

Both cell-autonomous mechanisms and hormones contribute to sexual development in vertebrates and insects

Ashley Bear and Antónia Monteiro

The differentiation of male and female characteristics in vertebrates and insects has long been thought to proceed via different mechanisms. Traditionally, vertebrate sexual development was thought to occur in two phases: a primary and a secondary phase, the primary phase involving the differentiation of the gonads, and the secondary phase involving the differentiation of other sexual traits via the influence of sex hormones secreted by the gonads. In contrast, insect sexual development was thought to depend exclusively on cell-autonomous expression of sex-specific genes. Recently, however, new evidence indicates that both vertebrates and insects rely on sex hormones as well as cell-autonomous mechanisms to develop sexual traits. Collectively, these new data challenge the traditional vertebrate definitions of primary and secondary sexual development, call for a redefinition of these terms, and indicate the need for research aimed at explaining the relative dependence on cell-autonomous versus hormonally guided sexual development in animals.

Keywords:

cell-autonomous; gonads; insects; sexual development; sexual dimorphism; sex hormones; vertebrates

DOI 10.1002/bies.201300009

Department of Ecology and Evolutionary Biology, Yale University, New Haven, CT, USA

Corresponding authors:

Ashley Bear
E-mail: ashley.bear@yale.edu
Antónia Monteiro
E-mail: antonia.monteiro@yale.edu

Abbreviations:

ESD, environmental sex determination; GSD, genetic sex determination.

Introduction

The evolution of sexual dimorphism in animals is believed to proceed via the action of sexual or natural selection acting differentially on each sex. Differential selection on each sex began with the origin of the sexes themselves, i.e. with the origin of anisogamy, when different individuals within a species began to invest differentially in their gametes. Some producing few sessile large gametes with nutrients to provision the offspring (the egg), and others producing many motile small gametes (the sperm) with the ability to find the larger gametes [1, 2]. Subsequently, in order to ensure successful reproduction, males and females evolved a series of additional sex-specific traits, such as external and internal genitalia, as well as morphological, physiological, and behavioral traits that not only facilitated wooing, winning, and mating with an animal of the opposite sex, but also promoted survival until reproduction (Fig. 1) [3]. Here, we turn our attention to the developmental mechanisms that underlie the differentiation of male and female specific traits in different animal groups.

Sexual development in vertebrates and insects has long been thought to proceed via distinct mechanisms. Traditionally, the development of sexual traits in vertebrates has been thought to consist of two distinct phases, known as primary and secondary sexual development. During primary sexual development, genetic or environmental factors determine the sex of the gonad, where sperm and eggs differentiate, whereas during secondary sexual development, the differentiated gonad secretes hormones that direct the development of all other sexually dimorphic aspects of the organism. In this case, individual cells outside the gonads gain a sexual identity by receiving a sex-specific hormonal signal. In contrast, sexual development in insects is thought to depend on a single mechanism known as cell-autonomous sexual development. Here, each cell in an organism relies on information from the sex chromosomes in order to develop the appropriate sexual phenotype and is thought to “know” its sexual identity” [4].

Recent studies, however, support the idea that both cell-autonomous and hormonal regulation of sexual development is found in vertebrates as well as insects. Here, we review

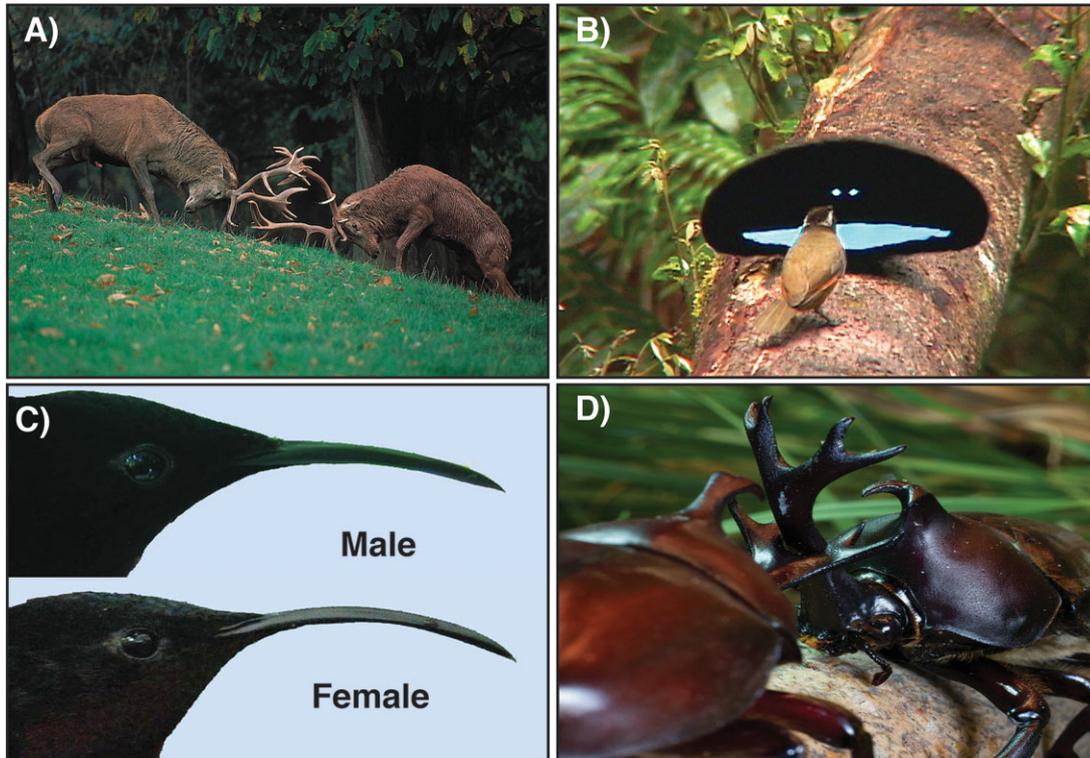


Figure 1. Secondary sexual traits are diverse and widespread throughout nature. **A:** Only male deer have antlers, which they use as weapons to fight for mates (photo from Encyclopedia Britannica Kids, Stefan Meyers GDT/Ardea London). **B:** The male “superb bird of paradise” (*Lophorina superba*) uses elaborate ornamentation in the form of iridescent plumage to attract the much drabber female (photo by E. Scholes/Maculay Library). **C:** Some animals are sexually dimorphic for ecological reasons, e.g. the purple throated carib hummingbird (*Eulampis jugularis*), in which male and female beak shape differs because each sex feeds on a different type of flower (photo by E.J. Temeles). **D:** Insects often use weapons to fight for females as well, an example being rhinoceros beetles, which use their sclerotized horns to do combat (photo by W. Freihofer and D. Emlen).

