

Testosterone, phenotype and fitness: a research program in evolutionary behavioral endocrinology

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Introduction

Birds vary both inter- and intra-specifically in their phenotypes in ways that appear adaptive, and some of this variation is mediated by hormones. The challenge is to account for how and why such variation arises, both mechanistically and in terms of evolutionary forces. In this paper we outline some of the contributions to be expected from a research program in *evolutionary behavioral endocrinology*. The evolutionary component of the program is based on qualitative concepts such as adaptation, exaptation, and constraint, and also on concepts from quantitative genetics. We emphasize behavior because it has received less attention from evolutionary biologists than has morphology, but we also stress that behavior, physiology, and morphology are inextricably linked. The endocrinological component is based on the signaling and inductive properties of hormones that serve to coordinate development and the simultaneous expression of suites of organismal characters. Hormones play a key role in translating genotype to phenotype at several levels and time scales, e.g., individuals and populations; within a lifetime and across generations. We illustrate from the literature and our own

20 Avian Endocrinology

work on a passerine bird, the dark-eyed junco, *Junco hyemalis* (Emberizidae).

Adaptation, exaptation and constraint

The study of adaptation was greatly aided by Gould and Lewontin's (43) criticism that by the 1970s too many researchers had begun to see organisms not as dynamic, interconnected and integrated systems, but rather as collections of traits, each individually perfected by natural selection (95). Because the evolutionary process is constrained by history and development, Gould and Lewontin (43) argued that individual traits are limited in the degree to which they can achieve adaptive modification (42). Conversely, they argued, a trait might appear to be an adaptation because it is advantageous and fits well with its environment, when in fact it is more probable that it is an exaptation. That is, if a trait arose not as the result of any advantage it provided when selection first acted, but as the result of its co-expression with some other trait that was acted upon directly by the environment (44, 60, 112), then it should not be treated as an adaptation. A frequently cited example of an exaptation is the penile clitoris of the female spotted hyena, which Gould and Vrba (44) saw not as the direct result of selection but as a side effect of selection on female aggressiveness (see also 39).

In response to these criticisms, students of adaptation re-examined their assumptions and generated more rigorous concepts and definitions. For example, modern definitions of adaptations specify not only that they be currently beneficial but also that they be characters that arose historically as the direct result of past selection for the function they presently serve (10, 25, 26, 44, 67, 102, 112). Further, most students of adaptation acknowledge the importance of constraints and seek to understand these limits to perfection, including genetic correlations among traits and life-history trade-offs (e.g. 7, 35, 71, 95, 102). The issue of exaptation remains elusive, because it is not clear how to determine whether a trait was the historical target of selection or whether it was co-expressed with a target trait at the time that selection originally acted (112). Nevertheless the concept of exaptation is useful because it places proper emphasis on the fact that many traits have common physiological causes, and selection on any one is likely to affect the evolution of others.

One indicator of the increased rigor in the study of adaptation is the number of experimental studies that have been performed in the field to test whether traits are currently beneficial (45, 61, 98, 103). Such studies ask whether fitness (relative survival and reproductive success) is diminished or enhanced when a normally occurring trait is altered. If it is

diminished, they conclude that the character is currently beneficial (e.g. 60, 106, see 61, 87, 103); if it is enhanced, the reason the character has not evolved to the altered experimental level may be that it is constrained (5, 18, 60). While representing progress, such studies have focused on single morphological traits rather than suites of correlated behavioral and physiological traits. Experimental manipulations of hormones are notable exceptions to this generalization (for recent summary, see 60, 103).

Hormones as a cause of correlated traits

Despite the complex nature of the relationship between hormones and phenotype, the fundamental notion of a hormone as a signal that produces multiple effects through its impact on an array of target tissues provides a useful working model for probing how selection might act on correlated behavioral and physiological traits (59, 60). We know that trait evolution can be constrained if the genes that underlie the expression of one trait also control the expression of another, and if the traits have opposing effects on fitness. Elsewhere we have argued that hormones can be viewed as physiological analogues of such pleiotropic genes (60), because hormones affect many different target tissues and, consequently, many different behavioral and physiological traits. If all of these effects are beneficial or neutral, then evolution may be hastened as variants expressing one or another trait are favored. If the effects are counteracting, i.e., some are disadvantageous, evolution of one trait may be slowed by the adverse consequences of enhancing others.

