Regulation of male traits by testosterone: implications for the evolution of vertebrate life histories

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Summary

The negative co-variation of life-history traits such as fecundity and lifespan across species suggests the existence of ubiquitous trade-offs. Mechanistically, trade-offs result from the need to differentially allocate limited resources to traits like reproduction versus self-maintenance, with selection favoring the evolution of optimal allocation mechanism. Here I discuss the physiological (endocrine) mechanisms that underlie optimal allocation rules and how such rules evolve. The hormone testosterone may mediate life-history trade-offs due to its pleiotropic actions in male vertebrates. Conservation in the actions of testosterone in vertebrates has prompted the 'evolutionary constraint hypothesis,' which assumes that testosterone signaling mechanisms and male traits evolve as a unit. This hypothesis implies that the actions of testosterone are similar across sexes and species, and only the levels of circulating testosterone concentrations change during evolution. In contrast, the 'evolutionary potential hypothesis' proposes that testosterone signaling mechanisms and male traits evolve independently. In the latter scenario, the linkage between hormone and traits itself can be shaped by selection, leading to variation in trade-off functions. I will review recent case studies supporting the evolutionary potential hypothesis and suggest micro-evolutionary experiments to unravel the mechanistic basis of life-history evolution. ***BioEssays*** **29:133–144, 2007.**

Introduction

Populations vary widely in life-history traits such as developmental rates, age at maturity, fecundity and lifespan.[1,2] Despite this variation, certain combinations of life-history traits are not found in nature.[3,4] For example, individuals within populations that maximize life-history traits such as fecundity typically cannot simultaneously maximize survival, and individuals within populations that maximize offspring size cannot maximize offspring number at the same time. These negative correlations between life-history traits imply the existence of ubiquitous trade-offs.[1–3] On a proximate level, life-history trade-offs are thought to result from limitations in the availability of critical resources such as energy, nutrients or time, necessitating decisions on the differential allocation of resources to costly traits.[4,5] For example, organisms that invest their resources primarily into reproductive function have fewer resources to invest in self-maintenance processes such as fat storage or immunity.[6] Selection is expected to favor the evolution of divergent optimal allocation rules in populations that live in different habitats. For example, populations experiencing low adult survival rates should invest more strongly in reproduction compared to populations with high adult survival rates.[1,2] Life-history theory also predicts that selection will promote efficient physiological mechanisms that mediate allocation strategies,[7] for example by linking life-history traits into trade-offs (e.g. via 'antagonistic pleiotropy'[8]).

What are the physiological mechanisms that underlie life-history trade-offs? Hormones are increasingly being considered to be mediators of life-history trade-offs.[5,8–14] Hormones are substances that are typically secreted into the general circulation and thus can reach all parts of the organism.[15] Furthermore, hormones transduce environmental information and regulate transitions between major life-cycle stages such as maturation, metamorphosis and reproduction, in which organisms face different trade-offs.[13,16] Finally and perhaps most importantly, most hormones have pleiotropic and often antagonistic effects on a variety of behavioral, physiological and morphological traits.[8,11,17,18]

The steroid hormone testosterone has become a prominent candidate for mediating male reproductive trade-offs in vertebrates.[7,10,19] Testosterone increases male reproductive success by promoting courtship and sexual behaviors, territorial aggression, secondary sexual characters and sperm

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production while often simultaneously decreasing fitness by suppressing traits such as immune function and parental care.[17,19,20] Such pleiotropic antagonistic actions of testosterone on male traits might be an important part of the mechanistic cascade that mediates reproductive trade-offs.

If testosterone represents a mechanism for mediating trade-offs across vertebrate taxa, how can different resource allocation strategies evolve? The observed evolutionary conservation in the regulation of traits by testosterone in male vertebrates could suggest that this system evolves as a complex, with the linkage between testosterone and male traits being inseparable (Fig. 1a).[17] This could be due to, for example, insufficient genetic variation among vertebrate taxa in the linkage between testosterone-signaling processes and target tissue responses, such that selection cannot dissociate individual traits from a control by testosterone (Fig. 1a). Hence, the evolutionary enhancement of one trait via an increase in the release of testosterone into the circulation would always have the consequence of suppressing another trait. This 'evolutionary constraint hypothesis' implies that circulating concentrations of testosterone are the primary target of selection, which is supported by the existence of large variations in circulating concentrations of testosterone among vertebrate species.[15,18]

An alternative hypothesis is that selection shapes the linkage between testosterone and male traits, with different components of the testosterone-signaling cascade evolving independently from each other, thereby altering the strength of the linkage or even dissociating certain traits from a control by testosterone (Fig. 1b, see also[8,11,21–23]). Hence, the pleiotropic actions of testosterone could evolutionarily be modified and would not constrain in the diversification of life histories. This 'evolutionary potential hypothesis' predicts that circulating concentrations of testosterone might be one target of selection, but many other processes in the testosterone signaling cascade, for example those that determine tissue responses can evolve equally likely. Because it assumes the linkage between testosterone and male traits to be evolutionarily plastic, the 'evolutionary potential hypothesis' predicts that other hormones (or non-hormonal factors) can regulate male traits involved in life-history trade-offs, either in combination or independent of testosterone. The 'evolutionary potential hypothesis' is supported by a number of recent studies demonstrating interspecific variation in the linkage between testosterone and male traits. For example, there exists large variation in the actions and specific pathways by which testosterone regulates several behaviors that enhance male reproductive effort, including vocalizations (song) and