these studies and how they influence our current understanding of the mechanisms of sexual development in birds, mammals, and insects. These studies also show that sex hormones can be synthesized in the gonads, as well as in other tissues, such as the brain [5–7]. In addition, in many vertebrates, the initial, “primary”, sex-determining mechanisms in the zygote are no longer exclusively involved in gonadal sexual development, but rather, also play a role in the development of other sexually dimorphic traits, which can sometimes develop before the gonads. In light of this information, we argue that the terms “primary” and “secondary” no longer accurately describe sexual development in vertebrates, that these terms should revert to an earlier definition based on the evolutionary significance of the sexual trait, and that future research in the field should focus on explaining the variable degrees of cell-autonomous versus hormonal mechanisms of sexual development used by each animal clade.

What are the mechanisms of primary sex determination in animals?

The first step in primary sex determination is the presence of sexually dimorphic factors in the developing zygote [8]. These factors can be genetic differences, in which case the organism relies on “genetic sex determination” (GSD), or environmental factors, in which case the organism relies on “environmental sex determination” (ESD) [9]. Both GSD and ESD lead to the differentiation of the gonads. In the case of GSD, males and females have specific sex chromosomes: XX and XY chromosomes in mammals and dipterans or ZW and ZZ chromosomes in birds and lepidopterans. The sex-specific genes that are present on these chromosomes determine whether an individual develops male or female gonads, and these genes differ across different taxa. For example, in mammals, the Y-linked “sex-determining region Y” gene (*sry*) is the initial sex-determining gene [10], whereas the W-linked *fem* gene is the primary sex-determining gene in some lepidopterans, such as the silkworm moth [11]. The sexual identity of the gonads then determines whether the germ line cells develop into sperm or eggs.

In contrast, ESD is a process in which environmental factors experienced by the individual, such as temperature, photoperiod, the presence of conspecifics, etc., determine either male or female gonadal development [9]. At this time, the molecular basis of ESD is not entirely understood and studies on temperature sex determination (TSD) in reptiles have shown that mechanisms of TSD alone vary considerably among different groups [12]. This variation makes a complete understanding of how temperature leads to male or female development elusive. Nevertheless, genes that

exhibit temperature-sensitive expression, including orthologs of mammalian sex-determining genes, appear to initiate gonadal development [13].

In summary, sexually dimorphic factors in the developing zygote, whether derived from genes, the environment, or a combination of both [14], initially establish the gonadal sex of the individual and determine whether that individual will produce male or female gametes: the only universally conserved feature that distinguishes males from females [3].

What are the mechanisms of secondary sexual trait development in birds?

Gonadal sex hormone secretions are known to play an important role in regulating secondary sexual development in birds. Regulation of secondary sexual development, specifically via gonadal sex hormones, is thought to be evolutionarily advantageous because these hormones are able to coordinate secondary sexual development with the maturation of the gonads. By communicating with other tissues in the body, sex hormones ensure that an animal develops certain sexual traits that are important for successful reproduction only at a time when the organism is physiologically capable of mating [15]. This is important because many sexual traits are costly and conspicuous and, thus, fitness-reducing when expressed before maturity.

Many male and female sexual traits are controlled by sex hormones such as estrogens and androgens. Though estrogens are usually considered the major sex hormones in female vertebrates and androgens (such as testosterone) the major sex hormones in male vertebrates, males and females make both types of hormones, and it is the ratio of these hormones in the body that can influence whether an individual develops male or female traits [16]. For instance, male birds injected with estrogens develop many of the sexual traits of females, while depletion of these hormones in female birds causes them to express male typical sexual traits [17]. Recent studies, however, have shown that secondary sexual development in birds also depends on cell-autonomous mechanisms and on sex hormones produced in tissues other than the gonads [5, 14, 18–20].

Cell-autonomous sex determination mechanisms for birds were first proposed upon the discovery of a zebra finch in which the right side of the body was genetically and phenotypically male and the left side of the body was genetically and phenotypically female [21] (Fig. 2A). This finch's phenotype is an example of gynandromorphy. Gynandromorphy typically occurs due to an error during cell division that results in an un-natural distribution of the sex chromosomes in the daughter cells such that one of the daughter cells gains a female genetic identity, while the other gains a male identity. The different sexual identities of these daughter cells will be passed on to their daughter cells, and so on, until part of the organism is female, while the other part is male. Thus, because half of this finch's cells contained female sex chromosomes, while the other half had male sex chromosomes, this finch displayed typical male plumage coloration on one half of its body and female plumage

