Influence of experimentally elevated testosterone on nest defence in dark-eyed juncos

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ABSTRACT

Testosterone affects the allocation of reproductive effort in male birds. Elevated testosterone causes male dark-eyed juncos, *Junco hyemalis*, to decrease care of dependant offspring, but this generalization is based largely on reduced provisioning rates by males treated with testosterone. Therefore, we used a predator model to explore the relationship between testosterone and nest defence, a more immediate measure of male parental effort. Control males (C-males) were more likely to respond within 10 min to a mount of an eastern chipmunk, *Tamias striatus*, placed at the nest than were testosterone-treated males (T-males). However, among males that did respond within 10 min, T-males initiated nest defence as fast as C-males and defended the nest with equal intensity. Females initiated nest defence more rapidly and struck the mount more often than their mates, regardless of the male's treatment. Overall, the decreased likelihood of T-males being present for nest defence (perhaps mediated by their large home ranges) may increase predation rates at their nests and represent an important cost of elevated testosterone levels.

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Testosterone has important effects on the allocation of reproductive effort in male birds (Wingfield et al. 1987, 1990; Ketterson & Nolan 1992; Ketterson et al. 1992, 1996). Experimental studies demonstrate that males whose testosterone levels have been elevated reduce their care of dependant offspring (Hegner & Wingfield 1987; Silverin 1980; Oring et al. 1989; Ketterson & Nolan 1992) and put greater effort into aggressive and sexual behaviours such as increased song rates (Ketterson et al. 1992) and larger home ranges (Chandler et al. 1994). The generalization that testosterone reduces investment in dependant offspring is based largely on observations that males with elevated testosterone reduce provisioning of nestlings (e.g. Hegner & Wingfield 1987; Ketterson et al. 1992). However, provisioning is only one possible measure of male parental effort. The instantaneous costs and benefits to a male that reduces provisioning of young are relatively small; reduced condition in nestlings or appreciable time available for other activities by the male accrue over a period of hours or days. It is unknown

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whether testosterone might affect other, more immediate measures of male parental effort.

Nest defence is one such measure. The cost of unsuccessful nest defence is the immediate loss of a brood; defence of the young against a predator may carry a substantial risk of death for an adult (e.g. Myers 1978; Denson 1979). The purpose of our study was to assess whether testosterone affects male investment in instantaneously risky nest-defence behaviour in a manner similar to the way it affects provisioning rates. Specifically, we elicited nest defence in male dark-eyed juncos, *Junco hyemalis*, by presenting a mounted specimen of a known nest predator, an eastern chipmunk, *Tamias striatus*, at junco nests. Because male juncos with experimentally elevated testosterone range further from the nest (Chandler et al. 1994) and provision nestlings less often (Ketterson et al. 1992), we predicted that testosterone would result in males responding more slowly and more weakly to a nest predator. We made these predictions because the larger home ranges of testosterone-treated males should result in their being further from the nest, and their lower provisioning rates implies they will devote less effort to other aspects of parental care as well. We asked (1) do male juncos with elevated testosterone levels defend their nests less intensely than do control males, and if so, (2) do females compensate for any possible reductions in male defence?

METHODS

Study Site

Our study was conducted at the University of Virginia's Mountain Lake Biological Station, Giles County, Virginia (37°22′N, 80°32′W) during May and June 1993. Situated at approximately 1100 m in the Allegheny Mountains of southwestern Virginia, the study site is dominated by montane deciduous forests (Chandler et al. 1994) in which dark-eyed juncos, *J. h. carolinensis*, are abundant. Since 1983 almost all juncos on the study site have been uniquely colour-ringed as part of long-term studies of their breeding biology (Wolf et al. 1990; Ketterson et al. 1991, 1992; Ketterson & Nolan 1992; Chandler et al. 1994, 1997).

Hormone Manipulation

During April, prior to territory formation, we captured 83 male juncos (in Potter traps and mist nets) and implanted them subcutaneously along the flank with two 10-mm lengths of silastic tubing. Testosterone-treated males (T-males) received implants filled with crystalline testosterone; control males (C-males) received empty implants. Implants were sealed at either end with silastic glue. The testosterone treatment prolongs naturally occurring spring levels of testosterone throughout the breeding season (Ketterson et al. 1991; Ketterson & Nolan 1992; Chandler et al. 1997). All males were captured at the end of summer for implant removal; all the males included in this study had retained their implants throughout the summer. We returned males to the site of capture within 30 min of implantation, and subsequently monitored territory formation, pairing and reproductive success.

Predator Presentation

We presented a taxidermic mount of an eastern chipmunk at nests that contained nestlings 7–9 days old. Chipmunks are frequent predators of junco eggs and nestlings on our study site, and adults defend the nest intensely against live chipmunks (Ketterson et al. 1996; personal observation). The chipmunk was placed within 0.5 m of the nest and covered with a small (30 by 30 cm) piece of camouflage cloth connected to a long string, one end of which the observer retained. The observer retreated at least 15 m to natural cover and was covered with camouflaged cloth as well. After the mount and observer were covered, adult juncos quickly (within 10 min) returned to provisioning nestlings and did not direct antipredator behaviour at the mount or observer.

