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Dmrt genes in the development and evolution of sexual dimorphism

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Abstract

Most animals are sexually dimorphic, yet different taxa have different sex-specific traits. Despite major differences in the genetic control of sexual development among animal lineages, the *Dmrt* family of transcription factors has been shown to be involved in sex-specific differentiation in all animals studied so far. In recent years, the functions of *Dmrt* genes have been characterized in many animal groups, opening the way for a broad comparative perspective. In this review, I focus on the similarities and differences in the functions of *Dmrt* genes across the animal kingdom. I highlight a number of common themes in the sexual development of different taxa, discuss how *Dmrt* genes have acquired new roles during animal evolution, and show how they contributed to the origin of novel sex-specific traits.

Dmrt genes: a common theme amidst diversity

Sexual dimorphism (phenotypic differences between males and females of the same species) is one of the most pervasive and diverse features of animal morphology, physiology, and behavior. The demands of sexual reproduction and competition for mates have led each animal lineage to evolve its own suite of sex-specific characters. Lion manes, butterfly wings, and bird songs seem to have nothing in common beyond the fact that they differ between males and females. The molecular mechanisms responsible for sexual dimorphism are almost as diverse, ranging from cell-autonomous, splicing-based sex determination in insects to gonad-dependent endocrine control of sexual traits in mammals and other vertebrates [1,2]. For a long time, these disparities encouraged a narrow, taxon-by-taxon approach to the study of sex-specific development. While evolutionary biology has provided a universal framework for understanding the evolution of sex in all organisms, such broad approach has been slow to take hold in developmental biology.

In recent years, however, some common themes in the development of sex-specific traits in different animal lineages have started to emerge. Central to this trend has been the discovery of the *doublesex/mab-3 related* (*Dmrt*) family of transcription factors [3] (Boxes 1, 2). *Dmrt* genes share a common DNA-binding domain (DM domain) but otherwise show little sequence conservation, making their phylogenetic relationships obscure. Members of this ancient gene family shape sexual dimorphism in organisms as diverse as mammals, insects, and nematodes. Initially their roles seemed very different in different taxa: acting in a global alternative splicing cascade in *Drosophila*, making the choice between testis and ovary

development in the vertebrate gonad, or cell-autonomously controlling sensory organ differentiation in *Caenorhabditis elegans* [4,5,6]. But upon closer examination, these differences may hide deeper similarities. It now appears that in all animals, *Dmrt* genes work as tissue-specific developmental regulators that integrate information about sex, position, and time to direct narrow populations of cells toward male or female fates. The most striking taxon-specific functions of *Dmrt* genes – such as alternative splicing in insects or primary sex determination in some vertebrates– are derived from this common ancestral function.

The molecular functions of *Dmrt* genes are well understood (Boxes 1, 2), and their roles in specific animal groups or developmental processes have been the subject of several recent reviews [7,8,9,10,11]. However, accumulating evidence from a variety of models opens the way for a broader comparative perspective. In this paper, I focus on the similarities and differences in the roles of *Dmrt* genes across the animal kingdom. The two complementary goals of this analysis are to identify common themes in the sex-specific development of different taxa and to examine lineage-specific changes in the development and evolution of sexual dimorphism.

A deeply conserved role in gonad development

Although virtually every animal lineage has evolved its own somatic sex-specific characters, the one trait most animals have in common is the presence of sexually dimorphic gonads. Despite profound differences in gonad structure and development among animal phyla, *Dmrt* genes are specifically expressed in the developing gonads of almost all animals: in vertebrates including mammals [4,12,13], birds [7], turtles and alligators [14,15], amphibians [9], and teleost fishes [8]; in arthropods including *Drosophila* [16] and diverse crustacean taxa [17,18,19]; and in different classes of mollusks [20,21]. A *Dmrt* gene is also present in the coral *Acropora millepora*, where its increased expression coincides with seasonal sexual reproduction [22]. The only known exception is *C. elegans*, where several *Dmrt* genes are involved in the sexual differentiation of somatic tissues but are dispensable for gonad development.

The main function of *Dmrt* genes in the gonad is to promote male-specific and repress female-specific differentiation. This function is best understood in the case of mouse *Dmrt1*, but the roles of other family members are also beginning to be characterized [23,24]. The vertebrate gonad is bipotential: the same embryonic cell lineages give rise to either ovaries or testes depending on the sex of the animal. Once established, sex-specific gonad differentiation must be actively maintained during embryonic and postnatal development. In mice, neither *Dmrt1* nor other *Dmrt* genes are involved in the initial sex determination; however, *Dmrt1* is essential for maintaining testis identity. Differentiated states of the testis and ovary are controlled by a genetic circuit centered on the competition between two transcription factors: *Foxl2* (female-specific forkhead box L2), which promotes the female-specific granulosa and theca cell fates, and *Sox9* (Sry-related box 9), which promotes the male-specific Sertoli cell fate [25,26,27]. During embryonic development, a sex-determining signal, which can be either genetic or environmental, directs gonad development down the male or female pathway. In most mammals, the Y-linked *Sry* (sex-determining region on the Y chromosome) gene is necessary to induce testis differentiation in males, while the absence of *Sry* leads to ovary development [2,26]. *Dmrt1,* along with *Sox9*, is one of the key SRY targets in mammalian testis development. In early mouse embryos, *Dmrt1* is expressed in the genital ridge of both sexes before any overt signs of sex-specific differentiation [4]. Later, *Dmrt1* expression declines in the ovary but is maintained in the testis, where it becomes restricted to germline and Sertoli cells [28]. Loss of DMRT1 function in Sertoli cells in the postnatal testis results in the loss of *Sox9* expression and the ectopic expression

of *Foxl2* and other feminizing genes, leading to the transdifferentiation of Sertoli into granulosa cells [27]. DMRT1 both activates testis-specific genes such as *Sox9* and *Sox8* and represses ovary-specific genes including *Foxl2*, the *Wnt4* and *R-spondin-1* signaling proteins, and estrogen receptors [27,29]. In addition to these regulators, DMRT1 controls hundreds of target genes in both Sertoli and germline cells, including genes involved in cell differentiation, cell cycle control, and pluripotency [29].

doublesex (*dsx*) function in arthropods shows interesting parallels with the vertebrate *Dmrt1*. In *Drosophila*, male and female gonads arise from the same somatic gonad precursor (SGP) cells. In males, *dsx* is expressed in SGPs and is required for the recruitment of additional precursors from the mesoderm, whereas no recruitment is observed in females [16,30,31,32]. In the crustacean *Daphnia magna*, loss of *dsx* transforms testes toward ovary-like morphology; conversely, ectopic *dsx* expression in female embryos can induce testis development [33]. Thus, in arthropods as well as vertebrates, the function of *Dmrt* genes is to promote testis and repress ovary development in bipotential primordia.

