Experimental elevation of testosterone lowers fitness in female dark-eyed juncos

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ABSTRACT

Testosterone (T) is often referred to as the "male hormone," but it can influence aggression, parental behavior, and immune function in both males and females. By experimentally relating hormone-induced changes in phenotype to fitness, it is possible to ask whether existing phenotypes perform better or worse than alternative phenotypes, and hence to predict how selection might act on a novel or rare phenotype. In a songbird, the dark-eyed junco (*Junco hyemalis*), we have examined the effects of experimentally elevated T in females on fitness-related behaviors such as parental care. In this study, we implanted female juncos with exogenous T and examined its effect on fitness (survival, reproduction, and extra-pair mating) to assess whether T-altered phenotypes would prove to be adaptive or deleterious for females. Experimental elevation of T decreased the likelihood that a female would breed successfully, and T-implanted females had lower total reproductive success at every stage of the reproductive cycle. They did not, however, differ from control females in fledgling quality, extra-pair offspring production, survival, or reproduction in the following year. Previous work in this system has shown that experimental elevation of T in males alters behavior and physiology and decreases survival but increases the production of extra-pair offspring, leading to higher net fitness relative to control animals. Our results suggest that increased T has divergent effects on male and female fitness in this species, and that prevailing levels in females may be adaptive for them. These findings are consistent with sexual conflict.

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Introduction

Testosterone (T) has a variety of behavioral and physiological effects, and is therefore likely to be involved in life history evolution (Finch and Rose, 1995). T is frequently considered to be a "male" hormone, and it unquestionably has numerous organizational and activational effects in male vertebrates (Adkins-Regan, 2005). Of course, T is not limited to males in terms of its production or its action; female vertebrates have detectable levels of circulating testosterone, and in some cases female T levels may approach those of males (Ketterson et al., 2005; Wingfield et al., 2000). T has been shown to affect a number of behavioral and physiological traits in females of a wide variety of taxa, including immune function, attractiveness, and sexual behavior (De Ridder et al., 2002; Eens et al., 2000; Langmore et al., 2002; Staub and De Beer, 1997). The ways in which testosterone can affect fitness in males are well characterized (reviewed in Ketterson and Nolan, 1999); relatively less work has been devoted to understanding this relationship in females (although see Ketterson et al., 2005; Staub and De Beer, 1997 and references therein).

Physiological mechanisms such as circulating levels of hormones relate to a suite of behavioral, immunological, and reproductive traits, and can frequently coordinate trade-offs between these traits (Finch and Rose, 1995; Hau, 2007; Ketterson and Nolan, 1992). Because testosterone can mediate a number of different phenotypic traits, each of which may be the target of selection, it is crucial not just to relate each trait to fitness independently, but to assess the net change in overall fitness that results from a change in hormone phenotype. One way to accomplish this is to use exogenous hormone implants to look at the effects of elevated testosterone on overall reproductive success over the course of a breeding season (Ketterson et al., 1996; Lynn et al., 2009; Marler and Moore, 1988; Silverin, 1991; Wingfield, 1984). In this study, we used exogenous testosterone implants to address three related questions: 1) Do males and females respond similarly to elevated T levels with respect to offspring production and survival? 2) Are current levels of T expression in females advantageous or detrimental relative to elevated levels? 3) Is selection on T phenotype in females likely to accelerate or constrain the evolution of T phenotype in males?

Previous work in our study system, the dark-eyed junco (*Junco hyemalis*), has shown that in males, experimental elevation of T decreases survival and the production of surviving within-pair offspring, but increases the production of extra-pair offspring, resulting in a net fitness benefit for implanted males relative to controls (Raouf et al., 1997; Reed et al., 2006). This suggests that selection may favor males with higher testosterone than is currently prevalent in the population. High levels of testosterone may be favored in female juncos as well. Junco females have relatively high levels of testosterone compared to other species of songbirds (Ketterson et al., 2005), and this testosterone peaks during the pre-breeding period, suggesting that these high levels may

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function in mate acquisition. Female juncos with experimentally elevated testosterone are also more aggressive (Zysling et al., 2006), which may confer a reproductive advantage, particularly in years with high intrasexual competition or predation pressure (Cain and Ketterson, 2012; Rosvall, 2013). In contrast, it may be the case that female fitness is relatively unaffected by high levels of testosterone. Because female parental care is critical to offspring survival (Wolf et al., 1988), the theory underlying the behavioral insensitivity hypothesis can be expanded to suggest that females should be relatively insensitive to any negative effects of testosterone on parental care during this period (Lynn, 2008; Lynn et al., 2002; Lynn et al., 2005).

However, there are also some indications in juncos and other species that selection on females may be constraining rather than accelerating the evolution of T in males, and thus that the sexes may be experiencing intersexual conflict (Chapman et al., 2003; Lande and Arnold, 1983; Price and Burley, 1993). Elevated testosterone levels delay clutch initiation or decrease fecundity in female zebra finches (Rutkowska et al., 2005), homing pigeons (Goerlich et al., 2009; Goerlich et al., 2010), red-winged blackbirds (Searcy, 1988), and spotless starlings (Veiga and Polo, 2008; Veiga et al., 2004), and a high level of T decreases incubation behavior and hatching success in tree swallows (Rosvall, 2013). In the spotless starling, T implants decrease female parental care, fledgling production, and attractiveness to males (García-Vigón et al., 2008; Veiga and Polo, 2008). However, they also increase a female's chances of acquiring and maintaining a position in a breeding colony over multiple years, resulting in no net difference between the lifetime reproductive success of T-implanted and control females (Veiga and Polo, 2008). In the dark-eyed junco, artificially elevated T levels in females result in a delay in egg-laying, decreased parental care during the nestling phase (but not during incubation), and overall lower rates of daily nest survival (Clotfelter et al., 2004; O'Neal et al., 2008). While these results certainly suggest that elevated T may be detrimental to female juncos, and thus may potentially constrain the evolution of male T, measures of overall reproductive success and survival are needed to fully address the net selective force experienced by females in this species.