Hormones and trade-offs

Recent experimental studies of trait evolution involving the manipulation of hormones (phenotypic engineering with hormones 59, 61) have found not only multiple effects, but also effects with contradictory influences on fitness. A now widely verified example is the testosterone (T)-mediated trade-off between mating effort and parental effort in birds (60, 62, 114, 115). Another well studied avian example of a hormonally mediated trade-off is that between sexually selected ornaments and immune function: some extravagant, sexually selected characters owe their development to T; yet T may compromise immunity (37, 38, 68, 110, 121; but see 48, 52, 86, 88). Examples from other taxa include (1) a T-mediated trade-off between reproduction and survival in lizards (73-76); (2) a follicle stimulating hormone (FSH)-mediated trade-off between egg size and egg number in lizards (e.g. 104, 105); and (3) a juvenile hormone (JH)-mediated trade-off between dispersal and reproduction in crickets (118-120) (for still more examples, see 60).

22 Avian Endocrinology

A research program in evolutionary behavioral endocrinology

Despite the growing literature on hormonal manipulations, relatively few studies have gone beyond establishing a connection between one hormone and one or a few closely related characters that might be expected to affect some component of fitness. The goal of our research over the past 13 years has been to produce a comprehensive account of the many and diverse phenotypic effects and fitness consequences arising from a change in circulating T in a male bird, the dark-eyed junco. Our primary goal has been to understand the evolution of complex, hormone-mediated traits, but we have also been interested in understanding how T exerts its effects on the male phenotype. Most recently we have considered how testosterone in the male affects the behavior of conspecifics by asking how experimental elevation of male T resonates to and through the phenotypes of mates and offspring (see next sub-section entitled, *The extended phenotype*). We have also begun to ask whether selection on hormonal (T) variation in the male might be expected to have genetically correlated consequences for the female phenotype, and, if so, whether those effects might be disadvantageous and thus constrain the evolution of males (see sub-section *Constraints on males; correlated response in females to selection on males*).

Phenotypic engineering on juncos

Since 1987, we have manipulated plasma levels of T in captive and free-living male dark-eyed juncos, a socially monogamous songbird that also engages in extra-pair copulations that lead to extra-pair fertilizations (EPFs). Our field site is near Mountain Lake Biological Station, Pembroke, Virginia USA. Half the males caught in spring before breeding are randomly assigned to receive subcutaneous silastic implants packed with T (experimental or T-males); the other half receive empty implants (control or C-males). The slow diffusion of exogenous T during the full breeding season prolongs the high physiological level that is present in unmanipulated males for only a brief period in early spring (59, 115). Our goals have been (1) to ascertain the hormone's diverse effects on a wide array of phenotypic traits and (2) to relate these effects to components of fitness.

In a series of papers (summarized in 60, 61), we have shown that experimentally elevated T enhances male song, home range size, and attractiveness to females and that it depresses male parental behavior, which in turn leads females to compensate for male deficiencies (21-23, 36, 51, 59, 99) (Fig 1). Physiologically, T supplementation elevates plasma corticosterone, corticosterone binding globulin, and response to stress (Fig 2). It also depresses spring body mass and fat (Fig 2) and suppresses prebasic (autumn) molt, but has no detectable effect on sperm numbers (55, 57, 63, 65, 84, 100).