After the adult or adults at the nest returned to apparently normal provisioning behaviour, we waited 10 min and exposed the chipmunk by pulling away the camouflaged cloth. The 10-min waiting period ensured that the mount was exposed without regard to the location or activity of the adults. After exposing the mount, we recorded the latency to respond to the mount, the number of swoops (i.e. a dive towards the mount without making contact) and contacts (hits) on the mount, and the number of nest visits (i.e. parent flies to the nest and checks the contents) by each parent. Intensity of defence was quantified as the number of swoops, hits, or nest visits divided by the duration of response (10 min minus latency). All trials lasted 10 min; adults that failed to show up during the trial were assigned a latency of 10 min. The observer was blind to the treatment of the male. No nest was used more than once to avoid problems of nonindependence and habituation or sensitization (Knight & Temple 1986).

Statistical Analysis

Chi-square tests were used to compare frequency of response between treatment groups. Because the distributions of response variables (latency, swoops, hits) were nonnormal, we used Mann–Whitney–Wilcoxon tests to compare the responses between treatment groups. We used Wilcoxon signed-ranks tests to compare responses between sexes. All tests were two-tailed; although we expected T-males to respond more slowly and weakly, results in the opposite direction were possible and would be of interest. All statistical analyses were conducted using SYSTAT (Wilkinson 1989).

RESULTS

Treatment Effects

Frequency of response

We presented the predator model at 18 nests of control pairs. Seventeen C-males (94%) appeared at the nest within the 10-min duration of the predator presentation. At the 15 nests of pairs whose males were testosterone-treated, 10 (67%) appeared within 10 min of the predator presentation. C-males were significantly more likely to appear than were T-males ($\chi^2=4.24$, $P=0.03$). Females were equally likely to appear ($\chi^2=0.60$, $P=0.44$), regardless of treatment of their mates (T-females: 13 of 15 nests, 87%; C-females: 17 of 18 nests, 94%).

Among T-pairs, in two cases both the male and the female failed to appear, and in three the male alone failed to respond. Among C-pairs, there was a single nest at which both the male and the female failed to respond.

Latency of response

When all males were ranked based on latency of response (males that failed to appear were considered tied at 10-min latency), T-males appeared to take longer to respond to a nest predator than C-males (especially given that males that failed to appear may have taken considerably longer than 10 min to respond) ($U=179.5$, $N=15$, 18, $P=0.10$). Half of all 18 C-males responded within approximately 1 min (median=0.9), whereas half of all 15 T-males responded within about 4 min (median=4.3). If we consider only males that actually responded within 10 min, latencies between T-males and C-males were similar ($U=92.0$, $N=10$, 17, $P=0.72$; Table 1). Thus, if T-males took longer to respond to a model of a nest

Table 1. Nest-defence behaviours ($\bar{X}\pm$SE) of control males and their mates (C-pairs) and testosterone-treated males and their mates (T-pairs). Only birds that returned to the nest during the 10-min trial are included

| | C-pairs | | T-pairs | |
	Male (N=17)	Female (N=17)	Male (N=10)	Female (N=13)
Latency (min)	1.87 (0.60)	0.76 (0.41)	2.14 (0.80)	0.73 (0.30)
Swoops/min	1.05 (0.44)	0.83 (0.30)	1.10 (0.48)	0.88 (0.37)
Hits/min	2.04 (0.62)	3.80 (0.75)	2.61 (1.16)	3.22 (0.95)
Nest visits/min	0.06 (0.02)	0.20 (0.06)	0.08 (0.05)	0.11 (0.03)
Total hits/10 min	47.2 (9.98)		40.13 (12.22)	
Total swoops/10 min	14.4 (5.51)		13.67 (5.27)	

predator, this was attributable to the larger number of individuals that failed to respond within 10 min.

There was no significant treatment effect on female latency, whether we considered all birds (U=153.0, N=15, 18, P=0.48) or only those that responded within 10 min (U=118.0, N=13, 17, P=0.73; Table 1).

Intensity of response

We compared intensity of nest defence only among those birds that appeared at the nest within the 10-min duration of the predator presentation. Treatment had no significant effect on swoops/min (U=86.0, N=10, 17, P=0.96; Table 1) or hits/min (U=92.0, P=0.72; Table 1) by male juncos. Similarly, swoops/min (U=115.5, N=13, 17, P=0.83; Table 1) and hits/min (U=93.5, P=0.48; Table 1) did not differ significantly between C-females and T-females. Treatment also failed to affect the number of nest visits/min by males (P=0.94; Table 1) or females (P=0.21; Table 1).

When we examined the effects of treatment from the perspective of the predator (total swoops or hits received in 10 min from both parents at all nests pooled by treatment), there was again no significant effect (hits: U=117, P=0.52; swoops: U=137.5, P=0.93; Table 1). On average, nest defence of T-pairs equalled that of C-pairs, but the probability of no male involvement was greater at nests of T-pairs.

