Prospects & Overviews

Function and evolution of sex determination mechanisms, genes and pathways in insects

Tanja Gempe and Martin Beye*

Animals have evolved a bewildering diversity of mechanisms to determine the two sexes. Studies of sex determination genes – their history and function – in non-model insects and Drosophila have allowed us to begin to understand the generation of sex determination diversity. One common theme from these studies is that evolved mechanisms produce activities in either males or females to control a shared gene switch that regulates sexual development. Only a few small-scale changes in existing and duplicated genes are sufficient to generate large differences in sex determination systems. This review summarises recent findings in insects, surveys evidence of how and why sex determination mechanisms can change rapidly and suggests fruitful areas of future research.

Keywords:
- evolution; evolutionary forces; gene duplication; sex determination; sexual differentiation and selection

Introduction

The regulation and evolution of sexual development have long been of central interest in developmental and evolutionary biology. One key question has been how sexual fate is determined and regulated, giving rise to the sexually dimorphic traits that play such a dominant role in animal evolution and behaviour. Much has been learned about how sex determination is realised and is integrated into the developmental program from model systems; the insect Drosophila melanogaster, the nematode Caenorhabditis elegans and the mouse Mus musculus [1–4]. These studies have not resolved the question of why the regulatory principles of sex determination are so bewilderingly different and how this diversity is generated molecularly.

Sex determination mechanisms can vary substantially between phylogenetically closely-related species [5–7] and even within a single species [8–10]. The same mechanism can apparently be regulated by different genes [11, 12]. This implies that these mechanisms evolve very rapidly, despite the antiquity of the two sexes.

Here, we review recent advances made in insects that have begun to uncover how diverse sex determination systems are generated and regulated [12–22]. The evolutionary and molecular routes taken broaden our understanding of how and why novel regulatory controls of a developmental process evolve. The key questions we address in this paper are: (i) How are differences in sex determination molecularly realized; (ii) how did differences in sex determination evolve from a pre-existing genetic repertoire; (iii) what forces drive sex determination systems to diverge?

Since the first reports of a sex determination mechanism [23] and of the inherited basis of sex determination [24, 25] – both made in insects – an astonishing diversity of mechanisms has been uncovered in a variety of species and phylogenetic lineages [5, 7, 26, 27]. Considering insects, classical genetic and cytological studies have identified a variety of genetic and environmental signals that determine the two sexes. In the fruit fly D. melanogaster, for instance, the double dose of X chromosomes [28] determines femaleness, a single dose maleness. Other dipteran insects (e.g. Musca domestica and Ceratitis capitata) employ a male-determining Y chromosome: females are XX and males are XY [13, 29]. Butterflies present the opposite scenario, possessing a female-determining W chromosome: females are ZW and males are ZZ [7]. In XY chromosomal systems the number of Y chromosomes can vary substantially [30]; for instance in some species of the
fruit fly *Anastrepha*, females are XX/XX and males XX/Y [26]. Other species employ male and female determiners with no visible chromosomal differences (e.g. phorid fly *Megaselia scalaris* [10], *Chironomus* [9, 31]). Male scale insects and white flies (both holometabolans), thrips (thysanopterans) and hymenopteran insects (wasps, ants and bees) are haploid and females are diploid [7]. The genetic basis of haplo/diploidy in several hymeopteran species is complementary sex determination; males are homo- or hemizygous and females heterozygous at a single locus [32, 33]. In another hymenopteran species, the parasitic wasp *Nasonia vitripennis*, sex determination is consistent with a maternal imprinting mechanism [22]. Differential elimination of sex chromosomes during the first stages of embryonic division is used as a sex determination mechanism in the fungus gnat *Sciara* [26]. Females develop when one paternal X chromosome is lost from the 3X:2A zygote and males arise when two paternal X chromosomes are lost. Maternally-derived signals (e.g. the blowfly *Chrysomya raffiaces*) or environmentally-derived signals, such as the temperature of egg incubation (e.g. the gall midge *Heteropeza* and the fungus gnat *Sciara*), are also utilised as sex determination signals.

Here, we wish to limit our review to sex determination signals of the pathways that have been identified in insects. From the phylogenetic relationships of hymenopteran insects, at the base of the holometabolous insect branch (Fig. 1F), we can retrace the ancestral components that were shared by common ancestors and then explore which components evolved and established new sex determination systems.

In the next section, we summarise the different ways in which sex determination is achieved molecularly to regulate the proportions of males and females. We then turn to the question of how novel sex determination systems can evolve from the ancestral genetic repertoire, before discussing the forces that drive divergence in sex determination. Finally, we highlight potentially fruitful directions for future research.

**Regulatory diversity and common principles of sex determination**

Sex determination systems use different genes and regulatory mechanisms to establish activities in either males or females (Fig. 1). These activities regulate *tra* genes, which are key, upstream components of an ancestral sex-determining pathway [12–15, 17, 20–22].

In *D. melanogaster*, the double dose of X chromosomes [28] establishes feminising activity (Fig. 1E). The X chromosome encodes several transcription factors (e.g. *runt*, *sisA*, *sisB*) that, through the double dose in females, activate the *Sxl* gene. Sxl proteins in females are splicing factors that splice *tra* mRNAs in females to produce Tra protein. A single X chromosome results in the absence of the Sxl protein and, as a consequence, male *tra* mRNAs with a premature stop codon are produced.

