Testosterone in Females: Mediator of Adaptive Traits, Constraint on Sexual Dimorphism, or Both?

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Online enhancements: appendix tables.

ABSTRACT: When selection on males and females differs, the sexes may diverge in phenotype. Hormones serve as a proximate regulator of sex differences by mediating sex-biased trait expression. To integrate these perspectives, we consider how suites of traits mediated by the same hormone in both sexes might respond to selection. In male birds, plasma testosterone (T) varies seasonally and among species according to mating system. When elevated experimentally, it is known to enhance some components of fitness and to decrease others. We report that female T also varies seasonally and co-varies with male T. Female T is higher in relation to male T in sexually monomorphic species and is higher absolutely in females of species with socially monogamous mating systems, which suggests adaptation. We also consider the effect of experimentally elevated T on females and whether traits are sensitive to altered T. We hypothesize that sensitive traits could become subject to selection after a natural change in T and that traits with opposing fitness consequences in males and females could constrain dimorphism. Results from birds, including the dark-eyed junco (*Junco hyemalis*), reveal many sensitive traits, some of which appear costly and may help to account for observed levels of sexual dimorphism.

Keywords: hormonal pleiotropy, correlated responses, sex differences, hormonal constraint, dark-eyed junco.

Evolutionary biologists have long recognized that traits that enhance fitness in males do not necessarily enhance fitness in females (Darwin 1871; Andersson 1994). Males typically invest more time and energy in attracting or pursuing mates; females invest more in caring for offspring. Among males, competition for mates often leads to a system in which a few males are highly successful and sire many offspring, while other males sire no offspring at all; reproductive success in females typically varies less than in males (Shuster and Wade 2003). As a consequence, traits that enhance mating success may be expressed only, or more strongly, in males than in females.

Sexual dimorphism can also be generated by sex differences in ecology. Selection will favor attributes that match each sex to its environment, and the favored attributes may differ by sex (Selander 1966; Ligon 1999; Karubian and Swaddle 2001). Conversely, if the sexes are quite similar in their ecology, selection that reduces competition may lead to character displacement, causing the sexes to diverge (Johnson and Macdonald 2001).

In addition, evolution of sex differences may be influenced by correlated responses to selection. If a trait that would be advantageous to one sex is detrimental to the other sex, evolution of the trait may be constrained, and the sexes may be more similar than would be "optimal" for either considered alone (Lande 1980, 1987; Lande and Arnold 1983). Correlated responses occur because some loci shared between the sexes influence trait expression in both males and females, and the selection that ensues after changes in gene frequency may be antagonistic or reinforcing (Rice 1996; Chippindale et al. 2001; Rice and Chippindale 2001). The balance between sex differences and resemblances that is achieved in any particular system will be determined by the interaction of viability, fecundity, and sexual selection and also by any correlated responses (see fig. 1).

Here a major objective is to relate this selective network and the resulting sexual dimorphism to proximate mechanisms that give rise to sex differences. While sexual dimorphism in animals is the product of numerous mechanisms (Nelson 2000), including maternal imprinting and sex-linked genes that act independently of hormones (Arnold 1996, 2004; Wade et al. 1999; Carrel and Willand 2005), hormones are a common cause. At the level of the organism, the steroid hormone testosterone is the most

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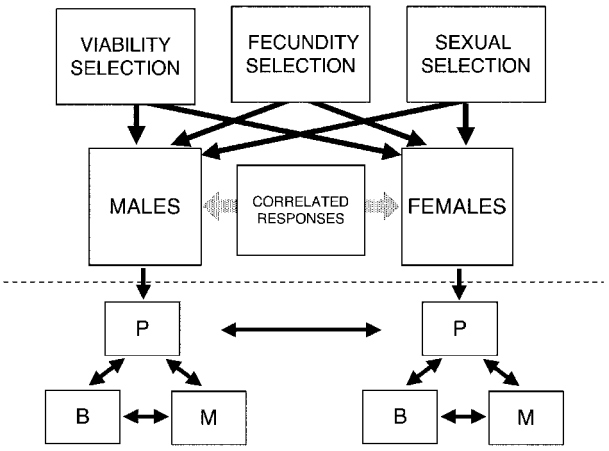

Figure 1: Hormones and the evolution and expression of sex differences and resemblances. Boxes and arrows above dashed line represent viability, fecundity, and sexual selection (*angled black arrows*) acting on males and females, and correlated responses to selection by both sexes (*gray two-headed arrow* connecting males and females). Boxes and arrows below dashed line represent within-generation variation in physiological (*P*), behavioral (*B*), and morphological (*M*) traits and interactions among them. Within each sex, *P*, *B*, and *M* traits mediated by the same hormone may be correlated in their expression (hormonal pleiotropy; see text). Between sexes, *P*, *B*, and *M* traits mediated by the same hormone may be hormonally correlated (see text). Owing to hormonal correlations, any form of selection acting on one trait may alter the outcome of selection on other traits in one or both sexes, and the net effect may be detected as sexual dimorphism.

important hormonal mediator of sex differences in vertebrate animals, and it is our central focus.

Testosterone, often regarded as the "male hormone," is synthesized primarily by the gonads, both testes and ovaries, but also by other tissues, for example, the adrenals and perhaps even the brain (Arnold 2004). Once secreted, testosterone enters the circulation, bound or not by carrier proteins, from which it may bind with androgen receptors and join with coactivators and corepressors to modulate gene transcription. Alternatively, it may be converted to other steroid hormones that interact with other receptors, activators, repressors, and genes. These receptors, cofactors, and conversions to and interactions with still other hormones allow testosterone to mediate numerous and varied phenotypic characters. In this respect, testosterone can be seen as analogous to a gene with multiple pleiotropic effects (hormonal pleiotropy) that also has epistatic interactions with other genes (physiological epistatsis; Cheverud and Routman 1995). Consequently, just as the evolution of multiple traits within a sex can be mediated by genetic correlations among the traits and the evolution of a particular trait in both sexes can be mediated by a genetic correlation between the sexes, multiple traits within a sex can be mediated by hormonal pleiotropy, and particular traits expressed in both sexes can be mediated by between-sex hormonal correlations (Finch and Rose 1995; Ketterson and Nolan 1999).

The boxes and arrows below the dotted line in figure 1 depict testosterone's multiple influences on the expression of the morphological (*M*), behavioral (*B*), and physiological (*P*) phenotypes. In males, these effects have been well characterized, whereas in females, the hormone's action on the phenotype is less well documented (but see Staub and De Beer 1997). The double-headed arrow connecting the sexes below the dotted line represents another important set of interactions beyond the scope of this article: testosterone-mediated traits in one sex can act as an environmental influence on hormonal and phenotypic expression in the other sex, even into the next generation (Ketterson et al. 2001; Gil et al. 2004).

The conceptual framework laid out in figure 1 leaves many unanswered questions, and we have identified two of particular interest. The first question is which aspects of the female hormonal phenotype might be the result of selection acting directly on the female phenotype and which might represent correlated responses to selection on males. The second question is how often existing male phenotypes might represent compromises between the advantages of testosterone-mediated characters in males and the costs of such characters in females. To begin to address these questions, we make two sets of predictions and present our findings in two parts. In part 1 ("Comparative Analyses of Female Testosterone"), we address the first question by focusing our attention on variation in plasma levels of testosterone (T) and testosterone-mediated traits in females, particularly female birds; the approach is comparative. In part 2 ("Experimental Analyses of Female Testosterone"), we address the second question by focusing on sex differences in sensitivity to T; the approach is experimental.

In part 1, we reason as follows. If female T is more often a reflection of male T and exists in females primarily because of correlated responses as opposed to direct selection, then we would predict across species that male and female levels of T would co-vary over time and in absolute levels. Further, we would expect variation among species to be better explained by factors that relate to male than to female biology. Within species, we would not expect female T to co-vary with female fitness.

Conversely, if female T is primarily the product of direct selection, we would not necessarily expect to find similar patterns of temporal variation in T in males and females or covariation in T between males and females across species. Rather, we would expect T or T-mediated traits to co-vary with female ecology and/or fitness. Thus, for example, across species we might predict higher female T in

species in which female-female competition is intense. Within species, we would predict covariation between T and fecundity or other components of fitness (e.g., Brown et al. 2005).

Variation in hormone responsiveness is at least as important to understanding sex differences as is variation in plasma hormone concentration, and in part 2 we look within species to consider sex differences in responsiveness to T. We argue that experimental manipulations of T can be used to help predict how a change in T might affect phenotypic evolution, and we collate examples from the literature of traits in females whose expression is or is not sensitive to experimentally altered levels of T.

