Proximate perspectives on the evolution of female aggression: good for the gander, good for the goose?

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Female–female aggression often functions in competition over reproductive or social benefits, but the proximate mechanisms of this apparently adaptive behaviour are not well understood. The sex steroid hormone testosterone (T) and its metabolites are well-established mediators of male–male aggression, and several lines of evidence suggest that T-mediated mechanisms may apply to females as well. However, a key question is whether mechanisms of female aggression primarily reflect correlated evolutionary responses to selection acting on males, or whether direct selection acting on females has made modifications to these mechanisms that are adaptive in light of female life history. Here, I examine the degree to which female aggression is mediated at the level of T production, target tissue sensitivity to T, or downstream genomic responses in order to test the hypothesis that selection favours mechanisms that facilitate female aggression while minimizing the costs of systemically elevated T. I draw heavily from avian systems, including the dark-eyed junco (Junco hyemalis), as well as other organisms in which these mechanisms have been well studied from an evolutionary/ecological perspective in both sexes. Findings reveal that the sexes share many behavioural and hormonal mechanisms, though several patterns also suggest sex-specific adaptation. I argue that greater attention to multiple levels of analysis—from hormone to receptor to gene network, including analyses of individual variation that represents the raw material of evolutionary change—will be a fruitful path for understanding mechanisms of behavioural regulation and intersexual coevolution.

1. Introduction: why individual variation and mechanisms matter

Evolutionary endocrinology employs both proximate and ultimate approaches to understand how phenotypes evolve [1–5]. Proximate mechanisms generate many of the constraints that underlie trade-offs, they create or constrain the potential for phenotypic plasticity, and they harbour the ‘pool’ of inter-individual variation that can be shaped by evolution [2–8]. In short, mechanisms determine what is possible in evolution in a number of ways, and, as a consequence, a mechanistic view is essential to a full understanding of the evolutionary process.

Several tools and perspectives from evolutionary biology and endocrinology are central to this ‘evo-endo’ approach. For one, manipulating a hormone-mediated trait with exogenous hormones or receptor antagonists is key to establishing the functional significance of hormone–phenotype relationships. Further, a focus on individual variation can shed light upon the proximate sources of natural phenotypic variation, because individual variation in quantitative traits is the raw material of phenotypic evolution. Endocrinology has traditionally ignored individual variation as technical or environmental noise [9], though it has received greater attention in recent years [6,7,10,11]. By illuminating covariation among endocrine parameters, phenotypes and Darwinian fitness, research on among-individual variation lends insights into how evolution can proceed [9]. For instance, if there is no inter-individual variability in some component of mechanism, then evolution cannot proceed via changes in that particular mechanism because the strength of...
2. A mechanistic approach to the evolution of female aggression

(a) Why female–female aggression, why testosterone?

The evo-endo approach just described can be profitably applied to the evolution of female competition and aggression, a topic that has received considerable recent attention [14–17]. Much of this research has emphasized ultimate perspectives, and it has become clear that female–female aggression and other exaggerated traits often function in competition over reproductive and social benefits. Female–female aggression has been shown to be beneficial in many contexts, including year-round territorial species, cooperative breeders and sex-role reversed species where females compete for rank, territories and access to mates. Even females in monogamous and polygynous species compete for paternal care, limited nesting sites or access to high-quality males [15,16]. Thus, female–female aggression is widespread in the animal kingdom, and given that it is often individually repeatable [18,19], this lays a strong foundation for aggression to be heritable and evolutionarily significant. The arguments and predictions below build upon this now well-supported view that aggression is often selectively advantageous to females.

In addition to direct selection on females themselves, female aggression and its underlying mechanisms ought to be shaped by correlated responses to selection shaping aggression in males, as well as coevolutionary dynamics between males and females. It was long assumed that exaggerated female traits and behaviours like female aggression were primarily non-adaptive by-products of genetic correlations with males [20]. After all, males and females share the vast majority of their genome [21], and so, phenotypes and mechanisms that are advantageous to males ought to be expressed in females as well. If the sexes differ in the degree to which these phenotypes or mechanisms are advantageous, then this sexual conflict will lead to coevolution between males and females [22]. Coevolution may take many different forms, including antagonistic directional change in one sex that is countered by the other sex (e.g. increase versus decrease in trait X) [23], as well as ‘palliative’ adaptations that break or minimize sexual conflict by reducing the deleterious consequences for one sex (e.g. one sex avoids the costs of an increase in trait X via some adaptation in trait Y) [24].

In investigating how these evolutionary processes act on mechanisms of aggression in males and females, the sex steroid hormone testosterone (T) is a logical starting point. The activational effects of T often masculinize behaviour, either directly or after T has been converted to other hormones, such as oestradiol (E2). It is also well established that T and its metabolites are proximate mediators of male aggressive behaviour, and more and more studies are investigating relationships between T and aggression in females [4,25]. Females naturally produce T in the gonads, brain and adrenals, and T is often highest in circulation in association with reproduction, in part because T is an intermediary on the pathway to produce E2 which is required for ovulation [26,27]. Furthermore, both sexes express androgen receptors (ARs) in a number of neural and peripheral tissues, and both sexes exhibit behavioural or physiological responses to T [26,27]. Thus, T is naturally elevated in females at certain times of the year, providing opportunities for selection acting directly on females to shape T-behaviour relationships in potentially adaptive ways.