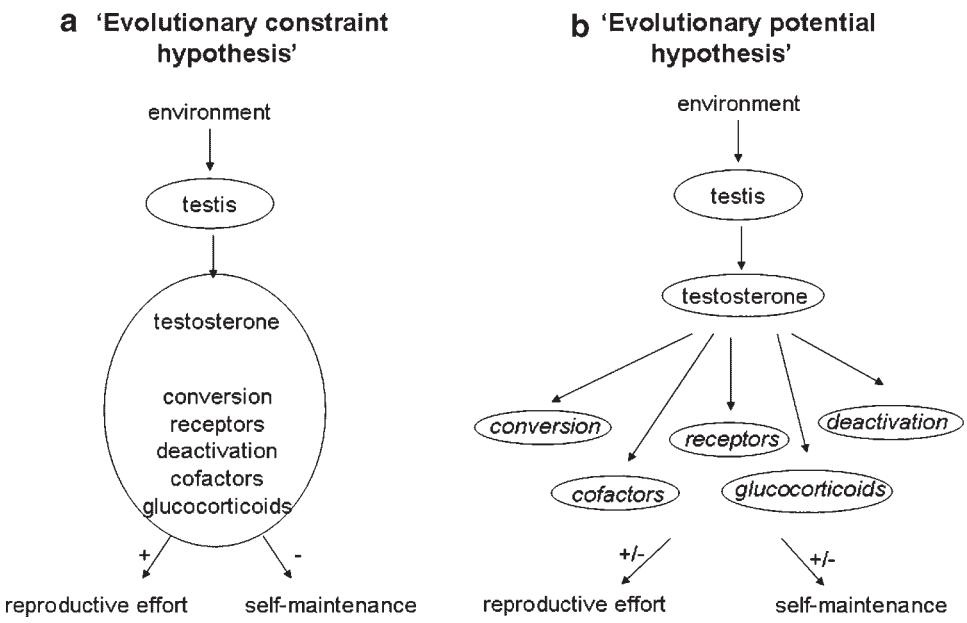

Figure 1. Physiological scenarios representing **a** the 'evolutionary constraint hypothesis' and **b** the 'evolutionary potential hypothesis' on the linkage between testosterone and of life-history traits in male vertebrates. Circles with solid lines indicate evolutionary units (i.e. units on which selection may act). In the 'evolutionary constraint hypothesis', testosterone synthesis, responsiveness at target tissues and life-history traits are tightly linked and co-evolve. In the 'evolutionary potential hypothesis', testosterone synthesis and mechanisms of tissue response can evolve independently from each other. Note that the various levels of endocrine organization can also interact with each other, but potential connections were not graphically depicted for clarity.

aggressive behavior,[24,25] as well as other components of fitness such as immune function.[26] For some behaviors such as paternal care, even closely related species differ in whether it is regulated by testosterone or not.[27]

The aim of this review is to examine the evidence for the 'evolutionary constraint hypothesis' versus the 'evolutionary potential hypothesis', focusing on the trade-off between reproductive effort and survival. Comparative studies on the regulation of life-history trade-offs by testosterone are still largely lacking. I will therefore discuss recent findings from case studies that examined the linkage between testosterone and individual traits. The focus will be on manipulative studies, as these most conclusively document the involvement of testosterone in controlling traits. I will discuss separately the two classical actions of steroid hormones with regard to the regulation of trade-offs: the permanent differentiation of traits during early development ('organizational effects') and the transient activation of traits during adulthood ('activational effects'). Finally, I will outline experiments that might help clarify how hormones and life-history traits evolve.

Testosterone and male reproductive trade-offs

Testosterone is a sex steroid mainly produced by the testes[15] (although recent research has provided strong evidence that other organs such as the adrenals and the brain also possess the enzymes necessary for the production of steroids including testosterone[28]). Gonadal testosterone secretion occurs typically at the beginning of the breeding season to support reproductive processes such as courtship and sexual behaviors, territorial aggression, the expression of secondary sexual characters and sperm production.[15] The classical way by which testosterone regulates traits is via genomic pathways.[23,29] Its most direct action is by binding to intracellular androgen receptors. This leads to the formation of a hormone–receptor complex, which then binds to hormone response elements in the promotor region of genes to modulate gene transcription. A second androgenic pathway consists of the conversion of testosterone to 5α-dihydrotestosterone (DHT) by the enzyme 5α-reductase (Fig. 2), which also acts via androgen receptors. A third, potent pathway is estrogenic; testosterone is converted by the enzyme aromatase (CYP19) into 17β-estradiol (E2) (Fig. 2), which then binds to estrogen receptors. The biological actions of any of these steroids can be further modified, for example by steroid-binding proteins affecting the availability of the hormone at target cells, steroid receptor co-factors altering the genomic actions of the hormone–receptor complex and other downstream mechanisms modulating the tissue response.[30,31] Recent research has shown that steroids can also exert non-genomic effects via membrane receptors or novel interactions with classical steroid receptors.[32]