We predicted that if events were to favor higher T in one sex, say males, and if females also responded with higher T, then this female response might in turn cause sensitive traits to be expressed, to be subjected to selection, and to potentially determine the evolutionary outcome. To extend this prediction, we would need to know not only whether traits are sensitive or insensitive but also how sensitive traits relate to fitness. If a trait is sensitive to altered hormone levels in both sexes and both sexes benefit independently from the alteration, then selection will be direct and the responses will reinforce each other, causing evolution to proceed more rapidly. If the sensitive trait influences the fitness of one sex but has no effect on that of the other, then the trait may be expressed in both sexes, but evolution may proceed less rapidly than it would if both sexes benefited. Also, if one sex benefits from the alteration while the other sex is harmed, then a compromise may be reached, and the sexes may constrain one another's evolution. Finally, if traits in one sex are insensitive to alterations in the level of T, such traits will be free to evolve independently and unconstrained in the sensitive sex so long as they are without fitness consequences for the insensitive sex.

After reporting information gathered in relation to these two sets of predictions in the form of a comparative analysis of among-species variation in female T and a summary of traits in females that have been reported to be sensitive or insensitive to experimentally altered T, we will review an experimental field study of T in males and females of a single songbird species, the dark-eyed junco, and conclude by returning to the issues raised in this introduction.

Comparative Analyses of Female Testosterone

Temporal Variation in T in Birds

Our first prediction was that if female T levels are primarily the result of a correlated response to selection on male T, then males and females should show similar patterns of temporal variation in T, and values should co-vary across species.

Males of many bird species show a peak in plasma T early in the breeding season at the time territories are being established, followed by lower levels later during breeding and still lower levels in the nonbreeding season (Wingfield et al. 1987, 1990; Goymann et al. 2004).

To assess whether female T varies independently of male T or is similar in its timing, we collected comparative data from the literature on males and females belonging to 44 species, nine orders, and 22 families (names and genera from Sibley and Monroe 1990; phylogeny from Sibley and Ahlquist 1990; see tables A1, A2 in the online edition of the *American Naturalist*). Female T levels and the ratios of male/female maximum levels were log-transformed for normality. We did not include phylogeny as a factor, but an initial analysis with nested ANOVA showed no effect of order or family on T levels (female T: $F = 1.06$, df $= 20, 41$, $P = .45$; ratio M/F: $F = 1.16$, df $= 21, 43$, $P = .32$), indicating that phylogeny is not likely to have had a large effect on our findings. As data on females accumulate, it will be important to return to this issue.

We found that circulating levels of T of female bird species varied significantly over the breeding cycle (SAS mixed model repeated-measurement ANOVA with Satterwaithe method to estimate the denominator degrees of freedom: $F = 16.41$, df $= 4, 22$, $P < .001$; fig. 2; cf. description in Wingfield and Farner 1993). As in males, in

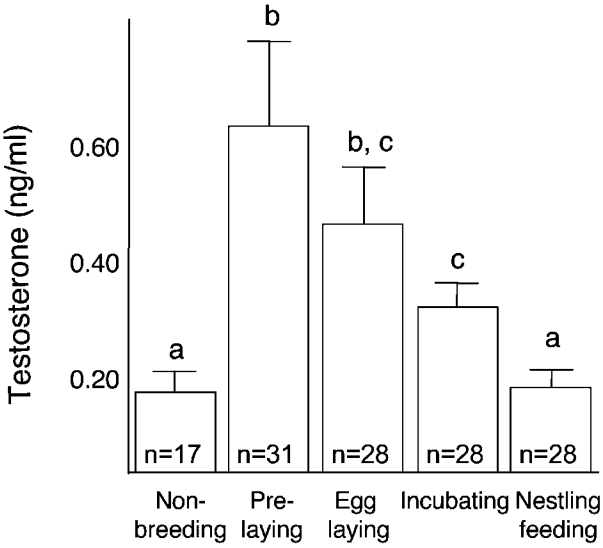

Figure 2: Plasma testosterone (mean + SE, ng/mL) in female birds according to stage of reproduction; see text and table A1 in the online edition of the *American Naturalist*. n = number of species for which testosterone was measured during at least three of the five stages; lowercase letters indicate statistically distinguishable stages.

females of the majority of species, levels of T peaked during the prelaying period (63% of all species) or the laying period (30%; fig. 2). The highest levels, and in many species the only time T was detectable, coincided with two important features in female reproduction, the acquisition of breeding resources (territory and mate) and ovulation.

In some species, female T is elevated for several weeks before egg laying, which indicates that high levels are associated with the period of territorial establishment, defense of nest sites, and defense of mate. Examples include two published studies of songbird species (red-winged blackbirds [*Agelaius phoeniceus*]: Cristol and Johnsen 1994; dark-eyed juncos: Ketterson et al. 2001) and one that is unpublished (European starlings [*Sturnus vulgaris*], M. Sandell, unpublished data). In many other species, the seasonal peak is during laying and is clearly related to egg production and ovulation. In laying hens, for example, the thecal layer of the developing follicle within the ovary is stimulated by luteinizing hormone (LH) to produce T (Johnson 2000), which is converted to an estrogen by adjacent granulosa cells (Etches and Duke 1984; Gomez et al. 2001). Testosterone also regulates the production of albumin by the oviduct (Yu and Marquardt 1973). Testosterone that is not converted to estradiol in the ovary, however, may enter the circulation and reach the brain, where it may have behavioral effects (e.g., Brenowitz and Arnold 1993). Some T is also deposited in egg yolk, where it influences development of the next generation (Schwabl 1993; Müller et al. 2002).

We next asked whether T levels co-varied in males and females across species. We determined maximum T for each sex in each species by noting the stage of reproduction at which average levels of T were the highest (see table A1) and found considerable variation in both sexes. In some species, female maximum T was low; for example, in some tropical species, female T was below the detection level of the assay used for analysis (Hau et al. 2004); in other species, it was quite high (e.g., the dark-eyed junco: Ketterson et al. 2001; range across species: 0.05–3.8 ng/mL). A visual comparison of the sexes (fig. 3) appears to indicate two sets of species, one in which maximum T of the sexes co-varies and another in which female T is low regardless of the level of male T. Statistically, the data reveal a novel result (fig. 3): female maximum levels were significantly correlated with male maximum levels ($r = .70$, $n = 44$, $P < .001$). The effect was particularly clear in the diverse order Ciconiiformes ($r = .77$, $n = 17$, $P < .001$; fig. 3), which is represented here by data from albatrosses, penguins, and waders; many Ciconiiformes are socially monogamous and sexually monomorphic, with males and females contributing about equally to parental care. The correlation also held in the Passeriformes ($r = .70$, $n = 16$, $P < .003$; fig. 3), although it was dependent on the presence of two outliers, the song sparrow and the dark-eyed junco, in which females had very high levels of testosterone.

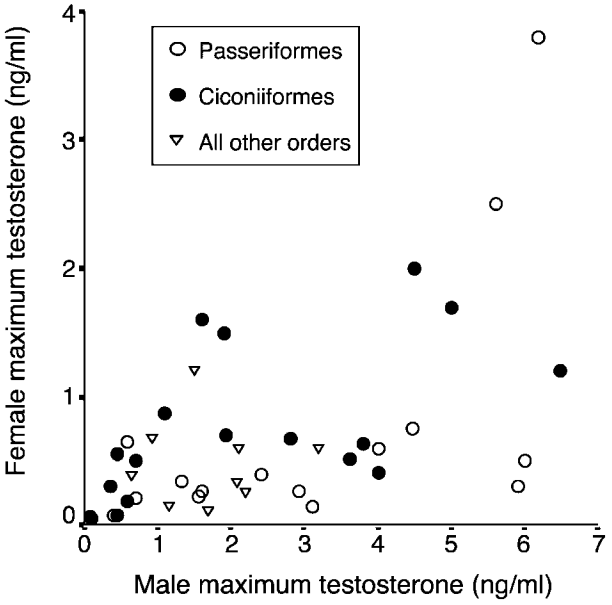

Figure 3: Covariation between maximum levels of plasma testosterone (stage of reproduction with highest mean level) in male and female birds. See text and table A1 in the online edition of the *American Naturalist.*

In sum, the functional role of the prelaying seasonal peak in female birds is not certain but may be related to direct selection on females, benefiting them in acquiring resources and mates (i.e., viability and sexual selection). The peak during laying is clearly associated with egg production (fecundity selection). The fact that female birds resemble males in seasonal profiles and seasonal maxima is consistent not only with a direct effect of selection on females but also with the indirect effect of hormonal correlations and associated correlated responses to selection.