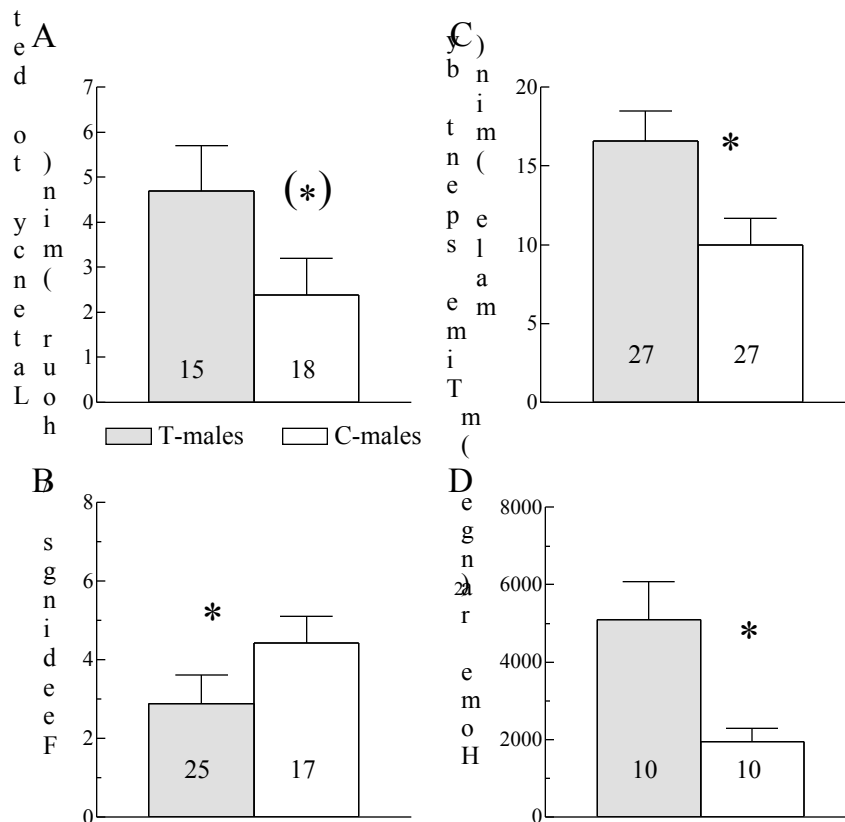


Fig. 1 Behavioral differences between testosterone-treated (T-males) and control (C-males) male dark-eyed juncos (mean ± s.e., n indicated in bars, asterisk indicates $P \leq 0.05$, asterisk in parentheses indicates $P \leq 0.1$): **A**, time to detect a simulated nest predator placed near the nest (figure redrawn from 21); **B**, rate of feeding nestlings (figure redrawn from 62); **C**, female preference as measured by mean time spent in close association with one or the other or two simultaneously presented males, one a T-male and one a C-male (figure redrawn from 36); and **D**, home range size after female has completed egg laying (figure redrawn from 23).

As for the effects that these (and possibly other) changes have on male fitness, T-males obtain *more* EPFs than C-males (i.e. fertilize more females that are mated to other males), but achieve *less* reproductive success with their own social mates (92). We have found no effect on survivorship (61, Ketterson, Nolan and Snajdr, unpublished data). Thus, to date, we have found that treatment with testosterone alters components of male fitness by affecting the balance of mating effort and parental effort, but these effects have been counteracting and have not led to a net decrease or increase in fitness.

