Behav Ecol Sociobiol (2000) 48:407–411

ORIGINAL ARTICLE

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Testosterone and the allocation of reproductive effort in male house finches (*Carpodacus mexicanus*)

Received: 6 June 2000 / Accepted: 17 July 2000

Abstract Testosterone has been proposed to serve as the mediator that controls the relative effort that an individual male bird will devote to mating effort versus parental effort. Here, we demonstrate a testosterone-influenced trade-off between parental and mating efforts in male house finches. Male house finches with experimentally elevated testosterone fed nestlings at a significantly lower rate, but sang at a higher rate than males without manipulated testosterone levels. Females mated to testosterone-implanted males fed nestlings at a significantly higher rate than females mated to males without testosterone implants, resulting in similar feeding rates for both treated and untreated pairs. The effects of testosterone on male house finches, however, were not as dramatic as the effects of testosterone observed in some other socially monogamous species of birds. Because extra-pair copulations are uncommon in house finches and males provide substantial amounts of parental care, these more modest effects may be due to differences in how the allocation of reproductive effort affects the costs and benefits of different reproductive behaviors.

Key words Reproductive effort · Testosterone · Parental care · Song rate · House finch

Introduction

In the majority of avian species, males provide some form of parental care (Ketterson and Nolan 1994; Ligon 1999), and for these species reproductive investment is composed of both mating effort and parental effort (Williams 1966; Trivers 1972; Johnson and Burley 1998). Mating effort in paired monogamous males is any effort needed to maintain a pair bond as well as the pursuit of extra-pair copulations. These latter efforts in particular require time and energy that often must be diverted from investment in young produced with a primary mate. Thus, parental behaviors and the pursuit of extra-pair copulations are often mutually exclusive. Males of monogamous species are expected to balance their mating and parental efforts in a manner that maximizes their reproductive success. A thorough understanding of how the allocation of reproductive effort to parental care and mating behaviors influences the evolution of mating systems requires an understanding of both the ultimate causes (Trivers 1972) and proximate mechanisms that control male reproductive behaviors.

Research in the last two decades has shown that seasonal testosterone profiles often vary among avian species with different mating systems. Polygynous males, which typically provide relatively little parental care, maintain high levels of testosterone throughout the breeding season while monogamous males exhibit testosterone peaks early in the breeding season but then maintain testosterone at much lower levels for the remainder of the season (Wingfield et al. 1990; Beletsky et al. 1995; see Ketterson and Nolan 1999 for a recent review). These observations have led to the hypothesis that testosterone plays an important role in mediating reproductive behaviors in male birds (Wingfield et al. 1990). Several studies have supported this hypothesis by demonstrating that elevated levels of testosterone increase behaviors associated with mating success, such as song rate (Silverin 1980; Ketterson et al. 1992; Enstrom et al. 1997), mate guarding (Saino and Møller 1995a) and territorial defense (Wingfield 1984; Hegner and Wingfield 1987). Testosterone has also been shown to reduce paternal care (Hegner and Wingfield 1987; Ketterson et al. 1992; Saino and Møller 1995b; Moreno et al. 1999).

The purpose of this study was to examine the relationship between testosterone and reproductive trade-offs in the house finch (*Carpodacus mexicanus*). Specifi-

Communicated by J. Höglund

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cally, we investigated how administration of exogenous testosterone to males would affect male, as well as female, parental efforts. Furthermore, we examined the effects of testosterone administration on male song rate. We predicted that if testosterone is responsible for mediating trade-offs in reproductive behaviors in male house finches, as it is in many other species, we would find that males with elevated testosterone would sing more but provide less parental care than controls.

Because female reproductive tactics are now recognized as possibly being as important as male tactics to the evolution of avian mating systems (Parker and Burley 1998), we also investigated nestling provisioning by female house finches mated to males with manipulated testosterone to see how females would respond to the altered behavior of their mates. Models of parental care predict that the most common response of one parent to the reduced parental care of the other should be an increase in parental effort (Chase 1980; Houston and Davies 1985; Winkler 1987). However, the response of females to the partially reduced parental efforts of their mates has been rarely investigated (e.g., Ketterson et al. 1992; Saino and Møller 1995b).