Female Testosterone in Relation to Sexual Dimorphism and Social System

We next asked whether female testosterone co-varies in an adaptive way with degree of sexual dimorphism or with mating system. Most previous analyses of avian testosterone in relation to sexual dimorphism and mating system have focused on males (Wingfield et al. 1990; Wingfield 1994; Hirschenhauser et al. 2003; Goymann et al. 2004). Male bird species vary in hormone responsiveness, the degree to which they elevate testosterone over baseline levels during breeding. This variation gave rise to the "challenge hypothesis" (Wingfield et al. 1990; Wingfield 1994), which describes the tendency of male testosterone levels

to rise in situations of social instability. A classic article showed that males of less aggressive species, which are socially monogamous and provide parental care, exhibit greater hormonal responsiveness than males of more aggressive, non-care-giving, polygynous species (Wingfield et al. 1990). More recently, hormonal responsiveness has been shown to relate more closely to mating strategies than to parental behavior (Hirschenhauser et al. 2003).

Strictly comparable analyses have not been conducted for female testosterone, at least in part because there are fewer data on seasonal profiles or the threshold levels of testosterone that are required to elicit female aggressive behavior, sexual behavior, or ovulation. Further, the literature is mixed as to whether females resemble males by elevating T in response to a challenge (Elekonich 2000; Langmore et al. 2002). Wingfield (1994) and Wingfield et al. (2000) circumvented some of these difficulties by focusing on the maximum levels of T in a broad range of species. They expressed the mean seasonal maximum in males and females as a ratio of male to female maxima (M/F), then related this ratio to an index of sexual dimorphism (an index that summarized male-female differences in body size, plumage, and social behavior). They found greater M/F ratios and greater variability in M/F ratio in more sexually dimorphic species, as opposed to monomorphic species. This pattern was more apparent in socially monogamous species than in polygynous and polyandrous species combined (Wingfield et al. 2000).

Like Wingfield (1994) and Wingfield et al. (2000), we also examined ratios of M/F maxima. However, we did not use the dimorphism index employed in the earlier studies because it reflected a blend of traits (body size, plumage, and behavior), only some of which may relate to testosterone in either sex. Instead, we related M/F maxima separately to mating system, breeding density, female song, plumage dimorphism, territorial behavior, and aggression (see tables A2, A3 in the online edition of the *American Naturalist*—63% of the citations overlap with those in Wingfield et al. 2000). We found no significant associations with any potential correlate of the M/F ratio except one: the greater the M/F ratio, the greater the degree of plumage dimorphism ($F = 3.49$, $\mathrm{df} = 2, 43$, $P = .034$; fig. 4). Within the two largest orders, the pattern remained significant (Ciconiiformes: $F = 7.24$, $\mathrm{df} = 1, 16$, $P = .017$; Passeriformes: $F = 4.66$, $\mathrm{df} = 2, 13$, $P = .03$). This finding, that the more the sexes differ in maximum T, the greater the difference in their appearance, contradicts a widely held view that sex differences in plumage are hormone independent (Owens and Short 1995). Testosterone's link to male plumage development varies across avian orders, however (Witschi 1961; Owens and Short 1995; Kimball and Ligon 1999), and the issue of whether and how generally T relates to plumage is unresolved.

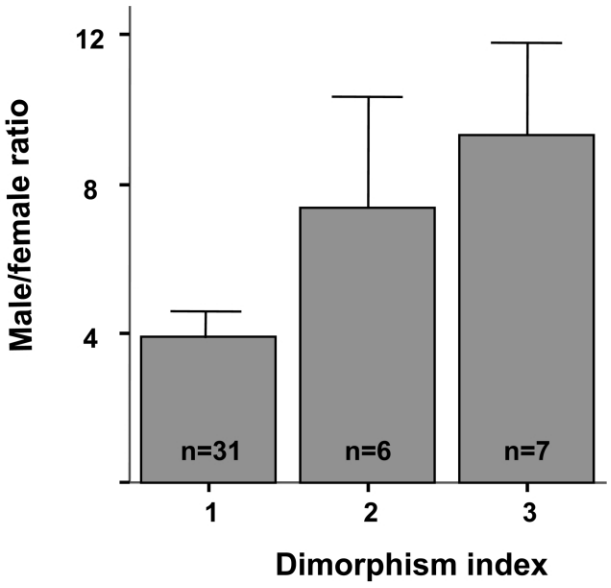

Figure 4: Ratio (mean + SE) of mean maximum plasma levels of testosterone in male and female birds in relation to sexual dimorphism in plumage and other ornaments. n = number of species; dimorphism index: 1 = monomorphic plumage, 2 = moderate dimorphism (e.g., eye, beak, leg color), 3 = great dimorphism. See text and tables A1, A2 in the online edition of the *American Naturalist*.

While helpful, analyses based on a ratio can be difficult to interpret because the ratio depends on both male and female levels; a low number, for example, would result whenever testosterone levels are similar in both sexes, whether the levels were alike in being high or low. Do the high M/F ratios of dimorphic species in figure 4 arise because male testosterone is relatively high or because female testosterone is relatively low? Consequently, we once more took a somewhat different approach and asked how maximum levels of T in females varied in relation to the same set of morphological and behavioral traits (see table A2). We also analyzed female testosterone in relation to mating system, dividing systems into four categories, social monogamy, polygyny (including facultative polygyny), polyandry, and cooperative breeding. As before, we constructed a conventional general linear model (GLM); maximum female testosterone levels were again log-transformed for normality.

Social mating system was significantly related to maximum female T (GLM: $F = 3.49$, $\mathrm{df} = 3, 41$, $P = .025$; fig. 5), a previously unreported finding that we suggest is quite interesting and important. Females of socially monogamous species, which might be expected to compete for mates and parental care, had significantly higher levels

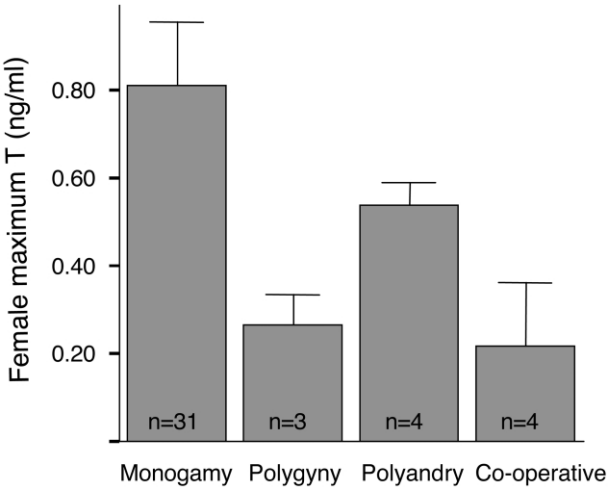

Figure 5: Maximum levels of plasma testosterone (mean + SE) in female bird species in relation to social mating system; n = number of species. See text and tables A1, A2 in the online edition of the *American Naturalist*.

of T than females from other social systems. Females of polyandrous species are known to be highly aggressive, so the comparatively lower levels in this small group of species as compared with monogamous species is surprising and unexplained. While maximum female T was related to mating system, it did not vary with breeding density, plumage dimorphism, female song, or whether females engage in territorial or social aggression ($P > .1$ in all tests).

We conclude this section by saying that the available data support both views of testosterone in females. Timing and maximum values of T co-vary in males and females in ways that suggest that current patterns may reflect past correlated responses to selection. Female T also varies among species in ways that suggest direct selection on female T.

Experimental Analyses of Female Testosterone

In this section, we return to the conceptual framework in figure 1 and to the second question raised in the introduction, which was how often existing male phenotypes might represent compromises between the advantages of T-mediated characters in males and the costs of such characters in females. To answer this question, the experimental breeder's approach might be to select on a T-mediated character and measure the evolutionary response in males. The breeder would compare the response in lines in which males are bred with females from their same lines (thus exposed indirectly to the same selection regime) and lines in which males are bred to females from unselected lines. Constraint would be indicated if males that were bred to unselected females evolved traits that were detrimental to females, while the males that were bred to indirectly selected females did not (Rice and Chippindale 2001). In natural populations, the approach would be to analyze a pedigree for between-sex genetic correlations in hormone-mediated characters and to determine whether fitness gradients were of the same or opposite sign in males and females.