This function of *Dmrt* genes remains conserved regardless of the mating system and the mode of sex determination. *Drosophila*, mammals, and birds have genetic sex determination, while in *Daphnia* and turtles, sex is determined by environmental factors. *Dmrt1* genes promote testis development both in the male-heterogametic (XY) mammals and medaka fish, and in the female-heterogametic (ZW) birds and frogs [7,8,9,27]. Fish studies are particularly illuminating due to the diversity of sexual lifestyles in this lineage. Sex determination in fish can be either genetic or environmental, and some species are gonochoristic while others are hermaphrodites. Despite these disparities, expression of *Dmrt* genes correlates with testis development in all teleosts. In the gonochorists, *Dmrt1* expression in the gonad is male-limited in some species and strongly male-biased in others. In hermaphroditic species, *Dmrt1* expression parallels the development of testes in protogynous, and their regression in protandrous, hermaphrodites [8].

Does the shared gonad function of *Dmrt* genes reflect conservation or convergence? Tissue- and sex-specific expression of *Dmrt* genes in the somatic gonads of phyla as divergent as chordates, arthropods, and mollusks suggests that they already functioned in testis development in the common Bilaterian ancestor. Conversely, the functions of *Dmrt* genes in the germline of some animals are far less consistent and may reflect independent cooption (Box 3).

Somatic sexual dimorphism – integration of sex and pattern

The nature and development of sex-specific somatic traits show great diversity among animals, but once again the *Dmrt* genes emerge as one of the few common features. Their roles in sexual differentiation are best understood in *Drosophila* and *C. elegans*, where sex determination is largely cell-autonomous (Box 1). In flies, recent work has shown that *dsx* is transcribed in tightly controlled spatial patterns. Most cells of both male and female flies do not express either *dsx* isoform, resulting in a complex mosaic of “sex-aware” and “sex-ignorant” cells [31,32].

The central function of *dsx* in somatic tissues is to induce localized sex-specific differentiation by integrating information about sex, position, and time. This role is perfectly illustrated by the *Drosophila* sex comb – a strictly male-specific organ that develops on the first, but not the second or third, pair of legs (Figure 1 A). Spatial control is accomplished in this case by the HOX genes while the sex-specificity is controlled by alternative *dsx* splicing (Box 1). During larval and pupal stages, *dsx* is expressed in the presumptive sex comb region and is necessary for its sex-specific differentiation [34]. *dsx* transcription is induced in the first leg by the HOX gene *Sex combs reduced (Scr)*, which is not expressed in the

more posterior segments, and further refined by intrasegmental positional cues. Ectopic expression of *dsxM* in male pupae induces extra sex combs in all three legs and outside of the normal sex comb position, while a switch from male to female isoform results in feminization without affecting the spatial pattern [34].

The role of localized *dsx* expression in sex-specific development can also be seen in other sexually dimorphic organs such as genitalia and central nervous system (CNS). *dsx* mutant flies develop both male and female genitalia, indicating that *dsx* acts as a switch between two alternative differentiation pathways but is not itself required to specify either [35,36,37]. In the CNS, *dsx* is expressed and required in discrete neuronal clusters that direct courtship song, ejaculation, and other sex-specific behaviors [38,39,40]. Here, *dsx* establishes sexual dimorphism by controlling neuroblast proliferation and death [40,41] and the differentiation of sex-specific neurons [38].

Unlike the insect mechanism where *dsx* plays active roles in both males and females, the *Daphnia dsx* is essential for male development but is dispensable in females. Males of *Daphnia magna* differ from females in having larger eyes, thoracic hooks, and longer antennae. All these structures express *dsx* in males but not females and are feminized by RNAi-induced *dsx* knockdown. Conversely, ectopic *dsx* expression in female embryos can masculinize these structures [33].

A similar pattern is observed in nematodes. In *C. elegans*, sexual differentiation is controlled by the transcription factor *transformer-1* (*tra-1*), unrelated to the *Drosophila tra* [42]. Males differ from hermaphrodites in several morphological traits that require *Dmrt* genes for male-specific differentiation. For example, *mab-23* promotes male development in the proctodeum, *dmd-3* is required for male tail and sensory organ formation, and *mab-3* is necessary for both proctodeum and tail development in addition to controlling gene expression in the intestine [43,44,45]. Similar to the *Drosophila* and *Daphnia dsx*, these genes are tightly regulated at the transcriptional level. All three have strictly male-limited functions and are dispensable in hermaphrodites [43,44,45].

The integration of sex and pattern by *Dmrt* genes in *C. elegans* is exemplified by *dmd-3* and *mab-3* (Figure 1 B) [43,46]. These genes are expressed sex-specifically in the male tail and can induce male morphogenesis when misexpressed in hermaphrodites. *tra-1* is the key sex-specific regulator that limits *dmd-3* and *mab-3* expression to males; their spatial pattern is established in part by posterior HOX genes and localized Wnt signaling; and the timing of their activation is determined by the heterochronic pathway that controls the general progression of worm development [43,46]. *dmd-3* and *mab-3* then jointly activate *eff-1*, a gene required for cell fusion, and other effector genes that mediate male morphogenesis (Figure 1 B). Thus, *dmd-3* and *mab-3* integrate sexual, positional, and temporal cues to initiate a sex-specific developmental program [43,46]. This is remarkably similar to the function of *dsx* in the *Drosophila* sex comb, where it integrates sex (through alternative splicing) with spatial information (from the HOX code) and timing (through an unknown but presumably ecdysone-dependent mechanism) to promote male-specific morphogenesis (Figure 1 A) [34].

