A general mechanism for conditional expression of exaggerated sexually-selected traits

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Sexually-selected exaggerated traits tend to be unusually reliable signals of individual condition, as their expression tends to be more sensitive to nutritional history and physiological circumstance than that of other phenotypes. As such, these traits are the foundation for many models of sexual selection and animal communication, such as handicap and good genes models. Exactly how expression of these traits is linked to the bearer's condition has been a central yet unresolved question, in part because the underlying physiological mechanisms regulating their development have remained largely unknown. Recent discoveries across animals as diverse as deer, beetles, and flies now implicate the widely conserved insulin-like signaling pathway, as a common physiological mechanism regulating condition-sensitive structures with extreme growth. This raises the exciting possibility that one highly conserved pathway may underlie the evolution of trait exaggeration in a multitude of sexually-selected signal traits across the animal kingdom.

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Abbreviations:
CHC, cuticular hydrocarbon; **FGFR1,** fibroblast growth factor receptor 1; **FOXO,** forkhead box-O transcription factor; **GH,** growth hormone; **hox,** homeobox; **IGF,** insulin-like growth factor; **IGFR,** insulin-like growth factor receptor; **ILP,** insulin-like peptide; **ILS,** insulin-like signaling; **InR,** insulin receptor; **JH,** juvenile hormone; **RNAi,** RNA interference; **UBX,** ultrabithorax.

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Keywords:
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Introduction

Some of the most visually striking products of evolution are the elaborate ornaments and weapons of sexual selection [1]. These traits are found across the animal kingdom with, at times, seemingly preposterous degrees of exaggeration in size, shape, and color (Fig. 1). Classic examples include the peacock's train [2], brightly colored dewlaps of anole lizards [3], sexual dichromatism in cichlid fish [4], antlers in deer [5], and the vast array of horns observed in scarab beetles [6]. Exaggerated traits are almost exclusively wielded by males, and their role is clear: enhancing reproductive access to mates.

Exaggerated traits can act as either ornaments for female choice, or as weapons in male-male disputes. In both contexts, exaggerated structures function as conspicuous visual signals of male condition (see Box 1 for working definition of condition), resolving competitive interactions among individuals [7]. For ornaments, such as the peacock's train, males differ in the relative magnitude of the trait, either in size, color, structure, or a combination of these factors. Females then use this variation as a basis for mate choice decisions [8–11]. Weapons, such as deer antlers (Fig. 1A), are used to establish dominance hierarchies, or to settle conflicts for mating resources and ultimately female access. Males use individual-differences in relative weapon size during stages of assessment preceding overt battle, and males with smaller weapons generally cede the contest without escalating into costly and dangerous fighting [12, 13]. However, basing crucial reproductive decisions on assessment of an exaggerated trait only works if the signal is a reliable indicator of the bearer's actual condition [7, 14, 15]. If low condition males produce signal structures similar to those of high condition males, then the value of the signal disappears. It would no longer benefit