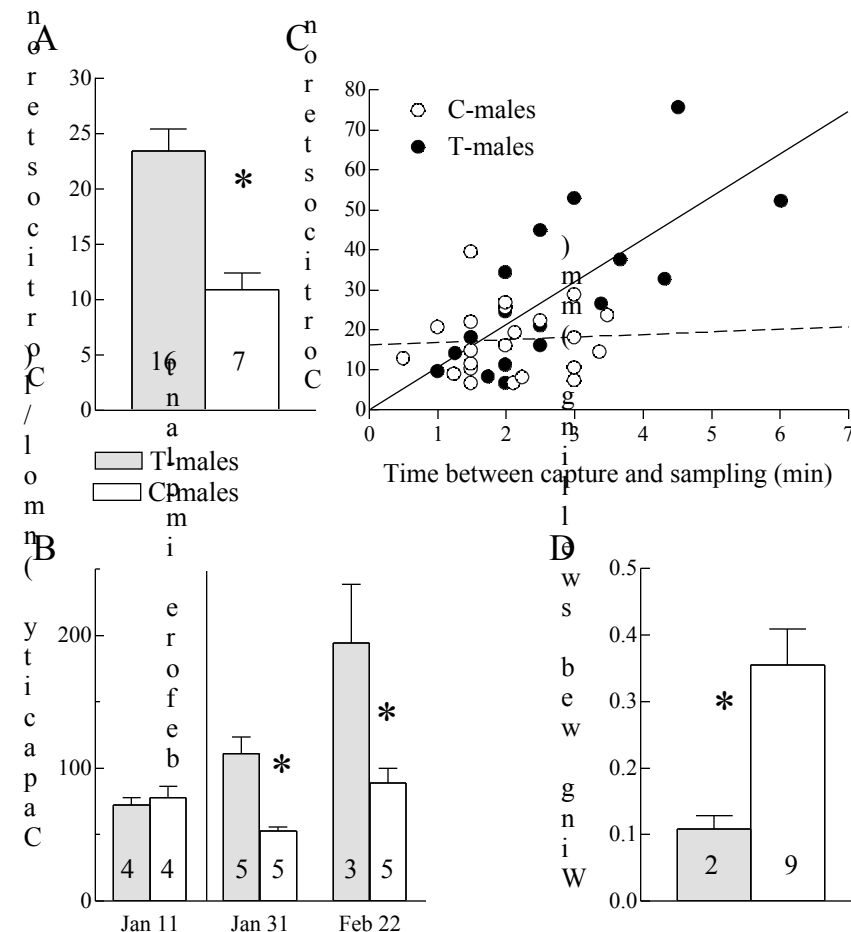


Fig. 2 Physiological differences between testosterone-treated (T-males) and control (C-males) male dark-eyed juncos (mean \pm s.e., n indicated in bars, asterisk indicates $P \leq 0.05$): **A**, resting levels of corticosterone (figure redrawn from 63); **B**, plasma concentration of corticosteroid binding globulin (figure redrawn from 65); **C**, rise in corticosterone in minutes immediately following capture (figure redrawn from 100); and **D**, cell-mediated immunity as measured by wing web swelling in response to injection with phytohemagglutinin (PHA) (19, 58).

Recent findings/future directions

More recently, in some cases we have identified additional direct effects of experimentally elevated T on the male phenotype, and in other cases have failed to find an effect, and we summarize these findings next. We have also asked *how* T might be having its effects and investigated whether T interacts with other hormones. Further, we have examined extended phenotypic effects of male testosterone-treatment on the

behavior and physiology of their social mates. Finally, we have collected preliminary data indicating that study of the levels of testosterone circulating in females is likely to be productive.

Effects of elevated testosterone on the male phenotype

T and immune function—Based on empirical data from other species (e.g. 37) and on theoretical predictions (e.g. 38), we might anticipate that treatment with T would suppress immune function. To investigate this question we exposed T- and C- males to an immune challenge, using two techniques: 1) injection of a plant lectin, phytohemagglutinin (PHA), into the wing to produce swelling (a measure of a cell-mediated immunity, procedures modified from 69); and 2) injection with sheep red blood cells (SRBC) and subsequent measurement of antibody production with a hemagglutination assay (a measure of humoral immunity, IgM, procedures of 49, 94). In free-living subjects (caught, treated, released, and later recaptured), we found less wing-web swelling in T-males than C-males (70% less, $p = 0.034$) (19, 58). Among captives, T-males were less effective than C-males at mounting a humoral response to SRBC (36% lower titers, one-tailed $p < 0.05$) (19, 58), but they did not differ from C-males in wing-web swelling (19, 58). Thus T suppressed cell-mediated immune function in free-living male juncos and humoral function in captive males, results that support the widely held view that T is immunosuppressive. However, T had no detectable effect on cell-mediated immunity in captives.

We suspect that T is less likely to have a suppressive effect on immune function in captives because in comparison to birds in the wild they are less active, have *ad lib* access to food, and (often) have fewer or less complex social interactions (compare 88). Consequently, we are continuing this project by comparing free-living T- and C-males for hemagglutination of SRBC and by employing additional antigenic challenges.