Methods

We conducted this study during the 1997 and 1998 breeding seasons on the campus of Auburn University in east-central Alabama, USA. We captured male house finches shortly before or early in the breeding season [18 February to 4 April in 1997, with most (38 of 49) in March, and from 5 January to 20 April in 1998, with most (63 of 92) in January and February] in hanging basket traps placed at established feeding stations. Following first capture, we gave each male a numbered aluminum U.S. Fish and Wildlife Service leg band and a unique combination of plastic colored leg bands so that birds could be identified in the field.

The vast majority of birds banded on our study site during this time period were never seen again (between approximately 4% and 25%, depending upon age class, are recaptured and even fewer remain to breed), so we were concerned that many of the implanted birds would not breed on site. In the event that this happened, we reasoned that, if necessary, we could include unimplanted birds in our control group because other studies have detected no effect of the implants per se on behavior (Saino and Møller 1995b; Hunt et al. 1997, 1999). Thus, we biased our assignment of treatments to insure that we had large numbers of testosterone-treated males (T-males). We did this by randomly assigning one male of every three captured to the control group (C-males).

We gave T-males two 10-mm-long Silastic tubing implants (Fisher Scientific, Pittsburg, Pa.; inner diameter 1.47 mm, outer diameter 1.96 mm) filled with crystalline testosterone (Sigma, St. Louis, Mo.). The actual portion filled with testosterone was approximately 7.5 mm/implant. Controls received identical but empty implants. To insert the implants, we anesthetized males with methoxyflurane (Metofane; Pitman-Moore, Mundelein, Ill.) and inserted the implants subcutaneously just above the left flank. Following implanting, we allowed each bird to recover for approximately 20 min before releasing it back onto the study site.

Parental effort

We used nestling provisioning rate, defined as the number of feedings/nestling per hour, as our index of parental effort. To record feeding rates, we video-taped behavior at nests when nestlings were 11 days old (P.M. Nolan, A.M. Stoehr, G.E. Hill, K.J. McGraw, unpublished data). On the Auburn University campus, house finches nest in areas of high human foot traffic and thus were not disturbed by the video-cameras (as evidenced by their willingness to resume feeding nestlings within minutes of our departure). In 1997, we video-taped parental behavior for 2 h, starting each taping between sunrise and 10:00 a.m. In 1998, we acquired new cameras that allowed us to video-tape for 8 h, again starting each taping between sunrise and about 10:00 a.m. When we had data from more than one nest for a single bird or pair within 1 year, we used the mean feeding rate.

We made observations of only three "true" control males (those with empty implants). The mean feeding rate for these males was 0.37 feedings/nestling per hour, compared to a mean of 0.29 feedings/nestling per hour for males with no implants. This difference was driven by the high feeding rate of one male (0.50 feedings/nestling per hour vs 0.32 and 0.28 for the other two), but because feeding rates as high as 0.50 were also observed in males with no implants and because this difference was not significant (Z=1.04, P=0.30), we felt justified in pooling all these males into one "control" group (hereafter referred to collectively as C-males). Furthermore, we found no significant effects of year on feeding rates for males ($F_{1,38}$=0.848, P=0.36), females ($F_{1,38}$=0.429, P=0.52), or pairs ($F_{1,38}$=0.066, P=0.80) so we pooled our data from both years prior to analysis. Although we often did not know whether a nest was the first or a subsequent nest of the season for all birds, a comparison of feeding rates for "earlier" versus "later" nests revealed no significant differences (Wilcoxon signed-rank tests that mean differences between early and late feeding rates equal zero resulted in P>0.70 for C-males, C-females, and T-males; for T-females, P=0.16). Furthermore, there was no significant correlation between date of observation and parental feeding rates (K.J. McGraw, P.M. Nolan, A.M. Stoehr, G.E. Hill, unpublished data). These observations suggest that prior breeding experience of which we were unaware or the time of the breeding season in which birds were observed did not substantially confound our results.

Mating effort

We used song rate as our index of mating effort. We quantified male song rates by walking the study site and observing singing males as they were encountered. All areas of the study site were checked regularly for singing males. We used this search method rather than targeting specific focal males because the unpredictable movements of nonterritorial male house finches made preselection of focal males unfeasible. Consequently, we were usually unable to determine with certainty how long a male had been singing before we observed him. Thus, while we recognize that investment in song is a function of both song rate and time spent singing, our measure considers only the former.