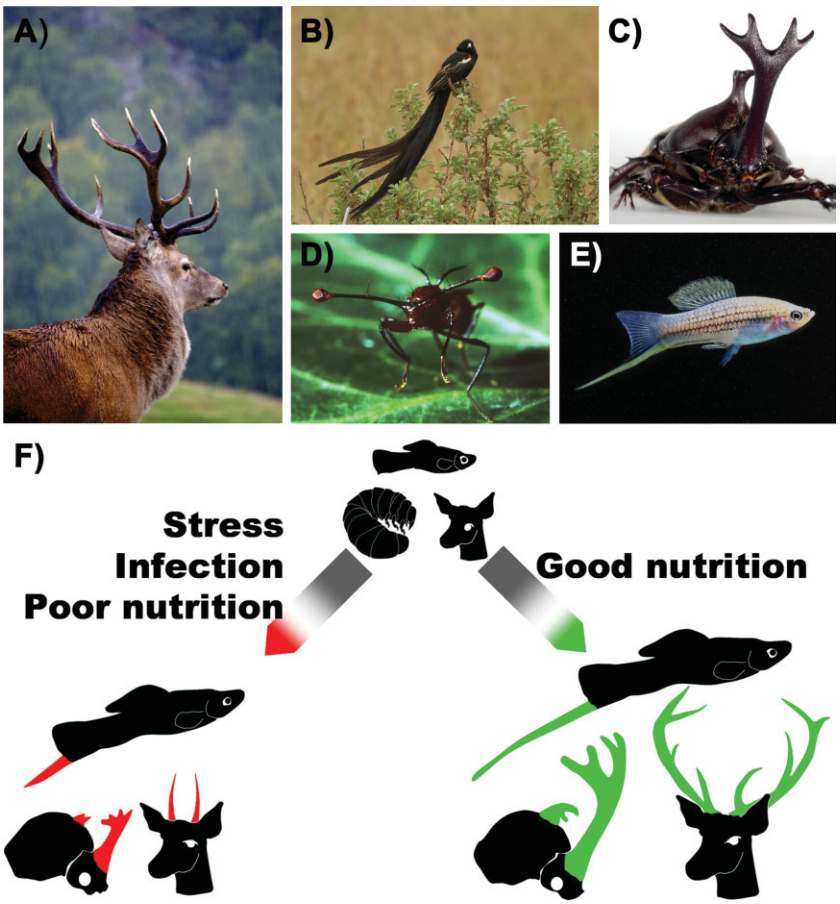

Figure 1. Examples of exaggerated traits. **A:** Red deer stag have large antlers used for male-male competition [5, 25] (photograph courtesy of Paul Barclay, www.pfg-photography.com). **B:** Female widowbirds prefer to nest with males with the longest tail [10] (photograph courtesy of Graham Searll). **C:** The exaggerated head horns of male rhinoceros beetles, *Trypoxylus dichotomus*, are used to settle disputes over resources and mating access [21, 22] (photograph by Doug Emlen). **D:** The stalk-eyed fly, *Teleopsis dalmanni* has eyes laterally displaced on long eye-stalks. Males with the longest eye-span reliably signal condition and are preferred by females [9] (photograph courtesy of Sam Cotton). **E:** Male swordtail fish (*Xiphorus* sp.), such as *X. nezahualcoyotl* pictured, have elongated caudal fins. Choosy females prefer males with the most elongated sword [35] (photograph courtesy of Kevin de Queiroz and Molly Morris). **F:** A simple model of growth trajectories based on condition for three different male animals with sexually-selected exaggerated structures – the horn of rhinoceros beetle, antlers of deer, and elongated caudal fin of swordfish. During the growth period of the sexually-selected exaggerated trait, individuals exhibit phenotypic plasticity in final adult trait size based on nutrition, infection state, and stress. The resulting adult structures show the three characteristics of a reliable signal: they are disproportionately large, are unusually variable in expression, and exhibit heightened sensitivity to condition. For our working definition of condition see Box 1.

females or rival males to pay attention to the trait, nullifying any rewards from bearing the trait.

Numerous studies of exaggerated sexually-selected traits suggest that they *are* reliable signals. In species ranging from birds [16, 17] to flies [18–20], beetles [21, 22], fish [23, 24], ungulates [5, 25], and crustaceans [26], expression of extreme traits is condition dependent, therefore tightly coupled with individual variation in traits such as age, size, parasite load, nutritional history, and genotype. For example, nutritional history is strongly linked to condition in horned beetles and males reared under low nutrition conditions produce horns that are either absent, or dramatically reduced in size compared to those of males reared under high nutrition conditions [22]. The near universality of these findings raises a critical question: *How* is reliability maintained? In other words, why don't poor condition males cheat?

Several theoretical models explore why poor condition males do not cheat by producing dishonest but attractive signals (reviewed in [7, 14, 15]). Briefly, either exaggerated traits are costly ("Handicap models"), or signal reliability is maintained via incorporation of existing physiological mechanisms linking trait expression to condition [27]. Handicap models propose that exaggerated traits are costly to bear and therefore only the best condition males can afford to possess them [1, 28, 29]. They predict that the cost of a trait will be higher for poor condition males than for high condition males, and as a result, faking an attractive signal will be cost-prohibitive. Studies have shown that exaggerated traits can have detrimental effects on locomotion and predation rates [30, 31], and their growth requires energetic investment which can stunt energy allocation to other structures [32]. However, there is also evidence that some signal traits – even exaggerated traits – are not sufficiently costly to maintain honesty [30, 33]. The apparent absence of costs may result from compensatory mechanisms having co-evolved with the signal trait, which ameliorate much of the locomotive costs of bearing the trait, but they may represent further energy costs for the bearer [30]. In other cases, the traits are often simple structures that appear remarkably "cost free" [30, 34] and in some instances, trait size is positively correlated with performance, such as in swordtail fish (Fig. 1E) where increased swimming performance is linked with increased tail length [35].

If the costs associated with growing or bearing an exaggerated trait cannot ensure an honest signal, the problem of how signal reliability is enforced remains: What maintains a robust link between condition and trait expression? In principle, if trait expression is mechanistically linked to the basic physiological processes of an organism, then it will provide a truly reliable signal of condition [27]. However, few studies have addressed the actual processes and pathways that could do this. For a small number of sexually-selected

Box 1

Defining "condition"

The words "quality" and "condition" are often loosely defined within sexual selection literature and are sometimes used interchangeably [27, 108]. The term "quality" can refer to genetic quality, phenotypic quality or both, and is often used interchangeably with many definitions of "condition". For this paper we define condition based on Hill's definition [27] as: the relative capacity of an individual to maintain optimal functionality of essential biological processes in the face of environmental challenges (e.g. variation in food resources and parasites). Under this definition, condition is the sum of the somatic (physiological) state, the genotype, and the epigenetic state of the individual [27]. Genetic quality is often causally related to condition in natural populations via genotype-environment correlations. (e.g. when quality parents place their eggs or young into better local environments and/or provision for them more effectively resulting in high quality offspring which are expected to outcompete lower quality rivals for access to critical resources such as nutrition and importantly, mates).

signals, such as brightly colored patches of skin or feathers infused with carotenoid pigments, the mechanisms have been examined [36, 37]. But the mechanisms responsible for variation in most exaggerated traits remained largely unexplored until recently. Advances in our understanding of nutrition-dependent regulation of overall body growth, and of the mechanisms regulating growth of individual tissues (e.g. mechanisms of allometry), provide a conceptual foundation for considering how exaggerated growth of particular traits might work [38, 39]. Focusing on traits with exaggerated growth (e.g. deer antlers and beetle horns) we review these mechanisms to suggest how condition dependence (and therefore signal reliability) of exaggerated morphological structures arises, and we discuss implications of these mechanisms for our understanding of the evolution of exaggerated traits. We then discuss how this mechanism may be applicable to other forms of exaggerated traits and displays, such as color, acoustic and behavioral traits, and pheromone signals.

What makes exaggerated sexually-selected traits reliable signals of male condition?