Many correlative studies have suggested that testosterone plays a role in reproductive trade-offs in male vertebrates, but the strongest and most-conclusive support comes from experiments that manipulated circulating testosterone concentrations in individuals ('phenotypic engineering').[33] These studies administered physiological doses of testosterone to free-living male vertebrates and estimated the resulting fitness effects on various traits (summaries in Refs 5,12,17,34). One of the earliest experimental demonstrations that testosterone mediates trade-offs in vertebrates was conducted in lizards.[9] In free-living male mountain spiny lizards (*Sceloporus jarrovi*), testosterone-implanted males showed increased territorial aggression,[35] but had reduced survival compared to males carrying an empty implant.[9] The most-comprehensive experimental studies on testosterone and life-history trade-offs have been conducted on a songbird species, the dark-eyed junco (*Junco hyemalis*).[17,33,34,36,37] Testosterone-implanted free-living male juncos performed more courtship displays, had larger home ranges, and obtained more extra-pair fertilizations. However, testosterone-implanted males also provided less parental care, had reduced nest defence, suppressed immune function, delayed molt and decreased survival compared to males carrying empty implants. Similar

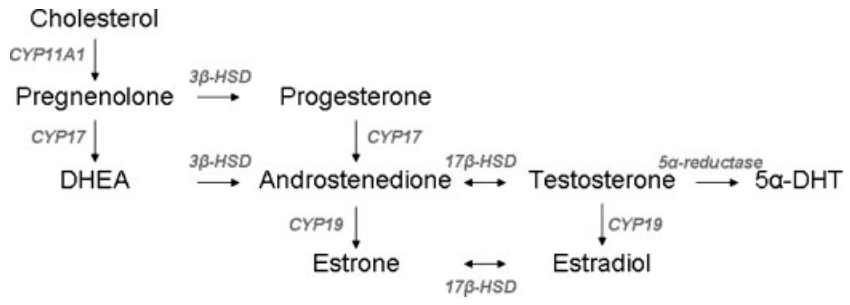

Figure 2. Schematic representation of sex steroid synthesis. Enzymes in italics are abbreviated as: CYP11A1, cytochrome p450 side-chain cleavage; CYP17, cytochrome p450 17α-hydroxylase/C17,20lyase; CYP19, P450 aromatase; 3β-HSD, 3β-hydroxysteroid dehydrogenase/Δ5-Δ4isomerase; 17β-HSD, 17β-dhydroxysteroid dehydrogenase. Hormone abbreviations are: DHEA, dehydroepiandrosterone; DHT, dihydrotestosterone. Modified from Ref. 100.

results have been reported in studies on several other avian species (reviewed in Ref. 17).

Testosterone might play a similar role in mediating life-history trade-offs in other vertebrate taxa, though the evidence currently is less complete. In mammals, testosterone boosts reproductive effort (sexual and aggressive behavior) across taxa[24] and appears to have a generally suppressive effect on immune function.[26,38] In fish and amphibian species, experimental androgen administration increases male reproductive and aggressive behavior across species (reviewed in Refs 39,40). However, the effects of androgens on immune function have so far rarely been tested in these taxa. Below I will discuss whether the linkage between testosterone and male traits in vertebrates constrains the evolution of life history trade-offs.

'Organization' of traits by testosterone during development

Sexual differentiation

The classical organizational effects of testosterone occur during early ontogeny, when this hormone permanently differentiates morphological, physiological and behavioral traits between sexes. For example, during embryonic development, the testes of male mammals begin to secrete testosterone, which organizes male accessory sex organs, body development and the central nervous system.[15,18] Such organizational actions of testosterone might generate a developmental constraint.[17] Indeed, in mammals, the permanent effects of testosterone on behavioral and physiological traits are pervasive and seemingly conserved,[15,18] suggesting that they indeed may constrain evolutionary variation. However, while the sexual differentiation by testosterone of individual traits is well-documented, the implications for life-history trade-offs have been rarely tested.