Sex Differences

Latency of response

Females had shorter latencies than males. If we consider both treatments combined, with all pairs included, males responded significantly more slowly than females (Z= − 2.43, P=0.02). When the treatments are separated, and all pairs are included in the analysis, males responded more slowly than females in each treatment (testosterone: Z= − 2.29, P=0.02; control: Z= − 1.84, P=0.07). If only pairs in which both birds responded within 10 min are included (N=17 C-pairs, 10 T-pairs), C-males appeared to respond more slowly than C-females (Z= − 1.84, P=0.07), while there was no difference between T-males and T-females (Z= − 1.52, P=0.13).

Table 2. Nest defence ($\bar{X}\pm$SE) for male and female dark-eyed juncos, treatments combined. Only those birds responding are included

	Males (N=27)	Females (N=30)
Latency (min)	1.97 (0.47)	0.76 (0.29)
Hits/min	1.97 (0.54)	2.95 (0.53)
Swoops/min	0.86 (0.26)	0.70 (0.22)
Nest visits/min	0.05 (0.02)	0.13 (0.03)

Intensity of response

Because we found no treatment-related differences in the intensity of the response of males or of females, we combined treatment groups to compare the sexes (Table 2). Although male and female juncos swooped at the mount with similar frequency (Z= − 0.83, P=0.41), females were more likely to hit the chipmunk (Z=1.96, P=0.05) and visited the nest more often (Z=2.25, P=0.03).

DISCUSSION

Our results demonstrate that male juncos with experimentally elevated levels of testosterone are less likely to respond to a nest predator within 10 minutes than are control males. This is consistent with the observation of Chandler et al. (1994) that T-males have significantly larger home ranges than C-males. The frequency with which T-males failed to show up to defend the nest (33% of 15 nests) may be a consequence (and a potentially important cost) of a large home range size. Increased home range size may provide benefits such as greater access to extrapair copulations (Raouf et al. 1997), but our results suggest that there is a clear trade-off between the investment in future young and the risk to existing young. In birds, this conflict may normally be reduced because testosterone levels are highest in early spring, when territory and pair formation are occurring, and they then decline (Wingfield et al. 1990; Ketterson et al. 1992; Chandler et al. 1997).

Although T-males were more likely to be absent during presentation of the predator mount, when they did appear for nest defence they arrived as quickly as C-males and defended the nest with equal vigour. These results are inconsistent with the prediction (based on provisioning

studies; Ketterson et al. 1992) that T-males would devote less effort to nest defence. They are also inconsistent with the possibility that T-males would defend more intensely because of the association between high levels of testosterone and increased levels of aggression (Balthazart 1983; Archawaranon & Wiley 1988). Instead, males from both treatment groups vigorously defended their nests, coming into frequent physical contact with the predator. It may be that the large immediate benefit of successful nest defence and the unpredictable timing of attacks on the nest combine to favour nest defence that is independent of current testosterone levels.

The ability to initiate prompt and effective nest defence against chipmunks is certainly relevant to the life history of juncos. In years when chipmunk density is high, as many as 75% of nests fail due to predation (Ketterson et al. 1996). However, in years when chipmunk density is low, nest-defence behaviours may be sufficient to allow the young to fledge. We know that nest defence sometimes is successful against live chipmunks because we have observed juncos defend their nests successfully. Even though juncos defend vigorously, the likelihood that they will succeed in protecting a nest until the young are independent may be as much a function of chipmunk density as junco nest-defence behaviours. Future studies should address the relationship between effort devoted to nest defence and chipmunk density.

Regardless of treatment, males and females responded differently to a nest predator. Females consistently arrived at the nest faster and made more frequent physical contact with the predator while defending the nest. This is similar to what is seen in other passerines, including yellow warblers, *Dendroica petechia* (Hobson et al. 1988; Gill & Sealy 1996), prairie warblers, *Dendroica discolor* (Nolan 1978), savannah sparrows, *Passerculus sandwichensis*, and song sparrows, *Melospiza melodia* (Weatherhead 1979, 1989). Montgomerie & Weatherhead (1988) presented several possible explanations for this difference in defence by males and females, including confidence of parenthood, life-history characteristics, perception of risk, renesting potential, and ability to raise young unaided. In dark-eyed juncos, females provide more care than males (they incubate, males do not; Ketterson et al. 1992) and thus may be expected to defend the nest (and their investment) more vigorously. In addition, paternity is uncertain due to extrapair copulations in this population (Raouf et al. 1997). This again would suggest that it is more to the female's benefit to engage in risky nest-defence behaviours. There may be other explanations as well; further study is needed to explain the difference between male and female nest defence in juncos.

In summary, there were few differences in nest defence between male dark-eyed juncos treated with testosterone and control males. Both treatment groups defended the young with equal vigour. However, testosterone-treated males were less likely to be present at the nest during a 10-min threat to the nest than their control counterparts. This decreased likelihood of being present for nest defence (probably mediated by the large home ranges of T-males) might increase predation rates at the nests of T-males and represent an important cost of elevated testosterone levels.

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