evolution. *Func. Ecol.*, **3**, 259–268.
- Titus, R. C. In press. Short-range and long-range song: use of two acoustically distinct song classes by dark-eyed juncos. *Auk*.
- Trivers, R. L. 1972. Parental investment and sexual selection. In: *Sexual Selection and the Descent of Man 1871–1971* (Ed. by B. Campbell), pp. 136–179. Chicago: Aldine.
- Westneat, D. F., Sherman, P. W. & Morton, M. L. 1990. The ecology and evolution of extra-pair copulations in birds. *Curr. Ornithol.*, **7**, 331–369.
- Wingfield, J. C. 1984. Androgens and mating systems: testosterone-induced polygyny in normally monogamous birds. *Auk*, **101**, 665–671.
- Wingfield, J. C. & Farner, D. S. 1975. The determination of five steroids in avian plasma by

- radioimmunoassay and competitive protein-binding. *Steroids*, **26**, 312–327.
- Wingfield, J. C., Hegner, R. E., Dufty, A. M., Jr & Ball, G. F. 1990. The 'challenge hypothesis': theoretical implications for patterns of testosterone secretion, mating systems, and breeding strategies. *Am. Nat.*, **136**, 829–846.
- Wingfield, J. C., Ball, G. F., Dufty, A. M., Jr, Hegner, R. E. & Ramenofsky, M. 1987. Testosterone and aggression in birds. *Am. Sci.*, **75**, 602–608.
- Zuk, M., Johnsen, T. S. & Maclarty, T. 1995. Endocrine-immune interactions, ornaments and mate choice in red jungle fowl. *Proc. R. Soc. Lond., Ser. B*, **260**, 205–210.