Available evidence related to testosterone shows that artificial and natural selection on males lead to correlated responses in females, although there is less evidence for intersexual conflict. For example, 10 generations of selection on male boars based on the increase in T levels they exhibited when challenged with gonadotropin-releasing hormone (GnRH) led to a threefold increase in male T in the high line versus the low line. Selection also produced a correlated response in litter size (Robison et al. 1994). Whether the increase in litter size was mediated by male or female fertility was not clear. In sheep, selection on male testis size led to a correlated response in female sensitivity to GnRH, as well as responses in several correlates of fecundity (lower female body weight, higher fertility; McNeilly et al. 1988; Haley et al. 1990). In mice (*Mus musculus*), artificial selection on male aggression led to correlated responses in maternal and predatory aggression in females (Sandnabba 1996). Finally, in an example from a natural population, in side-blotched lizards (*Uta stansburniana*), cyclical natural selection on throat color, a T-mediated character, produced correlated throat color responses in males and females (Sinervo and Zamudio 2001).

Here we suggest an additional approach, which is to measure the effect of experimentally elevated testosterone (EET) on the phenotype of each sex. In this approach, shared sensitivity to the hormone in each sex would be interpreted as analogous to a between-sex genetic correlation, and thus, as a predictor of a common evolutionary response. In contrast, if only one sex is sensitive to the manipulation, then the insensitive sex will be insulated, which would leave the sensitive sex freer to evolve independently according to costs and benefits accruing to it alone. While this approach vastly oversimplifies the endocrine system, just as genetic correlations fail to specify which genes are essential or how they interact, we hypothesize that hormonal correlations, like genetic correlations, have predictive power.

To assess the utility of this approach involving hormonal manipulations, we first sought evidence from the literature regarding female sensitivity to EET. We surveyed a wide array of species and traits (see table A3), classifying traits as sensitive or insensitive to manipulated T and as morphological, behavioral, or physiological, as in figure 1. We found a broad range of sensitive traits, including some

normally exhibited only by males, some normally exhibited by both sexes, and some normally exhibited only by females. In the first such category, sensitivity indicates that evolutionary changes in female T have the potential to masculinize females; in the second, the indication is that change might cause females to express traits at values more typical of males; in the third, the indication is that changes might be hyperfeminizing. We also found traits that are responsive to EET in males but are insensitive in females. Space limitations allow us to summarize only our findings about birds, but other taxa and details are included in table A3.

Traits Sensitive to EET in Female Birds

Morphology. Male and female European starlings (*Sturnus vulgaris*) and common moorhens (*Gallinula chloropus*) change the color and structure of their bills as breeding begins, and in both species, females proved sensitive to EET, indicating a role for T in the normal assumption of breeding morphology in both sexes (Eens et al. 2000; DeRidder et al. 2002). In the budgerigar (*Melopsittacus undulates*), bills of females and males normally differ in color, but female bills became more malelike in coloration after treatment with testosterone (Nespor et al. 1996). EET also induced increases in body mass in female European starlings (Eens et al. 2000) and muscle mass in ruffs (*Philomachus pugnax*: Lank et al. 1999), moving females toward the male mean. Conversely, EET decreased body mass in female dark-eyed juncos (Clotfelter et al. 2004) as it does in males (Ketterson et al. 1991), and it had no effect on mass in still other species (see "Insensitive Traits").

Behavior. Aggressive and courtship behavior are typically enhanced by EET in males and have been shown to be sensitive to EET in females (table A3). For example, EET induced male-typical courtship behavior in female budgerigars (Nespor 2000) and female ruffs (Lank et al. 1999). In the spotless starling (*Sturnus unicolor*), EET enhanced social status in the wild but only after the hormone treatment ended (Veiga et al. 2004). In female zebra finches (*Taeniopygia guttata*), under some experimental protocols, EET increased aggressive behavior (Adkins-Regan and Ascenzi 1987; Adkins-Regan 1999).

Males typically increase vocal behavior (song) in response to EET, and the same is true of females in bird species in which females do not ordinarily sing or sing only rarely. Sometimes induction of song by EET requires earlier exposure to an organizational steroid (not testosterone) treatment (zebra finch; Adkins-Regan 1999), but frequently it does not (white-crowned sparrow *Zonotrichia leucophrys*: Kern and King 1972; canary: Nottebohm 1980; song sparrow *Melospiza melodia*: Arcese et al. 1988; European starling: Hausberger et al. 1995; DeRidder et al. 2002; budgerigar: Nespor et al. 1996). In the canary, a species in which males sing more frequent and complex songs than females, the volume of the neural song control region is larger in males (Brenowitz and Arnold 1993), but EET has been shown to increase the volume of this region in female canaries (Nottebohm 1980) and, at a more cellular level, to enhance cholinergic innervation of the song control nuclei (Appeltants et al. 2003).

While the authors of the studies just reported labeled the behaviors they studied somewhat differently (e.g., as aggression, courtship, or song), the common theme is that females exposed to higher than normal levels of testosterone displayed an interrelated set of behaviors associated with resource defense and mate acquisition. The induced changes included both more frequent performance of behaviors that are part of the normal female repertoire and also performance of behaviors not normally performed by females. Additional study will be required to determine whether such changes would be beneficial or detrimental to females.

Physiology. EET has been shown several times to delay the onset of reproduction in females. Examples include female red-winged blackbirds (Searcy 1988), dark-eyed juncos (Clotfelter et al. 2004), zebra finches (Rutkowska et al. 2005), and European starlings (M. Sandell, unpublished manuscript; see table A3 for similar effects in a reptile and a mammal). Because T levels are naturally high in females birds before breeding (fig. 2) and in some species during periods of social unrest (Langmore et al. 2002), the natural early spring peak may serve to postpone breeding until social circumstances have stabilized and no longer promote secretion (Langmore et al. 2002). Speculating now, to the extent that the sexes are hormonally correlated, prolongation of the delay in females might be detrimental to them and retard evolution of testosterone-mediated traits in males.

Also sensitive to EET is the timing and duration of molt. Molt is delayed in response to EET in male European starlings (Dawson 1994) and dark-eyed juncos (Nolan et al. 1992), and the hormone has the same effect in females of both species (DeRidder et al. 2002; Clotfelter et al. 2004). How T delays molt is not known, but similar sensitivity in both sexes suggests that molt schedules have a common physiological-hormonal basis that could influence the coevolution of the annual cycles of males and females.

EET also suppresses immune function in males of some species, for example, dark-eyed juncos (Casto et al. 2001), but not others (red-winged blackbirds: Hasselquist et al. 1999; Westneat et al. 2003). Casto et al. (2001) have suggested that species' differences in male sensitivity to EET

may represent an evolved response that protects males from the immunosuppressive cost of testosterone in species in which exposure to elevated testosterone is prolonged, as it is in red-winged blackbirds (cf. Wingfield et al. 2001). EET has also been associated with suppressed immune function in females (European starling: Duffy et al. 2000; dark-eyed junco: Zysling et al. 2003) and with greater incidence of parasites and disease (common moorhen: Eens et al. 2000; European starling: DeRidder et al. 2002). If naturally elevated T were to reduce immune function and increase mortality in females without compensatory gains in mating success or fecundity, the result might be to retard the evolution of testosterone-mediated characters in males. Future work should address the links between testosterone, immune function, and fitness in females.

Insensitive Traits

According to our working hypothesis, if a trait is sensitive to a hormone in only one sex, then the sexes are freer to evolve independently. We turned to the literature (see table A3) to see whether it would be possible to generalize about traits in females that were insensitive to experimentally elevated testosterone (for males, cf. Hunt et al. 1999; Lynn et al. 2002, 2005).

Examples of insensitivity included certain secondary sex characteristics in female ruffs (Lank et al. 1999), aggressive behavior in European robins (*Erithacus rubecula*: Kriner and Schwabl 1991) and European starlings (DeRidder et al. 2002), aspects of vocal behavior in European starlings (DeRidder et al. 2002), and aspects of immune function in common moorhens and European starlings (Eens et al. 2000; DeRidder et al. 2002).