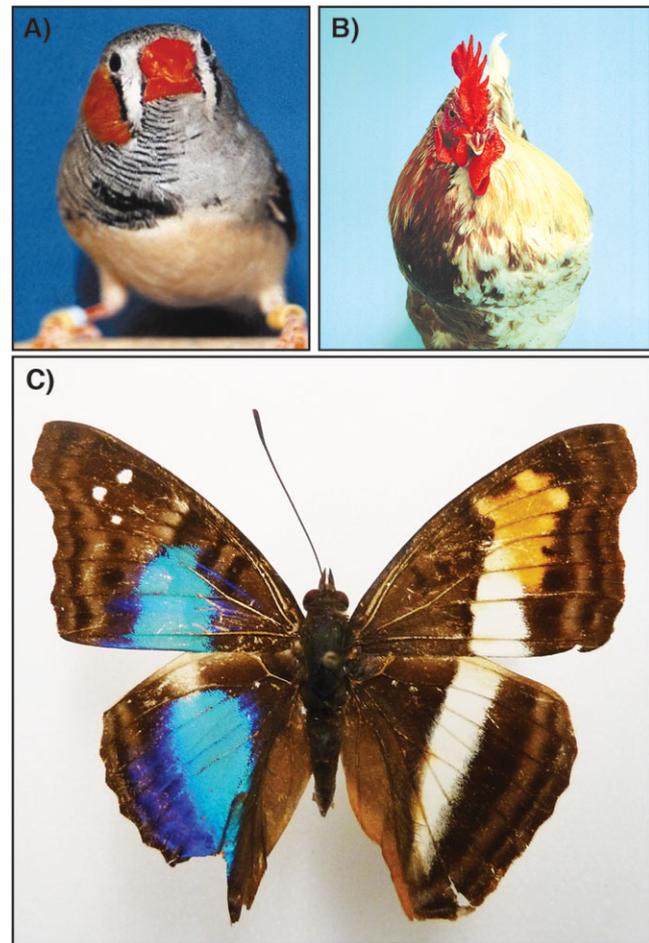


Figure 2. Animals that are half male and half female (gynandromorphs) usually indicate cell-autonomous mechanisms of sexual development. **A:** The gynandromorphic zebra finch that exhibits a male phenotype on its right side and a female phenotype on its left side (photo by R.J. Agate). **B:** A gynandromorphic chicken in which the left side exhibits a male phenotype and the right side exhibits a female phenotype (photo by M. Clinton, courtesy of the The Roslin Institute, The University of Edinburgh). **C:** A gynandromorphic butterfly (*Doxocopa* sp.), in which the right side of the body exhibits a female phenotype and the left side of the body depicts a male phenotype (specimen from the Peabody Museum of Natural History, photo by A. Monteiro).

coloration on the other half. The finch's brain was also part male and part female, with areas of the brain associated with song production exhibiting a male phenotype on the right side of the brain, and a female phenotype on the left side of the brain. These differences cannot be easily explained by the presence of a gynandromorphic gonad because an imbalance of the normal male and female hormone ratios produced by such a gonad would have produced an intersexual individual, with intermediate traits, which was not the case. These differences suggested instead, that brain and epidermal cells generating plumage established their sexual identity directly from the sex chromosomes, as is often the case in insects.

Since this landmark study, researchers have established further support for a role of cell-autonomous secondary sexual development in birds by examining the chromosomal identity of chicken gynandromorph tissues, by performing tissue transplants into hosts of a different sex, and by identifying sexually dimorphic gene expression in the brain in a variety of bird species. For example, chicken gynandromorph tissues exhibiting female-like traits, such as brown plumage and a small wattle (the red fleshy tissue hanging from the throat region), were predominantly made of cells containing female sex chromosomes, while tissues exhibiting male-like traits, such as white plumage and a large wattle, were predominantly made of cells containing male chromosomes [22] (Fig. 2B). These same results were confirmed with transplantation experiments using green fluorescent protein (GFP)-labeled “donor cells” from either male or female embryos [22]. Furthermore, the putative female-determining gene, *Hintw*, as well as the putative male-determining gene, *Dmrt1*, were found to be differentially expressed in female and male chicken embryonic brains before the development of the gonads, showing that this sexually dimorphic pattern of gene expression could not be influenced by gonadal sex hormone secretions [23].

Additional experiments in birds have shown that while some sexual traits *do* depend on hormones to direct their development, these hormones *do not* necessarily originate from the gonads. For instance, brain tissue transplants from female Japanese quail (*Coturnix japonica*) into a male host led to adult behavior that was not typical of males [24]. Presumably, these bird brains were insensitive to the influence of male gonadal hormone secretions, yet the physiological reason for this is unclear. One possibility is that female behavior in Japanese quail is primarily dependent on cell-autonomous mechanisms (i.e. the read-out of the sex chromosomes). Another possibility is that these cell-autonomous mechanisms in the brain lead to local synthesis of sex hormones. We derive the latter hypothesis from the fact that the bird brain is known to convert androgens into estrogens through the local activity of the enzyme, aromatase, and these neural estrogens play a powerful role in the regulation of sexually dimorphic behaviors in birds [5]. Therefore, we speculate that differences in aromatase expression in the female brain, as compared to the male brain, might limit the influence of male gonadal hormone secretions on behavior.

All these studies demonstrate that secondary sexual development in birds does not depend only on sex hormones produced by the gonads. These data challenge the traditional view of secondary sexual development in birds and demonstrate that the initial factors that determine primary sexual development, in this case genetic factors, are also directly involved in secondary sexual development. Thus, secondary sexual development in birds should no longer be considered to depend exclusively on sex hormones produced in the gonads.

What are the mechanisms of secondary sexual trait development in mammals?

Though there is ample evidence that sex hormones secreted by the gonads play a major role in mammalian secondary sexual

development, new data show that, as in birds, cell-autonomous mechanisms and extra-gonadal sex hormone secretions also play a role [7, 25, 26]. Recent studies have shown that secondary sexual development in mammals can begin before the development of the gonads, refuting the long held idea that vertebrate embryos are gender neutral until the development of the gonads and their subsequent secretion of sex-specific hormones. For example, the development of the scrotum or mammary glands in marsupials is determined before the development of the gonads, as is the sex-specific neural development of embryonic brain tissue from male and female rats cultured in vitro under identical conditions [7, 27, 28].

In addition, mammalian organs other than the gonads have been shown to produce sex hormones, such as the brain, adrenal gland, breast tissue, fat, kidney, liver, bone, lung, thymus, and skin [12]. Hormones, such as estrogen, can be synthesized in certain brain regions and play a role in establishing male and female specific behaviors [7]. Thus, it is possible that sex-specific neural development in pre-gonadal embryonic brains could result from cell autonomous mechanisms, neural sex hormone synthesis and secretion, or a combination of both.