Three properties of sexually-selected traits with exaggerated growth make them reliable signals of individual condition [14]. First, these traits are disproportionately large compared to the rest of the body (and compared to other traits such as wings and legs). Their extreme size causes them to be highly conspicuous and renders them effective signals [14, 15]. Second, exaggerated traits are unusually variable in their expression from individual to individual (unlike other body parts, the sizes of these traits range from tiny or absent, to extreme). Small increases in body size can be associated with much more dramatic, and visible, increases in trait size. Hypervariability makes exaggerated traits very precise tools for judging otherwise-subtle differences in body-size [14, 15]. Finally, exaggerated sexually-selected traits are exquisitely sensitive to condition. Their extreme growth is tightly linked to a male's nutritional state, stress level, dominance status, or health (such as parasite or pathogen load) [17, 18]. Together, these three properties make exaggerated sexually-selected traits reliable metrics of male condition because subtle differences among individuals will be revealed as conspicuous differences in the relative size of the trait. Ideally, candidate mechanisms for growth regulation of these structures will help explain how each of these critical properties is generated, and why they frequently occur in tandem.

Insulin/insulin-like signaling (ILS) pathways and condition-dependent growth

The ILS pathway (Fig. 2) is a well-studied, highly conserved and central physiological pathway involved in a range of functions including metabolism, aging, reproduction, and growth [40–42]. The exact nature of the pathway varies between vertebrates and invertebrates, although the downstream targets and effects are highly similar (Fig. 2). In vertebrates, insulin-like growth factor 1 (IGF1), and to a lesser extent IGF2, are the main circulating hormones from this pathway that affect growth. Artificially reducing circulating levels of IGF in transgenic mice causes a 30% reduction in body size compared to control littermates [43]. Studies in a broader range of species show circulating IGF levels correlating positively with growth differences, including dog breeds [44], rats [45], several species of fish [46], as well as snakes [47], ducks [48], and humans [49].

Drosophila melanogaster provides the most detailed study of the invertebrate ILS pathway. Here, seven interacting insulin-like peptides (ILPs) are present that act on a single insulin receptor (InR) [50]. Manipulation of the ILS pathway in *D. melanogaster* also results in changes in body size. For example, removal of InR results in dramatic reductions in growth, and similar effects are seen when expression of specific ILPs is removed [50]. ILPs are ubiquitous within insects and have been, and continue to be, characterized in sequence and function in other species such as silkmoths, butterflies and mosquitoes [51].

A key characteristic of the ILS pathway is that it tracks the nutritional state of each individual [40–42], and responds to both stress [52] and infection [53]. Well-fed and less stressed individuals (e.g. in good condition) will have increased levels of IGFs/ILPs relative to poorly fed, diseased, or stressed individuals, resulting in differential growth. Therefore, the ILS pathway integrates physiological condition and metabolism with growth in a condition-dependent manner.