Another example of insensitivity is partner preference in female birds. Females treated with T as adults may exhibit malelike courtship (zebra finches, budgerigars), but given a choice, they nevertheless prefer to pair with males, not females (Mansukhani et al. 1996; Adkins-Regan 1999; Nespor 2000; Nespor et al. 2000). Only when female zebra finches are subject to organizational hormone manipulations early in development do they show a preference for females, and even that preference may depend on whether they were reared with males or females (Mansukhani et al. 1996; Adkins-Regan and Wade 2001). Partner preference appears to be an organized trait determined early in development that differs qualitatively between the sexes and is not likely to serve as a constraint on male and female coevolution.

Parental behavior in females has received less attention in relation to T than has sexual behavior, but it is of special interest because a robust effect of EET in male birds is the suppression of parental behavior (summarized in Ketterson and Nolan 1999; but see Lynn et al. 2005; Schwagmeyer et al. 2005). Two recent investigations of females found no evidence that elevated T interferes with incubation (dark-eyed junco: Clotfelter et al. 2004; European starling: M. Sandell, unpublished manuscript) or feeding of offspring (European starling, M. Sandell, unpublished manuscript). If borne out by additional studies, these findings suggest that female birds may be insulated from the disruptive effects that testosterone has on male parental behavior (see below).

Although traits found to be insensitive to EET might also be insensitive to natural alterations in T as hypothesized, caution is required. It is quite possible that insensitive traits would have been sensitive under different experimental protocols (e.g., dose, timing, presence/absence of additional hormones) and thus would also be sensitive to natural alterations. Insensitive traits should be interpreted with care.

To conclude this section, testosterone plays a role in normal female functioning, but it can also cause females to become more like males in morphology, behavior, and physiology. Female birds exposed to EET begin to sing or sing more than before. They may also become more aggressive or assume malelike appearance. However, studies to date also suggest that they will continue to pair with males, lay eggs, and develop brood patches, although perhaps more slowly. They will also care for their offspring at typical female levels. Future research should focus on the effect of T in females on a wider array of traits and organisms. More studies are also needed of variation in responsiveness to T in females, including distribution of androgen receptors, thresholds, and phenotypic response to antiandrogens (Astiningsih and Rogers 1996; Wingfield et al. 2001). In addition, obviously, more information is needed on the relationship of fitness to plasma levels of T and responsiveness to T.

Experimentally Elevated Testosterone and the Dark-Eyed Junco

This section focuses on studies of EET in a single songbird species, the dark-eyed junco. The goal is to understand whether testosterone in female juncos is best seen as a mediator of adaptive traits, a constraint on sexual dimorphism, or both. The research we review is ongoing, and the conclusions reached are necessarily tentative.

Earlier work (Ketterson and Nolan 1992, 1999; Ketterson et al. 1996, 2001; Casto et al. 2001; W. L. Reed, M. E. Clark, P. G. Parker, S. A. Raouf, N. Arguedas, D. S. Monk, E. Snajdr, V. Nolan, and E. Ketterson, unpublished manuscript) explored the effect of EET on the behavior, physiology, and fitness of male dark-eyed juncos and showed that testosterone mediates a trade-off between

mating effort and parental effort. T-treated males courted females more than did controls given empty implants and were also more attractive to females (Enstrom et al. 1997), but they provided less care for offspring (Ketterson et al. 1992). T-treated males were also more successful than controls at siring young through extra-pair fertilizations (Raouf et al. 1997; W. L. Reed et al., unpublished manuscript). In addition, T suppressed immune function (Casto et al. 2001) and reduced adult survivorship (W. L. Reed et al., unpublished manuscript). Paradoxically, the net effect of EET was to elevate the fitness of T-treated males above that of controls (W. L. Reed et al., unpublished manuscript), leading us to ask what might be constraining the evolution in males of a natural counterpart to the experimental phenotype. Could the answer lie in the possibility that T levels are hormonally correlated in male and female juncos and that the consequences of higher levels would be disadvantageous to females?

In free-living male and female juncos, patterns of variation in circulating levels of T according to stage of reproduction are similar but not identical (Ketterson et al. 2001; fig. 6). Most notable is the peak in testosterone levels in both sexes before nest building/egg laying. The source and function of this early-season T in females is not known, but interestingly, T and corticosterone levels are correlated, suggesting that female T in early spring may be associated with greater energy expenditure or perhaps social stress and that the adrenal gland might be the source of the testosterone (E. Ketterson, C. Ziegenfus, J. Cawthorn, T. Johnsen, and V. Nolan, unpublished data). The similarity of males and females at some stages of reproduction is consistent with a past correlated response, but the differences between the sexes at other stages suggest that T can be expressed independently in males and females. The nearly identical early-season maxima, combined with the paradoxical finding of greater fitness in T-treated males, led us to ask what the consequences would be of extending the early season maximum experimentally in females as we had earlier done in males.

We began elevating T experimentally in females by capturing and implanting them in early spring (at Mountain Lake Biological Station, VA, 2000–2002), releasing them at their capture sites and monitoring their behavior. A dose that was half that used with males (5-mm implants) induced physiological maximal levels in female plasma, also elevating testosterone in the yolk of eggs laid (Clotfelter et al. 2004).

The effects on traits expected to be shaped by fecundity selection include the following (fig. 1). EET delayed reproduction slightly but significantly: after nest completion, T-females took 1 day longer to lay their first egg (2.6 days) than did control females (1.6 days). There were no detectable effects on clutch size or egg dimensions (Clotfelter et al. 2004) and no influence on the proportion of time females spent incubating (observed by videotaping, T-females 70% vs. control females 65%; fig. 7*A*; Clotfelter et al. 2004) or on nest defense against a common nest predator, a mounted chipmunk *Tamias striatus* (Clotfelter et al. 2004; fig. 7*B*).

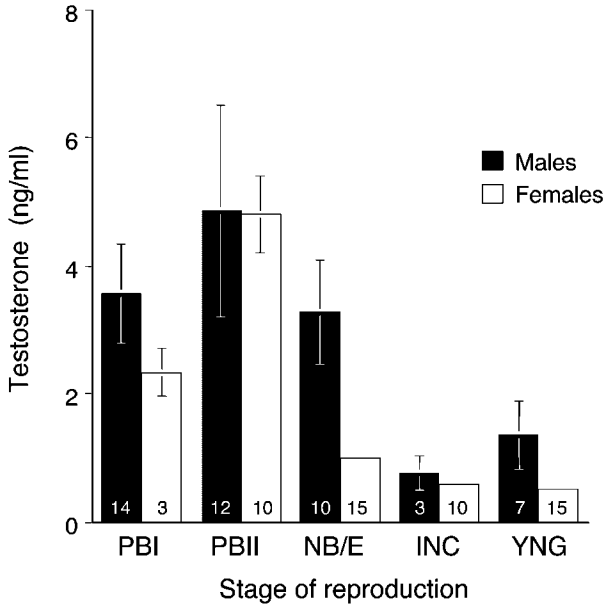

Figure 6: Plasma testosterone in the dark-eyed junco, compared by sex and stage of reproduction (*solid bars*, males; *open bars*, females; ANOVA, stage [$P = .095$], sex [$P = .029$], date [$P = .005$], breeding time [$P = .043$]; ANOVA without date, stage [$P = .000$], sex [$P = .008$]). Stages are prebreeding I (*PBI*), early April; prebreeding II (*PBII*), late April; nest building/egg laying (*NB/E*); incubation (*INC*); and tending young (*YNG*). Testosterone varies with sex and date or stage. Numbers indicate sample size; data shown are mean ± SE.

In relation to traits expected to be shaped by sexual selection, we compared the effect of EET on female attractiveness to males. Whereas, in males, EET increased caged male attractiveness to caged females (Enstrom et al. 1997), in this experiment, males spent more time near control females than T-treated females (one-tailed *t*-test, $P = .035$; fig. 7*C*), suggesting a reduction in female attractiveness (I. Parker-Renga, K. Jones, and E. Ketterson, unpublished manuscript). Interestingly, testosterone also affected female mate choice and discrimination among males. When T-treated and control females were given the opportunity to choose between a T-treated and control male, control females showed a preference, whereas testosterone females spent equal time with the males of both treatment groups (McGlothlin et al. 2004; fig. 7*D*).