In vertebrates, although the gonad plays the central role in sexual differentiation, nongonadal cells also have an intrinsic sexual identity [7,47]. Many vertebrate *Dmrts* are expressed in somatic tissues including the brain [48], and there are tantalizing hints that some of them may have sex-specific functions. In *Dmrt4* mutant mice, for example, a substantial proportion of males display same-sex copulatory behavior, raising the possibility that *Dmrt* genes act in the nervous system to control sexual behavior in vertebrates as well as in insects and nematodes [24]. However, it remains to be seen whether the vertebrate

Dmrt genes play any cell-autonomous roles in the sexual differentiation of non-gonadal tissues.

Non-autonomous control of sexual dimorphism

Precise spatial regulation of *Dmrt* genes is essential for normal development in animals as diverse as insects, vertebrates, and nematodes. In all these systems, most cells do not express any *Dmrt* genes. However, the relatively small populations of *Dmrt*-expressing cells can have a profound influence on sexual differentiation in the rest of the body. The most obvious of these roles is the endocrine control of vertebrate sexual differentiation. For example, the mammalian Sertoli cells, whose development depends on *Dmrt1* expression [27,28], orchestrate the hormonal control of sex-specific development in non-gonadal tissues both by secreting sex hormones themselves and by controlling the development of other hormone-producing cell types in the gonad [2] (Figure 2 A).

Dmrt genes have a widespread non-autonomous role in germline sexual dimorphism. Although mice have cell-autonomous germline sexual identity and require *Dmrt1* expression in germline cells, *Dmrt1* function in Sertoli cells is necessary for germline cell maintenance and meiotic progression [27,28,49] (Figure 2 A). In medaka fish, where *Dmrt1* is only expressed in Sertoli cells, it is required for mitotic arrest in the primordial germ cells [50]. *Drosophila*, like mouse, has intrinsic germline sexual identity, but *dsx* is not expressed in germline cells [16]. Rather, sexual differentiation of the germline is controlled by a combination of *dsx*-independent intrinsic mechanisms and *dsx*-dependent signals from the somatic gonad [51] (Figure 2 B). In adult testes, *dsxM* continues to be expressed in the "hub", a key part of the somatic niche that maintains germline stem cells [16,32].

dsx also acts non-autonomously in the *Drosophila* somatic gonad, where signaling from *dsx*-expressing gonad precursor cells is necessary to recruit additional groups of male-specific precursors from the mesoderm [16,30,31]. Similarly, in the external genitalia *dsxM* and *dsxF* organize the global morphogenesis of the entire tissue by controlling the activation of several long-range signaling pathways [36] (Figure 2 B). In these and other examples, *Dmrt* genes control sexual differentiation far beyond their own expression domains – in fact, some of the most dramatic aspects of sexual dimorphism are due to the non-autonomous functions of *Dmrt* genes.

Evolutionary takeovers of sex determination

Sex determination signals and mechanisms evolve so rapidly that the master gene rarely stays at the top of the hierarchy for very long. For example, *Sry* does not exist outside of mammals [52], while *Sex-lethal (Sxl)* (Box 1) exists but does not play a sex-determining role outside of drosophilids [10,11]. In other lineages, sex-determining mechanisms turn over even more rapidly and can differ within species or between sibling species [53,54]. In vertebrates, the gonad controls sex-specific differentiation in the rest of the body through endocrine signaling [2], although non-gonadal cells have intrinsic sexual identity as well [7,47]. Male- and female-specific steroid hormones secreted by somatic gonad cells bind to nuclear hormone receptors in other tissues to promote sexually dimorphic development. One consequence of this mechanism is that the gene located at the top of the gonad-determining hierarchy essentially becomes the main sex-determining gene.

Dmrt1 and its paralogs have taken over this role in several vertebrate lineages. One of these regulatory coups has occurred recently in the evolution of medaka fish. In the model species *Oryzias latipes, Dmrt1* has undergone a duplication and one of the newly derived paralogs, called *Dmrt1bY* or *Dmy* became the dominant male-determining gene [55,56]. Other

Oryzias species lack *Dmy*, underscoring the recent origin of this sex-determining system [57].

In the frog *Xenopus laevis*, sex determination is female-heterogametic (ZZ males and ZW females). *X. laevis* is tetraploid and so has two *DMRT1* co-orthologs. Similar to medaka, one of the *DMRT1* genes underwent an additional duplication and one of the duplicates (called *DM-W*) is located on the W chromosome [58,59]. *DM-W* has acquired a dominant female-determining function by repressing the transcriptional targets of *DMRT1* and tilting the balance of the bipotential gonad toward ovary development [9,58,60]. As in medaka, this sex-determining role is a recent innovation: closely related species such as *X. tropicalis* (also known as *Silurana tropicalis*) lack the *DM-W* gene [59].

Birds are also female-heterogametic, but unlike the dominant-W mechanism of *X. laevis*, sex in birds appears to be controlled by Z chromosome dosage [7]. In all birds, *DMRT1* is located on the Z chromosome but is absent from the W. In chicken embryos, it is expressed in the early bipotential gonad and shows higher expression in males than in females [13]. As in the mouse, *DMRT1* promotes testis development by activating *SOX9* and repressing female-specific genes [61]. Chicken sex is determined, at least in part, by *DMRT1* dosage: the two copies present in males are sufficient to turn on the testis specification pathway while the single dose in females fails to override ovary development [7].

Thus, *Dmrt1* genes have moved up the regulatory hierarchy, from downstream positions in gonad differentiation to the top sex-determining role, in at least three distantly related clades. Why *Dmrt* rather than, say, *Sox9* or *Foxl2*? One possible answer may lie in the large number of target genes they control and in their ability to either activate or repress transcription [29]. These features may place structural or regulatory changes in *Dmrt1* outside the buffering capacity of the finely balanced gene circuit that controls testis versus ovary decision, leading to a rapid evolutionary takeover of sex determination.

Sex-specific splicing – an evolutionary switch in regulatory activity

A comparison of *Dmrt* genes from different animal lineages reveals important differences in their molecular functions despite their conserved roles in sexual differentiation. The mouse DMRT1 is a bifunctional regulator, activating some direct targets and repressing others [29]. The molecular basis of this versatility is unknown, but may involve the recruitment of different cofactors (coactivators *vs* corepressors) to the enhancers of different DMRT-regulated genes. The nematode MAB-3 is only known to act as a transcriptional repressor [62]. The most peculiar regulatory switch, however, occurred in insects.