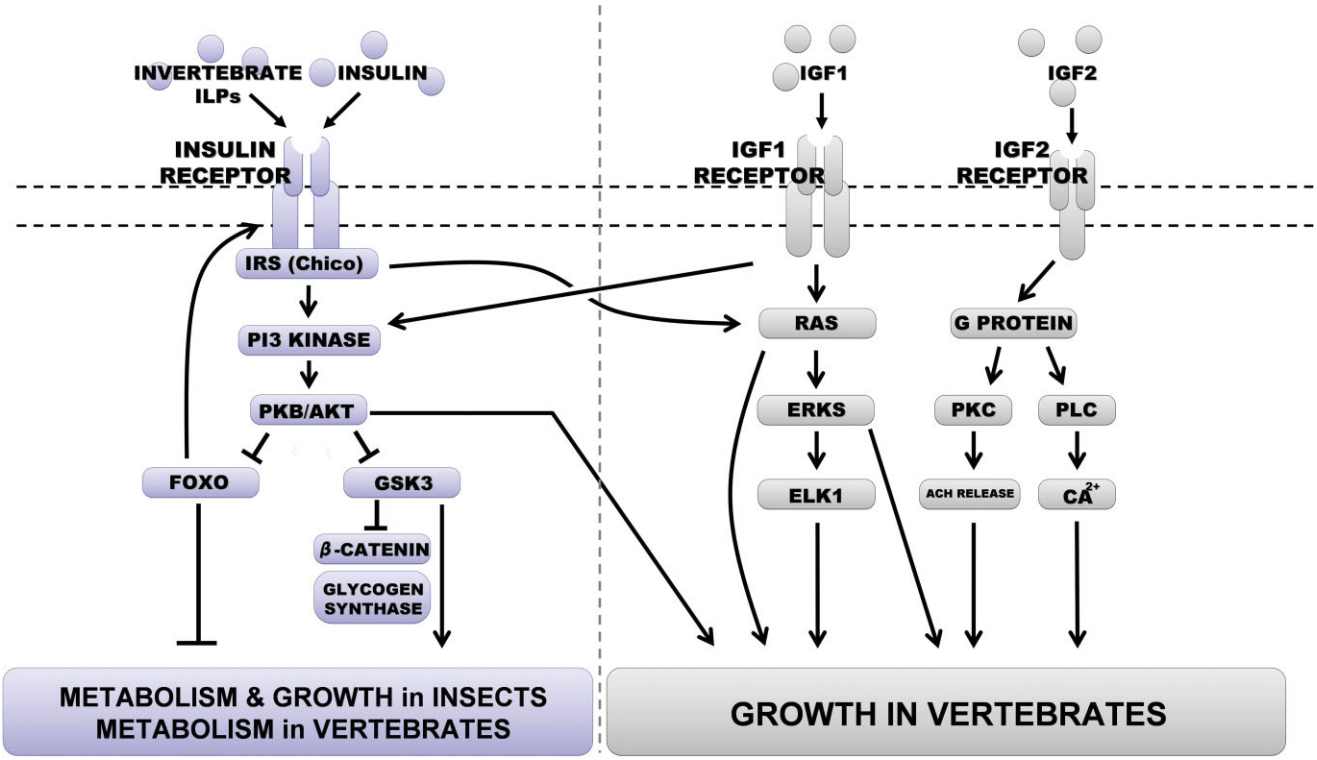

Figure 2. A generalized view of the insulin/ILS pathways for vertebrates and insects. The vertebrate pathway has three circulating insulin-related peptides: insulin itself and two insulin-like growth factors (IGF1 and IGF2). Each peptide has a corresponding receptor: Insulin receptor InR, and two insulin-like growth factor receptors (IGF1R and IGF2R). The primary role of insulin is in metabolism, whereas IGF1 and IGF2 are more directly involved in growth [40–42]. The primary site for production of insulin is the pancreas and IGF1 is the liver, whereas IGF2 is more commonly seen in early development. In contrast, insects (and invertebrates in general), typically have one InR but have numerous (seven in *D. melanogaster*) circulating ILPs binding to InR. The primary secretion site for ILPs is the brain and in insects this pathway regulates both metabolism and growth, although the distinct role of each ILP is still being elucidated [50]. Both IGFRs and InR are found in almost all tissues, and ILS pathways, in particular those involving IGFs and ILPs, can affect a range of physiological processes including reproduction, lifespan, and growth [40–42]. Binding of IGFs or ILPs to the IGFR or InR, respectively, initiates a highly conserved signaling cascade in both vertebrates and insects [40–42]. The members of the pathway include: InR substrate (IRS or *chico* in *D. melanogaster*), phosphoinisitide 3-kinase (PI3K), and protein kinase B (PKB/Akt) that regulate downstream factors, such as the transcription factor *forkhead box-O* (FOXO). The end result is the regulation of protein synthesis, cell and organ growth. ILPs and IGFs along with their receptors also activate other conserved pathways involved in protein translation, autophagy, apoptosis, oxidative stress, gene transcription through the mTOR pathway, Ras kinase pathways, and G-protein pathways, of which further details can be found in [40–42, 118] along with further details of the ILS pathways. (ERK, extra-cellular related kinases; PKC, protein kinase C; ACH, acetylcholine; PLC, phospholipase C).

Downstream signaling cascades of the ILS pathway can act in a tissue- and cell-specific manner [40–42], and are therefore capable of generating different trait allometries with body size in response to condition. In *D. melanogaster*, wing growth is sensitive to nutrition levels whereas male genitalia growth is not [51]. These trait differences in nutrition sensitivity translate into striking differences in trait allometry in Drosophila populations: wings scale positively and proportionally with variation in body size, while male flies produce similar genitalia sizes regardless of adult body size [54]. Tissue specific differences in sensitivity to ILS are responsible for the divergent patterns of wing and genitalia growth [54, 55], and manipulation of a downstream member of the ILS pathway, forkhead box-O transcription factor (FOXO), can decrease the sensitivity of wing growth to nutrition [54]. Because ILS pathway activity can diverge from tissue to tissue, co-option of this pathway during the evolution of trait exaggeration is a plausible route to the evolution of exaggerated growth.

Insulin/insulin-like signaling (ILS) pathways regulate growth of condition-dependent sexually-selected traits

Evidence implicating the ILS pathway in the evolution of extreme trait growth has begun to accumulate in several vertebrate and invertebrate species. In vertebrates, deer (Fig. 1A) provide the best-studied system, where many lines of evidence link ILS pathways and antler size in males [5, 25]. In both white-tailed deer [56] and red deer stags [57], IGF1 levels are positively correlated with body size, and antler size. In white-tailed deer antler size and IGF1 levels correlate with age, with IGF1 levels initially increasing, before decreasing above a specific age threshold [56]. A functional link between IGF1 and antler growth comes from in vitro studies of antler

cells, where IGF1 application stimulates cell growth [58]. In the swordtail fish, *Xiphophorus* sp. (Fig. 1E), growth of the elongated tail has been linked to co-option of a gene network that includes *fibroblast growth factor receptor 1* (*FGFR1*) [59]. Interestingly, it has been demonstrated in rabbits that IGF can up-regulate FGFR1 expression [60], suggesting that ILS signaling may regulate tail elongation, although this link remains to be tested in swordtail fish.

Within invertebrates, increased expression of InR, the target receptor for ILPs, is observed within the soon to be terminated horn primordia of hornless dung beetles (*Onthophagus nigriventris*) [61], and in the developing eye-stalks of female stalk-eyed flies, *Teleopsis dalmanni* (Fig. 1D), which do not grow exaggerated eye-stalks [62]. These results are consistent with inactivation of the ILS pathway within these tissues, as would be seen when exaggerated traits were not being grown, resulting in the upregulation of InR by feedback from the downstream target FOXO (Fig. 2) [6, 61, 63]. In crustaceans, a large body of research has focused on the androgenic gland and the hormone it secretes ("androgenic hormone"), which regulates growth of sexually dimorphic morphological and behavioral traits, including the enlarged chelae of male crabs and shrimp [64]. Crustacean androgenic hormone was recently identified as an insulin-related ligand [64, 65] and knock-down of the hormone itself affects body growth and molting [65], indicating that the ILS pathway may control condition-dependent growth of exaggerated sexually-selected traits in crustaceans.