When a singing male was encountered we determined how many songs he sang during the observation period, thus enabling us to calculate his song rate as the number of songs/min while engaged in a song bout. House finch songs are discrete, which eliminated any chance that we were unable to determine the number of songs a male sang. There was a slight tendency for shorter observation bouts to result in higher song rates (R^2=0.17), so we eliminated all bouts under 100 s, after which the relationship between observation period length and song rate was virtually nonexistent (R^2=0.04; range of observation bout length 100–487 s). When we quantified song rates of individual males on multiple occasions, we calculated mean song rates. The mean date of observation for the groups did not differ significantly ($F_{2,34}$=1.021, P=0.37). As with parental care, we had observations from few true control males (n=2) but these males had similar song rates (5.52 songs/min) to males with no implants (5.68 songs/min) so we included all of these males in one "control" group. Again, we found no significant effect of year on song rates ($F_{1,38}$=2.036, P=0.16) so we pooled our data for both years. No male was represented more than once in the data set.

Blood collection and hormone analysis

We captured birds in traps randomly throughout the breeding season to inspect implants and collect blood samples. Usually within 5 min, and always within 10 min of approaching our traps, we collected from each male finch a small (approximately 150 µl) blood sample in heparinized capillary tubes by puncturing the brachial vein with a hypodermic needle. We kept blood samples on ice until they were centrifuged. Plasma samples were stored at –20°C for later hormone analysis. All blood samples were collected before 11:00 a.m.

We assayed plasma samples to determine testosterone levels following the protocol detailed in Mendonça et al. (1996). We incubated thawed plasma samples with approximately 1,000 cpm of tritiated testosterone for 1 h before extracting them with 3 ml of diethyl ether (Aldrich, Milwaukee, Wis.). We then dried down the extracted samples under nitrogen gas and resuspended them in phosphate buffer. The resuspended samples were allowed to equilibrate at room temperature for 1 h. We then incubated duplicate aliquots of the samples with tritiated testosterone (NEN, Boston, Mass.) and a testosterone antibody (Endocrine Sciences, Casablanca, Calif.) for 4 h at room temperature. We used a third aliquot to determine percent extraction efficiency, which was found to average 83%. Inter- and intraassay variation averaged 21% and 26%, respectively. The sensitivity of the assays was approximately 10 pg/ml. The testosterone antibody we used cross-reacts (44%) with dihydrotestosterone, thus making our assay technically a total androgen assay.

Results

Effectiveness of testosterone implants

Over the course of the study, we collected blood samples from 16 different males (one to seven samples/male) implanted with testosterone. The implants raised androgen levels to a mean of 15.00±1.63 ng/ml (*n*=16). We collected blood samples from 43 different C-males (one to seven samples/male), which averaged 1.05±0.21 ng/ml (*n*=43). The highest androgen level recorded from a C-male during the course of this study was 6.6 ng/ml. However, in 1999, testosterone levels as high as 15 ng/ml were recorded from free-living, unimplanted males (R. Duckworth, unpublished data). In many cases, the breeding status of birds sampled was unknown because blood samples were taken from all birds captured, not just those breeding on the study site. Furthermore, specific birds could not be targeted for capture because male house finches do not respond to playbacks of conspecific song, and capturing them at nests would be impossible without causing substantial stress to the birds, thus altering hormone levels. For this reason, we were unable to create an accurate seasonal androgen profile for male house finches and these results are presented simply to show that the testosterone implants did have the desired effect of raising androgen levels.

Behavioral observations

In all cases but one (female feeding rates), the data did not differ significantly from a normal distribution and independent-sample *t*-tests were used to test for differences in means. In the case of female feeding rates, a Wilcoxon rank-sums test (using a *z*-approximation for large samples) was used. Sample sizes are given in the figure captions. That the sample sizes for song rate and parental feeding rates are similar is coincidental: these are two separate subsets of the house finch population.