(b) Mechanistic sources of variation

In general, T affects behaviour through a cascade of interconnected mechanisms (figure 1 represents a simplified version), and this cascade can be parsed in different ways to study how each contributes to behavioural variation [10,30]. Here, I focus on three components of this cascade for which there are abundant data from males and females in relation to aggressive behaviour: (i) Behavioural variation may relate to variation in hormone production (figure 1a). The major source of T in circulation is the gonad, and gonadal T production is regulated by the hypothalamo-pituitary-gonadal (HPG) axis, which is activated by external stimuli (day length, conspecifics, etc.). Gonadotropin-releasing hormone (GnRH) is released from the hypothalamus (H in figure 1a), and acts on the pituitary (P) to release gonadotropins...
including luteinizing hormone (LH). LH binds LH-receptors in the gonad (G) to stimulate steroid biosynthesis and T release. (ii) Individuals also may differ in target tissue sensitivity or metabolism of hormones (figure 1b), due to variation in the location or abundance of nuclear hormone receptors (e.g. ARs, oestrogen receptors (ERs)) or enzymes involved in local steroid synthesis or metabolism, such as aromatase (AROM), which converts T to E2. Notably, many of the behavioural effects of T are generated by E2 after local conversion via AROM [10]. (iii) Individuals also may differ because different genes are ‘turned on’ by the hormone–receptor complex, i.e. downstream genomic responses (figure 1c), such that certain types of cellular and physiological effects are brought about by a hormone in one sex or one individual, and not in another. When T or E2 bind nuclear receptors, the hormone–receptor complex interacts with the genome to cause massive changes in transcription and translation, ultimately affecting phenotype [31]. Owing to interactions among these endocrine components, they should not be considered mutually exclusive (e.g. high T can upregulate target tissue expression of AR, behaviour can feed back upon any of these endocrine parameters, etc.). This list and schematic are also not exhaustive (e.g. see especially [28,29]), but they represent mechanisms that have been most thoroughly studied in both sexes.

(c) Hypotheses and predictions regarding adaptive mechanisms for females

By investigating these mechanisms in females and how they vary from T-mediated mechanisms of behaviour in males, we can gain unique insights into the role of sex-specific adaptation in intersexual coevolution and the mechanisms by which it occurs. Accordingly, the principal goal of this article is to assess the degree to which females use sex-specific mechanisms that are adaptive in the light of female life history.

To generate hypotheses regarding the nature of adaptive mechanisms of aggression for females, I begin by considering T-mediated mechanisms of aggression in males, and the degree to which they are well suited to females. In males, aggression is often associated with higher T in circulation and greater sensitivity to T in the brain. Binding of T to AR initiates a suite of transcriptional effects that are thought to be advantageous in the face of protracted social competition, e.g. by shifting resources towards greater activity and energy mobilization [8,32]. On the other hand, elevated T can be costly, e.g. in terms of immune function, stress physiology or trade-offs with parental care [33]. Notably, these costs have been repeatedly shown to be quite detrimental to females, e.g. by delaying breeding, inhibiting maternal care or interfering with mate choice [34–37]. Combined with observations from §2a that (i) female aggression is selectively advantageous in a number of competitive contexts, (ii) females are naturally capable of producing and responding to T and (iii) these mechanisms are probably influenced by coevolutionary processes with males, a series of clear predictions emerge regarding how selection might facilitate aggression in females without some of these costs of T.

In short, if mechanisms of aggression in females are adaptive (to females), then selection ought to modify the connections between behaviour and the mechanisms from figure 1 to minimize the costs of T [33]. There are several ways that this might occur, including lower T levels in circulation in females (figure 2a). A number of palliative adaptations that minimize the costs of high T on females also should be favoured (figure 2b,c), including reduced sensitivity to T in certain tissues (e.g. downregulation of AR expression in brain areas controlling maternal care), increased sensitivity to T in other tissues (e.g. in those controlling aggression), greater use of neurosteroidogenesis or local conversion to synthesize T within target tissues, or otherwise altered behavioural response to T via several mechanisms, such as sexually dimorphic transcriptional responses to T.

Notably, several studies of male aggression have demonstrated apparently adaptive seasonal changes in these very mechanisms. For example, it is thought to be costly for male birds to maintain high T outside of the breeding season, and many male songbirds do have lower T in the non-breeding season [33]. However, non-breeding season aggression can still occur, and it is facilitated by seasonal shifts in properties of neural target tissues, including altered neurogenomic responses to social challenges, upregulation of AR, and greater conversion of hormone precursors into T within the brain [38–40]. The important insight from these examples is that divergent behavioural mechanisms can exist within the same genome, suggesting it is reasonable to hypothesize that males and females also may differ in these same mechanisms despite their near-identical genomes.