In *Drosophila*, sex-specific splicing of *dsx* is the central event in sexual differentiation (Box 1), as the male- and female-specific Dsx isoforms exert distinct effects on the development of sexual traits. For example, the *yolk protein* (*yp*) genes are transcribed at a basal level in *dsx* mutants; DsxM further represses their transcription, while DsxF causes activation above the basal level [63,64]. *desaturase-F (desat-F)*, which encodes an enzyme involved in pheromone synthesis, is activated by DsxF but not affected by DsxM [65]. Microarray experiments also reveal genes that are repressed by DsxF and/or activated by DsxM, but it is unclear whether any of these genes are direct transcriptional targets of Dsx [66]. Recently, a large number of potential direct targets have been identified by chromatin profiling [67]. Analysis of these genes should elucidate the activator and repressor functions of the male- and female-specific Dsx proteins.

dsx is spliced sex-specifically and has distinct DsxM and DsxF regulatory activities in other dipterans, moths, beetles, and Hymenopterans [68,69,70,71]. In all these insects, *tra* is also spliced sex-specifically and controls the sex-specific splicing of *dsx*, leading to the idea that

the mechanism of sex determination based on the alternative splicing of *dsx* is common to all insects [10,11]. However, only holometabolous insects have been studied to date. Holometabola are a monophyletic group within insects that represents only a subset of order-level diversity. In *Daphnia*, the closest relative of insects examined so far, neither *dsx* nor *tra* show any evidence of sex-specific splicing, and *dsx* does not play any role in female development. Moreover, RNAi knock-down of the *Daphnia tra* has no effect on sex-specific development or *dsx* expression [33,72]. Similarly, the nematode and vertebrate *Dmrt* genes are regulated sex-specifically at the transcriptional level but do not show sex-specific splicing. Thus, although more basal insects and other arthropods need to be examined, the sex-specific splicing of *dsx* and its active role in female sex differentiation appear to be insect-specific (or even Holometabola-specific) innovations. An intriguing possibility is that DsxM and DsxF have subdivided the regulatory activity of the ancestral Dsx, leading to a more flexible control of sex-specific development.

Origin and evolution of new sex-specific traits

One of the most fascinating features of animal evolution is the rapid turnover of sex-specific traits. Both lions and gazelles are sexually dimorphic, but the traits distinguishing males from females are clearly different between the two. This simple observation implies that new sexual characters are gained, and old ones are lost, during the evolution of any animal lineage. The molecular mechanisms of this turnover are poorly understood, but recent evidence suggests that *Dmrt* genes may play important roles in this process.

The role of *Dmrt* genes in the evolution of sexual dimorphism may be particularly prominent in organisms like insects, where the sex of most cells is determined autonomously. For any sex-specific structure to evolve, *dsx* must be expressed in the cells that either give rise to this structure, or induce this structure through cell-cell signaling. In tissues that already express *dsx*, sexual dimorphism can originate if Dsx acquires new downstream targets through *cis*-regulatory changes that create new, or higher affinity, Dsx binding sites [73]. If, on the other hand, the tissue is ancestrally monomorphic and does not express *dsx*, changes in the spatial regulation of *dsx* that result in its de novo expression in this tissue must be a necessary first step [34].

Recent work shows that both of these mechanisms operate during the evolution of sexual dimorphism. For example, *dsx* and the HOX gene *Abdominal-B (Abd-B)* control sex-specific pigmentation in *D. melanogaster* by regulating the expression of *bab1* and *bab2*. Sexually dimorphic pigmentation is a recent evolutionary innovation: in most *Drosophila* species, males and females are pigmented identically. The origin of this novel pattern was caused by *cis*-regulatory changes in a *bab* enhancer, which changed the way in which the *bab* genes are regulated by Dsx and Abd-B [74,75]. Similarly, the evolution of sex-specific pheromone profiles in *Drosophila* reflects multiple gains and losses of Dsx binding sites in the enhancer of *desat-F* [65].

Sex comb evolution provides the clearest example of association between changes in the spatial expression of *dsx* and morphological diversity. The sex comb is another recent innovation restricted to a single lineage within *Drosophila*; outside of this lineage, males and females have identical leg bristle patterns [76]. In species that have sex combs, *dsx* is expressed in the presumptive sex comb region, where it is activated by *Scr*, while *Scr* expression is in turn regulated by *dsx* and is sexually dimorphic (Figure 3 A). However, in species that primitively lack sex combs, *dsx* is not expressed in the homologous region, and *Scr* expression is monomorphic [34,77]. Thus, sex comb evolution was associated with the origin of novel regulatory interactions between HOX and sex determination genes, leading to the gain of a new *dsx* expression domain. Two other, distantly related lineages in the

family Drosophilidae that independently evolved new male-specific structures also show recent evolutionary gains of *dsx* expression in the corresponding tissues (Figure 3 A) [34].

In the parasitoid wasp *Nasonia*, wing size and shape are sexually dimorphic in all species but to a different extent. In *N. vitripennis*, males have much smaller wings than females and are flightless, while in *N. giraulti* the dimorphism is only mild, and the males can fly (Figure 3 B). Greatly reduced male wings are a recently derived trait [78]. One of the loci responsible for the difference in male wing size between *N. giraulti* and *N. vitripennis* maps to the noncoding region immediately upstream from *dsx*, and the *N. vitripennis* allele associated with smaller wings increases *dsx* expression in the larval wing primordium more than two-fold (Figure 3 B) [78]. These observations in flies and wasps suggest that changes in *dsx* regulation may be a key mechanism that enables the evolution of new sex-specific traits in insects and other arthropods, contributing to some of the most dramatic examples of phenotypic diversification in nature.

Concluding remarks and future perspectives

Despite the endlessly diverse manifestations of sexual dimorphism and the profound differences between the mechanisms that specify it in different phyla, *Dmrt* genes provide a common framework for understanding the development and evolution of sex-specific traits. In all animals studied to date, members of the *Dmrt* family are expressed in tightly restricted spatial patterns in association with the development of sex-specific organs. Superficial differences between the roles of these genes in different phyla hide deeper similarities. In *Drosophila*, recent discoveries have demoted *dsx* from a global sex-determining gene to a tissue-specific regulator of cell differentiation. The fact that most cells in *Drosophila* lack *dsx* expression shows that only those cells that need to differentiate in a distinct male- or female-specific way are provided with the molecular machinery necessary to make the right choice. In this respect, the role of *dsx* in *Drosophila* is remarkably similar to the roles of *Dmrt* genes in nematodes and vertebrates. The canonical *Drosophila* mechanism of sex determination based on sex-specific splicing is an insect-specific innovation that was superimposed on a more ancestral mechanism based on localized transcription. Similarly, the role of *Dmrt1* genes as the master sex switches in several vertebrate taxa is also a recent innovation that can ultimately be traced to their localized activation in crucial cell types in the somatic gonad.