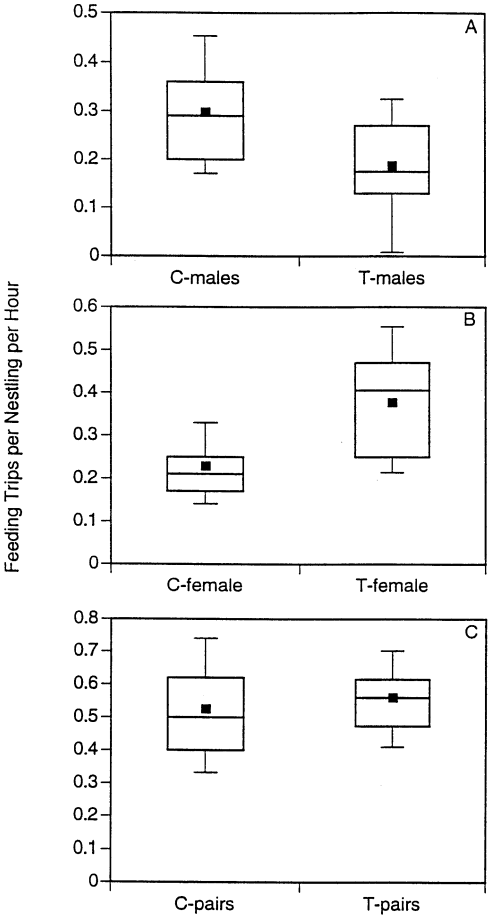

Fig. 1 Feeding rates of males (**A**), females (**B**), and pairs (**C**). C-males are males without implants or with empty implants. C- and T-females are females mated to C- or T-males, respectively. C-pairs and T-pairs (**C**) shows the combined feeding rates of both parents. For all panels, *n*=26 for controls (C-males, C-females, and C-pairs) and *n*=16 for treatment birds (T-males, T-females, and T-pairs). The box-and-whisker plots illustrate the 10th, 25th, 50th, 75th, and 90th percentiles as *horizontal lines*, and the means as *solid black boxes*

Parental feeding rates

Males with testosterone implants (T-males) fed nestlings at a significantly lower rate than C-males (0.19±0.03 vs 0.30±0.02 feedings/nestling per hour; t_{40}=3.321, *P*=0.002; Fig. 1A). Females mated to T-males (T-females) fed at a significantly higher rate than C-females (0.37±0.03 vs 0.23±0.02; *Z*=3.236, *P*=0.001; Fig. 1B). Thus, ultimately, the feeding rates for T-pairs and C-pairs did not differ significantly (0.56±0.03 vs 0.52±0.03; t_{40}=0.767, *P*=0.48; Fig. 1C).

Song rates

T-males sang at a significantly higher rate than C-males (7.25±0.53 vs 5.67±0.19 songs/min; t_{38}=3.489, *P*=0.001; Fig. 2).

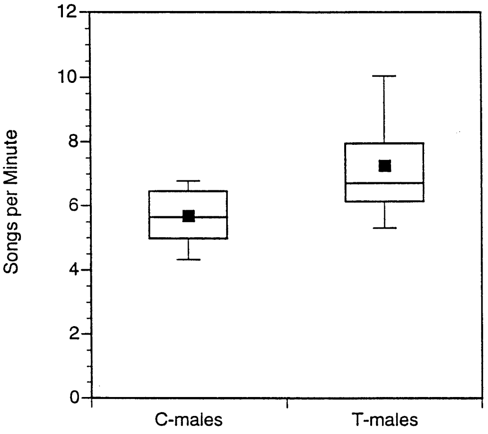

Fig. 2 Song rates for C- and T-males. Song rate is the number of songs/min while a male was engaged in a song bout (see Methods). Sample sizes are n=28 for C-males and n=12 for T-males. The box-and-whisker plots illustrate the 10th, 25th, 50th, 75th, and 90th percentiles as *horizontal lines*, and the means as *solid black boxes*

Discussion

Our observations in this study support the hypothesis that testosterone is at least partly responsible for the allocation of reproductive effort in male house finches. Male house finches with experimentally elevated testosterone increased their song rates, but they fed their nestlings less often than controls. Similar effects of testosterone on parental effort, mating effort, or both have been found in other species (Silverin 1980; Hegner and Wingfield 1987; Ketterson et al. 1992; Saino and Møller 1995b). Thus, in several species of socially monogamous birds, males may partially offset the costs of testosterone-induced changes in some behaviors through the benefits gained by testosterone-induced changes in other behaviors (Raouf et al. 1997).

The modest response of our male house finches to testosterone treatment is interesting given the high levels of testosterone produced by the implants – the highest level recorded for male house finches, far above the mean level recorded for unimplanted males. Males of different species might respond differently to elevated testosterone when the relative costs and benefits of reduced parental effort and increased mating effort differ. For example, male dark-eyed juncos given testosterone doubled their song rates during some phases of the nesting cycle (Ketterson et al. 1992) and decreased their paternal care by over 65% (Schoech et al. 1998). Paternal care decreased similarly in testosterone-treated pied flycatchers (Silverin 1980), house sparrows (Hegner and Wingfield 1987), and barn swallows (Saino and Møller 1995b). In the polygynous spotless starling, testosterone-implanted males fed young at roughly one-fourth of the rate of controls (Moreno et al. 1999). The responses to testosterone treatment in all of these species were greater than those we observed in male house finches.

