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# RNAi Doxxes Segregation Distorters on the X

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## Abstract

Species with chromosomal sex determination are susceptible to an evolutionary tug-of-war over sex chromosome segregation. RNA silencing has been proposed to play a role in this intragenomic conflict. Reporting in *Developmental Cell*, Lin et al. (2018) demonstrate that RNA interference is key to this conflict as a genome defender.

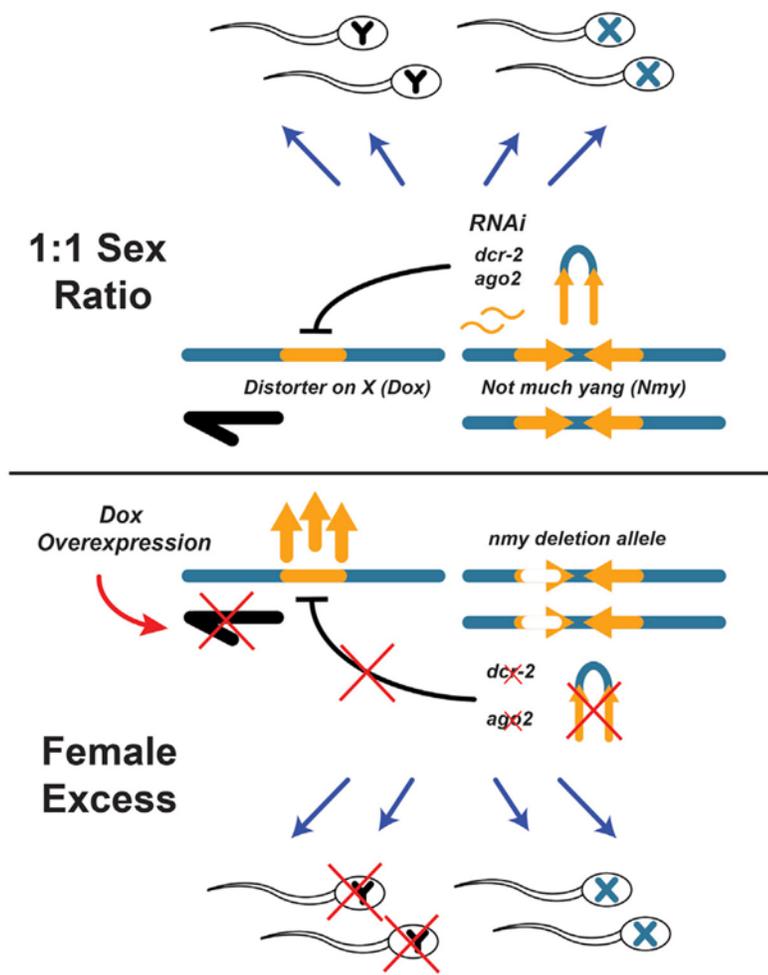
Adult sex ratios in natural populations vary widely, although many populations have approximately equal numbers of males and females. This variation is generated by many ecological and organismal factors, including the extent of inbreeding, population structure, and sex determination system (Hamilton, 1967). It is also generated by genetic parasites that reside on sex chromosomes and cheat meiotic segregation to increase their frequency in the population. In species with XY sex determination, such as mammals and fruit flies, alleles residing on an X chromosome gain an evolutionary advantage if they impair sperm carrying the Y chromosome, even if they reduce male fertility. Such alleles, known as segregation distorters, can skew population sex ratios. According to the Düsing-Fisher principle, selection will favor alleles that restore an even sex ratio. This is because, in a population with a skewed sex ratio, the rarer sex has more mating opportunities, leading to greater average fitness. Consequently, parents that produce the rarer sex have more grandchildren. Thus, as a distorting sex chromosome sweeps

through a population, there will be strong selection for alleles residing on other chromosomes that can suppress the distorter. Once cycles of distortion and suppression begin, they may quickly escalate to the point where unsuppressed distorters cause sterility. Such an escalation has been proposed as an explanation for Haldane's Rule, the observation that unisexual sterility in interspecific hybrids is almost invariably found in the heterogametic sex (XY males or ZW females), since hybrids of the heterogametic sex are more likely to encounter incompatibilities between sex chromosome distorters and suppressors that have diverged between species (Frank, 1991; Hurst and Pomiankowski, 1991).