In non-mammalian vertebrates, sexual differentiation appears less dependent on sex steroids than in mammals, thus reducing the potential for testosterone to represent a constraint. In birds, early estradiol exposure influences the sexual differentiation of some traits, but direct genetic processes that are independent of gonadal sex steroids might be equally important for the sexual differentiation of many traits.[41,42] For example, in Japanese quail (*Coturnix japonica*), early estradiol administration feminizes sexual behavior of males (such as attraction to the opposite sex, mounting and copulation). In contrast, in zebra finches (*Taeniopygia guttata*), early exposure to estradiol can masculinize behavior of females (song and sexual partner preference), but testosterone exposure has little effect.[43] Thus, where organization of sexually dimorphic traits by sex steroids has been shown in non-mammalian vertebrates, there exists large variation in how these hormones affect the phenotype.[42] Research on the organization of traits by steroid hormones in lower vertebrates is still in its infancy compared to mammals or birds, but so far the majority of sex-specific traits appear to be activated rather than organized.[18]

Alternative phenotypes

Recently, organizational actions of sex steroids in addition to sexual differentiation have been demonstrated, such as the differentiation into alternative phenotypes within one sex. Such phenotypes are genetically determined in some of the vertebrate species studied (e.g. alternative phenotypes in birds[44]) while, for other phenotypes, it is still unclear whether they have a genetic basis (e.g. alternative phenotypes in lizards[45]). How general are such 'non-traditional' organizational effects across vertebrate species and do they affect reproductive trade-offs?

In several species, males assume phenotypes (or morphs) with divergent reproductive strategies. For example, a territorial morph may defend a territory to attract females while a satellite or sneaker morph may forgo territory establishment and attempt to sneak copulations. Moore and colleagues proposed that, in species with morphs that display fixed (permanently differentiated) reproductive strategies, morph-specific traits are organized by steroid hormones.[46] Indeed, in fixed morphs of the tree lizard (*Urosaurus ornatus*) early exposure to testosterone (or progesterone) of males can alter their morphological phenotype (color of the dewlap and body size), which closely corresponds with the behavioral phenotype (territorial versus non-territorial behavior).[47] However, effects of testosterone on life-history trade-offs, for example permanent changes in immune function, have not yet been tested in this species. Nonetheless, one could expect such effects because, in the common lizard (*Lacerta vivipara*), prenatal testosterone exposure decreases measures of immune function.[48]

Maternal effects

Exposure to steroid hormones in utero or in ovo can have major effects on phenotypes and may organize life-history trade-offs as well. Whether these effects have a genetic basis is still largely unclear. One of the most notable examples for an organizational effect in utero in mammals is the masculinization of female hyena (*Crocuta crocuta*) genital morphology. Hyena fetuses are exposed to large amounts of maternal androstenedione in utero which is converted by the placenta into testosterone.[49] This process was earlier presumed to cause the masculinization of female genitalia. However, recent research suggests that other, probably genetic, factors are responsible for the expression of a pseudopenis in females and that androgen exposure during development merely alters the shape of the structure to more closely resemble that of males.[50] Nevertheless, maternal androgens might organize aggressive and sexual behavior in hyena offspring,[51] thus potentially influencing reproductive trade-offs.

In litter-bearing rodents, the proximity of a fetus in utero to male or female siblings determines its exposure to sex steroids during early development.[52] Offspring that develop between two males are exposed to more testosterone during development—secreted by the testes of their male siblings during late gestation—than offspring situated between a male and a female or two female siblings. Such differential intrauterine exposure to testosterone due to positioning affects aggression and parental care, and alters life-history traits such as the timing of puberty, life-time fecundity and offspring sex ratio of individuals in adulthood.[52]

In oviparous species, the yolk surrounding the developing embryo contains considerable amounts of androgens. In avian species, experimental testosterone administration to egg yolk enhances nestling begging behavior, food competitiveness and growth while, at the same time, reducing immune function.[53] These studies suggest a potential for yolk steroids to permanently alter reproductive trade-offs.

Conclusion

From the studies above, it appears that organizational effects of testosterone during sexual differentiation may have the potential to pose developmental constraints, especially in mammalian taxa. However, it appears unlikely that those are 'absolute constraints' (sensu[54]) as they might be offset by epigenetic organizational effects in utero. Rather, the organizational actions of testosterone might pose 'relative constraints' that introduce a bias in the evolution of life-history trade-offs,[54] essentially slowing down the rate of evolutionary change instead of making it impossible. In birds, interspecific variation exists in whether and how sex steroids organize sex-specific behaviors, such as between members of different clades like non-passeriform (e.g. non-passeriform taxa like quail versus passeriform taxa like zebra finches). In other non-mammalian vertebrates, testosterone seems to have only limited potential to organize traits and to influence trade-offs. Taken together, although the available evidence is still sparse, the existing data provide only limited support for the 'evolutionary constraint hypothesis'.

Activation of traits by testosterone in adulthood

Across vertebrates, testosterone promotes traits that enhance short-term reproductive success, for example by boosting behaviors such as courtship, copulation, song and territory defense.[7,15,17] In many species, testosterone appears to suppress traits such as parental behavior and immune function at the same time, perhaps corroborating the 'evolutionary constraint hypothesis'. However, detailed studies in recent years have documented considerable evolutionary variation in the existence and strength of the linkage between testosterone and several traits involved in reproductive trade-offs. A comprehensive review of these findings is beyond the scope of this article, therefore I focus on studies that illustrate compellingly the existence of such evolutionary variation in three exemplary traits: aggressive behavior, parental behavior and immune function. Similar evolutionary variation in the linkage between testosterone and other traits likely exists as well.