Studies that separate gonadal sex and genetic sex have also found that mammalian secondary sexual development can be independent of the gonadal sex of the individual [29]. For instance, genetic female mice that have testes, because of a transgenic autosomal mis-expression of *sry*, were still more likely to be inflicted with diseases that typically inflict females more than males, including anterior neural tube closure, multiple sclerosis, and lupus. This was because susceptibility was found to depend on the number of X chromosomes in the affected tissues, not on gonadal hormones [30, 31]. Conversely, knockdown of *sry* in the brains of adult mice led to male-specific behavioral impairment even though the gonads were intact [32]. Moreover, mice that are genetically male but gonadally female (and vice versa) have brain development and behavior that matches their genetic sex not their gonadal sex [29]. Also, a recent study that divorced gonadal and genetic sex demonstrated that sex differences in obesity can be attributed to the X chromosome [26]. These data suggest a role for cell-autonomous mechanisms and *sry* in regulating sexual development in these mice.

Taken together, the recent findings described above provide evidence that the chromosomal sexual identity of cells, in addition to gonadal and extra-gonadal secretions of sex hormones, have an impact on secondary sexual development in mammals. Specifically, mammals exhibit sexual development before the development of the gonads, and sex-specific traits are influenced by the sex chromosomes independently of gonadal sex. Furthermore, sex hormones can be synthesized in extra-gonadal tissues in mammals. Thus, as in birds, gonadal sex hormone secretion should no longer be defined as the only essential step in secondary sexual development, but rather as one of many factors that can play a role in regulating these traits. These findings make the classical definition of primary and secondary sexual development confusing because we now know that “secondary” sexual development can occur before “primary” sexual

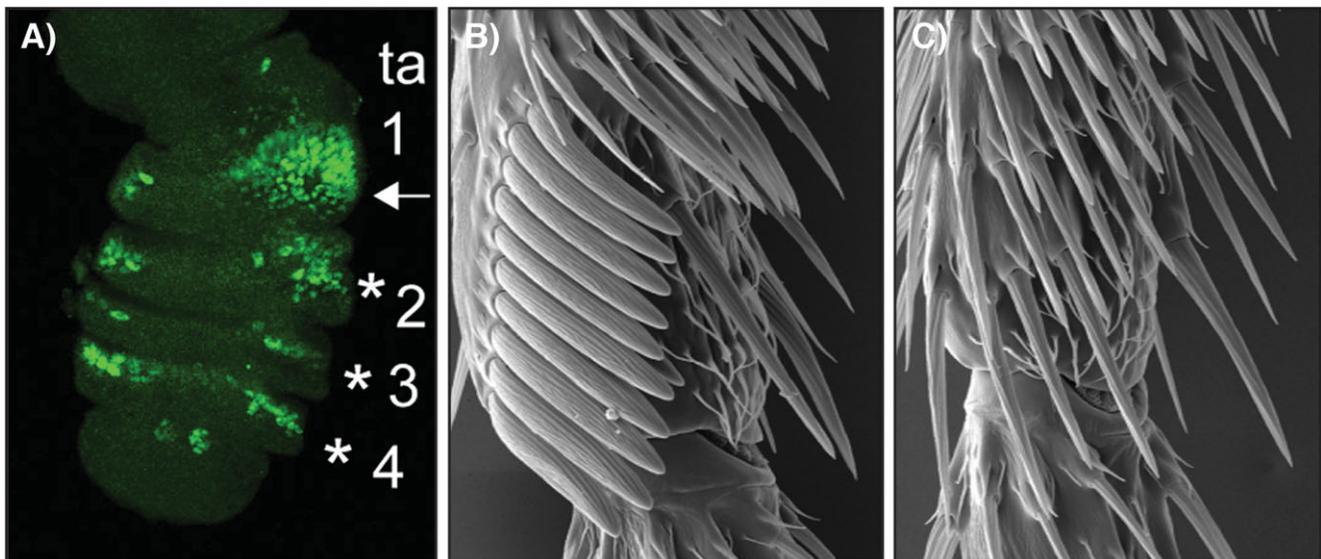


Figure 3. A conserved protein of the sex differentiation pathway, Doublesex (*Dsx*), is present primarily in cells in the fly tarsus that will become sexually dimorphic. **A:** Immunostaining (in green) shows that *Dsx* is strongly expressed on the ventral-anterior side of distal tarsus (*ta*), but is absent in most other leg tissues. Numbers indicate tarsal segments. Arrow points to future location of the sex combs that develop only in male legs and are used to grasp females during mating. **B:** SEM image of male distal tarsus with sex combs and **C:** female tarsus lacking these modified bristles (photos from Tanaka et al. [39]).

in certain neurons in the brain, and in sexually dimorphic tissues such as the external genitalia, pigmented areas in the abdominal tergites, and tarsal segments on the legs of *Drosophila* [4, 38–40]. Nevertheless, there are also many *D. melanogaster* cells that do not express *dsx*, and these cells are sexually neutral [38, 40] (Fig. 3). So, the gynandromorphic fly described by Morgan, despite its outward half male, half female appearance, had many tissues that were not reading their sexual identity from the sex chromosomes.

development, and both aspects of sexual development can depend on the same mechanisms.

Several lines of evidence suggest that hormones may also be playing a role in regulating sexual development in insects. In most of these cases, however, the data are tentative rather than conclusive. These data come from hormone titer measurements in males and females, manipulations of endocrine signaling, and studies on the influence of *Wolbachia* infection on insect sex ratios. These studies are described in the following paragraphs.

What are the mechanisms of sexual trait development in insects?