In relation to traits expected to be shaped by viability selection, we examined the effect of EET on the female

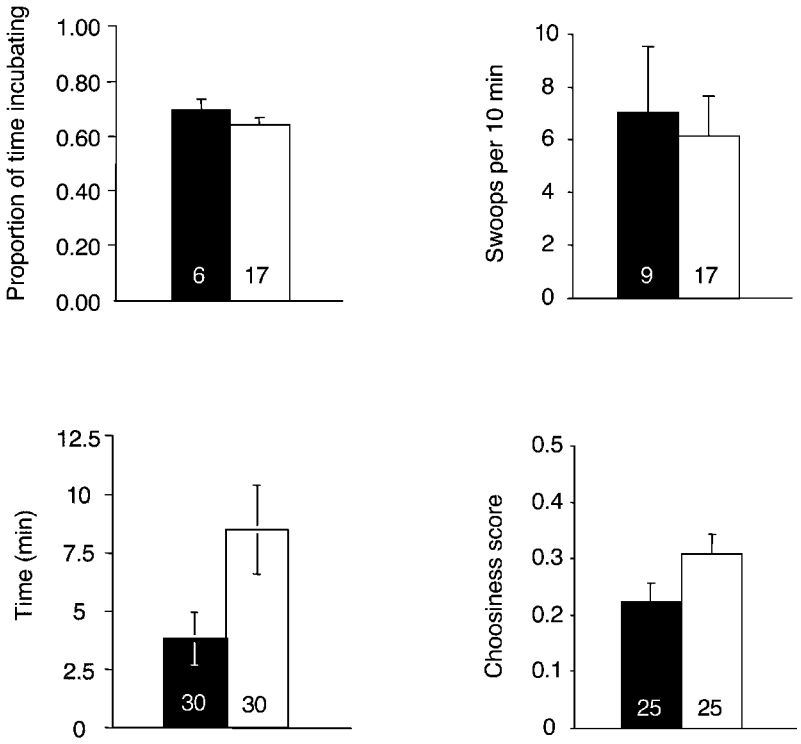

Figure 7: Experimental elevation of testosterone (T) in female dark-eyed juncos affects *A*, parental behavior (proportion of time spent in incubation; after Clotfelter et al. 2004); *B*, nest defense (swoops toward mounted nest predator; after Clotfelter et al. 2004); *C*, attractiveness to males (time spent by male adjacent to T-treated or control female; I. Parker-Renga, K. Jones, and E. Ketterson, unpublished manuscript); and *D*, discrimination between males in mate choice (based on a choosiness score, irrespective of which male was preferred; score is 0.50 if all time spent with one male, 0.0 if equal time spent with each male; after McGlothlin et al. 2004). *Solid bars* = T-treated females, *open bars* = controls; numbers indicate sample size; data shown are mean ± SE.

stress response (measured as the rise in plasma levels of corticosterone in response to handling), immune function, and aggressiveness (Zysling et al. 2003). In T-treated females, corticosterone rose in response to handling more rapidly than in controls, suggesting greater susceptibility to environmental stressors (Zysling et al. 2003). (However, levels of cortiocosteroid-binding globulin were also higher in treated females, clouding easy interpretation of this result; D. A. Zysling, T. J. Greives, C. Breuner and E. D. Ketterson, unpublished data.) Treated females also mounted a less robust cell-mediated immune response and behaved more aggressively toward an intruder female placed into their cage (Zysling et al. 2003). We also determined that EET delayed molt in captive females as it did in males (Clotfelter et al. 2004).

Interpretation of these initial experimental results with regard to the key question—the effect of EET on fitness in females—necessarily must await further experiments. Clearly, a number of the traits that are sensitive to testosterone could have an effect on fitness; possibly among them are the number of surviving young, the number of young sired by extrapair matings, survival of nests against predation, and proportion of females returning to breed in later years. So far, the only difference discovered is that nests of T-treated females are more subject to predation during incubation (D. O'Neal, E. Snajdr, and E. Ketterson, unpublished data).

Comparing initial female results with our earlier findings for males shows that males and females were similarly sensitive to T in at least five traits related to sexual and viability selection (see fig. 1): attractiveness, corticosterone response to handling, level of corticosterone-binding globulin, immune function, and molt. Of these five, response to stressors, impaired immune function, and delayed molt are potentially detrimental to females as well as males, thus suggesting that the sexes may be similarly constrained by hormonal pleiotropy. Selection for elevated T would be expected only if the benefits outweigh the costs as the result of hormonal correlations and trade-offs. In males, the enhancement of attractiveness appears to be a benefit that more than compensates for the costs enumerated above. In females, however, we know of no compensatory benefit

in mating success, but this is something that remains to be tested. Thus, this potentially negative consequence of enhanced testosterone might constrain male evolution.

In contrast, aggressiveness toward same-sex intruders may be useful to females in circumstances in which the same is true of males, which would predict more rapid evolution of this trait in each sex than would occur if it were beneficial in only one sex (reinforcement). Importantly, females, unlike males, were insensitive to T with respect to parental behavior, which predicts that so far as that trait is concerned, a beneficial rise in testosterone in males could proceed independently of the fact that a correlated rise in T was taking place in females. In sum, based on the data currently available, in juncos, depending on the trait under consideration, female testosterone may act both to mediate adaptive behavior and to constrain the male phenotype.

Conclusions and Future Research

The introduction to this article considered the various forms of selection—viability, fecundity, and sexual—and the existence of correlated responses in the opposite sex as they might interact to determine sex differences and resemblances. Because of testosterone's role in the induction of primary and secondary sex characteristics in males, this hormone is often invoked as a leading proximate cause of sexual dimorphism. Aware that T also has a role in female biology, we began to ask how selection acts on female T. We were also curious about a second question, which was whether the hormone's effects in females might sometimes constrain male evolution (and thus sexual dimorphism) through correlated responses to selection.

We made several sets of predictions, preliminary and simplistic but clear. If female testosterone is the outcome of correlated response to selection on males, then among species it should co-vary with male levels and be unrelated to female ecology; if, however, female T is the product of direct selection on females, then among species it should co-vary with female ecology. We found evidence for both predictions. Interspecifically, female T co-varied with male T across species. But females of socially monogamous species had higher levels of T than females of other social systems, and females of species dimorphic for plumage had lower T in relation to their males than did females of monomorphic species.

Within-species correlated responses to testosterone would require genetic and/or hormonal correlations between the sexes, while patterns arising from direct selection acting independently on each sex would not. We predicted that traits that were sensitive to T in females would be subject to selection as either direct or correlated responses; traits that were T-insensitive would not. The literature revealed that a wide array of traits in females are sensitive to elevated testosterone and thus might be expected to a respond to direct selection on females or as correlated responses to selection on males as well. Other traits were insensitive, thus predicting evolutionary independence for them.

Experimental manipulations of single species also hold promise in relating sensitivity and insensitivity to fitness. Studies comparing the fitness of phenotypes produced through EET with the fitness of normal control phenotypes may detect the presence or absence of constraints. Adequate experimental testing of the predictions presented here requires more data on the relationship between T-mediated traits and fitness in females, and an objective of this article is to stimulate pursuit of these questions.

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Literature Cited

Adkins-Regan, E. 1999. Testosterone increases singing and aggression but not male-typical sexual partner preference in early estrogen treated female zebra finches. Hormones and Behavior 35:63–70.

Adkins-Regan, E., and M. Ascenzi. 1987. Social and sexual behaviour of male and female zebra finches treated with oestradiol during the nestling period. Animal Behaviour 35:1100–1112.

Adkins-Regan, E., and J. Wade. 2001. Masculinized sexual partner preference in female zebra finches with sex-reversed gonads. Hormones and Behavior 39:22–28.

Andersson, M. 1994. Sexual selection. Princeton University Press, Princeton, NJ.

Appeltants, D., G. F. Ball, and J. Balthazart. 2003. Song activation by testosterone is associated with an increased catecholaminergic innervation of the song control system in female canaries. Neuroscience 121:801–814.

Arcese, P., P. Stoddard, and S. Hierbert. 1988. The form and function of song in female song sparrows. Condor 90:44–50.

Arnold, A. P. 1996. Genetically triggered sexual differentiation of brain and behavior. Hormones and Behavior 30:495–505.

———. 2004. Sex chromosomes and brain gender. Nature Reviews Neuroscience 5:701–708.

Astiningsih, K., and L. J. Rogers. 1996. Sensitivity to testosterone varies with strain, sex, and site of action in chickens. Physiology and Behavior 59:1085–1091.

Brenowitz, E. A., and A. P. Arnold. 1993. Hormone accumulation in song regions of the canary brain. Journal of Neurobiology 23:871–880.

Brown, C. R., M. B. Brown, S. A. Raouf, L. C. Smith, and J. C. Wingfield. 2005. Effects of endogenous steroid hormone levels on annual survival in cliff swallows. Ecology 86:1034–1046.