In the following sections, I test these predictions regarding adaptive mechanisms of female aggression by focusing on T-mediated mechanisms, with an emphasis on birds and other systems in which these mechanisms have been well studied in an evolutionary/ecological context in both sexes.

I draw many examples from the dark-eyed junco (Junco hyemalis), the songbird that I have been studying for the past few years, because T-mediated mechanisms and their phenotypic and evolutionary consequences have been particularly well documented in both sexes in this system [5,27].

I first examine sex differences in T production (§3) and the degree to which the relationship between T and behaviour is similar or different in the two sexes, focusing on the behavioural effects of experimental elevation of T (§4) and social modulation of T (§5). I then examine the degree to which aggression is mediated by variation in target tissue...
sensitivity to T (§6) and whether molecular mechanisms of aggression and hormone response are similar or different in the two sexes (§7). In each section, I relate findings on females to comparable findings on males, and I assess evidence for female-specific adaptation by relating findings to female life history. Whenever possible, I emphasize individual-based approaches that facilitate conclusions as to whether evolution might change aggression via the same or different mechanisms in the two sexes. In closing, I considering findings in the larger context of molecular and behavioural evolution (§8), and I outline critical next steps in this field of research (§9).

3. Testosterone production in males and females

(a) Do the sexes coevolve in hormone production?

Male and female animals often differ in levels of T in circulation, providing one possible way for the sexes to differentially modulate behaviour. Comparative analyses demonstrate that female T levels covary with male T levels (birds: [27,41], fishes: [42]), and these positive correlations between male and female T levels are consistent with the hypothesis that T levels in one sex reflect correlated evolutionary responses between the sexes. If true, then another prediction is that males and females will have arrived at these correlated T levels due to shared underlying changes along the HPG axis. For example, we might expect males and females to be correlated in (i) abundance of GnRH-producing neurons in the hypothalamus, (ii) secretion rates of LH from the pituitary and/or (iii) abundance of gonadal LH-receptors or steroid biosynthetic enzymes, to name a few possibilities.

When colleagues and I tested this prediction in the dark-eyed junco, however, it was not fully supported. We found that males from two phenotypically divergent subspecies of junco differed in gonadal sensitivity to LH (LH-receptor mRNA abundance) and hypothalamic sensitivity to androgen-mediated feedback (AR mRNA abundance), but females from the two subspecies did not differ in these or other HPG-related parameters that we assessed [43,44]. Thus, males from these two subspecies appear to have diverged in HPG axis regulation, but females did not show correlated changes. On the other hand, the mechanisms that differentiate ‘high T’ individuals from ‘low T’ individuals appear to be similar in the two sexes [43,44]. For instance, among individual within each sex, we found that endocrine parameters were not correlated across multiple tiers of the HPG axis (e.g. a ‘high T’ individual was not a ‘high LH’ individual or a ‘high LH-receptor’ individual). Further, an individual junco produces roughly the same amount of T when the pituitary is stimulated with an exogenous dose of GnRH as he or she does when the gonad is stimulated with a standardized dose of LH. Thus, something downstream of the pituitary (i.e. something about the gonad) holds the key to individual differences in T production, and our analyses suggest that it is the expression of steroid biosynthetic enzymes. Because the gonad is the most sexually differentiated organ, these findings also suggest that one or another sex could alter T levels via changes that are localized to the gonad, rather than also requiring concomitant changes in the pituitary and hypothalamus, therefore permitting somewhat independent changes in hormone secretion in the two sexes. Greater attention to individual variation in more species will elucidate whether females tend to diverge in the same ways as males, or whether sex-specific changes along the HPG axis (possibly enabled via organizational effects) facilitate somewhat independent mechanisms in males and females.

(b) Does female life history predict variation in T?

Male T levels tend to covary with mating system and parental investment at an evolutionary scale, and T also varies with season, being higher in association with mating or male–female aggression and lower in association with paternal care [32,45–47]. Similar to these patterns in males, several lines of evidence from birds lend support to the hypothesis that female T levels are shaped by selection acting directly on females. First, female T levels peak early in the breeding season, either prior to laying when female competition is most intense, or during egg laying when both T and E2 are high in association with ovulation/egg production [27]. Second, female T levels are higher in monogamous versus non-monogamous species [27], and they are higher in colonial versus non-colonial species [41], demonstrating elevated T in two mating systems where female–female competition plays an important role (e.g. in defense of monogamy or nesting sites, respectively). Perhaps surprisingly, T levels are relatively low in females from polyandrous species [27]. However, if competition is especially intense among these females, then selection may promote behavioural mechanisms that do not require high T in circulation. This prediction is supported by findings from sex-role reversed species in which females typically have lower T than males [48], but females may instead have heightened sensitivity to T in areas of the brain associated with aggression [49]. Thus, in addition to any correlated responses to selection acting on males, selection also appears to act directly on females to alter T levels in association with female–female competition.