Insects produce androgens and estrogens as do vertebrates [41] but they do not use them as sex hormones, and it is unclear what these hormones actually do. Instead, insects appear to use the same hormones that control molting and growth [45], the ecdysteroid hormones (ecdysone and 20-hydroxyecdysone) and juvenile hormones (JH) to influence secondary sexual development [6, 33]. Ecdysteroids are known to affect sexual development, such as yolk protein production in females, as well as male courtship behavior [42–44]. The presence of different ecdysteroid titers (and differential expression of enzymes that synthesize ecdysteroids) in male and female insects points to a likely involvement of these hormones in male and female sexual development. De Loof [45] posited that 20-hydroxyecdysone (20E) might act as the major sex hormone in female insects, whereas ecdysone (E), the precursor to 20E, may act as the male sex hormone. Each of these ecdysteroids is known to regulate the expression of different genes in larval *D. melanogaster* [46]. To test if ecdysteroids could be sex-hormones in insects, Briers and De Loof used an antibody specific to 20E and found that 20E titers were much higher in females of four fly species and two beetle species than in males [51, 47]. Other studies found that injection of 20E in

The idea that insects use exclusively cell-autonomous mechanisms for sexual development has prevailed for many years. Originally, this notion came from a description by Morgan in 1919 of a *Drosophila melanogaster* gynandromorph [33, 34]. This discovery led researchers to conclude that a half male/half female gynandromorphic phenotype could only develop if each cell had its own individual sexual identity (Fig. 2C). Thus, sexual development in insects was thought to depend on the expression of male or female sex-determining genes present on the sex chromosomes in every cell in the body. Below, we review evidence that sexual development in insects is not regulated via a cell-autonomous mechanism in all tissues and that insects, like vertebrates, also rely on sex hormones for sexual development.

Male and female insects appear to be mosaics of an amalgam of tissues, some sex-neutral, others with a clear sexual identity [4]. Studies on the expression of the gene *doublesex* (*dsx*), a gene belonging to a family of highly conserved DM domain genes that play a known role in sex determination in vertebrates as well as invertebrate [35–37], showed that *dsx* is expressed in the somatic gonadal tissues,

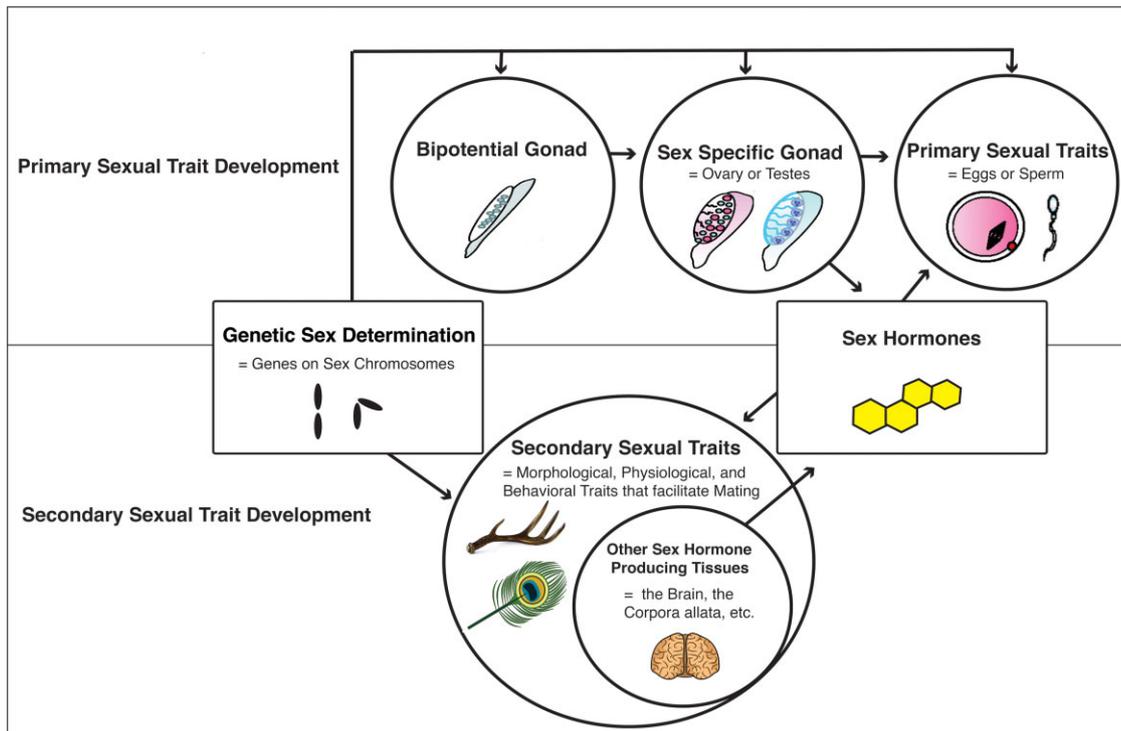


Figure 4. Summary of the interconnectivity of developmental mechanisms that produce primary sexual traits (the gametes) and secondary sexual traits (all other sexually dimorphic traits). The development of sexual traits (depicted in circles) is either directed by cells reading the information contained in their sex chromosomes locally, or directed by the action of sex hormones secreted in one part of the body and impacting sexual trait development in other parts of the body (both cell-autonomous and hormonal factors are depicted in boxes). We do not specifically address mechanisms of ESD in this figure because many aspects of ESD development are unknown.

male flies stimulated vitellogenesis, an exclusively female trait [48–50]. Though De Loof's hypothesis is intriguing, more research is needed to determine whether the insect hormones 20E and E could be acting as the counterparts of the vertebrate sex hormones. Two recent studies in *D. melanogaster*, however, present evidence that ecdysteroids may play an important role in the development of male courtship behavior. In both of these studies, reduced neural expression of the ecdysteroid receptor (EcR) in flies, an essential mediator of ecdysone signaling, led to aberrations in courtship behavior and indicated that ecdysteroid signaling mediated fly courtship [43, 44]. Comparatively, little data is available for the role of JHs in secondary sexual trait development. However, these hormones also influence sex-specific processes such as vitellogenesis in females, sex pheromone production in males, and female sexual behavior in several species of insects [6].