T, corticosterone (CORT), and immune function— T-implants significantly elevate CORT in house sparrows (*Passer domesticus*), European starlings (*Sturnus vulgaris*), and house finches (*Carpodacus mexicanus*) (31, 37, R. Duckworth personal comm), as well as in juncos captured in the field (58, 99) or held isolated as captives (63; Fig. 2). In other bird species (10, 11, 110), endogenous T co-varies with CORT. Returning to juncos, T-implants also engender an increase in corticosterone binding globulin (CBG) (63) and tend to produce a stronger stress response (99). Because CORT is often regarded as immuno-suppressive (6, 52, 79, 97), any effects of T on immune function may be mediated through CORT (16, 37). On the other hand, Breuner (this volume) has shown that free CORT differs less between T-

26 Avian Endocrinology

and C-male juncos than total CORT, so the physiological significance of the patterns we have observed requires further investigation.

T and molt—Testosterone is known to delay or prevent molt (e.g. 29, 32), and we have shown previously that it does the same in free-living juncos (84). To investigate this phenomenon in greater detail, we elevated T of captive males at the beginning of autumn and measured the timing and extent of molt in T- and C-males. For those males that underwent partial molt (rather than none), testosterone delayed time of onset and rate of molt but had no effect on time of termination (56, Jones, Schoech, McNabb, Nolan and Ketterson, unpubl. data).

T and coccidial function, overwinter survival— Juncos suffer from coccidiosis, a wasting disease caused by the protozoan *Isospora* spp. when it infects the epithelial lining of the gut and interferes with nutrient uptake (pers. obs.). However, we have uncovered no treatment-related difference in the incidence of coccidial oocysts in the feces of T- and C-males (54), nor have we detected any difference in overwinter survival in the two treatment groups (61, Ketterson, Nolan Snajdr, unpublished data).

T and components of male parental behavior— T's suppressive effect on male parental behavior (feeding of nestlings) has been observed in a wide variety of bird species (summary in 60), including juncos (62, 99). To examine the behavioral mechanisms responsible for suppression of nestling feeding by male juncos, we asked whether T reduces motivation to forage, but found instead that captive T-males consume more food than C-males and also choose larger food items (both groups held on long days) (24). In a related project currently being conducted in the field, we are asking whether T affects the size or number of prey items delivered to nestlings (Clotfelter, Ketterson and Nolan in prep.).

T and metabolic rate—The literature is conflicting on whether T elevates metabolic rate in songbirds, and the answer may depend on whether the measure under consideration is standard metabolic rate, in or out of the thermoneutral zone, during the active phase of the diurnal rhythm or the resting phase (113). Using doubly labeled water, we have measured daily energy expenditure in T- and C-males held captive and have found no detectable effect of testosterone on metabolic rate (72, see also 30). In keeping with lack of difference in metabolic rate, we have also found no difference in levels of thyroid hormone during molt (see below, Jones, Schoech, McNabb, Nolan and Ketterson, unpubl. data, 55). Among the captives, foraging was more frequent by T- than C-males (24, 56, see also 72) and T-males were more active (72). Both observations predict elevated metabolism, so the failure to see a difference in metabolic rate remains a paradox.

How does experimentally elevated testosterone affect the phenotype?: interactions with other hormones and neuroanatomy

Understanding the evolution of T-mediated traits (or traits mediated by any other hormone) will require more than enumeration and quantification of the phenotypic effects of experimentally elevated plasma levels. It will also require knowledge of mechanisms, since it is these that must vary and be acted upon by selection if hormonally mediated traits are to evolve. The relationship between hormones and the total phenotype is obviously hugely complex, and selection might act on variation in any of the following: (1) responsiveness to stimuli that promote or suppress hormone secretion, (2) transport mechanisms and half-life of the hormone in the circulation, (3) localization and activity of enzymes responsible for steroid synthesis, (4) localization, capacity, or affinity of hormone receptors, and (5) interactions with other hormones. Thus, in nature, if selection were acting on a trait whose expression was enhanced by testosterone, e.g., song, it might equally favor individuals whose song control nuclei were increased in size (with effects on song characteristics) owing to higher plasma levels of T or greater responsiveness to a given level of T, or it might favor individuals whose song was enhanced owing to reduced inhibition of song control nuclei by another hormone (e.g. melatonin, 13, 14).