(c) Individual variation in T

Contrasting males and females in patterns of individual variation can tell us whether there is existing variation in T that could be shaped by selection. Techniques such as injections with GnRH permit precise assessment of an individual’s physiological capacity to produce and secrete T [50]. Importantly, T produced in response to this ‘GnRH challenge’ is repeatable in individual males and females [43,44,50], building an essential foundation upon which further questions about the functional consequences or mechanistic sources of these stable individual differences can be built. For example, one key question is whether individual differences in T are more likely to relate to aggression in males than in females. In dark-eyed juncos, variation in T produced in response to a GnRH challenge is positively correlated with individual differences in aggression within each sex [51,52], but in the White’s skink (Egernia whitiwi), circulating T is related to aggression in males but not in females [53]. These are only two examples, and many more studies will need to consider individual variation if we are to determine whether the sexes differ in the degree to which levels of circulating T predict behaviour.

4. Behavioural response to exogenous testosterone

(a) Control versus testosterone-treated

Experimental elevation of T (EET) via implants or injections [27] often leads to an increase in aggressive behaviour in
males [4,25,33]. However, not all species follow this pattern, and hypotheses proposed to reconcile these mixed results have invoked species differences in the relative benefits of aggression and paternal care (reviewed in [54]). Selection is thought to favour behavioural insensitivity to T (or, lack of behavioural response to T) in species in which (i) breeding seasons are short and temporal partitioning of mating effort and parental effort may be advantageous (‘single-brood’ or ‘short season’ hypotheses), or where (ii) male care is especially important for offspring survival (‘essential parental care’ hypothesis).

Because females often invest relatively more in parental care than do males [55], this logic might also apply to females that are faced with trade-offs between maternal care and aggression. Provided that T is detrimental to maternal care, then females ought to evolve behavioural insensitivity to T, such that EET will not impact care or aggression. On the other hand, if T is beneficial because it facilitates adaptive physiological or behavioural responses to female–female competition, then selection may favour behavioural sensitivity, with females responding to EET with an increase in aggression and a reduction in care [35]. A third alternative would invoke adaptive modularity of aggression and care, allowing females to retain sensitivity to T in relation to aggression but not provisioning [4,5], possibly by altering receptor abundance in some brain areas and not others.

In most species studied to date, female breeding season aggression increases in response to EET (see electronic supplementary material, table S1). In some species, certain aspects of aggression are affected, whereas others are not (e.g. displays versus overt attacks), suggesting that T influences aggression, but other mechanisms fine-tune expression of one or another behaviour (e.g. European robins (Erithacus rubecula) [56], bank voles (Clethrionomys glareolus) [57]). In songbirds, EET typically increases the incidence of spontaneous song or other vocalizations (e.g. European starling (Sturnus vulgaris) [58]), though these findings should not necessarily be viewed as increased aggression without contextual information about song use [56,59]. Interestingly, some exceptions to the general EET-increase in female aggression suggest potential links between function and mechanism. For example, female rats (Rattus norvegicus) are not very aggressive except in the context of maternal aggression (defense of young), and they exhibit less female–female aggression in response to EET [60]. Thus, one possibility is that T-independent mechanisms may be more applicable when female–female aggression functions in defense of young (instead of in defense of social or reproductive benefits), a hypothesis that is ripe for more rigorous experimental testing as additional species with more diverse life histories are studied.

Thus, evolution has largely not produced sex-specific behavioural insensitivity to T in spite of the fact that T can be particularly costly to females and despite evidence (from males) that behavioural sensitivity/insensitivity to T is fairly evolutionarily labile. But, is this broad pattern adaptive? When these data are paired with data on the effects of EET on parental behaviour, an apparently adaptive view does begin to emerge. For example, provisioning and incubation are not affected by EET in female juncos [34,61], but aggressive behaviour is increased [62]. Combined with evidence that male provisioning is reduced by EET in this species [63], this example shows female-specific adaptive modularity of behavioural sensitivity to T, suggesting that behavioural mechanisms have been shaped by direct selection on females. In other species, such as the tree swallow (Tachycineta bicolor), females are behaviourally sensitive to EET both in terms of (increased) aggression and (reduced) incubation behaviour [35]. Female incubation is essential in this species, and so this behavioural sensitivity to T seems maladaptive in light of the essential parental care hypothesis. However, if we instead apply the assumption typically applied to males, i.e. that T-mediated aggression is advantageous because it facilitates exclusive access to a mate and/or territory [8,32,33], then behavioural sensitivity to T seems adaptive in light of the life history of female tree swallows: heightened female aggression increases the likelihood of obtaining a nesting cavity [19] and deters floater females that can kill or evict resident females [64]. Under these circumstances, any female that is able to increase aggression in response to either social or seasonal changes in T ought to have a fitness advantage over a behaviourally insensitive female.

An important caveat is that EET studies run the risk of falsely concluding that elevated plasma T is the source of female aggressiveness for three reasons. (i) Some studies use male-like T doses, and other studies do not report T levels, drawing into question whether all of these manipulations should be seen as ecologically relevant. (ii) Some studies use short-days as a means of hormonally castrating animals, but enlarged steroid-synthesizing gonads are not the only difference between short- and long-day animals, and seasonal neural plasticity may also affect how T is processed in the brain [65]. (iii) EET studies can tell us that aggression is mediated by T to some degree, but they cannot address whether natural variation in T in circulation relates to female aggression. For example, due to extensive interactions among components of the endocrine system, EET might lead to enhanced aggression by upregulating AR expression, increasing testosterone-AR binding, or affecting titers of other hormones that directly mediate aggression [1]. Greater use of sex-appropriate doses and other experimental manipulations, such as receptor antagonists or AROM inhibitors, will be essential to contextualizing these findings in the future.