Insect hormones can be synthesized in a variety of different tissues, including the gonads. The ecdysteroids are synthesized in the prothoracic endocrine gland in response to secretion of the prothoracic hormone by the brain during the larval stage [51]. However, during pupal and adult

development (and possibly larval development as well), ecdysteroids are synthesized in the gonads in several major insect groups (Dermaptera, Orthoptera, Dictyoptera, Isoptera, Hemiptera, Coleoptera, Hymenoptera, Lepidoptera, Diptera) [42–44]. This implies that these hormones may be playing the role of gonadal sex hormones. In contrast, the JHs are produced in an endocrine gland adjacent to the brain known as the corpora allata. Thus, insects like birds and mammals, use extra-gonadal structures, as well as the gonads, to synthesize hormones that influence sexual trait development (Fig. 4).

Additional support for the hypothesis that ecdysteroids act as sex hormones in insects comes from the parasitic bacterium, *Wolbachia*. Studies have found evidence that the feminizing role attributed to *Wolbachia* may be due to manipulation of ecdysteroid synthesis, and/or signaling in its insect hosts [47]. *Wolbachia* is only transmitted from mothers to their offspring, and this mode of transmission has selected for *Wolbachia* to promote the development of female offspring by the feminization of male offspring [52]. *Wolbachia* seems to only infect arthropods and nematodes, which are two groups that regulate molting of their exoskeleton via ecdysteroid and ecdysteroid-like hormones, respectively. As a result, researchers have speculated that ecdysteroid signaling underlies the sex-ratio manipulations [53]. Further support for this idea comes from the detection of *Wolbachia* in the fat body of insects, a tissue with a role in steroid hormone synthesis [54], and from the migration of the bacteria (after injection into *D. melanogaster*) towards stem cells of another tissue that is involved in ecdysteroid biosynthesis, the follicular epithelium [55]. Interestingly, partial curing of *Wolbachia* infection in feminized, genetically male insects produces intersexes that have both male and female secondary sexual traits [56–58]. Because intersex phenotypes should not occur

if each cell makes its own sexual decision, these experiments challenge the long accepted notion that insects use exclusively cell-autonomous mechanisms to determine their sexual identity.

The terms “primary” and “secondary” should revert to an evolutionary definition

It is clear from the results of the studies reviewed here that the traditional vertebrate definitions of primary and secondary sexual development are no longer accurate given that the development of the gonads in birds and mammals is not a necessary “primary” event before differentiation of other “secondary” sexual traits can begin. Thus, it is time to consider whether the terms primary and secondary should be abandoned altogether or merely redefined. We propose that these historic terms are still useful as long as they revert to a definition that accounts for the evolutionary significance of different sexual traits.

Historically, the terms “primary” and “secondary” were first used to describe the function of a sexual trait and later the process of sexual development [59–61]. Initially, Darwin, who was interested in explaining the evolution of sexual dimorphism, used “primary” to describe sexual traits that consisted of male and female “organs of reproduction”, including gonads and genitalia, that were directly connected with the act of reproduction, whereas he used the term “secondary” to describe traits that aided in the process of reproduction such as “... the greater size, strength, and pugnacity of the male, his weapons of offense or means of defense against rivals, his gaudy coloring and various ornaments, his power of song, and other such characters” [60]. It should be noted, however, that Darwin expressed concern over this functional definition because it included genitalia as a primary sexual trait even though he recognized that some animals use non-genital structures to transfer gametes (such as pedipalps in spiders). Given this concern, Darwin went so far as to say that “... unless indeed we confine the term “primary” to the reproductive glands, it is scarcely possible to decide which (traits) ought to be called primary and which secondary” [62]. Later, the functional definition of primary and secondary sexual traits was confounded with a novel ontogenetic definition. In the ontogenetic literature, the development of primary sexual traits, namely the gonad, was considered the first step in sexual development, upon which secondary sexual trait development depended [63]. Specifically, gonad development was thought to be the primary step in sexual development, and hormones secreted by the gonads then directed the development of secondary sexual traits. So, the vertebrate definition of primary and secondary sexual traits included part of Darwin’s functional definition as well as an ontogenetic definition for these traits.

Our current knowledge of vertebrate sexual development challenges the ontogenetic definition of these terms and, by Darwin’s own admission, the functional definition is also flawed. Nevertheless, we maintain that the terms primary and secondary are still useful when considered in the light of

evolution. From an evolutionary perspective, the differently sized gametes, which fundamentally define sexual identity, are the major driving force behind the evolution of sexual dimorphism [1]. Thus, the male and female gametes can be considered primary sexual traits, in that they evolved first, while other sexually dimorphic traits can be considered secondary in that they evolved because of the selective forces imposed by anisogamy [1–3]. In the same vein, primary sexual development would refer to the development of the gametes, whereas secondary sexual development would pertain to the development of all other sexually dimorphic traits. Maintaining an evolutionary definition helps to separate the primary cell types, or traits, that define male and female individuals (sperm and eggs) from other traits that help males and females survive and achieve successful reproduction.

Conclusion

It has long been thought that vertebrates and invertebrates employed fundamentally different mechanisms to differentiate sexually dimorphic traits. However, we have detailed recent studies in birds, mammals, and insects that suggest there is more overlap than previously thought. These studies provide evidence that both hormone secretions (produced in the gonads or in other organs) and cell-autonomous mechanisms play important roles in sexual development in vertebrates and insects. Remaining open questions include discovering (1) how the tremendous diversity of the initial sex-determining factors (e.g. different genes or environments) converge on producing a conserved cell type, eggs or sperm, (2) why (or whether) gonadal hormones remain the predominant mode of secondary sexual trait development in mammals while cell-autonomous mechanisms remain the predominant mode in insects, (3) cementing the role of ecdysteroids and JHs as potential sex hormones in insects, and (4) discovering why and when different hormones took over this role in insect and vertebrate lineages.

Acknowledgments

We thank Bethany Wasik and two anonymous reviewers for their suggestions to improve the manuscript, the Peabody Museum for access to their collection of gynandromorph butterflies, and The Roslin Institute, The University of Edinburgh, Roslin, Scotland, UK, for the photograph of the chicken gynandromorph. This research was funded with the support of NSF IOS 1146933 and NSF DDIG IOS-1110523.