With respect to interactions among hormones, we have considered how plasma levels of testosterone might affect secretion of and responsiveness to other hormones (see analogy with *physiological epistasis*, 60). For example, the testosterone-corticosterone interactions just described indicate that some of the effects of T on male juncos are likely to reflect T's effect on CORT. Similarly, we asked whether T's suppressive effect on parental behavior might be mediated through an interaction with prolactin (PRL), and we found no difference between T- and C-males in plasma levels of PRL or in the binding of PRL by the preoptic area (99). A comparable inquiry into whether experimentally elevated T suppresses molt by lowering plasma levels of T_3 and T_4 showed that T had no such lowering effect (Jones, Schoech, McNabb, Nolan and Ketterson, unpubl. data). Likewise, experimentally elevated T had no effect on plasma levels of LH (T-males = 0.97 ng/ml, C-males = 1.32 ng/ml, $n = 10, 12$, $p = 0.256$, Schoech, Nolan and Ketterson, unpubl. data).

In another mechanistic study whose intent was to explore the mechanisms underlying testosterone's phenotypic effects, we asked whether the greater home range size that characterizes T-males might be mediated by a T-induced increase in hippocampal volume, but found no such effect (107).

The extended phenotype

Defining the boundary of the phenotype is a controversial topic. According to Dawkins (28), the “conventional phenotype” is a special case in which a gene’s effects are confined to individual organisms; but more typically, he argued, the effects of genes extend to other organisms and to the physical environment (e.g., a bird’s nest is a manifestation of the bird’s “extended phenotype”). Extrapolating from the effects of genes to those of hormones, the extended phenotype can be described as all of the effects of a hormone - not simply those detectable in the organism that produces it - but also those affecting other individuals (60). If these effects are substantial, then the hormone extends to, becomes part of the environment of, other individuals and may affect their behavior and physiology. Applying this argument to our inquiry, when attempting to predict the possible evolutionary consequences of enhanced T in males, we must know whether the enhancement indirectly affects females or offspring in ways that might react on male fitness. As an example, which we have already investigated, if females mated to T-males were less likely to re-mate with them following nest failure, this result could lower male reproductive success. We found no such effect of T on the pair bond (62), but the possibility illustrates the potential impact of the male’s extended phenotype on his own fitness.

Application of the extended phenotype concept to hormones

This concept of the extended phenotype of a hormone is closely related to a fundamental precept of behavioural endocrinology, which is that “hormones affect behavior, and behavior affects hormones” (e.g. 81, p.16). The physiology of male birds, for example, is influenced by the behavior of the female (e.g. 96, 109), and many studies (but not all, e.g. 96) indicate that females respond to T-mediated attributes of males. Thus, female canaries (*Serinus canaria*) spend more time nest building if they hear recorded male song (53) and Bentley *et al.* (15) have recently shown that ovarian follicles grow more rapidly and more eggs are laid when female canaries are exposed to male song. In what may be a related phenomenon of response to a visual stimulus, Gil *et al.* (41) found that when female zebra finches are mated to attractive males (i.e. males wearing red leg bands), they deposit higher levels of androgens in their eggs than do females mated to less attractive males. What follows is a summary of recent findings regarding the influence of a male junco’s hormonal state on the behavior and physiology of his mate.

Egg size, egg steroids

In nature, egg size of female mates of T- and C-males (hereafter *t- and c-females*) differs: egg volume increases with laying order in t-females but not in c-females, and the first- and second-laid eggs of t-females are

significantly smaller than those of c-females (20, Casto, Ketterson and Nolan unpublished data). Interestingly, this effect on egg size is stronger early in the season when weather and food supply are less favorable (20, Casto, Ketterson and Nolan unpublished data unpublished data). Recalling that T-male juncos are more attractive to females than are C-males (36, 51), we predicted that the concentration of androgens in yolks produced by t-females would be higher than in yolks of c-females (compare 41), but we found no such effect (20, Casto, Ketterson and Nolan unpublished data).

Female home range size

Females mated to C-males (i.e. c-females) are more likely than t-females to rear young that are sired by EPFs, and the EPF-sires are more likely to be T-males than C-males (92). We therefore attempted to determine whether c-females were more likely than t-females to make movements off the territories of their social mates during the period when they were building nests and laying eggs, i.e. when presumably they were fertile. Radio-tracking of 13 females, however, did not reveal any more off-territory movements by c-females than t-females, leaving open the question of whether it is the behavior of males or females that is decisive in determining extra-pair paternity and also the question of how high testosterone increases male success in achieving EPFs (Neudorf, Nolan and Ketterson unpubl. data).

