## COMMENTARY ON *J. GENET.* CLASSIC

# Sex determining signal in *Drosophila melanogaster*

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A N U R A N J A N   A N A N D

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Sexual dimorphism is the most striking naturally occurring phenotypic variation that is a direct outcome of a simple Mendelian segregation. Molecular genetic dissection of mechanisms underlying sexual development in organisms ranging from the flies to humans has been a subject of extensive research during the last two decades. Our knowledge about the genetic regulatory machinery that underlies sex determination and differentiation has gone farthest in flies. Almost eighty  years after discovery of the chromosomal basis of sex determination in flies, we are now in a position to understand as to how this sex determining signal works at the molecular level.

Bridges (1921) established that sex in Drosophila melanogaster is determined by the ratio of the number of X chromosomes to the number of sets of autosomes rather than the absolute number of X chromosomes and that the Y chromosome has no role in sex determination. He analyzed the effect of varying X/A ratios on sexual development and found that genotypes 2X:2A (ratio=1) and 3X:3A (ratio=1) develop as females, 1X:2A (ratio=0.5) are males and 2X:3A (ratio=0.67) are intersexes containing patches of female and male tissues. Bridges suggested that sex determination results from two sets of counter-acting factors, with the X chromosome carrying 'female determiners' and the autosomes, 'male determiners'.

The first attempts at localization of the X-linked female determiners were made by Dobzansky and Schultz and results of these elegant genetic experiments were published seventy years ago in the Journal of Genetics (see, accompanying classic article). Dobzansky and Schultz added duplications of the X chromosome to the 2X:3A intersexes. Intersexes were used for such studies because small variations in their X:A ratio produce noticeable changes in the sexual phenotype. Addition of almost any segment of the X chromosome produced a shift towards femaleness. The extent of shift in the female direction produced by various duplications was on the whole proportional to their cytological lengths. On this basis, they concluded that sex de-

termining role of this chromosome was due to a co-operative effect of numerous female determiners located in all regions of the chromosome except the proximal inert region.

It is now well established that the X/A signal acts to set the functional state of a key regulatory gene, Sex-lethal (Sxl) (Bell *et al* 1988). Through its interaction with the downstream sets of genes, Sxl regulates developmental pathways leading to somatic sexual development, germline sexual development, dosage compensation (reviewd in Cline and Meyer 1996) and formation of sexual and courtship behaviours (reviewed in Hall 1994). Mutations that result in complete loss-of-function of Sxl are recessive female-specific lethal. These mutations have no detectable effect in males. Sxl gain-of-function mutations that are dominant male-specific lethal are known and these mutations rescue female lethality resulting from loss of Sxl functions. An X/A ratio of 1 results in activation of Sxl and this is essential for female development. When this ratio is 0.5, Sxl is not activated and male development occurs (Cline 1978, 1979 a , b, Keyes *et al* 1992). Initiation of Sxl activity occurs at the level of transcription (Parkhurst *et al* 1990, Keyes *et al* 1992).  There are embryo-specific transcripts of Sxl that utilize a female-specific early-acting promoter of the gene and not the late-acting sex non-specific promoter. Most of the genes known to form the X/A signal encode proteins containing DNA binding and dimerization motifs such as those found in transcription factors. Once Sxl has been activated, the X/A ratio signal is not necessary for maintenance of its activity during subsequent development because female-specific activity of Sxl is autoregulated via alternative splicing (Bell *et al* 1991). Molecular mechanisms underlying early embryonic activation of Sxl are the key to the understanding to how the X/A sex determining signal is formed, measured and conveyed to Sxl.

Four genes, sisterless-a (sis-a; Cline 1986, Erickson and Cline 1993), sisterless-b (sis-b; Cline 1988, Torres and Sanchez 1989), sisterless-c (sis-c; Sefton *et al* 2000) and runt (Duffy and Gergen 1991), are known to form female determiners of the X/A ratio signal. These genes are func-

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tionally related because a defect in one of them can be partly offset by a duplication of one of the remaining genes. These genes cause female lethality and/or show dose-dependent, reciprocal and sex-specific interactions among themselves and with the target gene Sxl (Cline 1988). Sex-specific activation of Sxl is evident by the blastoderm stage and it seems to occur in an all-or-none fashion. The early transcripts are detected in female embryos but not in male embryos. An interesting feature of the X:A signal in wild type flies is that the 2-fold difference in dosage of X-linked female determiners elicits a binary, 'male' or 'female', decision and this mechanism converts the two-fold difference into an all-or-none response. This comes about due to dose-dependent and co-operative association between the transcription factors forming the X signal, their multiple binding sites for activation, and male determiners modulating activity of relevant transcription factors acting on early Sxl promoter to amplify two-fold difference, between males and females, in the dosage of female determiners.

X-linked female determiners are 'measured' in reference to the autosomal male determiners (Bridges 1921). Denominator elements by negatively regulating Sxl would appear as masculinizing genes that could antagonize the action of the feminizing elements. Early attempts to localize male determiners on autosomes were not successful. None of the autosomal duplications tested was able to masculinize the phenotype of triploid intersexes nor did deficiencies feminize them. Hence, these experiments provided little evidence for the existence of specific male-determining elements on autosomes (Pipkin 1947, 1960). More recently, deadpan (dpn) has been identified to play the role of a male determiner, though its effect does not seem to be as strong as that of the female determiners (Younger-Shepherd et al 1992, Barbash and Cline 1995). Loss of dpn activity causes modest suppression of Sxl and the time course of expression of dpn and of Sxl in dpn mutant backgrounds suggests that dpn is required in males only during later stage of the X/A signaling process to prevent inappropriate expression of Sxl in face of increasing dosage levels of female determining gene products. It appears that if there are additional male determiners, their individual contributions are less than dpn (Barbash and Cline 1995). Regarding male determiners, suggestions made by Dobzansky and Schultz in this paper are very interesting. They suggested sex determining role of autosomes without assuming any autosomal male determiners and raised the possibility that sex may be determined by the ratio between the number of X-chromosomes present in the cell and the size of that cell. Indeed somewhat similar conclusion seems to have been reached after extensive genome-wide screening for the male determiners. It is also believed that male determiners may not be present in a specialized sense as female determiners. Autosomal component of the X/A ratio may correspond to mass of DNA that constitutes autosomes, non-coding DNA sequences or cell size etc.

This work by Dobzansky and Schultz represents a significant step towards addressing the problem of the distribution of female determiners in *Drosophila melanogaster*. It makes an interesting reading, trying to fit in the female determiners, as we understand them today, in relation to the genetic and cytological landmarks used then, and their phenotypic effects on the sexual development. Dobzansky and Schultz conducted these experiments at California Institute of Technology, USA during 1928-33 and going through this work is like a journey back in time to the formative years of fly genetics. I hope you enjoy it!

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