The breadth of these examples provides exciting circumstantial evidence for involvement of the ILS pathway in the evolution of exaggerated sexually-selected animal structures. However, none of these studies compare the relative sensitivity of traits to test whether exaggerated structures are more sensitive to ILS signaling than other traits, as predicted if the evolution of exaggeration arose through increased tissue sensitivity to the ILS pathway. The first direct evidence for this hypothesis comes from the sexually dimorphic head horns of the Asian rhinoceros beetle, *Trypoxylus dichotomus* (Fig. 1C) [21, 22, 66]. Male rhinoceros beetles wield a forked horn on their heads that: exhibits disproportionate growth compared to other body parts; is hypervariable among males dependent on body size; and exhibits heightened sensitivity to condition [21, 22, 66]. Males use horns to resolve competition at mating sites [21, 22, 66]. We tested whether rhinoceros beetle horns were more sensitive to ILS during growth than wings or genitalia using RNA interference (RNAi) to knockdown gene function of the InR just prior to pupation, a critical period for organ proliferation [66]. The InR is a keystone within the ILS pathway as it is the binding site on the cells of growing organs to which ILPs bind in order to stimulate growth (Fig. 2). Reducing InR expression affects the entire pathway by blocking ILP signals to the cells. We measured adult structures of treated and control animals and found dramatic variation between the three traits in their sensitivity to ILS, including heightened condition sensitivity for head horns (Fig. 3). Genitalia did not respond to RNAi knockdown, whereas both wings and horns did. The resulting reduction in horn size was eight times greater than that observed in wings, consistent with the evolution of disproportionate or exaggerated weapon size resulting from enhanced tissue-specific sensitivity to the ILS pathway.

Increased sensitivity to ILS should cause traits to be reliable signals of condition

Earlier, we outlined three key features of sexually-selected exaggerated traits that make them reliable signals of condition (extreme size, high variability in size, and heightened sensitivity to condition), and the results described above indicate the ILS pathway could be a common mechanism for providing them. Circulating levels of IGFs/ILPs are tightly linked to the condition of an individual, therefore providing a precise metric of condition circulating within animals that can be interpreted directly by growing traits. Furthermore, growth responses to IGFs/ILPs are tissue specific, and trait sensitivity to the ILS pathway can be adjusted by selection according to a trait's function (Fig. 3). For example, strong stabilizing selection exists on genitalia in many insects to maintain a constant absolute size (due to a variety of factors including mechanical constraints, as well as sexual conflict [67–69]), and this may explain their relative insensitivity to the ILS pathway. Wings and legs are required for locomotion and scale proportionately with body-size, and this is achieved by moderate sensitivity to ILS pathways. Sexually-selected exaggerated traits often communicate an authentic and precise measure of their bearer's condition, and we now suspect that this is achieved by heightened sensitivity to the ILS pathway. Low condition males have low circulating levels of IGFs/ILPs and the signal trait grows very little in response. High condition males have high circulating levels of IGFs/ILPs and this causes disproportionately large amounts of signal trait growth. Subtle differences in individual condition are thus amplified in the signal trait due to its heightened sensitivity to the ILS pathway. Indeed, evolutionary increases in the sensitivity of a trait to ILS should generate all three of the properties of a reliable signal of male condition (Fig. 3).

In the remainder of the review we discuss the effects of these findings on the evolution of sexually-selected traits. We also discuss the interactions of ILS pathways with other endocrine pathways, as well as possible implications for other forms of exaggerated sexually-selected traits. Finally, we discuss future directions for study in order to further test and develop our hypothesis.

Implications for the evolution of trait exaggeration

Even this preliminary understanding of the mechanistic processes regulating growth of exaggerated structures has important ramifications for understanding the evolution of sexually-selected traits. ILS has coupled tissue growth with nutrition, stress, and physiological condition in metazoans for at least half a billion years [40–42]. The near universality of this mechanism means that it likely regulated the growth of almost all adult structures in animals. That is, morphological structures would already have been sensitive to these signals, and among-individual variation in body and trait sizes would