Progeny sex ratio

To determine whether differences in attractiveness of males would affect the sex ratio of young produced by females (as has been found to be true of other species, e.g. 17, 34, 101), we compared brood sex ratios of t- and c-females during the breeding seasons of 1995-1998. We extracted 204 blood samples from 51 full broods (broods with no egg or nestling mortality) and found no difference in the proportion of female offspring (t-broods, 0.507; c-broods, 0.505) (46).

Female stress response, parental behavior, and immune function

Females mated to T-males (i.e. t-females) tend to exhibit a steeper initial rise in CORT in response to handling than c-females (100). They also feed offspring more frequently as they compensate for the reduced parental care of their mates (62). Both observations raise the possibility that being mated to a T-male might lead to suppression of a female's immune function, and when we assessed cell-mediated immune status by injection with PHA, we found greater wing-web swelling in c- than t-females (Casto, Parker-Renga, Ketterson and Nolan, unpubl. data)

In sum, aspects of a female junco's phenotype are affected by the hormonal status of her mate, and these include egg dimensions, response to stressors, parental behavior, and immune status. For other aspects -

30 Avian Endocrinology

egg steroids, offspring sex ratio, and home range size - we were not able to detect any effect. Whether we would expect male fitness to be influenced by the female's responses depends upon a number of unknowns, including whether the detected effects have fitness consequences for females, whether they affect the bond between males and females, and whether female mates are so readily replaceable that new, unaffected mates are quickly acquired. Regardless, it is clear that selection for elevated testosterone in males would be likely to have indirect effects on the behavior and physiology, and possibly the fitness, of females.

Constraints on males; correlated response in females to selection on males?

Theoretically, natural or sexual selection on testosterone-mediated traits in males could affect females in ways that go beyond modifying their environment and thus the phenotypes they express. Supposing that females prefer T-mediated traits in males, as studies suggest they do (3, 36, 40, 51, 92, 114, 122), then, if genetic correlations between the sexes are strong, sexual selection on males might be expected to engender a correlated genetic response in females. If, further, the response in females is detrimental to them, e.g., if the selection were to masculinize females in disadvantageous ways, then further evolution of sexual dimorphism could be stalled (8, 66, 78, 90, 91).

A number of studies of birds have asked whether male-like traits are beneficial, neutral, or detrimental when expressed in females (e.g. 4, 27, 50, 80, 89, 93). These studies were not mechanistic however, and most of them were of plumage traits whose physiological basis is poorly understood but not likely to involve testosterone (64). It appears that, except in model species, very little attention has been devoted to determining whether traits that are testosterone-mediated in males are also testosterone-mediated in females and whether they have fitness consequences for females.

A first step in a research program focused on testosterone in females would be to describe natural variation in T. For example, several workers have noted natural, age-related variation among females in either testosterone or the degree of expression of male-typical traits (83, 85, 108). Among the important questions for which more information is needed are these: Do females exhibit seasonal profiles in testosterone like those characterizing males? Given that seasonal profiles of testosterone in males are affected by a species' mating system (115), is the same true of females? Similarly, are plasma levels of T higher in females of species in which females are highly aggressive or

ornamented, as compared to species in which females are less aggressive or ornamented?

In juncos, which we think of as typical north-temperate passerines, the seasonal pattern of plasma T is similar in males and females (Ketterson and Nolan unpubl. data, Fig. 3). It is highest in the early spring during territory establishment and pair formation and prior to nest building, declines during egg laying and incubation, and rises again during the nestling phase. Whether females of many other species resemble juncos is difficult to know, because T-levels are less often reported for females than for males, perhaps because they are often so low as to be undetectable with current assay techniques. But for those species that have been studied, peaks in T near the onset of breeding have been reported for both males and females (e.g. 47, 70, 77, 116).