In the honeybee *Apis mellifera*, heterozygosity of the complementary sex determining gene (*csd*) establishes feminising activity [33, 34] (Fig. 1A). *Csd* allelic proteins derived from heterozygous *csd* activate the feminizer gene (*fem*) by directing splicing to form the *female fem* mRNAs that encode the Fem protein. Proteins derived from hemizygous (haploid, unfertilised eggs) or homozygous *csd* genes are non-active. As a result, the *fem* mRNAs are spliced into the male configuration that contains a premature translation stop codon. *fem* is apparently an orthologue of the *tra* genes [12].

The housefly, *M. domestica*, exhibits a number of different sex determination systems [8, 29, 35, 36] that co-exist in this species. There is a classical system (Fig. 1B) in which a dominant male-determiner on the Y chromosome provides a masculinising activity (males are X/M-Y and females X/X; the molecular nature of M has yet to be identified). Other systems can have the dominant male-determiners *M* on any of the five autosomal chromosomes, and even on the X chromosome. Furthermore, a dominant female-determiner (*F0*) exists in this species that establishes feminising activity. Females are F0/M/FM and males are FM/FM (Fig. 1C). *F0*, the dominant female-determiner, is an allelic variant of the *Md-tra* gene [15] that produces female *tra* mRNAs and active Tra protein, even in the presence of the male-determiner *M*. In the absence of the *F0* allele, the male-determiner *M* ensures male-specific splicing of other *Md-tra* alleles and, consequently, the absence of active Md-Tra protein in males.

The medfly, *C. capitata*, also possesses a dominant male-determiner *M* on the Y chromosome that is responsible for masculinising activity [13, 17, 37] (Fig. 1D). The molecular nature of *M* is yet to be identified. In the presence of *M*, male *Cc-tra* mRNAs, but not *Tra* proteins, are produced. In the absence of *M*, maternally-derived *Tra* proteins direct splicing into productive female *Cc-tra* mRNAs.

These results imply that sex determination mechanisms in insects are used in two ways (Fig. 2); sex determination mechanisms in the zygote produce either feminising activities (*D. melanogaster/A. mellifera*) that switch *tra* genes ON, or they generate masculinising activities (*C. capitata* and *M. domestica*) that switch *tra* genes OFF. In the absence of these signals the pre-zygotic state of *tra* (‘default’ OFF or ON) is executed, resulting in male (*D. melanogaster/A. mellifera*), or female (*C. capitata and M. domestica*) development. *tra* gene regulation in *N. vitripennis* apparently follows this latter rule [22].

How do *tra* genes implement female and male development? *Tra* proteins are members of SR-type splice regulators that control female splicing of the *dsx* gene (Fig. 1). In the absence of *Tra* proteins, *dsx* mRNAs are produced. This regulatory principle is shared among dipteran and hymenopteran insects [12–22, 38, 39] implying that this is an ancestral principle of sexual regulation in holometabolous insects (hymenopteran insects are at the base of holometabolous insects [40]). *tra* gene regulation involves a positive feedback loop in females that generates even more *Tra* proteins and, thereby, a stable female state throughout development [13–15] (Fig. 1). The feedback activity of *tra*, and its’ role in germ cell differentiation, have been co-opted in *D. melanogaster* by the *Sxl* gene, the next upstream component [1, 41, 42] (Fig. 1), suggesting that the *Drosophila* model system is derived in these respects.

*Sex-specific splicing of the dsx gene has been identified in other phylogenetic lineages, including the lepidopteran insects [16, 17, 43–51]. The sex-specific, spliced *dsx* transcripts encode transcription factors of the DM type that have an atypical zinc-finger domain. The proteins differ in females and males at their C-terminal ends [1], which control transcription of target genes differently [52]. The role of
**Figure 1.** Sex determination in insect model species, with their phylogenetic relationships representing ~300 million years of evolution. The fruit fly *D. melanogaster*, the housefly *M. domestica*, the medfly *C. capitata* and the honeybee *A. mellifera* share a common pathway (indicated by a grey box) composed of the transformer (*tra*) gene and its downstream target doublesex (*dsx*). Female spliced *tra* transcripts (*traF*) give rise to Tra proteins that direct splicing of *dsxF* mRNAs, production of DsxF proteins and female development. When *tra* is spliced into the male variant, no Tra proteins are produced. This results in splicing of male *dsxM* mRNAs, DsxM proteins and male development.

**A:** Sex in the honeybee (*A. mellifera*) is determined by the dosage of X chromosomes [1, 28]. Double doses of X in females activate the doublesex (*dsx*) gene, directing production of more female transcripts (*dsxF*). Female spliced *dsxM* mRNAs, production of DsxM proteins and female development. In females, different Csd proteins, derived from a heterozygous *csd* gene, direct the processing of female *fem* mRNAs (*femF*) [14]. The *fem* gene is apparently an orthologue of the *tra* gene [12]. Fem protein regulates female splicing of *dsx*, but also self-sustains the female splicing of *fem*. In males, inactive Csd proteins that are derived from the same alleles (homo- or hemi-zygosity of the *csd* gene) result in a default splicing of *fem* (*femM*).