Aggressive behavior

Aggressive behavior serves to establish dominance relationships among animals and can generate fitness benefits by ensuring access to resources (i.e. food, territories and mates). In the majority of male non-tropical vertebrates, circulating concentrations of testosterone increase during the breeding season to promote the expression of aggressive behavior (recent summaries in Refs 55–57). However, in birds, recent comparative studies documented that tropical species have lower peak testosterone concentrations during the breeding season compared to non-tropical species, and that seasonal fluctuations in testosterone can be slight or even absent.[58–60] Detailed experimental studies, the year-round territorial spotted antbird (*Hylophylax n. naevioides*), suggest that testosterone can regulate aggressive behavior even in a species with low circulating concentrations of this hormone, as the pharmacological manipulation of testosterone actions did modulate aggressive behavior.[61] However, the regulation of aggressive behavior by testosterone in male spotted antbirds differs from what is known for most temperate birds in at least two ways.[62,63] First, instead of increasing circulating testosterone concentrations during the breeding season, male spotted antbirds generally maintain low testosterone concentrations and only increase plasma levels during high-intensity aggressive interactions lasting longer than two hours.[64] Second, in many temperate-zone male birds the seasonal regulation of testosterone concentrations and brain sex steroid receptor expression are temporally linked, both being elevated during the breeding season.[65,66] In contrast in male spotted antbirds, the seasonal regulation of circulating testosterone concentrations and sex steroid receptors in certain brain areas is uncoupled such that receptor expression is increased during the non-breeding season when circulating testosterone concentrations are lowest (Fig. 4, see also Box 1).[67] These findings in spotted antbirds strongly suggest evolutionary independence in testosterone signaling (secretion rates) versus response mechanisms (receptor dynamics).

In another tropical species, the rufous-collared sparrows (*Zonotrichia capensis*) from Ecuador, male peak testosterone concentrations are similar to those of higher latitude *Zonotrichia* congeners.[68] However, experiments in which the actions of testosterone were pharmacologically manipulated have not indicated that testosterone is involved in regulating male aggressive behavior in these birds.[69] Further experiments are needed to conclusively determine in this species

Box 1. The difference between the sexes.
Comparing the two sexes can be a powerful approach to study the linkage between testosterone and traits, and advance our understanding of life history evolution.[36,85] Males and females share large parts of their genome, raising the possibility that selection on traits in one sex constrains the evolution of the same traits in the other sex.[36,85] For example, selection pressures favoring a control of aggressive behavior by testosterone in males could have led, via correlated evolution, to a corresponding linkage between testosterone and aggressive behavior in females. Indeed, in many vertebrate species, females are as aggressive as males. Peak plasma testosterone concentrations of males and females during the breeding season are correlated in many species.[36,85,86] Furthermore, females possess androgen receptors in brain areas associated with aggressive behavior, and experimental administration of testosterone often enhances female aggressive behavior.[87] These findings support the hypothesis of correlated evolution. However, a number of experimental studies in female birds found overall low testosterone concentrations and a lack of testosterone increases during aggressive behavior.[88–90] In some species females may be highly sensitive to such low concentrations of testosterone by having an increased number of androgen receptors in the brain.[91] In other species, females may involve other steroids in the regulation of aggressive behavior. For example, female mountain spiny lizards (*Sceloporus jarrovi*), show elevated plasma concentrations of both testosterone and estradiol at times of the year when aggressive behavior is highest.[92] Removal of ovaries decreases, and testosterone implantation only partially restores, aggressive behavior, suggesting that estrogens are involved in controlling aggressive behavior.[93] Likewise, in female rats, ovariectomy decreases aggressive behavior (towards a female intruder), and only a combined administration of testosterone and estradiol restores it.[94] Overall, in female rats, aggressive behavior appears to be regulated by multiple steroid hormones, with progesterone having an inhibitory effect.[95] Thus, even though only few detailed studies exist,[36] the available evidence suggests that selection shapes the endocrine mechanisms that control behavioral traits in male and female vertebrates separately.

whether aggressive behavior is indeed dissociated from a control by testosterone. Still, the studies on rufous-collared sparrows illustrate that the linkage between testosterone and aggressive behavior can vary even within a genus.

In many rodent species, testosterone controls male aggressive behavior.[15] However, recent studies showed that Syrian (*Mesocricetus auratus*) and Siberian hamsters (*Phodopus sungorus*) display increased aggressive behavior under short-daylength conditions of winter, when their gonads are regressed and plasma testosterone concentrations are low.[24] Interestingly, administration of exogenous testosterone to Siberian hamsters at this time of year reduced aggressive behavior.[70] Instead, the indoleamine hormone melatonin produced in the pineal gland appears to support aggression during the non-breeding season in hamsters, perhaps indirectly via actions on adrenocortical hormones.[24] Thus, in these rodents, the linkage between testosterone and aggressive behavior has changed, and other hormones have become more important in regulating this trait.