Carrel, L., and H. R. Willand. 2005. X-inactivation profile reveals extensive variability in X-linked gene expression in females. Nature 434:400–404.

Casto, J. M., V. Nolan Jr., and E. D. Ketterson. 2001. Steroid hormones and immune function: experimental studies in wild and captive dark-eyed juncos (*Junco hyemalis*). American Naturalist 157:408–420.

Cheverud, J. M., and E. J. Routman. 1995. Epistasis and its contribution to genetic variance components. Genetics 139:1455–1464.

Chippindale, A. K., J. R. Gibson, and W. R. Rice. 2001. Negative genetic correlation for adult fitness between sexes reveals ontogenetic conflict in Drosophila. Proceedings of the National Academy of Sciences of the USA 98:1671–1675.

Clotfelter, E. D., D. M. O'Neal, J. M. Gaudioso, J. M. Casto, I. M. Parker-Renga, E. A. Snajdr, D. L. Duffy, et al. 2004. Consequences of elevating plasma testosterone in females of a socially monogamous songbird: evidence of constraints on male evolution? Hormones and Behavior 46:171–179.

Cristol, D. A., and T. S. Johnsen. 1994. Spring arrival, aggression and testosterone in female red-winged blackbirds (*Agelaius phoeniceus*). Auk 111:210–214.

Darwin, C. 1871. The descent of man and selection in relation to sex. J. Murray, London.

Dawson, A. 1994. The effects of daylength and testosterone on the initiation and progress of moult in starlings, *Sturnus vulgaris*. Ibis 136:335–340.

DeRidder, E., R. Pinxten, V. Mees, and M. Eens. 2002. Short- and long-term effects of male-like concentrations of testosterone on female European starlings (*Sturnus vulgaris*). Auk 119:487–497.

Duffy, D. L., G. E. Bentley, D. L. Drazen, and G. F. Ball. 2000. Effects of testosterone on cell-mediated and humoral immunity in non-breeding adult European starlings. Behavioral Ecology 11:654–662.

Eens, M., E. Van Duyse, L. Berghman, and R. Pinxten. 2000. Shield characteristics are testosterone-dependent in both male and female moorhens. Hormones and Behavior 37:126–134.

Elekonich, M. M. 2000. Female song sparrow, *Melospiza melodia*, response to simulated conspecific and heterospecific intrusion across three seasons. Animal Behaviour 59:551–557.

Enstrom, D. E., E. D. Ketterson, and V. Nolan Jr. 1997. Testosterone and mate choice in the dark-eyed junco. Animal Behaviour 54: 1135–1146.

Etches, J. R., and C. E. Duke. 1984. Progesterone, androstenedione and oestradiol content of theca and granulosa tissues of the four largest ovarian follicles during the ovulatory cycle of the hen (*Gallus domesticus*). Journal of Endocrinology 103:71–76.

Finch, C. E., and M. R. Rose. 1995. Hormones and the physiological architecture of life history evolution. Quarterly Review of Biology 70:1–52.

Gil, D., G. Leboucher, A. Lacroix, R. Cue, and M. Kreutzer. 2004. Female canaries produce eggs with greater amounts of testosterone when exposed to preferred male song. Hormones and Behavior 45:64–70.

Gomez, Y., P. N. Velazquez, I. Peralta-Delgado, M. C. Mendez, F. Vilchis, M. Juarez-Oropeza, and E. Pedernera. 2001. Follicle stimulating hormone regulates steroidogenic enzymes in cultured cells of the chick embryo ovary. General and Comparative Endocrinology 121:305–315.

Goymann, W., I. T. Moore, A. Scheuerlein, K. Hirschenhauser, A. Grafen, and J. C. Wingfield. 2004. Testosterone in tropical birds: effects of environmental and social factors. American Naturalist 164:327–334.

Haley, C. S., G. J. Lee, M. Ritchie, and R. B. Land. 1990. Direct responses in males and correlated responses for reproduction in females to selection for testicular size adjusted for body weight in young male lambs. Journal of Reproduction and Fertility 89:383–396.

Hasselquist, D., J. A. Marsh, P. W. Sherman, and J. C. Wingfield. 1999. Is avian humoral immunocompetence suppressed by testosterone? Behavioral Ecology and Sociobiology 45:167–175.

Hau, M., S. T. Stoddard, and K. K. Soma. 2004. Territorial aggression and hormones during the non-breeding season in a tropical bird. Hormones and Behavior 45:40–49.

Hausberger, M., L. Henry, and M. Richard. 1995. Testosterone-induced singing in female starlings (*Sturnus vulgaris*). Ethology 99:193–208.

Hirschenhauser, K., H. Winkler, and R. F. Oliveira. 2003. Comparative analysis of male androgen responsiveness to social environment in birds: the effects of mating system and paternal incubation. Hormones and Behavior 43:508–519.

Hunt, K., T. Hahn, and J. C. Wingfield. 1999. Endocrine influences on parental care during a short breeding season: testosterone and male parental care in Lapland longspurs (*Calcarius lapponicus*). Behavioral Ecology and Sociobiology 45:360–369.

Johnson, A. L. 2000. Reproduction in the female. Pages 569–596 *in* P. D. Sturkie, ed. Avian physiology. Springer, New York.

Johnson, D., and D. Macdonald. 2001. Why are group-living badgers (*Meles meles*) sexually dimorphic? Journal of Zoology (London) 255:199–204.

Karubian, J., and J. Swaddle. 2001. Selection on females can create "larger males." Proceedings of the Royal Society of London B 268: 725–728.

Kern, M., and J. King. 1972. Testosterone-induced singing in female white-crowned sparrows. Condor 74:204–209.

Ketterson, E., V. Nolan Jr., J. Casto, C. Buerkle, E. Clotfelter, J. Grindstaff, K. Jones, et al. 2001. Testosterone, phenotype and fitness: a research program in evolutionary behavioral endocrinology. Pages 19–38 *in* A. Dawson and C. Chaturvedi, eds. Avian endocrinology. Narosa, New Delhi.

Ketterson, E. D., and V. Nolan Jr. 1992. Hormones and life histories: an integrative approach. American Naturalist 140(suppl.):S33–S62.

———. 1999. Adaptation, exaptation, and constraint: a hormonal perspective. American Naturalist 154(suppl.):S4–S25.

Ketterson, E. D., V. Nolan Jr., L. Wolf, C. Ziegenfus, A. M. Dufty, G. F. Ball, and T. S. Johnsen. 1991. Testosterone and avian life histories: the effect of experimentally elevated testosterone on cor-

ticosterone and body mass in dark-eyed juncos (*Junco hyemalis*). Hormones and Behavior 25:489–503.

Ketterson, E. D., V. Nolan Jr., L. Wolf, and C. Ziegenfus. 1992. Testosterone and avian life histories: effects of experimentally elevated testosterone on behavior and correlates of fitness in the dark-eyed junco (*Junco hyemalis*). American Naturalist 140:980–999.

Ketterson, E. D., V. Nolan Jr., M. J. Cawthorn, P. G. Parker, and C. Ziegenfus. 1996. Phenotypic engineering: using hormones to explore the mechanistic and functional bases of phenotypic variation in nature. Ibis 138:70–86.

Kimball, R. T., and J. D. Ligon. 1999. Evolution of avian plumage dichromatism from a proximate perspective. American Naturalist 154:182–193.

Kriner, E., and H. Schwabl. 1991. Control of winter song and territorial aggression of female robins (*Erithacus rubecula*) by testosterone. Ethology 87:37–44.

Lande, R. 1980. Sexual dimorphism, sexual selection, and adaptation in polygenic characters. Evolution 34:292–305.

———. 1987. Genetic correlations between the sexes in the evolution of sexual dimorphism and mating preferences. Pages 83–95 *in* J. W. Bradbury and M. B. Andersson, eds. Sexual selection: testing the alternatives. Wiley, Chichester.

Lande, R., and S. J. Arnold. 1983. The measurement of selection on correlated characters. Evolution 37:1210–1226.

Langmore, N., J. Cockrem, and E. Candy. 2002. Competition for male reproductive investment elevates testosterone levels in female dunnocks, *Prunella modularis*. Proceedings of the Royal Society of London B 269:2473–2478.

Lank, D., M. Coupe, and K. E. Wynne-Edwards. 1999. Testosterone-induced male traits in female ruffs (*Philomachus pugnax*): autosomal inheritance and gender differentiation. Proceedings of the Royal Society of London B 266:2323–2330.