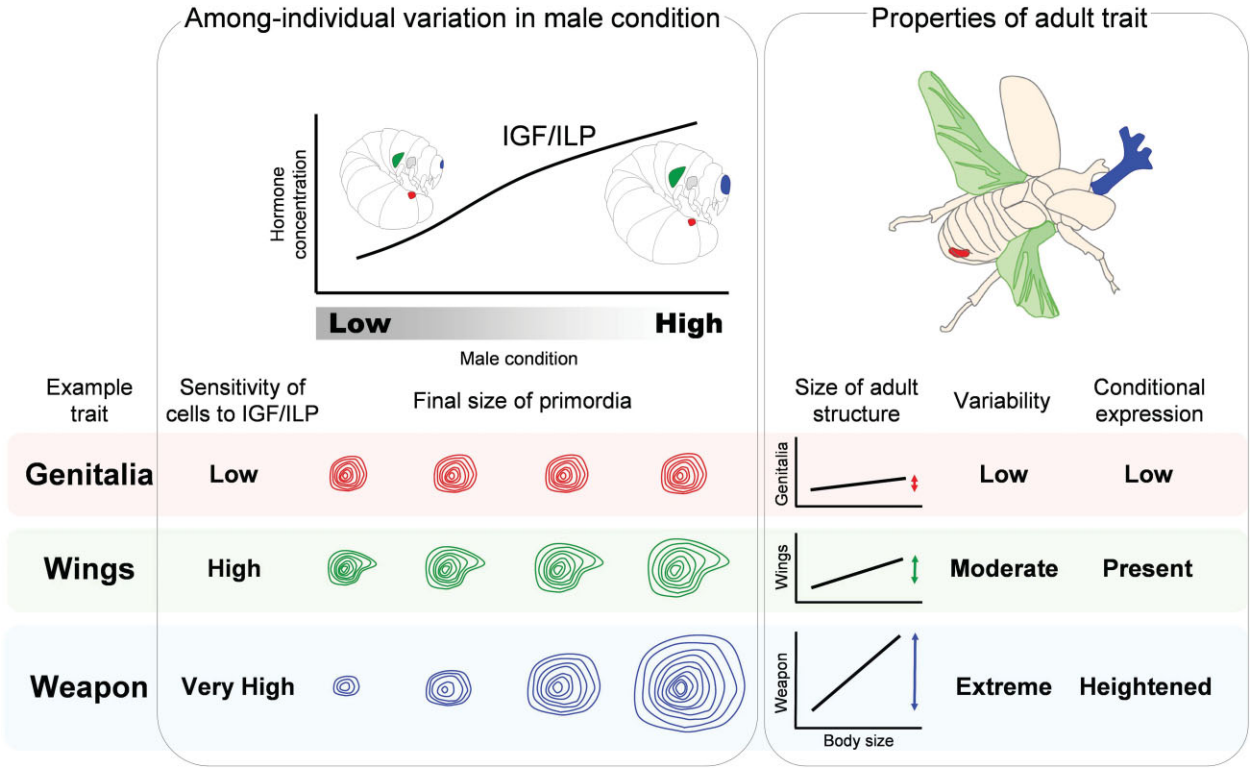

Figure 3. A mechanism for the evolution of trait exaggeration through increased cellular sensitivity to the ILS pathway shown for the imaginal disc primordia of insects. Circulating concentrations of insulin, ILPs and/or IGFs reflect nutritional state and physiological condition of an individual. Growth of each of the trait primordia is modulated by these signals but traits exhibit different sensitivities to these circulating signals. For example, some traits such as male genitalia (red) are less sensitive to the same signal than other traits such as wings (green), which exhibit nutrition-dependent phenotypic plasticity and among individual variability. Any increase in the sensitivity of cells within a particular trait, such as horns (blue) would lead to disproportionately rapid growth of that trait in the largest, best condition individuals and smaller trait sizes in low condition individuals. Figure adapted from [66].

likely have existed due to these mechanisms. Because ILS pathways were already active in tissues during growth, increases in the sensitivity of cells in a specific structure to these signals need not have been a difficult step. Indeed, studies in Drosophila suggest that subtle changes to expression of any of numerous ILS pathway elements could accomplish this outcome (such as by resulting in overgrowth of a specific structure [54, 55, 66]).

Not only should increases in sensitivity to ILS have been easy to attain, but once established, alleles enhancing ILS sensitivity may have experienced positive selection in the context of male-male competition or mate choice. Mutations increasing the sensitivity of a structure to the ILS pathway would have generated individuals with unusually large versions of the structure (Fig. 3). But, given the way these mechanisms work, only high-condition animals would express the enlarged trait because poor condition individuals inheriting the same mutation would express the original sized structure, or even a diminutive version of the trait. Enlarged structures would be highly conspicuous, increasing the chances that other individuals in the population would notice them, and the intrinsic reliability of these structures as signals of male condition would have favored the evolution of receivers who responded to them, reinforcing the evolution of both signal and response.

Finally, as these structures evolved to ever-larger sizes, their utility as signals should have continued to increase, since ever increasing ILS pathway sensitivity would be coupled with increased trait size, amplification of trait variability, and increased condition-dependence – all three parameters of a reliable signal. Thus, the properties of this mechanism may have predisposed populations to the rapid evolution of extreme trait size.

We suspect that the intrinsic reliability of exaggerated structures arising from increased sensitivity to ILS may have facilitated early stages in the evolution of extreme trait size in ornaments and weapons of sexual selection. However, this does not exclude the evolution of cheaters. We already know that it is possible for structures to evolve in sensitivity to ILS signaling [54, 55], and these changes should uncouple growth of a structure from among individual variation in male condition. Once a signal trait has evolved, mutations causing growth of the structure to be insensitive to ILS could invade a population, and spread. Individuals bearing such mutations would produce large structures regardless of their condition. However, the spread of these alleles would also erode the efficacy of the trait as a signal, since trait size would be decoupled from male condition. Similar to the cycling of body size proposed by Maynard Smith [70], we suggest that this cycle of escalation and evolution of extreme trait size, followed eventually by erosion of condition-dependence for

the traits, may be a typical dynamic in clades of ornamented or weaponed species, perhaps accounting for the widespread patterns of both gains and losses of these extreme structures [64–66].

ILS pathways do not act alone in regulating condition-dependent traits

ILS pathways may be involved in the development of diverse sexually-selected exaggerated traits, but they are unlikely to act alone. In both vertebrates and invertebrates, ILS pathways interact with a host of other pathways to regulate growth [38, 40], and therefore control condition-dependent growth through a variety of mechanisms.

In vertebrates, IGF interacts with growth hormone (GH) signaling, and together they are sometimes referred to as the IGF-GH growth axis [40]. In addition to its regulation of IGFs, GH itself can have direct effects on growth (e.g. [71]). The ILS pathway also appears to interact with both androgens (including testosterone) and glucocorticoids [40]. For example, circulating levels of testosterone are increased by IGF1R activity acting specifically in testicular cells, predicted to be brought about by the mitogenic effects of IGF1R [72]. In deer antlers, in vitro studies have shown that both testosterone and IGF1 are capable of promoting antler growth (for review see [58]). Precisely how these two hormones interact to affect antler growth remains unclear [58], but under certain in vitro conditions testosterone can only promote antler growth in the presence of IGF1 [73].