Paternal care

Testosterone may mediate the trade-off between current and future reproductive effort in male vertebrates by stimulating aggressive and sexual behavior and suppressing parental behavior.[10,12,71] Indeed, in many species, circulating testosterone concentrations during the breeding season drop precipitously when males become parental, and experimental testosterone administration suppresses parental behavior in the majority of avian species studied so far.[71] This trade-off has so far best been studied in avian species in which males contribute substantially to parental care. Such suppression of parental behavior can decrease fitness by reducing the number of offspring that survive to fledging.[27]

However, recent work in several bird species showed that testosterone administration during the parental phase does not always suppress paternal behavior, even though it does increase sexual behavior.[72,73] Variation in the suppressive effects of testosterone on paternal care exists even among closely rated species: testosterone administration suppresses paternal care in snow buntings (*Plectrophenax nivalis*), but not in two closely related longspur species (*Calcaricus spp*).[27,72] The 'essential parental care hypothesis' proposes that the necessity for biparental care to successfully raise the offspring in certain environments selects for an uncoupling of parental care from the suppressive effects of testosterone.[27]

Even though males of most lower vertebrates show a decrease in circulating androgen concentrations during the parental phase, testosterone administration does not suppress parental care in several reptile, amphibian and fish species (for summaries see Ref. 74). In mammals, experimental evidence for a suppressive effect of testosterone on parental care is also mixed. As in many birds, testosterone administration to male Mongolian gerbils (*Meriones unguiculatus*) suppressed, while castration increased, paternal behavior.[75] However, in other rodent species, castration either failed to alter, or even reduced, paternal behavior.[76]

Similarly, in the monogamous California mouse (*Peromyscus californicus*) testosterone via conversion into estradiol promotes paternal behavior.[77,78] Post-partum mating in these rodents requires males to display sexual and paternal behaviors at the same time, perhaps selecting for an insensitivity of paternal behavior to the suppressive effects of testosterone.[77,78]

Immune function

The long-known suppressive effects of testosterone on aspects of immune function have sparked the idea that testosterone might be the physiological mechanism that mediates the trade-off between reproductive effort and survival by impairing immune function.[6,19] There exists both correlative and experimental evidence across vertebrate species that testosterone suppresses aspects of immune function.[19,79] For example, early studies on laboratory models like rats and chicken demonstrated that male vertebrates have lower immune responses than females. Also, removal of the testes stimulates and administration of testosterone suppresses immune function in males of these species. Furthermore, vertebrate immune tissues express androgen and estrogen receptors suggesting a responsiveness of immune function to sex steroids.[79]

Recent studies in wild vertebrates confirmed the immunosuppressive actions of testosterone in a number of species.[37,38] However, there also exist a number of studies that fail to show a suppressive effect of testosterone administration on immune function. A recent meta-analysis on reptile, birds and mammal studies concluded that testosterone administration generally decreases immune function, but that its effects varied considerably between and within taxa, and also with type of immune response measured.[26] One way in which these findings could be interpreted is that there exists evolutionary variation in the strength of the connection between testosterone and immune function. This

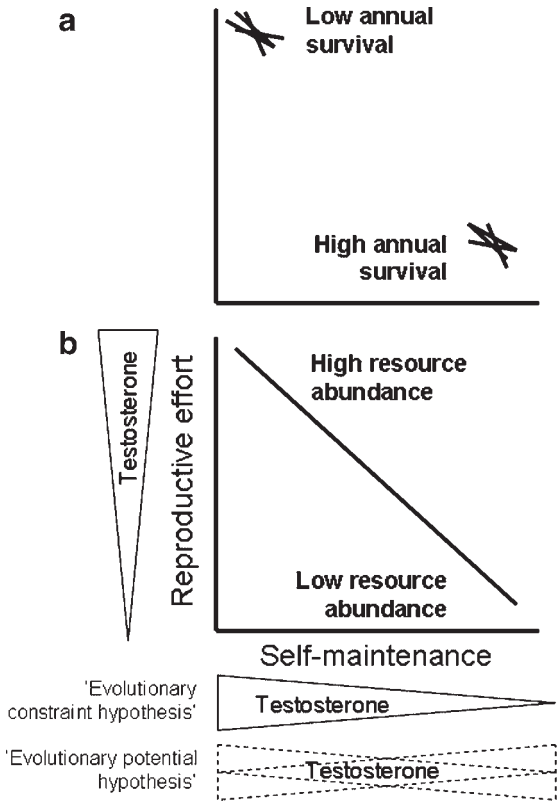

Figure 3. Hypothetical variation in the trade-off between reproductive effort and self-maintenance **a** between different species and **b** within an individual. **a** Species that differ in annual adult survival rates may also differ genetically in the way they allocate resources to traits that increase reproductive effort versus those that promote self-maintenance. Solid lines represent different responses of individuals (genotypes) within species to environmental conditions. **b** Plasticity in trade-off functions within an individual in response to environmental conditions, for example in food abundance.-The predicted associations of life-history traits and testosterone with respect to the 'evolutionary constraint' and 'evolutionary potential' hypotheses are indicated in triangles, with dashed lines indicating flexibility in (or lack of) a linkage. Note that both hypotheses suggest an increase in reproductive effort with testosterone, but diverge in their predictions regarding the link between testosterone and other life-history traits such as immune function (a self-maintenance trait).