Ligon, J. D. 1999. Avian breeding systems. Oxford University Press, Oxford.

Lynn, S., L. Hayward, Z. Benowitz-Fredericks, and J. Wingfield. 2002. Behavioural insensitivity to supplementary testosterone during the parental phase in the chestnut-collared longspur, *Calcarius ornatus*. Animal Behaviour 63:795–803.

Lynn, S. E., B. G. Walker, and J. C. Wingfield. 2005. A phylogenetically controlled test of hypotheses for behavioral insensitivity to testosterone in birds. Hormones and Behavior 47:170–177.

Mansukhani, V., E. Adkins-Regan, and S. Yang. 1996. Sexual partner preference in female zebra finches: the role of early hormones and social environment. Hormones and Behavior 30:506–513.

McGlothlin, J. W., D. L. H. Neudorf, J. M. Casto, V. Nolan Jr., and E. D. Ketterson. 2004. Elevated testosterone reduces choosiness in female dark-eyed juncos (*Junco hyemalis*): evidence for a hormonal constraint on sexual selection? Proceedings of the Royal Society of London B 271:1377–1384.

McNeilly, J. R., M. Fordynce, R. B. Land, G. B. Martin, A. J. Sringbett, and R. Webb. 1988. Changes in the feedback control of gonadotrophin secretion in ewes from lines selected for testis size in the ram lamb. Journal of Reproduction and Fertility 84:213–221.

Müller, W., C. Eising, C. Dijkstra, T. Groothuis, and G. Sageder. 2002. Sex differences in yolk hormones depend on maternal social status in leghorn chickens (*Gallus gallus domesticus*). Proceedings of the Royal Society of London B 269:2249–2255.

Nelson, R. J. 2000. An introduction to behavioral endocrinology. 2nd ed. Sinauer, Sunderland, MA.

Nespor, A., M. Lukazewicz, R. Dooling, and G. Ball. 1996. Testosterone induction of male-like vocalizations in female budgerigars (*Melopsittacus undulatus)*. Hormones and Behavior 30:162–169.

Nespor, A. A. 2000. The neuroendocrine basis of sex differences in vocal production, reproductive behavior, and neuroanatomical components of the budgerigar (*Melopsittacus undulatus*). PhD thesis. University of Maryland, College Park.

Nespor, A. A., R. J. Dooling, and G. F. Ball. 2000. Effects of exogenous testosterone on partner preference and courtship behaviors in adult female budgerigars (*Melopsittacus undulatus*). Proceedings of the International Conference on Hormones, Brain and Behavior, Madrid, Spain. Trabajos del Instituto Cajal 38:284–286.

Nolan, V. J., E. D. Ketterson, C. Ziegenfus, D. P. Cullen, and C. R. Chandler. 1992. Testosterone and avian life histories: effects of experimentally elevated testosterone on prebasic molt and survival in male dark-eyed juncos. Condor 94:364–370.

Nottebohm, F. 1980. Testosterone triggers growth of brain vocal control nuclei in adult female canaries. Brain Research 189:429–436.

Owens, I. P. F., and R. V. Short. 1995. Hormonal basis of sexual dimorphism in birds: implications for new theories of sexual selection. Trends in Ecology & Evolution 10:44–47.

Raouf, S. A., P. G. Parker, E. D. Ketterson, V. Nolan Jr., and C. Ziegenfus. 1997. Testosterone affects reproductive success by influencing extra-pair fertilizations in male dark-eyed juncos (Aves: *Junco hyemalis*). Proceedings of the Royal Society of London B 264:1599–1603.

Rice, W. 1996. Sexually antagonistic male adaptation triggered by experimental arrest of female evolution. Nature 381:232–234.

Rice, W., and A. Chippindale. 2001. Intersexual ontogenetic conflict. Journal of Evolutionary Biology 14:685–693.

Robison, O. W., D. Lubritz, and B. Johnson. 1994. Realized heritability estimates in boars divergently selected for testosterone levels. Journal of Animal Breeding and Genetics 111:35–42.

Rutkowska, J., M. Cichon, M. Puerta, and D. Gil. 2005. Negative effects of elevated testosterone on female fecundity in zebra finches. Hormones and Behavior 47:585–591.

Sandnabba, N. 1996. Selective breeding for isolation-induced intermale aggression in mice: associated responses and environmental influences. Behavior Genetics 25:477–488.

Schwabl, H. 1993. Yolk as a source of maternal testosterone for developing birds. Proceedings of the National Academy of Sciences of the USA 90:11446–11450.

Schwagmeyer, P. L., H. G. Schwabl, and D. W. Mock. 2005. Dynamics of biparental care in house sparrows: hormonal manipulations of paternal contributions. Animal Behaviour 69:481.

Searcy, W. 1988. Do female red-winged blackbirds limit their own breeding densities? Ecology 69:85–95.

Selander, R. 1966. Sexual dimorphism and differential niche utilization in birds. Condor 68:113–151.

Shuster, S., and M. Wade. 2003. Mating systems and strategies. Princeton University Press, Princeton, NJ.

Sibley, C., and B. L. J. Monroe. 1990. Distribution and taxonomy of birds of the world. Yale University Press, New Haven, CT.

Sibley, C. G., and J. E. Ahlquist. 1990. Phylogeny and classification of birds: a study in molecular evolution. Yale University Press, New Haven, CT.

Sinervo, B., and K. R. Zamudio. 2001. The evolution of alternative reproductive strategies: fitness differentials, heritability, and genetic correlations between the sexes. Heredity 92:196–205.

Staub, N. L., and M. De Beer. 1997. The role of androgens in female vertebrates. General and Comparative Endocrinology 108:1–24.

Veiga, J. P., J. Vinuela, P. J. Cordero, J. M. Aparicio, and V. Polo. 2004. Experimentally increased testosterone affects social rank and primary sex ratio in the spotless starling. Hormones and Behavior 46:47–53.

Wade, J., D. Swender, and T. McElhinny. 1999. Sexual differentiation of the zebra finch song system parallels genetic, not gonadal, sex. Hormones and Behavior 36:141–152.

Westneat, D., D. Hasselquist, and J. Wingfield. 2003. Tests of association between the humoral immune response of red-winged blackbirds (*Agelaius phoeniceus*) and male plumage, testosterone, or reproductive success. Behavioral Ecology and Sociobiology 53: 315–323.

Wingfield, J. C. 1994. Hormone-behavior interactions and mating systems in male and female birds. Pages 303–330 *in* R. V. Short and E. Balaban, eds. The differences between the sexes. Cambridge University Press, Cambridge.

Wingfield, J. C., and D. S. Farner. 1993. Endocrinology of reproduction in wild species. Pages 164–327 *in* D. S. Farner, J. R. King, and K. C. Parkes, eds. Avian biology. Academic Press, London.

Wingfield, J. C., G. F. Ball, A. M. Dufty Jr., R. E. Hegner, and M. Ramenofsky. 1987. Testosterone and aggression in birds. American Scientist 75:602–608.

Wingfield, J. C., R. E. Hegner, A. Dufty Jr., and G. F. Ball. 1990. The "challenge hypothesis": theoretical implications for patterns of testosterone secretion, mating systems, and breeding strategies. American Naturalist 136:829–846.

Wingfield, J. C., J. D. Jacobs, A. D. Tramontin, N. Perfito, S. Meddle, D. L. Maney, and K. Soma. 2000. Toward an ecological basis of hormone-behavior interactions in reproduction of birds. Pages 85–128 *in* K. Wallen and J. E. Schneider, eds. Reproduction in context: social and environmental influences on reproduction. MIT Press, Cambridge, MA.

Wingfield, J., S. Lynn, and K. Soma. 2001. Avoiding the "costs" of testosterone: ecological bases of hormone-behavior interactions. Brain Behavior and Evolution 57:239–251.

Witschi, E. 1961. Sex and secondary sexual characters. Pages 115–168 *in* A. J. Marshall, ed. Biology and comparative physiology of birds. Academic Press, New York.

Yu, J. Y. L., and R. R. Marquardt. 1973. Synergism of testosterone and estradiol in the development and function of the magnum from the immature chicken oviduct. Endocrinology 92:563–572.

Zysling, D. A., T. J. Greives, G. E. Demas, and E. D. Ketterson. 2003. Effects of testosterone on female aggression and immune function: possible evidence of intersexual conflict in dark-eyed juncos (*Junco hyemalis*). Integrative and Comparative Biology 43:927.

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