In metamorphic insects, two interacting endocrine pathways are well known to be involved in trait growth and trait exaggeration: the sesquiterpenoid juvenile hormone (JH) and the steroid molting hormone ecdysone (Box 2). Both have critical roles in the development of adult insect organs, which develop during the late larval and early pupal stages (Box 2) [74]. Interestingly, there is evidence that both JH and ecdysone signaling pathways interact with the ILS pathway [75–78]. In insects, JH signaling can both upregulate, and be upregulated by, the ILS pathway [75, 76]. Furthermore, manipulation of expression of ILS pathway genes within the ring gland and prothoracic glands in Drosophila resulted in variation in the size of the glands, and variation in the ecdysone levels they produced [78, 79]. When the expression of non-nutrition dependent genes involved in size regulation (*myc* and *cyclinD/Cdk4*) were manipulated, variation in prothoracic gland size was again observed, but without variation in ecdysone signaling [78]. Another example is from the silkmoth, whose ILP bombyxin promotes release of ecdysone resulting in molting and metamorphosis [80]. Taken together, these studies suggest that ecdysone signaling can be regulated through the ILS pathway to generate exaggerated trait growth.

These studies clearly show that the ILS pathway interacts with several other endocrine pathways in ways relevant to the growth of extreme structures. In particular, ILS may integrate information from a breadth of physiological pathways, reinforcing the idea that the ILS pathway acts as a central mechanism for regulating condition-dependent, sexually-selected exaggerated trait growth.

Box 2

Juvenile hormone (JH), ecdysone, and trait exaggeration in insects

Endocrine pathways in insects are responsible for a wide range of physiological processes from the control of molting to development and growth. The sesquiterpenoid JH and the steroid molting hormone ecdysone are well studied and interacting endocrine pathways, critical for the transition of insects through their developmental life-stages [74]. Pulses of ecdysone are responsible for the transition of developing larvae through developmental stages, when JH levels are high, the transitions occur between larval instars. Just prior to pupation, JH levels drop and the subsequent ecdysone pulse causes larval gut-purge followed by pupation and regulates cell proliferation within the imaginal disks [109]. The involvement of JH and ecdysone in exaggerated trait development has also been well studied. Elevated JH signaling has been shown to be responsible for the development of the exaggerated, sexually dimorphic horns of dung beetles *Onthophagus taurus* [110, 111], enlarged mandibles of the male stag beetle *Cyclommatus metallifer* [112] and horn growth in the broad-horned flour beetle *Gnatocerus cornutus* [113]. In the stalk-eyed fly, *Teleopsis dalmanni*, JH analog (JHA) application can increase relative eye-stalk size [114]. In addition to its significant role in exaggerated trait growth, JH may also be involved in coordinating and distributing the limited resources available to individual traits during pupal growth of adult structures [113]. An illustrative example is from the sexually dimorphic broad-horned flour beetle, *G. cornutus*, which has enlarged male mandibles and a male head horn. Application of JHA resulted in increased mandible growth along with traits for their mechanistic support. In contrast, wings responded to JHA by becoming relatively smaller, and legs exhibited no response [113]. Differences in ecdysone levels correlate with the expression of beetle horns in *O. taurus* [110]. In addition, results from the stalk-eyed fly, *T. dalmanni* (Fig. 1D), suggest ecdysone signaling is involved in trait exaggeration [115, 116]. Microarrays [115] and quantitative trait locus mapping [116] have identified candidate genes involved generating increased eyespan, amongst these are *ecdysone-induced protein 75B* (*Eip75B*) and *ecdysone-inducible gene L2* (*eimpl2*), which are both downstream targets of ecdysone [117]. However, this link remains to be tested causally.

ILS pathways and other types of sexually-selected signals

So far we have focused on traits that are exaggerated in their size or growth. However, mate preference can also be based on

other signals that follow the three basic principles for signal reliability (e.g. highly conspicuous/detectable, extremely variable, and condition-dependent expression) [8, 14, 15]. Behavioral mating displays and rituals provide highly informative indicators of individual condition [81]. These include the acoustic signals of frogs, crickets, and birds which have been shown to be reliable signals of male condition [82–84]. In songbirds, studies [85, 86] have found evidence of differential regulation of ILS correlated to male courtship song. In zebra finches and canaries, increased IGF2 expression is present in the areas of the brain critical for the production of male courtship song, and is correlated with periods of rapid neuronal growth within these regions [85]. Using a microarray approach, male junco songbirds were found to differentially express insulin-like growth factor receptor (IGFR1) and phosphoinisitide 3-kinase along with other members of the ILS pathway (Fig. 2) in the brain region responsible for song generation when compared to female juncos [86]. These examples provide correlative links for the role of ILS in reliable sexual signals of condition.

In addition, bright coloration (e.g. in sticklebacks [87] and the colorful plumage in birds [36, 37]) indicate condition of the male. In the freshwater prawn, *Cherax quadricarinatus*, a male-specific soft red claw patch is linked to male condition [88]. Removal of the androgenic gland, and therefore the insulin-like androgenic hormone, results in a reduction of patch size [89]. Finally, the level and compositions of pheromonal cues can indicate condition (e.g. in *D. melanogaster* [90, 91], and the ornate moth [92]). Recent studies in Drosophila link the ILS pathway with cuticular hydrocarbon (CHC) production and attractiveness [90, 91]. In these flies, levels of CHCs were negatively impacted by dietary restriction, which affected an individual's attractiveness to potential mates [90]. Furthermore, when expression of members of the ILS pathway were individually removed, the effects of poor nutrition were replicated resulting in an individual's attractiveness being reduced [90, 91]. Further studies will be needed to test for roles of ILS pathways in additional sexual signals, including especially bright colors and elaborate courtship dances, but accumulating evidence now hints that the ILS pathway may be critical in generating a plethora of condition-dependent sexually-selected signals.