At the other extreme, male Lapland longspurs with elevated testosterone did not show decreased paternal care late in the nestling cycle (Hunt et al. 1999). These authors suggested that this is because male Lapland longspurs lack the opportunities for extra-pair copulations at that time of the year. Breeding is highly synchronous for these longspurs, so that after an initial burst of nesting activity, no females are fertile. Thus, testosterone-induced reductions in paternal care and increases in mating effort would have costs but no benefits (Hunt et al. 1999). Studies of dark-eyed juncos also support the hypothesis that behavioral responses to testosterone may be influenced by the importance of extra-pair fertilizations: increased testosterone depressed male parental care and decreased apparent reproductive success at nests (Raouf et al. 1997), but because females were more attracted to males with high testosterone (Enstrom et al. 1997), these males gained more extra-pair fertilizations (EPFs) than controls (Raouf et al. 1997). (In dark-eyed juncos, 28% of young are the result of EPFs; Ketterson et al. 1998). In the end, high testosterone and control males had equal reproductive success (Raouf et al. 1997).

The hypothesized relationship between differential response to testoserone and EPFs may explain our results. House finches have a low frequency of EPFs. Of 119 nestlings from a Michigan population, only 10 (8.4%) were the result of EPFs (Hill et al. 1994). Male house finches make, on average, slightly over 50% of the feeding visits to their young. Furthermore, house finches may fledge several broods (up to four in some years) over the course of their very long breeding season. The large parental investment made by male house finches may mean that the costs to males of reduced parental care generally outweigh the slight benefits they might gain from EPFs through increased mating effort. This may explain why the effects of testosterone on parental care in male house finches were intermediate between those on male parental care in dark-eyed juncos, barn swallows, and house sparrows and those in Lapland longspurs: EPFs during the nestling phase of breeding are less likely to occur in house finches than in dark-eyed juncos, but more likely than for Lapland longspurs.

The above argument is strengthened by, but not dependent upon, the assumption that male song functions in mate attraction. Male house finches do not defend territories (Hill 1993), so song is unlikely to function in intrasexual interactions. On the other hand, both paired and unpaired males spend much of their time singing. While no tests of the function of song in the house finch have yet been conducted, it seems reasonable to assume that if song does not function in intrasexual interactions, and males spend much time singing during the breeding season, then song functions in mate attraction. Such intersexual display may be for the few available extra-pair matings, but it may also function to maintain a current pair bond. Female house finches frequently leave their mates (Hill 1991) and continued signaling of health and vitality by the male may be required by the female to maintain a pair bond (see Møller 1991). Females may be monitoring both male parental contribution and male display and presumably a male should properly balance the two to insure that he maintains his mate. Thus, regardless of whether song functions to attract extra-pair mates or to maintain pair bonds (or even to announce to other males a willingness to guard mates), there is a trade-off between song rate and paternal care, and our observations and those from other studies suggest that testosterone serves as the proximate means for maintaining this balance.

That female house finches should be concerned with levels of male parental care is very likely considering that those mated to T-males fully compensated for the reduced parental care of their mates. While our study did not address the long-term effects of female compensation, there may be costs to males with reduced parental care because their mates may leave them, suffer from reduced condition, or die. In a species such as the house finch which rears several broods each season and often forms pair bonds that last several seasons, these costs are likely to be great.

Acknowledgements We would like to thank Paul Nolan, Kevin McGraw, Barbara Ballentine, Renee Duckworth, Anne Dervan, Lynn Seifferman, Steve Dobson, Mary Mendonça, Marlene Zuk, and anonymous reviewers for commenting on this manuscript. Assistance in the field was provided by Paul Nolan and Kevin McGraw, and Mary Mendonça provided help with hormone assays. Support for this work came from the National Science Foundation (Grant. No. IBN9722171 to G.E.H.) and from the American Ornithologists' Union, the American Museum of Natural History, the Association of Field Ornithologists, and the Auburn University Graduate School (to A.M.S.). Birds were collected under a State of Alabama permit (No. 12) and a federal permit (No. 784373). The procedures described here were approved on 25 February 1997 by the Auburn University Institutional Animal Care and Use Committee (PRN No. 001-R-0979).

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