The key function of *Dmrt* genes lies in the integration of spatial, temporal, and sexual information to direct male- or female-specific differentiation of a relatively small number of cell types. This function can be as minor as regulating a few enzymes or cytoskeletal molecules, or as major as controlling the deployment of long-distance morphogens or endocrine signals that induce drastically different patterns of tissue growth and differentiation. Characteristic features of *Dmrt* genes such as limitation to a small number of cell types, upstream co-regulation by global sex-determining pathways and local spatial activators, as well as the ability to promote sex-specific differentiation both autonomously and non-autonomously turn out to be surprisingly similar in widely diverged animal phyla.

In animals as diverse as vertebrates, arthropods, and mollusks, *Dmrt* genes are involved in gonad development, suggesting that they acted as selector genes that controlled testis versus ovary decision in the last Bilaterian ancestor, if not earlier. Starting from this ancestral role, changes in the transcriptional regulation of *Dmrt* genes could have led to their independent cooption for the development of other sex-specific structures in different lineages. In insects, this mechanism is still playing an important role in the evolution of sexual dimorphism. Every group of insects has evolved its own sex-specific structures, which often reach truly

bizarre proportions but have no clear homologues in other taxa. Many of these innovations may be due to the origin of new *dsx* expression domains.

Many unanswered questions remain both about the functions of *Dmrt* genes and about their roles in evolution (Box 4). However, the last few years have laid an excellent foundation for this work. Going forward, this unique gene family will be central to our understanding of sexual dimorphism throughout the animal kingdom.

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Glossary

Bilateria	a group of animal phyla that evolved from a common bilaterally symmetric ancestor; it includes all multicellular animals except the phyla Porifera, Placozoa, Cnidaria, and Ctenophora
Cell-autonomous gene function	developmental mechanism where the expression of a regulatory gene in a particular cell determines the fate or functional properties of that same cell
***cis*-regulatory changes**	nucleotide substitutions in non-coding gene sequences that result in altered expression of that gene in the course of evolution
Courtship song	in *Drosophila* males, a precisely modulated series of wing vibrations that produce species-specific sounds recognized by females
DM domain	a DNA-binding domain common to all DMRT transcription factors
***Dmrt* genes**	the family of *doublesex / mab-3* related transcription factors, characterized by the presence of the DNA-binding DM domain
Ecdysone	an insect hormone that controls molting, metamorphosis, and tissue remodeling
Gonochorists	species where each individual is either male or female, but not both
Granulosa cells	In vertebrates, somatic ovary cells closely associated with developing female gametes; granulosa cells produce female sex hormones from a precursor secreted by the theca cells
Hermaphrodites	species where a single individual can function as both male or female, either sequentially or at the same time
Heterochronic pathway	the genetic pathway that controls the timing of development in *C. elegans*
Non-autonomous gene function	developmental mechanism where the expression of a regulatory gene in some cells determines the fates of other cells that do not express this gene, usually through a signaling pathway
Orthologs	genes in different species that evolved from a common ancestral gene by speciation, e. g. *Dmrt1* in mouse and *Dmrt1* in human

Paralogs	genes that evolved by duplication of a common ancestral gene within a genome, e. g. *Dmrt1* and *Dmrt2* in mouse, or mouse *Dmrt1* and human *Dmrt2*
Primordial germ cells	embryonic stem cells that give rise to adult germline cells
Proctodeum	posterior ectodermal part of the digestive tract connecting to the hindgut
Protandrous hermaphrodites	sequential hermaphrodites where individuals function first as males, and later as females
Protogynous hermaphrodites	sequential hermaphrodites where individuals function first as females, and later as males
Sertoli cells	somatic testis cells associated with developing male gametes; Sertoli cells produce male sex hormones and control the development of germline cells and other hormone-producing somatic cells
Sequential hermaphrodites	hermaphroditic species where some or all individuals change sex at some point during adult life
Sexual dimorphism	phenotypic differences between males and females of the same species
Theca cells	In vertebrates, somatic gonad cells associated with ovarian follicles; theca cells secrete a steroid precursor that is converted into female sex hormones by the granulosa cells

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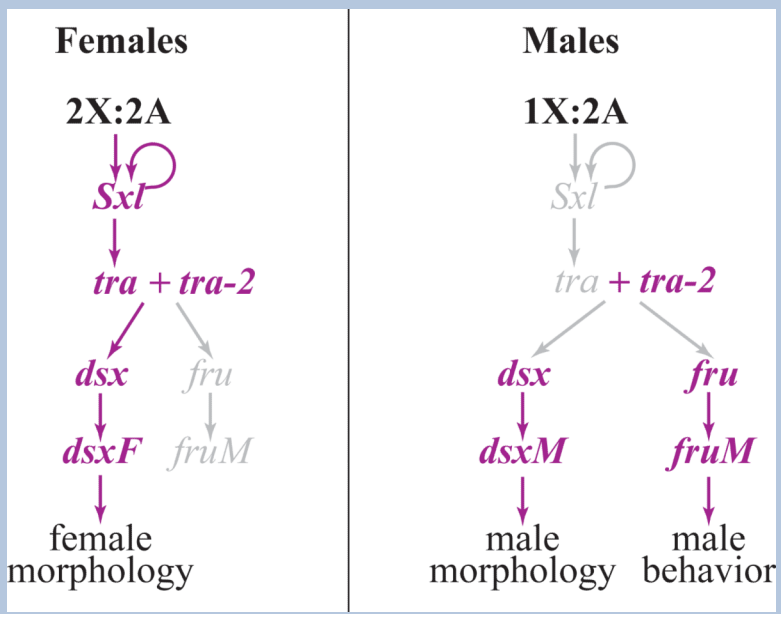
Box 1. Where it all began: *doublesex* and sex determination in *Drosophila*

doublesex (*dsx*), the founding member of the *Dmrt* gene family, was first identified as a mutation affecting sexual differentiation in *Drosophila* [35]. Its name stems from the intersexual phenotype of *dsx* mutants: some traits are intermediate between males and females, while in other tissues both male and female structures develop in parallel [35,37].