(b) Individual variation in behavioural response to T

At the inter-individual level, it is unclear how much variability there is in aggressive response to EET, though some individuals certainly seem to be more affected than others in terms of parental behaviour. For instance, EET leads to delayed breeding in female juncos, and some females implanted prior to egg laying fail to breed altogether [34]. Further, T-females have fewer nests than controls, and among those T-females who do have nests, they have fewer eggs and hatching than controls; however, among females who have chicks, there is no EET effect on likelihood of survival to fledging [36]. One explanation for these patterns is that only those T-females who were least sensitive to T were able to breed successfully, whereas females who were naturally most sensitive were unable to nest to begin with. Interestingly, offspring of T-females were more likely than those of control females to return the following year as adults, consistent with natural selection related to behavioural sensitivity to T. Future studies should attempt to uncover the proximate mechanisms that differentiate these most responsive and least responsive females, and alternatives will need to be explored (i.e. variation in T metabolism, implant placement, etc.).
5. Hormonal response to an aggressive challenge

Hormones may affect behaviour, and behaviour also may alter hormone secretion. Among the best-studied effects of behaviour on hormones is the transient rise in T associated with social instability in many male vertebrates (i.e. the challenge hypothesis [32,45–47]). T is thought to rise in response to social challenges in order to facilitate physiological and behavioural changes that are adaptive for prolonged social instability, such as altered stress reactivity, immune function, metabolism or aggression [33].

While the challenge hypothesis has been well supported in most vertebrate lineages, recent analyses in male songbirds suggest that many species do not modulate T in response to aggression [47,66]. Many of these exceptions occur in species that show a discrete shift from investment in mating effort to investment in parental effort, including species with only one brood, a short breeding season, and/or essential paternal care [47,66]. Therefore, the same life-history traits and hypotheses that account for behavioural sensitivity to T also may account for interspecific variation in T response to aggressive challenges (see §4). If these hypotheses are generally applicable and therefore apply to females, and if female mechanisms of behaviour are adaptive (for females), then these mechanisms should reflect the selective forces acting on female aggression and female life-history trade-offs as well [35].

A review of this literature in females reveals mixed results that provide key insights into the evolution of hormone–behaviour interrelationships (see electronic supplementary material, table S2). In some species, breeding density and/or the frequency of aggressive interactions is positively correlated with T levels in circulation or in egg yolks, suggesting that females may socially modulate T [67,68]. Notably, though, yolk and plasma T are not always correlated [69], and so apparent support for the challenge hypothesis assessed via indirect measures such as eggs should be interpreted with caution. When female T has been measured in circulation following an aggressive challenge and compared to unchallenged controls, plasma T are not always correlated [69], and so apparent support for the challenge hypothesis assessed via indirect measures such as eggs should be interpreted with caution. When female T has been measured in circulation following an aggressive challenge and compared to unchallenged controls, only a few species show a significant elevation: cichlids (Neolamprologus pulcher) [70], dunnocks (Prunella modularis) [71], marmosets (Callithrix kuhlii) [72] and buff-breasted wrens (Thryothorus leucotis) [73]. Several other studies show no change in T following aggressive encounters, and some species that do not elevate T instead show socially induced changes in progesterone (California mice (Peromyscus californicus) [74], African black coucals (Centropus grillii) [75], Galapagos marine iguanas (Amblyrhynchus cristatus) [76]) or corticosterone (mountain spiny lizards (Sceloporus jarrovi) [77], European stonechats (Saxicola torquata) [78]). Aggression-induced changes in progesterone are especially intriguing because they again suggest overlap with mechanisms of maternal aggression (see [79]). Another notable finding is that some females exhibit reduced plasma T levels after aggressive challenges (song sparrows (Melospiza melodia) [80], Eastern bluebirds (Sialia sialis) [69], Galapagos marine iguanas (A. cristatus), [76]), suggesting that T is actively removed from circulation during these encounters, probably via liver catabolism or conversion to other hormones in target tissues.

Even more revealing is that there are four examples in which males and females from the same species differ in their hormonal response to aggressive challenges (see electronic supplementary material, table S2), and three of four show that males elevate T and female do not. While this pattern may well suffer from publication biases, it suggests that evolutionary losses of social elevation of T might be more common in females than males, a result that is consistent with a predicted palliative adaptation permitting females to be aggressive independently of elevated T. The buff-breasted wren is the one exception where females elevate T and males do not [73,81], and several details suggest that female hormone–behaviour relationships are indeed adaptive to these female wrens [73]. First, T secretion is highly context-specific: females primarily elevate T levels after female or pair (female + male) intrusions during the pre-breeding season when territorial challenges from floater females are most frequent. Second, the hormonal response seen in females in the pre-breeding season dissipates in the nesting period, in accordance with the essential parental care hypothesis. This female-specific social modulation of T is particularly striking in light of the usual disconnect between T and aggression in other tropical (male) birds [4,82], further suggesting that this pattern of T secretion relates to selection acting directly on females.