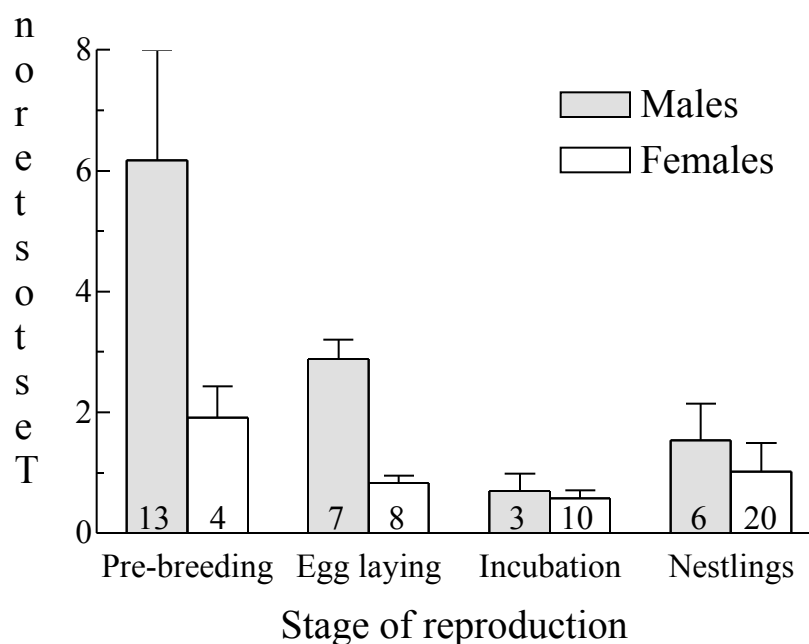


Fig. 3 Plasma concentration of testosterone in untreated male and female juncos captured at Mountain Lake Biological Station, 1989, and classified according to known stage of reproduction (Anova, stage $p = 0.000$, sex $p = 0.054$)(Ketterson and Nolan unpubl. data).

Having found evidence that plasma T varies seasonally in female juncos to produce a profile like the profile of males, next steps in a research program focused on whether hormonal effects in females might

32 Avian Endocrinology

constrain the evolution of males would be to establish (1) the degree to which testosterone-mediated traits are heritable and (2) the strength of any genetic correlations between the sexes. Outside of Galliformes (e.g. 82, 117), we have found surprisingly little information on the heritability of T or T-mediated traits in male birds, and no information on whether plasma levels or trait expression are correlated in male and female relatives. Because in birds females are the heterogametic, organized sex, we might expect them to be de-masculinized early in development and thereby insulated from the consequences of selection on male-typical traits. However, this preconception probably reflects the fact that research to date has emphasized traits that are sex-limited in their expression, e.g., mounting behavior in male Japanese quail (1, 9). Traits that are weakly dimorphic and considered masculine (e.g. greater activity levels and appetite) may be more likely candidates for modifying females by means of correlated selection, because while such traits are expressed at higher levels in males, they are also expressed in females. Consequently, selection on such traits in males may affect loci that are active in females as well. Still another way to address whether a response in females to selection on males might constrain males would be to manipulate testosterone in females, quantify any effects, and relate them to female survival and reproductive success (compare studies already performed on males as described above, see also 2, 33). Experimentally induced reductions in female fitness would be consistent with a hypothesis of constraint. We are presently conducting such studies.

Conclusion

An objective of this paper has been to consider how to strengthen understanding of the links between physiological mechanisms and adaptation and constraint. We have emphasized what we have learned from manipulating plasma T in male dark-eyed juncos and what we hope to learn from manipulating T in females. By tracing multiple and subtle effects of these manipulations, we hope to increase knowledge of how hormones affect the phenotypes and fitness of animals living free in nature. We expect animals that deviate from the norm to be selected against, but only by testing that idea in diverse ways, and over time, can we learn how robust it is.

Acknowledgments

We appreciate financial assistance from the National Science Foundation (most recently IBN-9728384 to EK and VN) and logistical support from the University of Virginia's Mountain Lake Biological Station. Many very able and energetic students and field assistants helped to collect the data described here, and we are grateful to all of them. We thank the Mountain Lake Wilderness Conservancy, the Mountain Lake Hotel, and

the Jefferson National Forest for permission to work on their property. Thanks also to Creagh Bruener and George Bentley for useful discussions and to Alistair Dawson for inviting us to contribute to this volume.

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