References

1. Cox PA. 2011. Introduction: the evolutionary mystery of gamete dimorphism. In Togashi T, Cox PA, eds; *The Evolution of Anisogamy: A Fundamental Phenomenon Underlying Sexual Selection*. Cambridge: Cambridge University Press. p. 262.
2. Parker GA. 2011. The origin and maintenance of two sexes (anisogamy), and their gamete sizes by gamete competition. In Togashi T, Cox PA, eds; *The Evolution of Anisogamy: A Fundamental Phenomenon Underlying Sexual Selection*. Cambridge: Cambridge University Press. p. 262.
3. Scharer L, Rowe L, Arnqvist G. 2012. Anisogamy, chance and the evolution of sex roles. *Trends Ecol Evol* 27: 260–4.

4. **Robnett CC, Vaughan AG, Knapp JM, Baker BS.** 2010. Sex and the single cell. II. There is a time and place for sex. *PLoS Biol* **8**: e1000365.
5. **Forlano PM, Schlinger BA, Bass AH.** 2006. Brain aromatase: new lessons from non-mammalian model systems. *Front Neuroendocrinol* **27**: 247–74.
6. **Wyatt GR.** 1997. Juvenile hormone in insect reproduction – a paradox? *Eur J Ent* **94**: 323–35.
7. **Callard GV, Petro Z, Ryan KJ.** 1978. Conversion of androgen to estrogen and other steroids in the vertebrate brain. *Am Zool* **18**: 511–23.
8. **De Loof A, Huybrechts J, Geens M, Vandersmissen T,** et al. 2010. Sexual differentiation in adult insects: male-specific cuticular yellowing in *Schistocerca gregaria* as a model for reevaluating some current (neuro) endocrine concepts. *J Insect Physiol* **56**: 919–25.
9. **Gamble T, Zarkower D.** 2012. Sex determination. *Curr Biol* **22**: R257–62.
10. **Sekido R, Lovell-Badge R.** 2009. Sex determination and SRY: down to a wink and a nudge? *Trends Genet* **25**: 19–29.
11. **Traut W, Sahara K, Marec F.** 2007. Sex chromosomes and sex determination in Lepidoptera. *Sex Dev* **1**: 332–46.
12. **Lance VA.** 2009. Is regulation of aromatase expression in reptiles the key to understanding temperature-dependent sex determination? *J Exp Zool A Ecol Genet Physiol* **311**: 314–22.
13. **Merchant-Larios H, Diaz-Hernandez V.** 2013. Environmental sex determination mechanisms in reptiles. *Sex Dev* **7**: 95–103.
14. **Barske LA, Capel B.** 2008. Blurring the edges in vertebrate sex determination. *Curr Opin Genet Dev* **18**: 499–505.
15. **Crews D, Moore MC.** 1986. Evolution of mechanisms controlling mating behavior. *Science* **231**: 121–5.
16. **Adkins-Regan E.** 2005. *Hormones and Animal Social Behavior*. Princeton: Princeton University Press.
17. **Smith CA, Sinclair AH.** 2004. Sex determination: insights from the chicken. *BioEssays* **26**: 120–32.
18. **Clinton M, Zhao D, Nandi S, McBride D.** 2012. Evidence for avian cell autonomous sex identity (CASI) and implications for the sex-determination process? *Chromosome Res* **20**: 177–90.
19. **Smith CA, Roeszler KN, Hudson QJ, Sinclair AH.** 2007. Avian sex determination: what, when and where? *Cytogenet Genome Res* **117**: 165–73.
20. **Arnold AP, Xu J, Grisham W, Chen X,** et al. 2004. Minireview: sex chromosomes and brain sexual differentiation. *Endocrinology* **145**: 1057–62.
21. **Agate RJ, Grisham W, Wade J, Mann S,** et al. 2003. Neural, not gonadal, origin of brain sex differences in a gynandromorphic finch. *Proc Natl Acad Sci USA* **100**: 4873–8.
22. **Zhao D, McBride D, Nandi S, McQueen HA,** et al. 2010. Somatic sex identity is cell autonomous in the chicken. *Nature* **464**: 237–42.
23. **Scholz B, Kultima K, Mattsson A, Axelsson J,** et al. 2006. Sex-dependent gene expression in early brain development of chicken embryos. *BMC Neurosci* **7**: 12.
24. **Gahr M.** 2003. Male Japanese quails with female brains do not show male sexual behaviors. *Proc Natl Acad Sci USA* **100**: 7959–64.
25. **Arnold AP.** 2012. The end of gonad-centric sex determination in mammals. *Trends Genet* **28**: 55–61.
26. **Chen X, McClusky R, Chen J, Beaven SW,** et al. 2012. The number of X chromosomes causes sex differences in adiposity in mice. *PLoS Genet* **8**: e1002709.
27. **Pilgrim C, Reisert I.** 1992. Differences between male and female brains – developmental mechanisms and implications. *Horm Metab Res* **24**: 353.
28. **Reisert I, Pilgrim C.** 1991. Sexual differentiation of monoaminergic neurons – genetic or epigenetic? *Trends Neurosci* **14**: 468–73.
29. **Arnold AP.** 2009. Mouse models for evaluating sex chromosome effects that cause sex differences in non-gonadal tissues. *J Neuroendocrinol* **21**: 377–86.
30. **Smith-Bouvier DL, Divekar AA, Sasidhar M, Du S,** et al. 2008. A role for sex chromosome complement in the female bias in autoimmune disease. *J Exp Med* **205**: 1099–108.
31. **Chen X, Watkins R, Delot E, Reliene R,** et al. 2008. Sex difference in neural tube defects in p53-null mice is caused by differences in the complement of X not Y genes. *Dev Neurobiol* **68**: 265–73.