Tests of the challenge hypothesis in females have been woefully biased towards species with fairly extensive maternal care, limiting ability to ask whether the extent of female care maps onto these mechanisms. Further study of species with different life histories is needed, as there are many open questions. Are socially induced T surges in females more common in species with limited female care? Are they more common when offspring are relatively resistant to parental neglect? Is social modulation adaptive in females, and does it prepare them for success under persistent social instability, as it is thought to do in males [32,33]? With strong evidence that female aggression is advantageous in a number of contexts, such as competition for rank, territories or access to mates, the solution to this issue may lie in finding the (possibly rare?) species in which female aggression has little to no benefit, and contrasting hormonal mechanisms with closely related species that benefit from female aggression.

6. T, sensitivity and natural variation in aggression

Experimental manipulations using receptor antagonists, RNA interference (RNAi), and knockouts widely support a causal relationship between aggression and AR, ER, AROM and other aspects of target tissue binding and processing of sex steroids [25]. Comparative studies in males [83] and male–female comparisons in sex-role reversed species [49] likewise demonstrate that T and/or sensitivity to T can diverge alongside aggressive behaviour. Comparative analyses of T, AR and aggression in both sexes are rare, though both T and hypothalamic AR abundance track species and sex differences in aggression in Sceloporus lizards [84]. Collectively, these studies suggest that target tissue sensitivity to T is likely to play a role in the expression of aggression behaviour, though until recently, there was limited direct evidence to extend these findings to explain individual differences (but see [85–88]), which is a necessary step in understanding how aggression might evolve. What evidence is there that individuals within a sex naturally vary in hormone sensitivity in target tissues, and does this variation predict functional variation in behaviour that could be used by selection?
Using dark-eyed juncos, colleagues and I contrasted males and females in the degree to which T levels and neural sensitivity to T predict individual differences in aggression [88]. Using plasma and tissues collected from free-living males and females just after a short simulated territorial intrusion, we showed that neural gene expression for AR, ERs and AROM predicted individual differences in aggression in both sexes, but circulating T levels related to aggression only in males. In addition, the sexes did not differ in variance in transcript abundance in any gene or brain area, but male T levels had significantly higher variance than female T levels. Building upon an evolutionary framework in which variance in quantitative traits is proportional to the potential strength of selection, these data support the view that the potential for selection to change aggressiveness via shifts in T levels may be higher in males, whereas the potential for selection to change aggressiveness via shifts in AR, ERs or AROM expression may be equal in the two sexes. Future studies should extend this line of research to investigate (i) how sources of variation in female aggression change as populations diverge and (ii) the degree to which mechanisms of female aggression can change independently of mechanisms of male aggression. Some of our recent work on male juncos suggests that the endocrine mechanisms that account for subspecies differences in aggression are not simply extensions of intra-populations mechanisms [89], and similar comparative approaches applied to females should be quite informative in understanding how mechanisms of behaviour evolve.

7. Downstream genomic mechanisms

Changes in the regulation of gene expression are thought to be major drivers of phenotypic evolution [90]. Gene regulation (transcription) can be influenced by experience or by organizational effects of hormones, so differential gene expression is important in the context of sexual conflict because it allows potentially adaptive trait modification over the backdrop of the same genome [91,92]. Indeed, sex differences in gene expression are a common solution to sexual conflict [21,93], one that is mediated by sex steroids to some degree [94]. Genes with sexually dimorphic expression also tend to evolve more rapidly that those without sex biases [95,96], and this accelerated evolution supports an important stipulation of sex-specific mechanisms of behaviour because it permits molecular mechanisms to diverge (adaptively) between the two sexes.

(a) Molecular mechanisms of aggression

Whole genome expression studies on males demonstrate a strong connection between aggression and gene regulation at short time-scales (i.e. immediate response to a rival) and at evolutionary time-scales (i.e. between species comparisons) [97,98]. Artificial selection further confirms that divergence in aggression is accompanied by major changes in gene expression, including genes whose products have known roles in mediating aggression (serotonin receptor) or hormonal response (ER-related transcription repressor) [99,100]. An elegant set of studies on Drosophila showed that only 8% of genes differed consistently between replicate lines selected for aggression [99], and only 10% of the aggression-associated genes from this artificial selection paradigm were also differentially expressed among wild-derived lines that naturally varied in aggressiveness (a result not significantly different from the null expectation) [101]. While all of these genes may not play a direct role in aggression, experimental manipulations suggest that many do [101], and these studies collectively suggest that, among males, there are multiple molecular routes to greater aggressiveness.