interpretation is supported by recent findings in song sparrows (*Melospiza melodia*) showing that the immunosuppressive effects of testosterone are mediated via conversion into estrogens (at least during the non-breeding season).[20]

Several recent studies suggest that some of the immunosuppressive effects of testosterone might be mediated via glucocorticoid hormones.[80] It remains to be determined whether testosterone generally affects immune function via glucocorticoids or whether it affects some immune components directly and others indirectly. The presence of both glucocorticoid and sex steroid receptors in immune tissue[79] lends support to the latter possibility, which could add considerable evolutionary plasticity to the connection between testosterone and immune traits.

Testing the evolutionary linkage between testosterone and trade-offs

The data discussed above suggest that the linkage between testosterone and male traits is plastic, but the degree of evolutionary plasticity seems to depend on the type of hormone action (organizational versus activational), taxonomic group and evolutionary time scale considered. In mammals, the organizational effects of sex steroids might constrain evolutionary variation in trade-offs than in non-mammalian taxa. But, even in mammals, hormonal effects in utero might alter traits that are organized as part of sexual differentiation. If organizational effects of sex steroids in utero were found to have a genetic basis, they would have the potential to evolutionarily offset possible constraining actions of sex steroid actions during sexual differentiation. In contrast, the activational effects of testosterone are unlikely to present evolutionary constraints, even on short evolutionary time scales (i.e. within families or genera).

Further studies are needed to understand the microevolutionary plasticity in the connection between testosterone and life-history traits, for example within genera or even subspecies. Understanding microevolutionary processes will be important in light of the ongoing anthropogenic alteration of climatic conditions and habitat availability, which will affect the abiotic and biotic environment of populations and consequently the nature and shape of life-history trade-offs.[81]

To distinguish between the 'evolutionary constraint hypothesis' and the 'evolutionary potential hypothesis' over short evolutionary time scales, comparative studies within a life-history context are needed. A promising approach would be to study closely related species, or populations of the same species, that differ in life table variables such as annual adult survival rates. Life history theory predicts that populations with low annual adult survival rates should invest strongly into each reproductive effort and to a lesser extent in self-maintenance traits. Conversely, species with high annual adult survival rates should invest relatively more into self-maintenance function and relatively less into each reproductive effort.[1,2,4] The 'evolutionary constraint hypothesis' would predict that reproductive trade-offs are controlled by similar endocrine control mechanisms in all populations or species, regardless of their respective life-history strategies (Fig. 1a). Conversely, the 'evolutionary potential hypothesis' would predict that endocrine control mechanisms of traits are shaped by selection and therefore could vary between species experiencing different survival rates. In some species, life-history traits may be linked into trade-offs via testosterone-signaling processes, in other

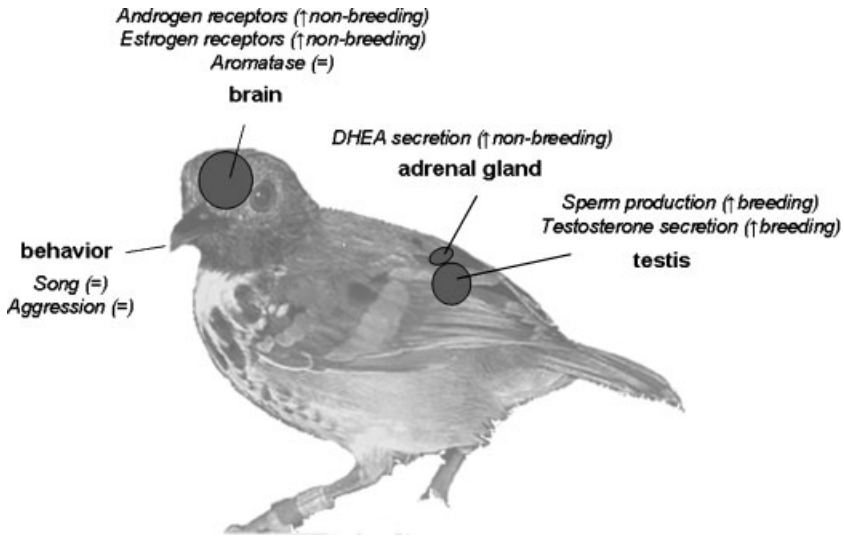

Figure 4. Schematical representation of seasonal variation in several behavioral and endocrinological traits of male spotted antbirds. Trait may lack seasonal variation (=), or show seasonally increased expression (↑). Data from Refs. 61,62,67,84)

species the same traits may be dissociated from a control by testosterone (Fig. 1b).