**B and C:** Models of two alternative sex determination systems that co-exist in *M. domestica* populations [15, 29]. **B:** Sex is determined by the absence/presence of an unidentified male-determiner *M*. In the absence of *M*, maternally-derived Md-tra gene products establish an auto-regulative loop in females in which Md-Tra protein mediates the production of more female Md-tra mRNA. Presence of *M* impairs this tra auto-regulatory loop and also mediates the splicing of male Md-tra mRNAs. **C:** Sex in *M. domestica* can also be determined by a female determiner. Presence/absence of a tra allele, Md-traD (=Ftr), determines sexual fate. In females, the presence of Md-traD leads to female splice products and Md-Tra protein, even in the presence of the male-determiner *M* [15]. In males, the male-determiner *M* mediates male Md-traM mRNAs in the absence of the Md-traD allele. **D:** Sex in *C. capitata* [13, 17] is determined by presence/absence of *fru*, thus far, unidentified male-determiner *M*. In the absence of *M*, maternally-derived Cc-tra gene products appear to establish a Cc-tra auto-regulative loop [13]. Presence of *M* mediates the splicing of male Cc-tra transcripts (*traM*). **E:** Sex in *D. melanogaster* is determined by the dose of X chromosomes [1, 28]. Double doses of X in females activate the Sxl gene and expression of Sxl proteins. Sxl proteins direct splicing of female *traF* mRNAs that give rise to functional proteins. Sxl proteins also establish an auto-regulatory feedback loop by directing splicing of productive female *SxM* mRNAs, which maintain the female state throughout development. In addition, there is an additional feedback activity in which Tra proteins stimulate Sxl positive auto-regulation [98]. In males, the single dose of X chromosomes does not direct early Sxl protein expression. As a consequence the downstream regulatory decisions do not occur and male *dsxM* is produced. **F:** The evolutionary relationship of the species used in the comparison with their approximate time scale of divergence [40, 99].

---

**Evolutionary origin of novel sex determination mechanisms**

Functional and evolutionary analyses of sex determination genes (Fig. 1A,C,E) have revealed that small-scale mutational changes in the nucleotide sequence from existing and from duplicated copies of genes can generate novel sex determination mechanisms (Fig. 3).
The evolutionary origin of the X dosage mechanism in *D. melanogaster* is associated with the evolutionary rise of the *Sxl* gene. The *Sxl* gene encodes an RNA-binding protein that originated in the dipteran lineage by gene duplication from a copy of the ancestral CG3056 gene [69] (Fig. 3A). The regulatory relationship of *Sxl* to the sex determination cascade arose through the evolution of *Sxl* protein-binding sites (poly-Uridine tracts between five and eight nucleotides in length) in the *tra* gene. Signalling of the double dose of X chromosomes by transcription factors (e.g., transcription factors *runt*, *sisA*, *sisB*) has apparently evolved through changes in the *cis*-regulatory control of *Sxl* transcription.

The complementary sex determination gene in honeybees (*Apis*) evolved through gene duplication of the ancestral copy of the *fem/tra* gene within the last 60 million years [12] (Fig. 3B). The evolutionary rise of *csd* was accompanied by the insertion of a novel hyper-variable region that consists of asparagine/tyrosine-enriched repeats [70, 71] and by single nucleotide replacement changes that produced a novel coiled-coil motif [12]. The hyper-variable region is thought to play a role in the recognition process of allelic differences (*csd* alleles are only active in the heterozygous state), whereas the putative coiled-coil domain appears to encode allelic protein-binding properties (Otte and Beye, unpublished results).

The novel, dominant female-determiner *F^D* (*Md-tra^D*) evolved in the housefly, *M. domestica*, from multiple small deletions and insertions in intron sequences of the *tra* gene [15] (Fig. 3C). The *Md-tra^D* allele is constitutively spliced in the productive female mode in the presence of a male-determiner *M* (Fig. 1C). *Md-tra^D* is a natural variant of the *tra* gene in housefly populations [29], suggesting that this mechanism originated recently.

These molecular analyses reveal how nucleotide changes in duplicated copies of genes and allelic variants can produce new sex determination genes and diverse mechanisms. Sex-determining variants, however, initially originated from rare mutational events in single individuals in a population. To understand how and why these initially rare variants became fixed in populations and species, we have to apply population genetic principles that account for the forces of evolutionary change. Multiple fixation processes through evolutionary time have generated the diversity of sex determination systems that we see today in the insects.

**Forces driving the divergence of sex determination systems**

Random genetic drift and directional (positive) selection are the evolutionary forces that can initially drive rare sex-determining variants throughout populations of any given species. Directional selection enhances the probability of fixation, given the random fluctuations of genetic variants in a population caused by genetic drift. We do not review here the body of theoretical work that has been done in the field of the evolution of sex determination, but rather focus on the empirical molecular evidence that has so far been documented.

Positive selection, driven by fitness gains in individual population members, is a plausible source for the fixation of new sex determination systems. For instance, phylogenetic surveys in hymenopteran species suggest that the complementary sex determination mechanism has been replaced in some highly inbreeding hymenopteran species (e.g., parasitic wasps) [22, 32, 72]. Complementary sex determination under inbreeding results in large numbers of diploid males that cannot reproduce (only haploid males are fertile); suggestive of an evolutionary advantage for alternative sex determination mechanisms in this case.