32. **Dewing P, Chiang CWK, Sinchak K, Sim H,** et al. 2006. Direct regulation of adult brain function by the male-specific factor SRY. *Curr Biol* **16**: 415–20.
33. **De Loof A.** 2008. Ecdysteroids, juvenile hormone and insect neuro-peptides: recent successes and remaining major challenges. *Gen Comp Endocrinol* **155**: 3–13.
34. **Morgan TH, Bridges CB, Sturtevant AH.** 1919. *The Origin of Gynandromorphs*. Washington: Carnegie Inst.
35. **Matson CK, Zarkower D.** 2012. Sex and the singular DM domain: insights into sexual regulation, evolution and plasticity. *Nat Rev Genet* **13**: 163–74.
36. **Williams TM, Carroll SB.** 2009. Genetic and molecular insights into the development and evolution of sexual dimorphism. *Nat Rev Genet* **10**: 797–804.
37. **Kopp A.** 2012. *Dmrt* genes in the development and evolution of sexual dimorphism. *Trends Genet* **28**: 175–84.
38. **Hempel LU, Oliver B.** 2007. Sex-specific DoublesexM expression in subsets of *Drosophila* somatic gonad cells. *BMC Dev Biol* **7**: 113.
39. **Tanaka K, Barmina O, Sanders LE, Arbeitman MN,** et al. 2011. Evolution of sex-specific traits through changes in HOX-dependent doublesex expression. *PLoS Biol* **9**: e1001131.
40. **Lee G, Hall JC, Park JH.** 2002. Doublesex gene expression in the central nervous system of *Drosophila melanogaster*. *J Neurogenet* **16**: 229–48.
41. **Mechoulam R, Brueggemeier R, Denlinger D.** 1984. Estrogens in insects. *Cell Mol Life Sci* **40**: 942–4.
42. **Brown MR, Sieglaff DH, Rees HH.** 2009. Gonadal ecdysteroidogenesis in Arthropoda: occurrence and regulation. *Annu Rev Entomol* **54**: 105–25.
43. **Dalton JE, Lebo MS, Sanders LE, Sun F,** et al. 2009. Ecdysone receptor acts in fruitless-expressing neurons to mediate drosophila courtship behaviors. *Curr Biol* **19**: 1447–52.
44. **Ganter GK, Walton KL, Merriman JO, Salmon MV,** et al. 2007. Increased male-male courtship in ecdysone receptor deficient adult flies. *Behav Genet* **37**: 507–12.
45. **De Loof A.** 2006. Ecdysteroids: the overlooked sex steroids of insects? Males: the black box. *Insect Sci* **13**: 325–38.
46. **Beckstead RB, Lam G, Thummel CS.** 2007. Specific transcriptional responses to juvenile hormone and ecdysone in *Drosophila*. *Insect Biochem Mol Biol* **37**: 570–8.
47. **Negri I, Pellecchia M, Grève P, Daffonchio D,** et al. 2010. Sex and stripping: the key to the intimate relationship between *Wolbachia* and host? *Integr Biol* **3**: 110–5.
48. **Huybrechts R, De Loof A.** 1977. Induction of vitellogenin synthesis in male *Sarcophaga bullata* by ecdysterone. *J Insect Physiol* **23**: 1359–62.
49. **Huybrechts R, De Loof A.** 1982. Similarities in vitellogenin and control of vitellogenin synthesis within the genera *Sarcophaga*, *Calliphora*, *Phormia* and *Lucilia* (Diptera). *Comp Biochem Phys B* **72**: 339–44.
50. **Bownes M, Blair M, Kozma R, Dempster M.** 1983. 20-Hydroxyecdysone stimulates tissue-specific yolk-protein gene transcription in both male and female *Drosophila*. *J Embryol Exp Morph* **78**: 249–68.
51. **Nijhout HF.** 1998. *Insect Hormones*. Princeton: Princeton University Press.
52. **Werren JH, Baldo L, Clark ME.** 2008. *Wolbachia*: master manipulators of invertebrate biology. *Nat Rev Microbiol* **6**: 741–51.
53. **Tzertzinis G, Egana AL, Palli SR, Robinson-Rechavi M,** et al. 2010. Molecular evidence for a functional ecdysone signaling system in *Brugia malayi*. *PLoS Negl Trop Dis* **4**: e625.
54. **Kamoda S, Masui S, Ishikawa H, Sasaki T.** 2000. *Wolbachia* infection and cytoplasmic incompatibility in the cricket *Teleogryllus taiwanemma*. *J Exp Biol* **203**: 2503–9.
55. **Frydman HM, Li JM, Robson DN, Wieschaus E.** 2006. Somatic stem cell niche tropism in *Wolbachia*. *Nature* **441**: 509–12.
56. **Tulgettske GM, Stouthamer R.** 2012. Characterization of intersex production in *Trichogramma kaykai* infected with parthenogenesis-inducing *Wolbachia*. *Naturwissenschaften* **99**: 143–52.
57. **Negri I, Pellecchia M, Mazzoglio P, Patetta A,** et al. 2006. Feminizing *Wolbachia* in *Zyginidia pullula* (Insecta, Hemiptera), a leafhopper with an XX/X0 sex-determination system. *P R Soc B Biol Sci* **273**: 2409–16.
58. **Kageyama D, Traut W.** 2004. Opposite sex-specific effects of *Wolbachia* and interference with the sex determination of its host *Ostrinia scapularis*. *Proc Biol Sci* **271**: 251–8.
59. **Jost A.** 1953. Problems of fetal endocrinology: the gonadal and hypophyseal hormones. *Recent Progr Horm Res* **8**: 379–418.
60. **Darwin C.** 1871. *The Descent of Man in Relation to Sex*. London: Murray.
61. **Eicher EM, Washburn LL.** 1986. Genetic control of primary sex determination in mice. *Annu Rev Genet* **20**: 327–60.
62. **Darwin C.** 1871. *Sexual Selection and the Descent of Man*. London: Murray.
63. **Jost A, Vigier B, Prepin J, Pichellet J.** 1973. Studies on sex differentiation in mammals. *Recent Prog Horm Res* **29**: 1.