As a first experimental step, administration of exogenous testosterone[33] could be used to test these predictions. One of the presumed functions of the systemic increase in testosterone during the breeding season is to signal the transition of the organism into the reproductive state. The 'evolutionary potential hypothesis' proposes that this systemic testosterone signal generates life-history trade-offs in species with low annual adult survival, perhaps via an antagonistic regulation of reproductive effort and immune function. Thus, in species with low annual survival rates, testosterone administration would be expected to boost reproductive effort (such as aggressive and sexual behavior) while at the same time suppressing immune function. In contrast, in species with high annual survival rates testosterone could still function as an internal signal to regulate the expression of morphological, physiological and behavioral traits associated with reproduction, but selection may have led to variation in the pleiotropic effects that testosterone exerts on other traits such as immune function. Thus, testosterone administration might still boost reproductive effort, but the suppressive effects on immune function might be blunted or absent.

The 'evolutionary constraint' and 'evolutionary potential' hypotheses can also be extended to predict the linkage between testosterone and male traits within individuals in a life-history context (see also Box 2, Fig. 3). Species with high survival rates may show a large degree of within-individual (i.e. phenotypic) plasticity as a mechanism to adjust reproductive trade-offs to variations in environmental conditions such as overall food abundance or predictability of food availability.[82] Hence males from species with high survival might seasonally vary the linkage between testosterone and traits (see Box 2, Fig. 4), thereby altering the nature and shape of life-history trade-offs at different times of year. In contrast, species with low annual survival rates may show a much-reduced plastic response to environmental conditions. In the latter species, testosterone might be tightly linked to male traits, thus ensuring a primary investment of available resources into reproductive effort. Likewise, ecological factors such as the predictability of resource availability can be expected to determine life-history trade-offs and thus the linkage between testosterone and male traits. As one example, animals living in arid habitats often face the unpredictable occurrence of rainfall, leading to an unpredictability in seasonal food abundance and hence reproductive opportunities.[83] Such environmental variability might necessitate the prompt and strict re-allocation of resources to reproductive processes and away from self-maintenance functions. Thus reproductive opportunists may show a strong linkage of traits by testosterone thereby promoting reproductive effort whenever environmental conditions allow. In contrast, animals inhabiting environments with more regular seasonal variations in resource abundance might show larger phenotypic

Box 2. Trade-offs and phenotypic plasticity.

Different seasonal activities (such as reproduction, dispersal, molt) necessitate that individuals adjust their allocation of resources seasonally (Fig. 3c).[16] The 'evolutionary constraint hypothesis' (Fig. 1) predicts that the primary mechanism of resource redistribution is via variation in plasma testosterone concentrations. Indeed, the majority of male vertebrates show large seasonal variations in plasma testosterone concentrations, with elevated levels during the reproductive season and low (or non-detectable) levels during the non-breeding season.[15,24] However, recent experiments revealed that vertebrates can seasonally alter the linkage between testosterone and certain life-history traits. Western song sparrows (*Melospiza melodia morphna*) vary the endocrine control of aggressive behavior between different seasons. During the breeding season, testosterone increases the frequency and intensity of male aggressive behavior.[96] However, during the non-breeding season when the gonads are regressed and plasma testosterone concentrations are low territorial aggression is regulated by estradiol.[97] Estradiol at this time of year is unlikely to come from the regressed testes, but instead may be synthesized in the brain from adrenal precursors such as dehydroepiandrosterone (DHEA).[98,99] Several enzymes required for the synthesis of active sex steroids from DHEA are present in the avian brain.[28] DHEA concentrations are elevated in song sparrows during the non-breeding season, and administration of DHEA stimulates song during aggressive encounters.[98,99] Alternatively, sex steroids might be synthesized entirely within the brainfrom cholesterol.[100] Likewise, year-round territorial tropical spotted antbirds show seasonal variation in the endocrine mechanisms that control behavior (Fig. 4).[63,67] Males display territorial aggression and sing year-round (Wikelski et al 2000). Testosterone appears to be involved in regulating territorial aggression year-round,[61] but its pathways vary seasonally. During the breeding season, direct androgenic actions of testosterone on aggressive behavior are likely.[61] During the non-breeding season, the expression of sex steroid receptors in brain nuclei associated with the regulation of aggressive behavior is increased, thereby likely increasing the brain's sensitivity to low concentrations of sex steroids.[67] Furthermore, plasma DHEA concentrations are elevated during the non-breeding season, perhaps as a substrate for the local production of sex steroids in the brain, similar to the situation in song sparrows.[62]

plasticity and regulate trade-offs more flexibly depending on environmental conditions. Thus in seasonal breeding species, the linkage between testosterone and male traits might vary depending on environmental or internal conditions (for example between seasons, Box 2, Fig. 4).

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