The central role in *Drosophila* sex determination is played by alternative splicing (Fig. I). *Drosophila* has male-heterogametic sex determination where sex depends on the X:autosome ratio: XX individuals are females and XY are males. The primary genetic switch that interprets X chromosome dose is the RNA-binding protein Sex-lethal (Sxl), which regulates both its own splicing and the splicing of another RNA-binding factor, *transformer* (*tra*); as a result, only females have functional Tra protein [79,80]. *dsx* and an unrelated transcription factor called *fruitless* (*fru*) lie at the bottom of this splicing cascade: *dsx* controls sexual differentiation in most somatic tissues, while *fru* functions in the nervous system [37,81,82]. In females, the presence of functional Tra causes *dsx* to be spliced into a female-specific isoform (*dsxF*), while the male-specific isoform (*dsxM*) is produced by default [1,6].

The two *dsx* isoforms have opposite effects on sex differentiation: *dsxM* promotes the development of male-specific structures and represses female-specific structures, while *dsxF* promotes female-specific and represses male-specific structures [37,83,84]. DsxM and DsxF are transcription factors that share a common N-terminal DNA-binding domain, but have different C-terminal domains with distinct effects on target gene expression [64,85,86].

Until recently, only a few direct Dsx targets had been identified. These include the *yolk protein* genes, which are expressed in females and deposited in oocytes; *bric a brac 1* (*bab1*) and *bric a brac 2* (*bab2*), which encode transcription factors that control sex-specific color patterns; and *desaturase-F*, which encodes an enzyme involved in the synthesis of sex-specific pheromones [64,65,75]. With the growing application of chromatin profiling techniques, this paucity of data is finally at an end. A recent paper lists several dozen likely direct targets of Dsx [67], and more genomic data are on the way.



Box 1 Figure I.

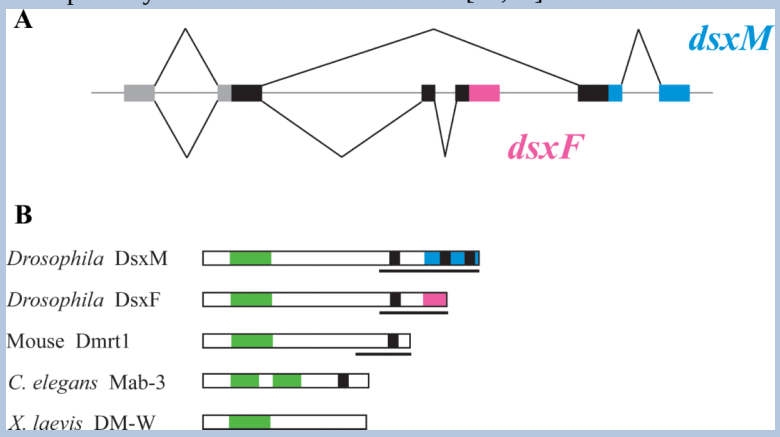
Sex determination pathway in *Drosophila.* Active gene products and gene interactions are shown in purple and inactive in grey. Primary sex determination occurs very early in embryonic development when zygotically transcribed genes located on the X chromosome and the autosomes activate *Sxl* in females but fail to activate it in males. Sxl makes sex determination permanent by establishing a positive autoregulatory loop in females and controls the splicing of *tra*. In females, the presence of functional Sxl and Tra proteins leads to female-specific splicing of *dsx* and suppresses male-specific splicing of *fru*. In males, the absence of Sxl and Tra allows the default splicing of *dsx* and *fru* to produce male-specific isoforms.

Box 2. The *Dmrt* gene family

A breakthrough discovery came when the *mab-3* gene of *C. elegans*, long known to control the development of male-specific sensory organs, was shown to encode a homologue of the *Drosophila dsx* [5]. A homologous gene was also identified in humans, where it was found to map to a chromosomal region associated with sex reversal [5]. Almost immediately, the mouse and chicken homologues of *dsx* and *mab-3* were shown to have gonad-specific expression and to correlate with testis development in particular [13] (Fig. I). The DNA-binding domain common to these genes was named the DM (Dsx/MAB-3) domain, and the family was named *Dmrt* (*doublesex/mab-3 related*) genes [5,13]. Outside of the DM domain, most *Dmrt* genes show little or no similarity across taxa [87].

In vertebrates, which have multiple *Dmrt* paralogs (e. g. *Dmrt1* through *Dmrt7* in mice and humans), *Dmrt1* plays a particularly important role in gonad development and sexual differentiation and has received the most attention, but other paralogs have also been characterized [23,24]. Orthology relationships between fish and mammalian genes are well supported, indicating that this family diversified prior to vertebrate radiation [87]. All invertebrates for which genome sequences are available also have multiple *Dmrt* genes; *C. elegans* holds the current record at 11. However, there is little sequence conservation between the vertebrate and invertebrate genes, making their relationships hard to determine. In particular, it is not clear whether *dsx, Dmrt1*, and *mab-3* are orthologous.

The DM domain is a zinc finger DNA binding motif that interacts with DNA in the minor groove [86]. The regulatory domains of DMRT transcription factors are located at their C-termini. Different DMRT proteins, such as DsxM and DsxF as well as different vertebrate DMRT paralogs, can bind DNA as either homo- or heterodimers [88,89]. DMRT proteins have fairly high specificity compared to other minor groove-binding proteins, but much of this specificity appears to be shared across the gene family. For example, the *Drosophila* Dsx and the *C. elegans* MAB-3 bind similar DNA sequences, and *dsxM* can partially substitute for *mab-3 in vivo* [89,90].



Box 2 Figure I.
The structure of *Dmrt* genes and proteins. **A**. Genomic structure of the *Drosophila dsx*. Coding sequences common to *dsxM* and *dsxF* are shown by thick black bars, transcribed untranslated regions by grey bars, and introns and intergenic regions by a thin grey line; splicing patterns are indicated by thin black lines above and below the gene region. *dsxM* and *dsxF* share the 5' exon that encodes the DNA-binding DM domain, but include mutually exclusive 3' exons that encode different dimerization/activation domains.

dsxM-specific exons are shown in blue and *dsxF*-specific sequence in pink. **B**. Protein structure of the *Drosophila* Dsx, mouse DMRT1, *C. elegans* MAB-3, and *Xenopus* DM-W. DM domains are shown in green and proline/serine-rich domains found in most DMRT proteins are in black. Pink and blue bars show the sex-specific domains of DsxF and DsxM, respectively. Lines under the protein schematics indicate characterized protein domains involved in transcriptional regulation. MAB-3 is unique in having two DM domains, and DM-W is unusual in lacking a C-terminal regulatory domain.