Genome-wide expression analyses also show some similarities in the molecular architecture of male and female aggression. In the same Drosophila experiment described above [99], there was strong evidence of correlated responses between the sexes, at both behavioural and molecular levels: female aggression also changed in response to artificial selection on males, and 96% of the genes associated with aggression in males were associated with aggression in females. Male and female zebrafish (Dato rio vento) likewise show similarities in the molecular signatures of aggression/dominance, layered atop sexual dimorphism in gene expression [102]. Notably, genes associated with the aggressive/dominant phenotype in both sexes include several related to HPG axis function and sensitivity to hormones (GnRH, AROM, AR and some ERs). In the cichlid fish N. pulcher, dominant females have high T and are also masculinized in neural gene expression, though they exhibit normal female reproductive behaviours [92]. Thus, the neurogenomic signature of the aggressive/dominant phenotype has strong overlap in the two sexes, and cluster analyses reveal sex-specific mechanisms as well. A similar conclusion was reached by comparing neural gene expression from one cichlid fish (Julidochromis transcriptus) that exhibits the typical pattern of male dominance and territoriality to a close congener, the sex-role reversed J. marlieri [103]. The large, aggressive dominant females from the sex-role reversed species were more similar in gene expression to the dominant males from the other species than they were to conspecific males or congeneric females. Again, differentially expressed genes include those known to affect aggression (isotocin, AROM), and the upregulation of neural AROM in role-reversed females lends support to the hypothesis that females may facilitate aggression by changes in target tissue processing of T.

(b) Transcriptional response to androgens

Comparing male and female transcriptional response to experimental androgen treatment can reveal whether sex-specific responses to hormones underlie sex-specific mechanisms of behaviour. Female and male mice that were gonadectomized (GDX) and treated with dihydrotestosterone (DHT), which is a non-aromatizable androgen with very high affinity for AR, however, show comparable transcriptional changes in liver and adipose tissues, compared to sex-specific controls [94]. Not surprisingly, many of the genes affected by DHT are related to lipid and steroid metabolism, and there is a strong overlap in the list of DHT-affected genes and sex-biased genes. Network coexpression analyses, which are representations of genes whose expression is correlated and therefore likely to be co-regulated, show that liver and muscle tissue have greater modularity in females than in males, demonstrating some degree of sex-specific gene regulation in response to hormones, possibly allowing females to regulate certain sets of genes independently of others. It may be exactly this sort of molecular mechanism that allows dominant N. pulcher females to exhibit largely male-like patterns of gene expression while maintaining normal female reproductive behaviours (i.e. shifts in an
‘aggression module’ of genes that occur independently of shifts in a ‘reproduction module’ [92].

Notably, the GDX male and female mice described above were treated with the same (male-like) androgen dose. While this approach can tell us how male-like hormone levels affect tissues with male and female genomic backgrounds, it cannot tell us whether androgen levels that are at the high end of the sex-appropriate physiological range also engender similar effects in the two sexes. Peterson and colleagues [104] reported such a study in the dark-eyed junco and come to a somewhat different conclusion, looking at neural tissue. T-treatment affected expression of many genes that have been functionally linked with behaviour, including AROM and monoamine oxidase A, among others, but males and females had very little overlap in the specific genes affected by T-treatment (<1%). While future studies are needed to explore alternative explanations for the difference between this study and the mouse study above (e.g. dosage effects, mammals versus birds, GDX versus intact, etc.), these results provide some of the first direct evidence that males and females can fine-tune behavioural mechanisms via sex-specific effects of T on particular genes. A key next question is whether these divergent transcriptional responses are adaptively suited to each sex.

(c) Individual variation in molecular mechanisms
There are hurdles to overcome in assessing how individual males and females vary in these molecular mechanisms. Most notably, the establishment of repeatable individual differences requires repeated sampling, but most studies use tissues that cannot be resampled (brain, liver, etc.). The use of commercial-grade arrays [104] and the discontinuation of the practice of pooling individuals [99] mean that we are well poised to learn from these individual differences, should we choose to analyze them. Cluster analyses and heat-maps often do reveal high variability among individuals within a population, sex or morph [92,102,104]. A handful of studies also report that individual variation is large compared to inter-population variation in gene expression, and at least some proportion of individual variation is genetic [105]. ‘Understanding what genes do in their natural context is a key issue’ [106, p. 10], and an increased focus on the individual at the molecular level will be critical to understanding how complex behaviours such as aggression evolve.

8. Do shared mechanisms represent homology?
As we attempt to synthesize the above observations that some hormonal and molecular mechanisms are the same in the two sexes, while others are different, another key question comes into focus: ‘What exactly do we mean by sameness?’ [107, p. 436]. When males and females repeatedly use the same mechanisms across a wide range of species, can we tell if the sexes have arrived at similar mechanisms because they share an ancient homology, because they have co-evolved using a derived mechanism, or because they have independently co-opted common mechanisms?

While there seem to be almost infinite mechanisms by which one or another trait might evolve, life repeatedly uses the same mechanisms [13,107–110]. Many of the studies reviewed here support this ‘toolkit’ or ‘hotspot’ view of evolution—aggressive behaviour has again and again been associated with variation in the same mechanisms, including T levels, abundance of AR, ER or AROM, and gene expression related to production of or sensitivity to sex steroids. Surely, it is most parsimonious to assume that sex similarities in mechanism are homologous. On the other hand, ‘mechanistic drift’ does occur in nature—even arthropod legs, which are well established to be homologous structures, are not produced via the same mechanisms in flies and beetles (for details, see [107]). Thus, if there is ample genetic variation in a particular gene in two lineages and the product of this gene is behaviourally relevant, then each lineage may independently arrive at similar mechanisms of behaviour [109,110].