While sex chromosome segregation distortion has been discovered in a wide range of species, we are only now beginning to identify the molecular mechanisms underlying cycles of drive and suppression. The fruit fly *Drosophila simulans* is one of the most important models for studying mechanisms and evolution of sex-ratio distorters. Natural populations of *D. simulans* harbor multiple genetically independent sex-ratio segregation systems of distorter and suppressor alleles. Some populations carry an X-linked haplotype of alleles that cause males to produce >80% daughters. One component of this haplotype consists of a partial deletion of the heterochromatin protein *HP1D2*, which disrupts segregation of Y chromatids in meiosis II, leading to an excess of X-bearing sperm among males carrying the driving *HP1D2* allele (Helleu et al., 2016). In this issue of *Developmental Cell*, Lin et al. (2018) utilize genetic, biochemical, transcriptomic, and transgenic analyses to demonstrate that in *D. simulans*, RNA interference (RNAi) plays a critical role in these intragenomic conflicts (Figure 1).

More than a decade ago, the evolutionary geneticist Yun Tao and his colleagues mapped two genes in *D. simulans* that are major components of sex-ratio distortion and suppression (Tao et al., 2007a, 2007b). *Not much yang* (*Nmy*) is an autosomal locus that harbors a dominant suppressor of the X-linked *distorter on the X* (*Dox*). Males homozygous for *nmy*, the non-functional allele of *Nmy*, produce an excess of daughters because they fail to suppress the function of *Dox*. This excess of daughters is caused by defective development of Y-bearing spermatids. Males carrying non-functional alleles at both loci have wild-type fertility and produce equal numbers of sons and daughters, suggesting that *Dox* and *Nmy* function solely in sex-ratio distortion and suppression. Both genes were mapped using naturally occurring alleles, indicating the existence of functional variation segregating at both loci.

Molecular analysis of these genes revealed that *Nmy* shares sequence homology with *Dox* and likely arose through retrotransposition of *Dox* itself. Subsequently, a duplication event resulted in the formation of an inverted repeat. Since inverted repeats can produce siRNAs, Tao proposed



**Figure 1. Sex-Ratio Distortion Is Suppressed by RNAi** — A wild-type *Nmy* hairpin locus generates endo-siRNAs via the RNAi pathway and represses *Dox*, enabling a normal sex ratio. In the absence of wild-type *Nmy* or the RNAi pathway, *Nmy* endo-siRNAs are not produced, *Dox* becomes overexpressed, and Y-bearing sperm fail to develop.

that suppression of *Dox* by *Nmy* could be mediated through RNA silencing. Whether this was the case has remained untested. In this issue of *Developmental Cell*, Lin et al. (2018) now show that endogenous siRNAs (endo-siRNAs) are produced from the *Nmy* hairpin RNA and that these siRNAs are absent in *nmy* genotypes that fail to repress *Dox*. Therefore, the RNAi pathway plays a critical role in intragenomic conflict over the sex ratio in *D. simulans*. Lin et al. (2018) also provide tantalizing evidence that the RNAi pathway may be important for the replacement of histones with protamines during spermatogenesis.

Lin et al. (2018) suggest that RNA silencing may have a specialized role in intragenomic conflict. In *D. melanogaster*, the X-linked *Stellate* repeats are suppressed by piRNAs derived from Y-linked *Su(Stellate)* repeats (Aravin et al., 2001). Though a role in segregation distortion is unproven, the absence of the *Su(Stellate)* repeats causes *Stellate* overexpression and defects in spermatogenesis. piRNAs have also been implicated in the *SD/Rsp* system of segregation distortion (Larracuenta and Presgraves, 2012). However, it is also clear that RNA silencing pathways can be recruited for functions other than intragenomic conflict. For example, piRNAs play an important role in sex determination in the silkworm (Kiuchi et al., 2014). RNA silencing, with its specialized ability to target specific sequences for repression, may easily be recruited for diverse functions, with intragenomic conflict being a fascinating example.