Future directions

The evidence presented here indicates that the ILS pathway may be a critical, and repeatedly used, pathway for generating condition dependent signals across a wide range of species. This hypothesis opens up several avenues of research, the most pressing is to explicitly confirm the ILS pathway's involvement in a wider range of species and differing types of condition-dependent sexually-selected traits (expanding on those in [66]). In the final sections of this paper we focus on two further future research areas that will robustly test and develop our hypothesis. The first is comparing different types of trait exaggeration (e.g. non-sexually-selected exaggerated traits), and the second is trying to identify exactly how heightened sensitivity to ILS pathways is generated.

Future direction one: Comparisons to non-condition dependent exaggerated traits

Not all exaggerated traits function as signals of sexual selection; e.g. the elongated hindlegs of jerboas [93], elongated foredigits in bats [94–96], and enlarged rear legs in crickets [97], result from selection on locomotion. Similarly many brightly colored traits, vocalizations, and behaviors exist that do not communicate condition, instead they act as warning signs and/or camouflage [98], such as the bright warning colors of poison arrow frogs and elaborately colored caterpillars. Since the purpose of these traits differs from condition-dependent sexually-selected traits, their expression does not follow the same pattern we have discussed. They are neither hypervariable nor condition dependent, and they are expressed by both males and females. For these reasons, they may act as ideal comparison studies for highly condition-dependent traits to further understand and validate the involvement of the ILS pathway in exaggerated sexually-selected trait evolution.

Studies into the development of several non-sexually-selected exaggerated traits indicate that multiple mechanisms not involving the ILS pathway regulate the development of these traits. The highly extended foredigits of bats result from changes in the expression of limb patterning genes [94–96]. Increased expression of bone morphogenetic protein 2 (BMP) [94], and a second wave of expression of the morphogen *sonic hedgehog* are observed in developing bat foredigits [94, 96]. In addition, application of *BMP* protein promotes exaggerated growth in bat embryonic foredigits [94]. Further strong evidence that limb patterning genes regulate limb elongation in bat foredigits comes from replacing the mouse homoeobox (hox) gene prx1 regulatory region with the bat version, resulting in foredigit elongation in transgenic mice [95].

Another example of how limb patterning genes are involved in trait exaggeration is from the elongated limbs of some insect species. Limb elongation in insects for improved locomotion is widespread with examples including exaggerated hindlegs in milkweed bugs and crickets [97], as well as the enlarged middle legs of water striders [99], which function as "oars" for propulsion. In all three examples, the hox gene *ultrabithorax* (*ubx*) has gained an expression domain within the exaggerated limbs during the later stages of trait growth and RNAi knockout phenotypes result in the exaggerated limb returning to the size of the non-exaggerated limbs [97, 99].

It is important to note that all sexually-selected structures, if they are body outgrowths, are likely to be patterned by hox genes and appendage patterning pathways [6, 100]. For example, studies of the development of dung beetle horns have identified functional roles for patterning genes in these growing structures (e.g. [101, 102]). But it remains unclear if or how expression of these genes contributes to exaggerated growth of these structures. We suspect that co-option of the appendage patterning pathway was a critical evolutionary step in the origin of beetle horns [6, 100], and that subsequent elaborations in weapon size – in particular, elaborations resulting in heightened condition-sensitive expression – resulted from increases in ILS pathway sensitivity within horn cells.

The relevant question becomes: once an appendage or outgrowth is present, how does it then evolve to extreme size? The answer, we now propose, depends on the nature of selection for exaggeration. Thus we predict that when natural selection (e.g. locomotion) drives increased size, exaggerated growth will be caused by further alterations in the expression of patterning genes. In contrast, if the exaggeration is used as a signal trait due to sexual selection, then we would predict exaggerated growth to be linked to alteration in the ILS pathway. Testing these hypotheses will shed more light on how exaggerated traits of all types evolve, in particular sexually-selected exaggerated traits.

Future direction two: How is increased sensitivity to ILS pathways generated?

The evidence described here implicates ILS pathways as a whole in trait exaggeration, but an important question that remains is how exactly heightened sensitivity to the ILS pathway is generated? The ILS pathway is complex, with many interacting components, with many isoforms, that may be incorporated into generating heightened condition sensitivity and extreme growth (Fig. 2). The transcription factor FOXO can generate insensitivity to ILP levels in Drosophila genitalia [54, 55]. This result, combined with its position as a downstream convergence point for the ILS pathway (Fig. 2) has resulted in FOXO being proposed as the key regulator of condition-dependent trait expression [103]. However, in those studies it was not possible to generate exaggerated growth, with both under- and over-expression resulting in insensitivity to the ILS pathway [54]. Furthermore, FOXO knockouts in horned beetles, while alleviating ILS pathway insensitivity in genitalia, only caused a modest increase in horn size [104], therefore we propose that other members of the ILS pathway may be responsible for heightened sensitivity. Previously, experiments that dissect apart signaling pathway members with fine detail were only possible in established model systems (e.g. Drosophila and mice). However, with the establishment of next generation sequencing, combined with the development of functional genomic tools in species with sexually-selected traits [66, 105–107] understanding exactly how heightened sensitivity to the ILS pathway is generated sits well within our grasp.

Conclusions and outlook

Extreme traits are intrinsically reliable because of the signaling pathway linking their extreme expression, increased between individual variability, and heightened sensitivity directly to condition. Here, we propose that the widely conserved insulin/insulin-like signaling (ILS) pathway provides a central mechanism for generating sexually-selected exaggerated traits. The possibility that reliable signaling arises as a by-product of this widespread and highly conserved growth mechanism goes far in explaining why trait exaggeration has evolved so many different times in the context of sexual selection.

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