Remarkably, once new sex determination mechanisms have evolved, nature does not stop generating new sex
Figure 3. Mutational routes for the evolutionary origin of novel sex determination functions. **A:** The origin of the Sxl gene by gene duplication of CG3056 in dipteran insects [69]. The encoding proteins are presented schematically. RRM 1 and RRM 2 denote the two RNA-binding domains. **B:** The origin of the csd gene by tandem gene duplication of the ancestral tra gene in the *Apis* lineage. The encoding proteins are presented schematically. The evolutionary rise of the csd gene was accompanied by the evolution of an asparagine/tyrosine-enriched repeat that varies in number in the different allelic specificities (denoted as HV: hyper-variable region) and with the origin of a putative coiled-coil domain (denoted as CC) possibly involved in protein binding [70, 71]. Adaptive evolution (directional selection) was involved in shaping the evolutionary rise of the csd gene [12]. The paralogous sister gene fem evolved under purifying selection, consistent with its ancestral function [100]. RS denotes the arginine/serine-enriched (RS) domain and PR denotes the proline-rich domain. **C:** The evolutionary origin of the Md-tra$^{D}$ allele from the ancestral Md-tra gene in *M. domestica* populations [15]. The Md-tra genome structure is presented schematically. Blue triangles denote nucleotide insertions and the green triangles represent nucleotide deletions in the Md-tra$^{D}$ allele that lost the ability to produce the male splice variant tra$^{M}$ in the presence of *M.*

determination favours directional selection of an alternative, novel sex determiner. The degeneration hypothesis implies an evolutionary paradox: although meiotic recombination and the mixing of genetic material require two sexes, the genes that establish them may lack the evolutionary advantage of sexual reproduction. The sex ratio drive hypothesis suggests that X-linked, meiotic drive factors increase in frequency by inducing the loss of Y chromosome-containing sperm [77, 78]. There is, thus far, no direct evidence that any one of these directional selection scenarios favoured the rise of novel sex determination systems. These hypotheses are also difficult to test, as it is not known whether fitness gains or losses currently observable arise from novel sex determiner genes themselves, or are associated with other, secondary effects (for instance, suppression of recombination around the sex determination gene or accumulation of sex-specific beneficial alleles) [84].

The function of sex determination hierarchies (Fig. 1) is inconsistent with the view that sex determination systems evolve because of sexual selection. The sex determination signals of *M. domestica*, *C. capitata* and *A. mellifera* all regulate tra genes that control sexual development in its entirety (Fig. 1). They do not specify a particular set of sexually-dimorphic traits on which sexual selection can operate.

There are also examples in which the evolutionary rise of a new sex determination system is inconsistent with the preconditions of any of the directional selection hypotheses. Multiple male-determiners, for instance, that co-exist in the dipteran insects *M. domestica* and *M. scalaris* [10, 35, 85], cannot be used to adjust sex ratios, nor do different male-determiners promote sexual conflict. It is not plausible that evolutionary degeneration of a preceding sex determiner (i.e. through the accumulation of deleterious mutations) would directionally select for multiple male sex determiners. Fitness differences of *M. domestica* populations that either have an autosomal *M* or a *Y-M* sex determination mechanism.
[86] can also be explained by secondary effects and genetic differences associated with X/Y chromosomal backgrounds.

Another explanation for the evolutionary rise of alternative male-determiners is genetic drift. A higher evolutionary origin rate by mutation will increase the probability of fixation (see [87] for a theoretical analysis of how genetic drift can drive genetic pathway evolution). Multiple male-determiners (>4) in M. domestica and M. scalaris populations could reflect the increased mutation rate by which new repressing activities of tra genes can evolve from the entire gene repertoire of the genome. A repressing activity can be established at different levels of tra gene regulation, suggesting a high rate of origin. In contrast, female-determiners evolve more rarely (indeed, only one female-determiner has been identified in M. domestica, but none in M. scalaris) because it is less likely that a new function arises by mutation that properly controls tra pre-mRNA splicing and protein production.

The null-hypothesis of genetic drift has been rejected in the case of the csd gene. An excess of non-synonymous over synonymous, neutral nucleotide changes has been identified during the evolutionary origin of csd (Fig. 3B) [12]. More amino acid-encoding nucleotides have been replaced directly after csd origin than neutral changes that solely became fixed by random genetic drift. Intriguingly, some of these new amino acids form a putative coiled-coil domain that appears to alter binding properties between CsD allelic proteins (Otte and Beye, unpublished results). The cause of directional selection during the evolutionary rise of csd is not understood and cannot be explained by an adjustment of sex ratios or an intra-locus sexual conflict. Complementary sex determination systems allow control over sex ratios (males derive from unfertilised eggs, females from fertilised eggs) and are not targets of intra-locus sexual conflict and sex ratio drive (as they are inherited in both sexes). Complementary sex determination systems can reside in genomic regions of substantially-reduced meiotic recombination [70, 88], implying that evolutionary degeneration may operate. Malfunction of the preceding complementary sex determiner could have selected for a new sex determiner; csd.

**Fruitful routes of future research**

There is a need to characterise more sex determination genes. Discovering other regulatory mechanisms and their routes of evolutionary origin will broaden our understanding of how and why these key controls of developmental processes have evolved. Characterisation of sex determiners will be greatly facilitated by next-generation sequencing technologies, especially for species in which sex-determining factors can be linked to short genomic regions of otherwise freely-recombining chromosomes (e.g. neo Y chromosomes) [9, 10, 31, 35, 85, 89]. Sequencing DNA from pools of male and female progenies of a single cross will identify sex-specific nucleotide differences. These sex-specific differences will identify candidate genes. The function of these genes can be further tested using RNAi-induced knock-down studies.

Nucleotide sequences of novel sex determiners will allow us to trace the evolutionary history [12, 15, 69] and the evolutionary forces that shaped their origin [12]. By applying population genetic tests on nucleotide sequences (i.e. dN/dS ratio, MacDonald Kreitman test, Tajima’s D, among others [90]), we can directly identify evidence for the cause of evolutionary change [12] in the responsible genes. This powerful approach has been widely neglected in evolutionary developmental biology.

