

## How is sex determined in insects? An epilogue

Although sex determination mechanisms and sex differentiation pathways have been elucidated in great detail in diverse insect taxa, a clear picture is yet to emerge as many questions remain unanswered. Some of these questions may possibly find answers in the coming years with ongoing comparative genetic analyses of insect genomes and many novel molecular tools on hand. Even in the model system *Drosophila*, a paradigm shift in the dogma concerning the primary signal X:A has happened very recently. Novel insights are required to clarify the number and identity of the direct targets regulated by the DSX and FRU transcriptional factors, which bring about sexual dimorphism in adult flies. The master gene *Sxl* produces many different protein isoforms in females, but whether or not they all have different functions remains to be understood. With the recent findings in *Ceratitis capitata*, *Musca domestica*, *Apis mellifera*, *Lucilia cuprina* and *Nasonia vitripennis*, it is clear that the intermediate genetic module  $tra^{ep}/tra-2^{aux-ep}$  is widely conserved, and that sex determination appears to revolve around this module. Despite the availability of the genome sequences of different diptera (from among > 100,000 dipteran species), only *Drosophila* have the primary signal and its pathway been deciphered in some depth. Concerning the more evolutionarily distant dipteran species such as *Anopheles* and *Aedes*, which also show sex-specific alternative splicing of both *dsx* and *fru*, no *tra* or *tra-2* functional orthologues have yet been identified, in contrast to hymenopteran species where *tra^{ep}* genes have been isolated and characterized.

The task of climbing up the sex-determining cascade without the use of an orthology-based approach has been achieved to some extent in *Bombyx mori*, in which a novel splicing regulator has been biochemically isolated recently. However, considering that it is expressed in both the sexes, its position in the sex-determination cascade seems akin to *tra-2* in dipterans. In this respect, determination of its link with upstream player(s) and of the interactive mechanism by which it associates with downstream targets would be central to our understanding of the mechanism of sex determination in this lepidopteran model system. The EST sequencing efforts in various species presently lack requisite molecular sophistication to give significant novel contributions to the study of sex determination, but the parallel ongoing genome projects perhaps will provide novel tools to reveal for example the molecular definition of the primary signals. In species such as *Sciara* in which imprinting plays a significant role in sex determination, isolation of *tra^{ep}* or *tra-2^{aux-ep}* orthologues will possibly clarify whether there also is an evolutionary divergence at this intermediate level of genetic control, or whether *tra^{ep}* also assumes a master control role in this insect similar to the one unravelled in *Nasonia vitripennis*. The emerging novel model systems such as *Tribolium castaneum* will, it is hoped, also soon begin to shed more light on evolution of sex determination in insects.

The future convergence of disciplines such as ecology, physiology and population genetics, together with evolutionary molecular genetics, will greatly help in drawing a refined picture of the evolution of sex determination, in response to both internal and external selection forces. The epigenetic nature of sex determination appears to be ancient as revealed by the existence of *tra^{ep}* in different insect orders, which needs to be further investigated in light of the presence of an analogous autoregulatory *Sxl*-based mechanism in *Drosophila*. Why have the mechanisms of alternative splicing and direct autoregulation of splicing-factor-encoding genes been used in different species to determine sex and to maintain memory? Why does an epigenetic master switch for sex determination encode the protein that shows the most divergent amino acid sequence even as it performs the same function? Sex-determination pathways seem to have evolved by a bottom-up mechanism as proposed by Adam Wilkins, which predicts that the downstream shared pathway existed as the rudimentary mechanism for sex determination in an ancient ancestor and that new regulatory signals have since been recruited upstream of the cascade during the course of evolution, thus rendering this layer of sex determination highly diverse. It is rather compelling also to surmise that the diverse sex-determination signals have been selected also to confer novel fitness advantages to the evolving species, especially when they control in parallel to *tra^{ep}*, other genetic pathways (female size, dosage compensation, male fertility, etc.). Nevertheless, 'up-bottom' changes are also required to change sexual differentiation, and hence sexual dimorphic traits in evolving species. More extensive *in silico* comparative analysis of ESTs in sexed individuals of different developmental stages using deep sequencing technologies will greatly contribute in future to understanding how the genetic network, upstream and downstream to the bottleneck represented by the genetic module  $tra^{ep}/tra-2^{aux-ep} > dsx/fru$ , both perform its functions and is modified.

Finally, the pursuit of molecular mechanisms of sex determination in insects has led also to unexpected practical applications, such as in the control of pests (e.g. *C. capitata*) and vectors of human diseases

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including malaria and dengue (e.g. *Anopheles gambiae*, *Aedes aegypti*), and in the breeding of economically important insects (e.g. *B. mori*). In all these cases, the attempts have been to obtain genetic sexing strains, by manoeuvring the chromosomes/genes involved in sex determination, with a view either to identify males from females for easy sex separation on a large scale or to obtain exclusively males by the killing of females at embryonic stage or reversing sex from female to male.

Perhaps too the insect studies will enable us to appreciate the ancient origin of the Yin/Yang forces, which are so pervasive in life manifestation on our planet. The genetic control of the innate program to court and to successfully copulate, which will be interrogated in *Drosophila* and comparatively studied in other insects, will offer us possibly a 'tantric' key to understand the selective pressures that shape this ultimate ancestral and highly complex phenotype that is sex.

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