

How is sex determined in insects?

Preface

Early observations that sex is associated with differences in chromosome constitution heralded the chromosomal theory of heredity. This year marks one hundred years since Thomas Hunt Morgan discovered a sex chromosome-linked mutation in *Drosophila melanogaster* which gave final proof to this theory. Morgan's student Calvin Bridges formulated his classic balance theory of sex determination in *Drosophila* on the basis of genotypes with variable X:A ratios, implying a counting mechanism for X-linked female determinants and autosome-linked male determinants. Later it was found that single-gene mutations can affect not only specific traits but also the entire sexual fate of an individual. In 1944 another of Morgan's students, Alfred Sturtevant, identified a recessive autosomal mutation in *D. melanogaster* that caused sex reversal of XX individuals into sterile males. This recessive loss-of-function mutation, named *transformer* (*tra*), did not have any effect in XY males, suggesting that the gene is required only in XX female flies. This was one of the first pieces of evidence that sex determination can be controlled by genes. Sturtevant also had an evolutionary perspective on the genic control of sex determination. He had the foresight that the *intersex* mutation, previously described in the distantly related species *D. virilis*, corresponds to the *D. melanogaster tra* gene. Indeed, almost 50 years later a conserved *tra* orthologue was isolated in *D. virilis* which functionally corresponds to the previously described *D. virilis intersex* mutation. During the 1980s, molecular cloning and characterization of this and other *Drosophila* sex-determining genes revealed that the decision whether to become male or female is conveyed very early in embryonic development by a primary signal which is transmitted through a cascade of regulatory networks which ultimately results in the production of two distinct sexes.

Surprisingly, other species, belonging to different animal phyla, provide puzzling examples of myriad sex-determining primary signals. The primary signal that provides the cue varies remarkably not only among taxa but also within taxa pointing to rapid evolution of sex-determining mechanisms. The insect order Insecta alone provides examples of astoundingly complex diversity of sex-determination mechanisms. In *Drosophila*, X-linked signal elements (XSEs) provide the signal to switch ON the top player of the sex-determination cascade, *Sex-lethal* (*Sxl*). This pathway consists of various regulatory genes that control not only the sex-determination cascade (*Sex-lethal*, *Sxl*; *transformer*, *tra*; *transformer-2*, *tra-2*), but also its maintenance (*Sxl*) as well as sexual differentiation (*doublesex*, *dsx*; *fruitless*, *fru*): XSE > *Sxl* > *tra*(+*tra-2*) > *dsx/fru*. These genes produce sex-specific alternatively spliced mRNAs, and encode splicing regulators (*Sxl*, *tra* and *tra-2*) or transcription factors (*dsx*, *fru*).

As homologous molecular players involved in the cascade were beginning to be uncovered in other species, it was soon realized that the top layer of the pathway is not conserved outside *Drosophila* and thus a wider evolutionary perspective on sex determination started to emerge. Dipterans exhibit consistent differences in regulation of chromosomal/molecular players involved in the sex-determination hierarchy as reported for example in the Mediterranean fruitfly (*Ceratitidis capitata*), housefly (*Musca domestica*), humpbacked fly (*Megaselia scalaris*), sheep blowfly (*Lucilia cuprina*) and *Sciara coprofila*. For example, XXY individuals are females in *D. melanogaster* because of the presence of two X chromosomes whereas in *C. capitata* XXY is male because of the presence of the Y chromosome. In *M. domestica*, the primary sex-determining loci vary in natural populations, and the presence of some of these loci convert autosomes to new sex chromosomes. A bizarre situation is encountered in *M. scalaris* where a low rate of transposition of male-determining factor from chromosome to chromosome creates a new sex chromosome each time it hops. Intriguingly, in *S. coprofila* elimination of the paternal derived chromosome occurs in a maternally controlled fashion. In the silkworm, *Bombyx mori*, a single W chromosome ensures female development even in the presence of triploid, tetraploid or hexaploid sets of autosomes and Z chromosomes, suggesting that the W chromosome harbours feminizing gene(s). At the next level of the sex-determination pathway, the well-studied RS protein-encoding gene *transformer* (*tra*) isolated in different dipteran species, offers an interesting example of partial conservation and functional divergence from *Drosophila*. Ever since the first discovery of its autoregulation in the Mediterranean fruitfly *C. capitata* (*tra^{ep}*, *tra* epigenetic), similar *tra* autoregulatory loops have been shown to be operative in all insects from which functional *tra* genes have been characterized.

The rapid divergence of molecular players at the top of the hierarchy is also evident in hymenopterans. In honeybee *Apis mellifera*, the allelic status of complementary sex determining (CSD) locus, a *tra^{ep}*-related

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gene, provides the initial cue. Bees heterozygous at the *csd* locus are females, whereas hemizygous or homozygous bees are males. The product of the *csd* gene sets up, during early embryogenesis, the initial activation or repression of *feminizer* (*fem*, equivalent of *tra^{ep}*), by controlling alternative splicing of latter's pre-mRNAs. The *fem* gene is able to maintain its activated state throughout development by an autoregulatory loop. The parasitic wasp *Nasonia vitripennis*, however, does not have a *csd* locus. Instead, this insect depends on a combination of maternal supply of *tra* mRNA (another *tra^{ep}*-equivalent gene that shows structural, regulatory and functional homologies to *tra^{ep}*) and a paternal genome set, to set up zygotic activity of *tra* through autoregulation of its own pre-mRNA splicing. In this species a novel way of *transformer* control in insect sex determination implies the action of maternal imprinting. In silkworm, attempts to discover the top molecular signals have remained elusive. All lines of evidence point to the presence of a female-determining gene on the W chromosome. Initial results suggest that this region codes for a set of zinc finger protein encoding genes but their mechanism of action has remained unclear. Interestingly, a gene encoding *P*-element somatic inhibitor (PSI) has been discovered recently but it is expressed in both sexes and abrogation of its function results in alteration in *doublesex* (*dsx*) splicing pattern.

Contrary to the top signals, *dsx*, which functions at the bottom of the sex-determination cascade, is very well conserved in almost all the insects examined to date as also in other taxa as well, where it has been shown to be essential for male determination. Thus, insects provide vivid examples of an astonishing diversity of primary signals of sex determination that not only vary between species but even within species, in contrast to terminal genes which are conserved across taxa.

Accordingly this special issue of *Journal of Genetics* is dedicated to provide an update of the data available from genetic studies of sex determination and of sexual differentiation in a wide range of insect species.

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