An open question is when did the key sex-determining functions of tra (Figs. 1, 3) evolve? tra genes apparently have no sex-determining function outside of insects (see the crustacean case Daphnia magna [91], but it is not known whether hemimetabolous insects use these tra key functions. The fast-evolving tra genes may be identified by a conserved 30 amino acid motif that has been identified in non-Drosophila species [12, 13, 15, 92, 93]. tra has not been identified in the sequenced B. mori genome [94, 95], which may reflect a lack of sufficient conservation, evolutionary loss, or a lack of corresponding sequence information.

What is widely unknown is how sexual dimorphic traits can evolve so rapidly by changes in the underlying developmental program. Reproductive structures, behaviours and secondary sexual characteristics are some of the most variable and changeable features among insects; some of which evolve through sexual selection (Darwin 1871). Morphological changes may evolve by the regulatory control of dsx target genes [by either modification, loss or novel origin of cis regulatory elements (CREs) [52]] or by recruiting other genes (i.e. transcription factors) to the sex-determining process. Investigation into what aspects of morphology dsx controls in different species could give insights into whether dsx is a conserved key determinant of sexual morphic differentiation. Transgenic studies have shown that at least some aspects of dsx sexual differentiation are conserved in the dipteran insect M. domestica [16] and the lepidopteran B. mori [55, 96]. Expression of male and female Dsx proteins, with the help of transgenic tools in a dsx null mutant background, would facilitate study into which aspects of sexual differentiation are controlled by dsx (RNAi-induced knockdown of dsx by injection procedures have, thus far, failed in several insects) and whether other key components in non-Drosophila species have yet to be identified. The evolution of abdominal pigmentation and pheromone production in some Drosophila species have been shown to be caused by small-scale evolutionary changes of CREs of Dsx protein target genes [52, 63, 97]. A way to identify putative shared and evolved dsx target genes is to characterise binding sites of Dsx proteins in informative insect species by chromatin immuno-precipitation (ChiP) and next-generation sequencing.

**Conclusion**

Recent studies in insects have shed some light on how and why sex determination systems evolve [1, 12, 15, 69]. Gain-of-function alleles of tra, the double dose of X-linked transcription factors that activate Sxl, or Csd proteins derived from a heterozygous csd gene are the molecular signals of diverse sex determination mechanisms, such as a dominant female-determining system, an X:A ratio and a complementary sex determination system. These organism-specific signals all share transduction of their activities to common tra genes; the upstream component of an ancestral pathway tra->dsx in
holometabolous insects. Diverse signals utilise two regulatory principles to determine the two sexes (Fig. 2): they either produce feminising activities that switch *tra* genes ON, or they produce masculinating activities that switch *tra* genes OFF. In the absence of these activities, the pre-zygotic activity of *tra* (‘default state’ either ON or OFF) executes male or female development. *tra* genes in most studied insects maintain the sexually-determined state epigenetically through a positive regulatory feedback loop of pre-mRNA splicing [13].

Diverse mechanisms can evolve through small-scale nucleotide changes in regulatory and coding regions from existing or from duplicated genes. From the ease and rate that novel sex determiners arise by such changes [12], even within a species [10, 15, 35, 85], we suggest that non-adaptive forces (mutation and genetic drift) are also possible sources of novel sex determination systems. We suggest that genetic drift should serve as a null hypothesis in future work.

An excess of non-synonymous changes over synonymous, neutral changes [12] in the *csd* gene of honeybees shows that directional selection can enhance the evolutionary rise of novel sex determinant genes [12]. Evolutionary degeneration, due to lack of recombination of the preceding complementary sex determinant gene, may have caused such directional selection [6, 12, 82, 83].

By applying molecular evolutionary and genetic approaches, we are just beginning to understand both the evolutionary routes and the molecular mechanisms that have generated the enormous diversity of sex determination mechanisms. These insights will greatly broaden our knowledge of how novel control mechanisms and regulatory principles evolve in developmental processes.

Acknowledgments

We very much thank Peter Dearden for his helpful comments on the manuscript; Daniel Bopp, Giuseppe Saccone and Walter Traut for discussion; and Robert Page (ASU) for hosting MB during spring break in 2009. We apologise to those whose work could not be cited due to space restrictions. This work was supported by grants from the Deutsche Forschungsgemeinschaft (DFG).