It seems reasonable to imagine that this logic could apply to males and females for whom parts of their genome are differentially regulated and may evolve somewhat independently. But, how does this fit with the coevolutionary dynamics and predicted palliative adaptation discussed above (§2)? If mechanisms are homologous in the two sexes, and represent correlated evolutionary responses, then the sexes should not simply share one determinant of phenotype (e.g. one gene, one receptor, or one hormone), but they also ought to share full networks of gene regulation [108] or multiple mechanisms along the cascade linking the endocrine system with behaviour [3,5]. Further analyses of individual variation in gene expression (and sex comparisons of that variation) in non-inbred organisms will be particularly important in resolving this issue, as will network analyses that assess interactions among co-regulated genes [105]. Deviations from full overlap at multiple levels of the endocrine system will be informative in understanding why evolution might use one mechanism instead of another. That is, if we accept that mechanistic drift is possible, then adaptive changes in mechanisms (‘mechanistic selection’) ought to occur to suit one or another sex, yielding neuroendocrine and genomic mechanisms of female behaviour that are adaptive for females, and vice versa. Notably, any palliative adaptation on the part of females might also lead to new dimensions of conflict and/or coevolution with males (figure 2, far right panel). If derived mechanisms of aggression break the cycle of conflict by reducing deleterious effects, then these adaptations should be advantageous for both parties and they should therefore take hold in the population [24]. Consistent with this view, many of mechanisms of aggression predicted to be beneficial for females have also been reported in males in contexts when aggression is advantageous but elevated T is not [38–40], though more research is needed to determine the sequence of adaptations leading to this outcome.

9. Conclusion
This review contrasts mechanisms of aggression in females with those of males, focusing on variation in T production, as well as target tissue hormonal and transcriptional responses. Because T can be particularly costly to females, but aggression has repeatedly been shown to be advantageous, I predicted that selection would favour female mechanisms of aggression that use variation at the level of target tissues (e.g. sensitivity, metabolism, or genomic responses) more than mechanisms that require systemically elevated T. The literature supported this prediction to some degree, though both sexes clearly mediate aggression at all of these levels. Some evidence suggested that female mechanisms track male mechanisms, consistent with correlated evolutionary responses between the sexes,
e.g. intersexual correlations in T levels, overlap in molecular mechanisms of aggression. But other patterns were more consistent with selection acting on females themselves, e.g. apparently adaptive context specificity of T levels or social regulation of T, behavioural modularity in response to EET, and some divergence in transcriptional responses to sex-specific elevations in T. I reviewed several studies on the organism that I study, the dark-eyed junco, combining results across multiple levels of analyses. These studies collectively showed that males and females share many of the same sources of hormonal and behavioural variation, but male and female juncos diverge in aspects of HPG axis regulation, in the degree to which circulating T relates to aggression, and in their response to T, both behaviourally and transcriptionally. These findings provide a novel dataset in support of the hypothesis that the sexes can implement somewhat independent mechanisms of behaviour, despite sharing the same genome.

A few limitations of this review highlight critical next steps for research. Most importantly, phylogenetic representation and diversity of female life history in study species is remarkably inadequate. Second, many questions have not yet been sufficiently asked or answered. Future studies should ensure that endocrine parameters and behaviour are assessed in an evolutionarily relevant context by carefully selecting the study system, dosage of hormone treatments and methods for assessing female--female aggression. Third, this review has addressed many connections between behaviour and the endocrine system, but it has not addressed them all. For example, do socially mediated T elevations in females enable physiological or behavioural priming for success in future social instability (as hypothesized in males), or have females found mechanisms to accomplish this endpoint without changes in circulating T, i.e. via neurosteroidogenesis, or modified transcriptional responses to social stimuli? Do the sexes differ in the degree to which rapid (non-genomic) effects [28] or binding globulins [29] modulate behaviour? Also needed are investigations of the mechanisms that engender these individual, sex, and species differences, and organizational effects of sex steroids are an obvious candidate [2].

Of particular note is the shortage of analyses that contrast the pool of individual variation in males and females, an approach that can reveal whether the endocrine system harbours the same raw material for phenotypic evolution in the two sexes. An individual-based approach is also essential to relating mechanistic variation to natural selection in the wild. In the junco, for example, individual differences in HPG axis reactivity [51] and gene expression related to neural sensitivity to T [88] predict individual differences in aggressiveness, and more aggressive females are more likely to successfully fledge offspring [51], suggesting a selection gradient on these endocrine parameters. I hope that this review will stimulate further use of these individual-based approaches to shed light on the mechanisms by which behaviour evolves. After all, 'real individuals are unique combinations of traits, some above and some below average. It is time to recognize the uniqueness of the individual and to turn it to our advantage as biologists.’ [9, p. 161].

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