In this study, we address the fitness consequences of chronically elevated T levels in female juncos. We address first the direct effects of T on fitness, i.e. a female's reproductive output and survival, by comparing the number of offspring produced at each stage of the breeding cycle by females treated with T as compared to controls. We also address whether the effects of T treatment have any long-term effects on female fitness; that is, whether elevated levels of T affect female reproduction in the following breeding season. Finally, we examine T's effects on a female's indirect fitness, i.e. the quality of her offspring. By comparing the effects of elevated maternal T not only on nestling quality, but also on nestling paternity (which has been shown to have effects on an offspring's reproductive success as adults, Gerlach et al., 2012) and juvenile survival and recruitment, we can examine the more subtle effects of testosterone on female fitness that extend over multiple generations.

Methods

Study system and general field methods

The dark-eyed junco is a socially monogamous songbird that breeds at high elevations throughout the southern Appalachian Mountains in eastern North America. This study was conducted on the population of juncos (*J. h. carolinensis*) breeding at Mountain Lake Biological Station in Giles County, Virginia (37°22′31″ N, 80°31′24″ W) (Chandler et al., 1994). This population has been studied since 1983, and almost all adult juncos in this population receive numbered USFWS aluminum bands as well as unique combinations of color bands for field identification.

Our field methods are described in detail elsewhere (Reed et al., 2006); in brief, adult juncos were captured at baited mist nets and potter traps during the pre-breeding and early breeding period (typically 15 April–15 May). At this time, each bird was banded, weighed, aged

based on its age at banding or by using plumage characteristics (Ketterson, 1979), and checked for reproductive condition (i.e. presence of a brood patch or cloacal protuberance). All birds also had a small blood sample (50–100 μL) collected via alar vein puncture. Blood samples were centrifuged and the plasma was drawn off and stored at −20 °C for later hormone analysis. Longmire's solution was added to the red blood cells for lysis and storage until DNA analysis (Longmire et al., 1988).

Experimental manipulation of testosterone

During the breeding seasons of 2001–2 and 2005–7, implants were given during the early part of the breeding season (during the spring census, 15 April–15 May, in 2001–2; shifted to 1 May–15 June in 2005–7 to focus implanting on resident females rather than late migrants). Captured females were anesthetized and had a 7 mm piece of silastic tubing implanted subcutaneously along their left flank. Implants were sealed at both ends with silastic glue, and contained either 5 mm of packed crystalline testosterone (= 0.01 g) (T-implants), or were empty (C-implants). In female juncos, levels of naturally-produced testosterone peak during the pre-breeding season (Ketterson et al., 2005); a T-implant of this size has been shown to maintain females at this peak level throughout the breeding season (Clotfelter et al., 2004; O'Neal et al., 2008).

Treatment group (T or C) was determined randomly by coin flip after blocking by site of capture within the study area. Females that had been implanted in previous years received the same treatment in each subsequent year they were captured. To avoid pseudoreplication, for females that were implanted in multiple years, we considered reproductive success from only the first year they received an implant. Our data set thus included 284 females; 142 with control implants and 142 with testosterone (2001: 16 C/16 T; 2002: 16 C/18 T; 2005: 52 C/47 T; 2006: 28 C/27 T; 2007: 30 C/34 T). Because adult site fidelity in this species is high (Reed et al., 2006; Gerlach et al., unpublished data), we measured survival based on whether a female was sighted and/or captured in our population in subsequent years. For those females that returned to the population and were not implanted in the year following their first implant (primarily females that returned in 2003 or 2008), we quantified annual reproductive success for that returning year in order to examine the potential carryover effects of testosterone treatment.

Nests

Each year we searched for nests daily between 15 May and 15 July. Once a nest was found, the female and the social father (the male that defended the nest and cared for the young) associated with the nest were identified using color bands. Nests that were found during building, laying, or incubation were checked every other day for progress. Once nestlings had hatched, the nests were checked on the day the eggs hatched (hatch day, day 0, D0), D3, D6, D9, and when the young left the nest (fledging; typically D11–12). Nestlings were weighed and measured on D0, D6, and at fledging. On the afternoon of D6, nestlings were individually banded, and a small (~50 μL) blood sample was collected by alar vein puncture for paternity analysis.

Adult females were recaptured at the end of the breeding season (15 July–early August). At this time a blood sample was taken to confirm plasma testosterone levels post-treatment, and their implants were removed. During this period, we also captured juveniles to determine fledgling survival to independence.

All procedures used in this study were approved by the Bloomington Institutional Animal Care and Use Committee.

Testosterone assays

Plasma was extracted with diethyl ether, and testosterone levels were measured using an enzyme immune assay (EIA), as described in Clotfelter et al. (2004). Each year was analyzed separately, and because