References

1. Cline TW, Meyer BJ. 1996. Vive la difference: males vs. females in flies vs. worms. *Annu Rev Genet* 30: 637–702.
2. Zarkower D. 2006. Somatic sex determination. *WormBook* 1–12.
3. Lovell-Badge R, Canning C, Sekido R. 2002. Sex-determining genes in mice: building pathways. *Novartis Found Symp* 244: 4–18.
4. Sekido R, Lovell-Badge R. 2008. Sex determination involves synergetic action of SRY and SF1 on a specific Sox9 enhancer. *Nature* 453: 930–4.
5. Marshall Graves JA. 2008. Weird animal genomes and the evolution of vertebrate sex and sex chromosomes. *Annu Rev Genet* 42: 565–86.
6. Graves JA. 2006. Sex chromosome specialization and degeneration in mammals. *Cell* 124: 901–14.
7. Bull JJ. 1983. *Evolution of Sex Determining Mechanisms*. Merlo Park, California: Benjamin/Cummings Publishing Company.
8. Franco MG, Rubini PG, Vecchi M. 1982. Sex-determinants and their distribution in various populations of *Musca domestica* L. of Western Europe. *Genet Res* 40: 279–93.
9. Thompson PE, Bowen JS. 1972. Interactions of differentiated primary sex factors in *Chironomus tentans*. *Genetics* 70: 491–3.
10. Traut W. 1994. Sex determination in the fly *Megaselia scalaris*, a model system for primary steps of sex chromosome evolution. *Genetics* 136: 1097–1104.
11. Matsuda M. 2005. Sex determination in the teleost medaka, *Oryzias latipes*. *Annu Rev Genet* 39: 293–307.
12. Hasselmann M, Gempe T, Schiott M, Nunes-Silva CG, et al. 2008. Evidence for the evolutionary nascent of a novel sex determination pathway in honeybees. *Nature* 454: 519–22.
13. Pane A, Salvemini M, Bovi PD, Politio C, et al. 2002. The transformer gene in *Ceratitis capitata* provides a genetic basis for selecting and remembering the sexual fate. *Development* 129: 3715–25.
14. Gempe T, Hasselmann M, Schiott M, Hause G, et al. 2009. Sex determination in honeybees: two separate mechanisms induce and maintain the female phenotype. *PLoS Biol* 7: e1000222.
15. Hediger M, Henggeler C, Meier N, Perez R, et al. 2010. Molecular characterization of the key switch F provides a basis for understanding the rapid divergence of the sex-determining pathway in the housefly. *Genetics* 184: 155–70.
16. Hediger M, Burghardt G, Siegenthaler C, Buser N, et al. 2004. Sex determination in *Drosophila melanogaster* and *Musca domestica* converges at the level of the terminal regulator doublesex. *Dev Genes Evol* 214: 29–42.
17. Salvemini M, Robertson M, Aronson B, Atkinson P, et al. 2009. *Ceratitis capitata* transformer-2 gene is required to establish and maintain the autoregulation of Cctra, the master gene of female sex determination. *Int J Dev Biol* 53: 109–20.
18. Alvarez M, Ruiz MF, Sanchez L. 2009. Effect of the gene doublesex of *Anastrepha* on the somatic sexual development of *Drosophila*, *PLoS One* 4: e5141.
19. Lagos D, Ruiz MF, Sanchez L, Komitopoulou K. 2005. Isolation and characterization of the Bactrocera oleae gene orthologous to the sex-determining Sex-lethal and doublesex genes of *Drosophila melanogaster*. *Gene* 348: 111–21.
20. Concha C, Scott MJ. 2009. Sexual development in *Lucilia cuprina* (Diptera, Calliphoridae) is controlled by the transformer gene. *Genetics* 182: 785–90.
21. Lagos D, Koukidou M, Savakis C, Komitopoulou K. 2007. The transformer gene in Bactrocera oleae: the genetic switch that determines its sex fate. *Insect Mol Biol* 16: 221–30.
22. Verhulst EC, Beukeboom LW, van de Zande L. 2010. Maternal control of haplodiploid sex determination in the wasp *Nasonia*. *Science* 328: 620–3.
23. Dzierzon J. 1845. Gutachten über die von Herrn Direktor Stöhr im ersten und zweiten Kapitel des General-Gutachtens aufgestellten Fragen. *Eichtädtler Bienenzeitung* 1: 109–113, 119–121.
24. McClung CE. 1902. The accessory chromosome – sex determinant? *Biol Bull* 3: 43–84.
25. Wilson EB. 1905. The chromosomes in relation to determination of sex in insects. *Science* 22: 500–2.
26. Sanchez L. 2008. Sex-determining mechanisms in insects. *Int J Dev Biol* 52: 837–56.
27. Schutt C, Nöthiger R. 2000. Structure, function and evolution of sex-determining systems in Dipteran insects. *Development* 127: 667–77.
28. Erickson JW, Quintero JJ. 2007. Indirect effects of ploidy suggest X chromosome dose, not the X:A ratio, signals sex in *Drosophila*. *PLoS Biol* 5: e332.
29. Dubendorfer A, Hediger M, Burghardt G, Bopp D. 2002. *Musca domestica*, a window on the evolution of sex-determining mechanisms in insects. *Int J Dev Biol* 46: 75–9.
30. Marin I, Baker BS. 1998. The evolutionary dynamics of sex determination. *Science* 281: 1990–4.
31. Kraemer C, Schmidt ER. 1993. The sex determining region of *Chironomus thummi* is associated with highly repetitive DNA and transposable elements. *Chromosoma* 102: 553–62.
32. Cook JM. 1993. Sex determination in the hymenoptera: a review of models and evidence. *Heredity* 71: 421–35.
33. Beye M. 2004. The dice of fate: the *csd* gene and how its allelic composition regulates sexual development in the honey bee, *Apis melifera*. *BioEssays* 26: 113–20.