Box 3. Germline functions of *Dmrt* genes

Anisogamy – a sexually dimorphic germline – is even more ancient than the gonads, predating animal multicellularity. Although gametes are probably the most sexually dimorphic cell type, in many animals (such as frogs, houseflies, or medaka fish) the germline lacks cell-autonomous sexual identity; sex-specific differentiation is imposed instead by the soma. Mice and *Drosophila*, by contrast, do have cell-autonomous germline sex but it is determined by mechanisms distinct from those operating in somatic cells [49]. *Dmrt* genes can be expressed in germline cells in both types of animals but, in contrast to the somatic gonad, their germline functions are not conserved.

In the mouse testis, *Dmrt1* is expressed in germline cells and is required cell-autonomously for their survival, migration, and the suppression of pluripotency and proliferation [28]. Its two major functions in the male germline are to repress the meiotic inducer *Stra8* and activate the spermatogonial differentiation factor *Sohlh1* [91]. *Dmrt1* function is different in the female germline, where it activates *Stra8* and is required for normal meiotic prophase [92]. In the testes of teleost fish, *Dmrt1* orthologs can be expressed in both somatic and germline cells (platyfish and catfish [93,94]), only in the soma (medaka, tilapia, and pufferfish [95,96,97]), or only in the germline (zebrafish and cod [98,99]). In species where *Dmrt1* is expressed in both sexes, female expression is usually restricted to the germline while in males it is seen in both germline and somatic cells.

Although *Drosophila*, like mouse, has cell-autonomous germline sex, *dsx* is not expressed in the germline, and the only function of *dsx* in germline development in flies is non-autonomous [49,51,100]. Interestingly, a *Dmrt* gene identified in the crustacean *Eriocheir sinensis* is expressed in both somatic and germline cells of the testis [18] – a situation closer to vertebrates than to *Drosophila*. In contrast to the near-universal conservation of gonad function, it is not clear whether the role of *Dmrt* genes in germline development is ancient or has evolved independently in different lineages.

Box 4. Unanswered questions

- How do *Dmrt* genes regulate their transcriptional targets? Can the *Drosophila* DsxM act as an activator, and DsxF as a repressor? How does mammalian DMRT1 achieve its dual function as both an activator and a repressor? What cofactors does it interact with to activate or repress transcription?

- Was the ancestral function of *Dmrt* genes restricted to males, or were they involved in gonad specification in both sexes? Is their function in germline cells ancestral, or were they independently recruited in germline development in some taxa?

- In vertebrates, what are the direct transcriptional regulators and targets of *Dmrt1* in the gonad? How evolutionarily labile are these interactions, and what changes in these interactions allow *Dmrt1* paralogs to acquire top positions in the sex-determining hierarchy?

- When and how did the splicing-based mechanism of insect sex determination evolve? What was the regulatory activity of arthropod Dsx before the evolution of sex-specific splicing? How did the newly independent DsxM and DsxF proteins acquire their distinct regulatory specificities?

- Do vertebrate *Dmrt* genes play cell-autonomous roles in sex-specific development outside of the gonad?

- How common is the evolution of new *dsx* expression domains in insects, and what role does it play in the origin of new sex-specific structures? Does a similar mechanism operate in other animal groups with cell-autonomous sex determination?

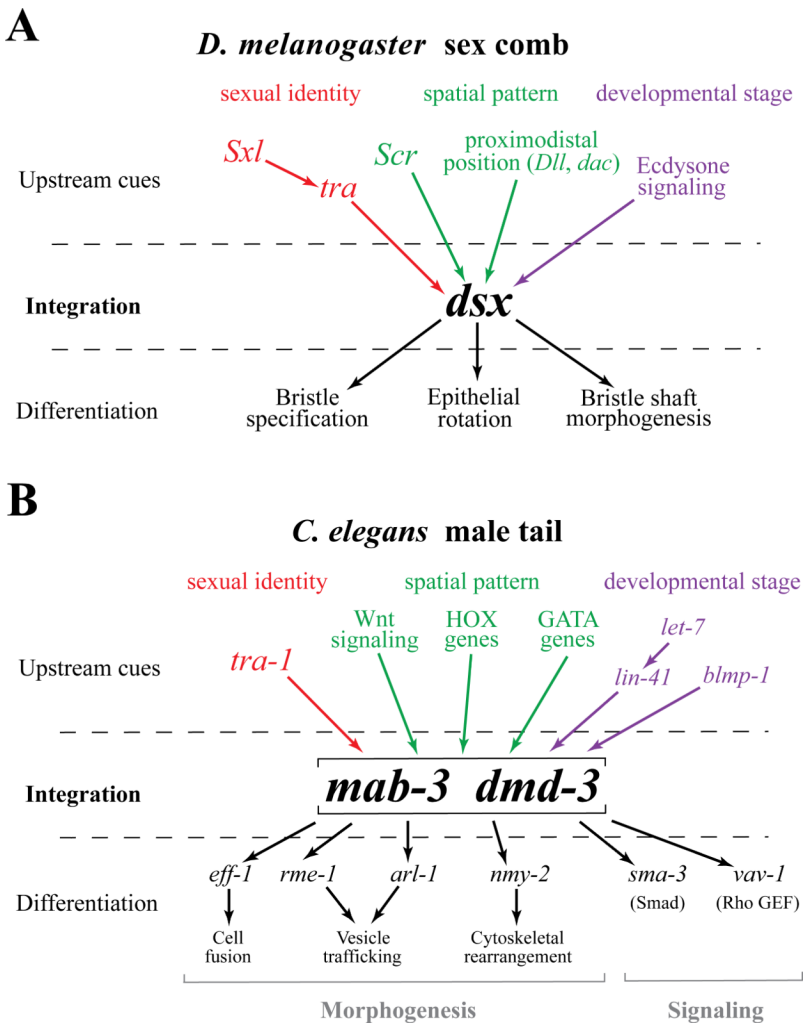


Figure 1.