By dissecting the molecular mechanism of drive and suppression, Lin et al. (2018) have also revealed links between different sex-ratio distortion systems. Three of these systems have been named in *D. simulans*: Winters, Durham, and Paris. Previous studies considered the Winters system, characterized by the *Dox* distorter and *Nmy* suppressor, to be distinct from the Durham system, in which an autosomal locus, *Too much yin (Tmy)*, suppressed the activity of an unknown distorter. Lin et al. (2018) now provide evidence that the Winters and Durham systems are connected. The genomic region where *Tmy* was originally mapped is highly repetitive and consequently was absent from genome assemblies for *D. simulans*. Using single-molecule long read sequencing, the authors resolved the genome sequence of the *Tmy* region and discovered a second locus encoding a hairpin structure that produces endo-siRNAs homologous to *Dox* sequences. They infer that this hairpin RNA (hpRNA) locus is *Tmy*, and it too suppresses *Dox*. Moreover, both *Nmy* and *Tmy* also appear to suppress *MDox*, a nearby *Dox* paralog that may also function as a distorter. Altogether, the Winters/Durham system appears to consist of four components that contribute to drive and suppression. This signature of recurrent amplification of these sequences hints at a history of iterated cycles of drive and suppression.

Interestingly, the evolution of this fascinating system coincides with the observation that an intact RNAi pathway is required for spermatogenesis in *D. simulans*. Null alleles of RNAi pathway components *dcr-2* and *ago2* cause a reduction of male fertility in *D. melanogaster*, which has been attributed to loss of endo-siRNAs derived from hpRNA loci that regulate endogenous genes. In *D. simulans*, mutations in these components of the RNAi pathway appear to cause complete sterility. It remains to be shown whether this sterility is solely driven by failure to suppress *Dox* and *MDox* via *Nmy* and *Tmy* endo-siRNAs. Alternately, sterility in *D. simulans* may be due to the combined effects of *Dox/MDox* expression and misregulation of other genes that

are the target of hairpin loci. In either case, this work from Lin et al. (2018) provides exciting new evidence that RNA silencing may play a special role as a genome defense against native genes gone rogue. It will be interesting to see how these evolutionary games mediated by RNA silencing influence germline evolution and the dynamics of speciation.

## References

- Aravin, A.A., Naumova, N.M., Tulin, A.V., Vagin, V.V., Rozovsky, Y.M., and Gvozdev, V.A. (2001). Double-stranded RNA-mediated silencing of genomic tandem repeats and transposable elements in the *D. melanogaster* germline. *Curr. Biol.* 11, 1017–1027.
- Frank, S.A. (1991). Divergence of meiotic drive-suppression systems as an explanation for sex-biased hybrid sterility and inviability. *Evolution* 45, 262–267.
- Hamilton, W.D. (1967). Extraordinary sex ratios. A sex-ratio theory for sex linkage and inbreeding has new implications in cytogenetics and entomology. *Science* 156, 477–488.
- Helleu, Q., Gérard, P.R., Dubruille, R., Ogereau, D., Prud'homme, B., Loppin, B., and Montchamp-Moreau, C. (2016). Rapid evolution of a Y-chromosome heterochromatin protein underlies sex chromosome meiotic drive. *Proc. Natl. Acad. Sci. USA* 113, 4110–4115.
- Hurst, L.D., and Pomiankowski, A. (1991). Causes of sex ratio bias may account for unisexual sterility in hybrids: a new explanation of Haldane's rule and related phenomena. *Genetics* 128, 841–858.
- Kiuchi, T., Koga, H., Kawamoto, M., Shoji, K., Sakai, H., Arai, Y., Ishihara, G., Kawakawa, S., Sugano, S., Shimada, T., et al. (2014). A single female-specific piRNA is the primary determiner of sex in the silkworm. *Nature* 509, 633–636.
- Larracuenté, A.M., and Presgraves, D.C. (2012). The selfish segregation distorter gene complex of *Drosophila melanogaster*. *Genetics* 192, 33–53.
- Lin, C.-J., Hu, F., Dubruille, R., Vedanayagam, J., Wen, J., Smibert, P., Loppin, B., and Lai, E.C. (2018). The hpRNA/RNAi pathway is essential to resolve intragenomic conflict in the *Drosophila* male germline. *Dev. Cell* 46, 316–326.
- Tao, Y., Masly, J.P., Araripe, L., Ke, Y., and Hartl, D.L. (2007a). A sex-ratio meiotic drive system in *Drosophila simulans*. I: An autosomal suppressor. *PLoS Biol.* 5, e292.
- Tao, Y., Araripe, L., Kingan, S.B., Ke, Y., Xiao, H., and Hartl, D.L. (2007b). A sex-ratio meiotic drive system in *Drosophila simulans*. II: An X-linked distorter. *PLoS Biol.* 5, e293.