34. Beye M, Hasselmann M, Fondrk MK, Page RE, et al. 2003. The gene *csd* is the primary signal for sexual development in the honeybee and encodes an SR-type protein. *Cell* 114: 419–29.
35. Inoue H, Fukumori Y, Hiroyoshi T. 1983. Mapping of autosomal male-determining factors of the housefly, *Musca domestica* L., by means of sex-reversal. *Jpn J Genet* 58: 451–61.
36. McDonald IC, Evenson P, Nickel CA, Johnson OA. 1978. Housefly genetics: Isolation of a female determining factor on chromosome 4. Ann Entomol Soc Am 71: 692–4.
37. Willhoft U, Franz G. 1996. Identification of the sex-determining region of the Ceratitis capitata Y chromosome by deletion mapping. Genetics 144: 737–45.
38. McKeown M, Belote JM, Baker BS. 1987. A molecular analysis of transformer, a gene in Drosophila melanogaster that controls female sexual differentiation. Cell 48: 489–99.
39. Butler B, Pirrotta V, Irrminger-Finger I, Nöthiger R. 1986. The sex-determining gene tra of Drosophila: molecular cloning and transformation studies. EMBO J 5: 3807–13.
40. Savard J, Tautz D, Richards S, Weinstock GM, et al. 2006. Phylogenomic analysis reveals bees and wasps (Hymenoptera) at the base of the radiation of Holometabolous insects. Genome Res 16: 1334–8.
41. Marsh JL, Wieschaus E. 1978. Is sex determination in germ line and soma controlled by separate genetic mechanisms? Nature 272: 249–51.
42. Bell LR, Horabin JJ, Schedi P, Cline TW. 1991. Positive autoregulation of Sex-lethal by alternative splicing maintains the female determined state in evolution in insects. Science 253: 610–23.
43. Traut W, Niimi T, Ikeo K, Sahara K. 2006. Phylogeny of the sex-determination gene doublesex across 250 Myr of insect evolution. Mol Biol Evol 23: 633–43.
44. Bertels R, van de Zande L, Beukeboom LW. 2009. The fruitless gene in Nasonia displays complex sex-specific splicing and contains new zinc finger domains. Mol Biol Evol 26: 1557–69.
45. Ruiz MF, Einir-Lopez JM, Stefani RN, Perondi AL, et al. 2007. The gene doublesex of Anastrepha fruit flies (Diptera, Tephritidae) and its evolution in insects. Dev Genes Evol 217: 275–31.
46. Ruiz MF, Stefani RN, Mascarhenas RO, Perondi AL, et al. 2005. The gene doublesex of the fruit fly Anastrepha obliqua (Diptera, Tephritidae). Genome 48: 84–92.
47. Che S, Huang ZY, Zhang J. 2007. Sex-specific splicing of the honeybee doublesex gene reveals 300 million years of evolution at the bottom of the insect sex-determination pathway. Genetics 177: 1373–81.
48. Dearden PK, Wilson MJ, Sablan L, Osborne PW, et al. 2006. Patterns of conservation and change in honey bee developmental genes. Genome Res 16: 1378–84.
49. Cristino AS, Nascimento AM, Costa LF, Simoes ZL. 2006. A comparative analysis of highly conserved sex-determining genes between Apis mellifera and Drosophila melanogaster. Genet Mol Res 5: 647–54.
50. Williams TM, Carroll SB. 2005. Genetic and molecular insights into the development and evolution of sexual dimorphism. Nat Rev Genet 10: 797–804.
51. Burtis KC, Baker BS. 1989. Drosophila doublesex gene controls somatic sexual differentiation by producing alternatively spliced mRNAs encoding related sex-specific polypeptides. Cell 56: 997–1010.
52. Coscigano KT, Wensink PC. 1993. Sex-specific transcriptional regulation by the male and female doublesex proteins of Drosophila. Genes Dev 7: 42–54.
53. Suzuki MG, Funaguma S, Kanda T, Tamura T, et al. 2003. Analysis of the biological functions of a doublesex homologue in Bombyx mori. Dev Genes Evol 213: 345–54.
54. Vincent S, Perkins LA, Perrimon N. 2001. Doublesex surprises. Cell 106: 399–402.
55. Kopp A, Duncan I, Godt D, Carroll SB. 2000. Genetic control and evolution of sexually dimorphic characters in Drosophila. Nature 408: 553–9.
56. Keisman EL, Baker BS. 2001. The Drosophila sex determination hierarchy modulates wingless and decapentaplegic signalling to deploy dachshund sex-specifically in the genital imaginal disc. Development 128: 1643–56.
57. Christiansen AE, Keisman EL, Ahmad SM, Baker BS. 2002. Sex comes in from the cold: the integration of sex and pattern. Trends Genet 18: 510–6.
58. Keisman EL, Christiansen AE, Baker BS. 2001. The sex determination gene doublesex regulates the A/P organizer to direct sex-specific patterns of growth in the Drosophila genital imaginal disc. Dev Cell 1: 215–25.
59. Sanchez L, Guerrero I. 2001. The development of the Drosophila genital disc. BioEssays 23: 698–707.
60. Sanchez L, Gorfinkel N, Guerrero I. 2001. Sex determination genes control the development of the Drosophila genital disc, modulating the response to Hedgehog, Wingless and Decapentaplegic signals. Development 128: 1333–43.
61. Williams TM, Selegue JE, Werner T, Gompel N, et al. 2008. The regulation and evolution of a genetic switch controlling sexually dimorphic traits in Drosophila. Cell 134: 610–23.
62. Ryner LC, Goodwin SF, Castrillon DH, Anand A, et al. 1996. Control of male sexual behavior and sexual orientation in Drosophila by the fruitless gene. Cell 87: 1079–89.
63. Demir E, Dickson BJ. 2005. Fruitless specifying male courtship behavior in Drosophila. Cell 121: 785–94.