Dmrt genes integrate sex-specific, spatial, and temporal cues to induce sexually dimorphic differentiation in restricted groups of cells. **A**. In the *D. melanogaster* sex comb, *dsx* is controlled by the splicing-based sex determination pathway (red), the HOX gene *Scr* and intrasegmental positional cues (green), and presumably by ecdysone signaling that regulates the timing of metamorphosis (purple). *dsx* induces sex comb development by controlling cell fate decisions and several distinct morphogenetic processes [34]. **B**. In *C. elegans, dmd-3* and *mab-3* are controlled by the sex determination pathway (red), by posterior group HOX genes, Wnt signaling and GATA transcription factors (green), and by the heterochronic pathway (purple). *dmd-3* and *mab-3* induce male-specific tail differentiation by regulating cell-autonomous morphogenetic processes as well as signaling pathways including TGF-β (*sma-3*) and Rho kinase (*vav-1*). In both organisms, arrows indicate either direct or indirect regulation [43,46].

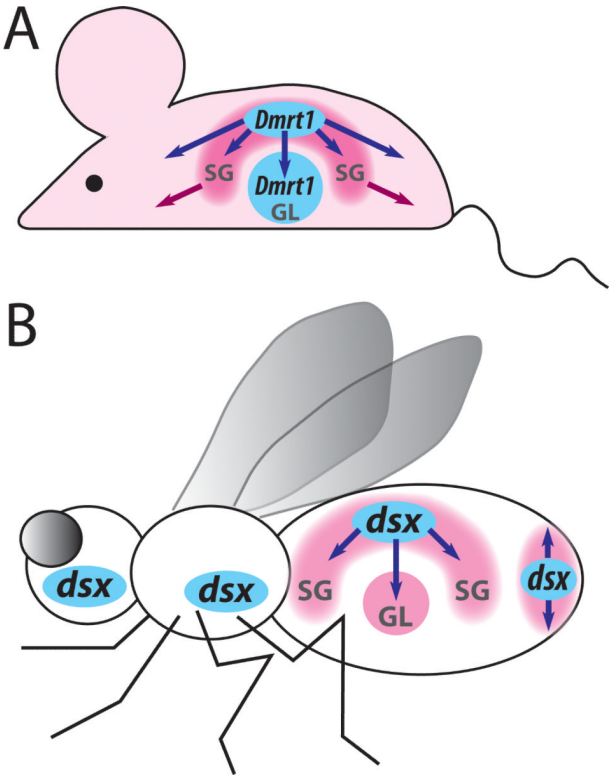


Figure 2.

Cell-autonomous and non-autonomous functions of *Dmrt* genes in mouse and *Drosophila*. Tissues where *Dmrt* genes are expressed and act cell-autonomously are shown in blue; tissues that do not express *Dmrt* genes but undergo sex-specific differentiation under the non-autonomous influence of *Dmrt* genes are in pink; sexually monomorphic cells are in white; arrows indicate non-autonomous functions of *Dmrt* genes. *GL*, germline; *SG*, somatic gonad. **A**. In the mouse, *Dmrt1* is expressed and acts cell-autonomously in germline cells and in the Sertoli cells of the somatic gonad. In addition, *Dmrt1* expression in Sertoli cells controls the development of the germline and other somatic gonad cells non-autonomously. Hormones secreted by the Sertoli cells (blue arrows) and other somatic gonad cells (purple arrows) regulate sex-specific development in many non-gonadal tissues. *Dmrt1* is not known to play any cell-autonomous roles in sexual differentiation outside of the gonad. **B.** In *Drosophila, dsx* is expressed in somatic gonad progenitor cells, which recruit additional cells into the gonad through cell-cell signaling. *dsx* is not expressed in germline cells, but its function in the somatic gonad is necessary for sex-specific germline development. *dsx* is expressed in a subset on non-gonadal cells, where it acts cell-autonomously to control somatic sexual differentiation. Some of these cells also induce sex-specific development in adjacent tissues by a signaling mechanism.

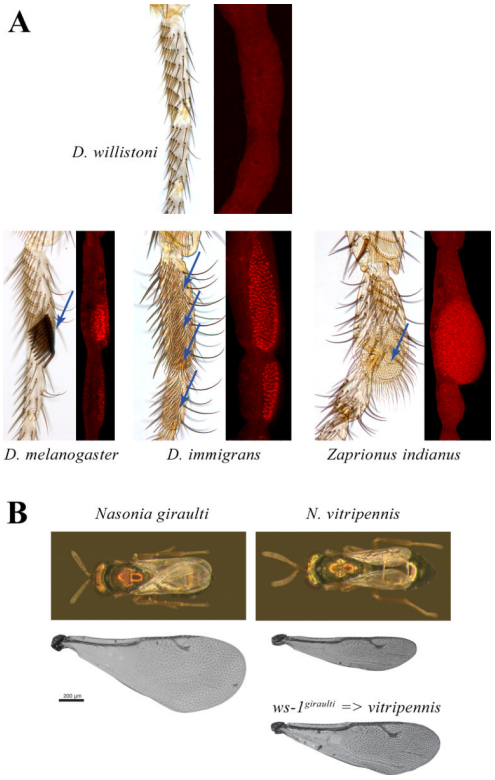


Figure 3.

Changes in *Dmrt* genes are responsible for the origin and evolution of sex-specific traits. Only males are shown for all species. **A**. In *Drosophila* and related genera, the first pair of legs is sexually monomorphic in the ancestral condition, illustrated here by *D. willistoni*, and *dsx* is not expressed during the critical stages of pupal leg development. Three different lineages of Drosophilidae have independently evolved new male-specific structures: sex comb in *D. melanogaster* and its relatives, a long brush of tightly packed bristles in the *immigrans* species group, and a tarsal bulge with a round brush of fine hairs in the genus *Zaprionus* (arrows in Fig. 2 A). In each case, the novel morphology is associated with a novel expression domain of *dsx* (Dsx antibody staining in red) (Modified from [34]). **B**. In the wasp *Nasonia*, male-specific wing reduction is much more pronounced in *N. vitripennis* than in *N. girault*i. Introgression of the *ws-1* genomic region, which spans the *dsx* locus, from *N. girault*i into *N. vitripennis* is sufficient to increase wing size [78] (modified from [78] with permission).