64. Rideout EJ, Doman AJ, Neville MC, Eadie S, et al. 2010. Control of sexual differentiation and behavior by the doublesex gene in Drosophila melanogaster. Nat Neurosci 13: 458–66.
65. Gailey DA, Billetter JC, Liu JH, Bauson F, et al. 2006. Functional conservation of the fruitless male sex-determination gene across 250 Myr of insect evolution. Mol Biol Evol 23: 633–43.
66. Beretta R, van de Zande L, Beukeboom LW. 2009. The fruitless gene in Nasonia displays complex sex-specific splicing and contains new zinc finger domains. Mol Biol Evol 26: 1557–69.
67. Traut W, Niimi T, Ikeo K, Sahara K. 2006. Phylogeny of the sex-determination gene doublesex across 250 Myr of insect evolution. Mol Biol Evol 23: 633–43.
68. Hasselmann M, Vekemans X, Pflugfelder J, Koeniger N, et al. 2008. Evidence for convergent nucleotide evolution and high allelic turnover rates at the complementary sex determiner gene of Western and Asian honeybees. Mol Biol Evol 25: 696–708.
69. Hasselmann M, Beye M. 2004. Signatures of selection among sex-determining alleles of the honey bee. Proc Natl Acad Sci USA 101: 4888–93.
70. Heimpel GE, de Boer JG. 2008. Sex determination in the hymenoptera. Annu Rev Entomol 53: 209–30.
71. Hamilton WD. 1967. Extraordinary sex ratios. A sex-ratio theory for sex linkage and inbreeding has new implications in cytogenetics and entomology. Science 156: 477–88.
72. Trivers RL, Willard DE. 1973. Natural selection of parental ability to vary the sex ratio of offspring. Science 179: 90–2.
73. Rice WR. 1986. On the instability of polygenic sex determination: the effect of sex-specific selection. Evolution 40: 633–9.
74. Van Doorn GS, Kirkpatrick M. 2007. Turnover of sex chromosomes induced by sexual conflict. Nature 449: 909–12.
75. Sandler L, Novitski E. 1957. Meiotic drive as an evolutionary force. Am Nat 41: 105–10.
76. Temin RG. 1991. The independent distorting ability of the Enhancer of Segregation Distortion, (ESD), in Drosophila melanogaster. Genetics 128: 339–56.
77. Poliankowski A, Nothiger R, Wilkins A. 2004. The evolution of the Drosophila sex-determination pathway. Genetics 166: 1761–73.
78. Singh RS, Arteri CG. 2010. Male sex drive and the maintenance of sex: evidence from Drosophila. J Hered 101 (Suppl 1): S100–6.
79. Singh RS, Kulathinal RJ. 2005. Male sex drive and the masculinization of the genome. BioEssays 27: 158–25.
80. Charlesworth B, Charlesworth B. 1978. 5X chromosomes: evolution of the weird and wonderful. Curr Biol 15: R29–31.
81. Denholm I, Franco MG, Rubini PG, Vecchi M. 1983. Identification of a male determinant on the X chromosome of housefly (Musca domestica L.) populations in south east England. Genet Res 42: 311–22.
82. Hamm RL, Gao JR, Lin GG, Scott JG. 2009. Selective advantage for IIM males over YM males in cage competition, mating competition, and pupal emergence in Musca domestica L. (Diptera: Muscidae). Environ Entomol 38: 499–504.
83. Lynch M. 2007. The evolution of genetic networks by non-adaptive processes. Nat Rev Genet 8: 803–13.
84. Hasselmann M, Beye M. 2006. Pronounced differences of recombination activity at the sex determination locus (SDL) of the honey bee, a locus under strong balancing selection. Genetics 174: 1469–80.
85. Gadad J, Gerloff CU, Kruger N, Chan H, et al. 2001. A linkage analysis of sex determination in Bombus terrestris (L.) (Hymenoptera: Apidae). Heredity 87: 234–42.
90. Excoffier L, Heckel G. 2006. Computer programs for population genetics data analysis: a survival guide. Nat Rev Genet 7: 745–58.
91. Kato Y, Kobayashi K, Oda S, Tatarazako N, et al. 2010. Sequence divergence and expression of a transformer gene in the branchiopod crustacean, Daphnia magna. Genomics 95: 160–5.
92. Kulathinal RJ, Skwarek L, Morton RA, Singh RS. 2003. Rapid evolution of the sex-determining gene, transformer: structural diversity and rate heterogeneity among sibling species of Drosophila. Mol Biol Evol 20: 441–52.
93. McAllister BF, McVean GA. 2000. Neutral evolution of the sex-determining gene transformer in Drosophila. Genetics 154: 1711–20.
94. Mita K, Kasahara M, Sasaki S, Nagayasu Y, et al. 2004. The genome sequence of silkworm, Bombyx mori. DNA Res 11: 27–35.
95. Xia Q, Zhou Z, Lu C, Cheng D, et al. 2004. A draft sequence for the genome of the domesticated silkworm (Bombyx mori). Science 306: 1937–40.
96. Suzuki MG, Funaguma S, Kanda T, Tamura T, et al. 2005. Role of the male BmDSX protein in the sexual differentiation of Bombyx mori. Evol Dev 7: 58–68.
97. Shirangi TR, Dufour HD, Williams TM, Carroll SB. 2009. Rapid evolution of sex pheromone-producing enzyme expression in Drosophila. PLoS Biol 7: e1000168.
98. Siera SG, Cline TW. 2008. Sexual backtalk with evolutionary implications: stimulation of the Drosophila sex-determination gene Sex-lethal by its target transformer. Genetics 180: 1963–81.
99. Yeates DK, Wiegmann BM, Courtney GW, Meier M, et al. 2007. Phylogeny and systematics of Diptera: two decades of progress and prospects. Zootaxa 1668: 565–90.
100. Hasselmann M, Lechner S, Schulte C, Beye M. 2010. Origin of a function by tandem gene duplication limits the evolutionary capability of its sister copy. Proc Natl Acad Sci USA 107: 13378–83.