Distorted Sex Ratios: A Window into RNAi-Mediated Silencing

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In 1925, Gershenson started laboratory cultures from 19 female Drosophila obscura that were collected from a forest near Moscow. After recounting his difficulties raising the flies (partial success achieved with a diet of potatoes and fermented raisins), he noted that progeny from most cultures contained an approximately equal sex ratio [1]. Several cultures, however, yielded progeny with highly skewed ratios, such as one group with 87 females and only 7 males. These deviations from the normal sex-ratio were so considerable that it seemed impossible to explain them by accidental causes, he wrote. Similar observations had been made by others, but Gershenson went on to perform a number of experiments and reached three important conclusions. First, sex-ratio distortion (referred to hereafter as sex-ratio) was associated with the X chromosome. Second, the expression of the phenotype was sex-limited, because it only occurred in the progeny of males carrying the causal X chromosome. And third, the low numbers of males did not appear to be caused by preferential death of male zygotes or their transformation into females. Rather, he concluded that “the greater part of the spermatozoa determining the development of males do not participate in fertilization”. Because females have two X chromosomes and males are XY, he further suggested that either Y-bearing sperm are less frequently produced by affected males than X-bearing sperm, or that Y-bearing sperm are less capable of achieving fertilization.

Numerous additional examples of sex-ratio have since been reported in other Drosophila species, but the identity of the causal genes has remained elusive. In this issue of PLoS Biology, Yun Tao and colleagues report the discovery and identity of an X-linked sex-ratio distorter from Drosophila simulans called Dox (Distorter on the X) [2]. In a second paper [3] they describe the identification of a dominant suppressor of Dox called Nmy (Not much yang).

The close association of distorting and suppressing genes, though not appreciated by Gershenson, is key to understanding the genetic basis and evolutionary dynamics of sex-ratio systems. The long-term prospects of sexually reproducing populations that contain predominantly one sex are dire, and theory (commonly attributed to R.A. Fisher, but see [4] for an alternative attribution) suggests that an equal sex ratio is generally the most stable ratio over evolutionary time. Genes causing sex-ratio are therefore selfish genes, good at promoting an increase in their own frequency while potentially driving their host species to ruin. The host species is thus predicted to evolve suppressing alleles in order to maintain an equal sex ratio. This battle between sex-ratio distorters and suppressors creates a genetic conflict and may lead to continual cycles of distortion and suppression.

One then expects that sex-ratio phenotypes may arise in progeny of crosses between populations or species, as distortion genes segregate away from their corresponding suppressors. Such phenotypes occurred when segments of the Drosophila sechellia genome were introgressed into its sister species, D. simulans [5]. Skewed sex ratios were observed in ~10% of such introgression lines. Tao et al. [3] chose one of these lines for further characterization, reasoning that an introgressed D. sechellia region was displacing a D. simulans sex-ratio suppressor and thus unleashing a D. simulans distortion gene. They found the suppressor gene, Nmy, but discovered that the absence of the suppressor was caused by a mutant allele, nmy, that is segregating within D. simulans populations, rather than being due to introgression from D. sechellia. While analyzing nmy, they also found a D. simulans strain that did not produce an altered sex ratio even when homozygous for nmy [2]. This strain turned out to carry a mutant allele, dox, that is incapable of distortion. The discovery of these mutant alleles dox and nmy allowed Tao and colleagues to map the corresponding genes to single-gene resolution.

Dox appears to be a transposition from a parental gene named by the authors as Mdox (Mother of Dox). Mdox partially overlaps the 3′ end of another gene, CG32702, but is transcribed on the other strand. Both Mdox and Dox have limited protein-coding potential, and the longer hypothetical open reading frames do not match any known protein sequences. Determining whether the distorting activity of Dox is mediated via an RNA or protein product will require future experimental analysis. Most intriguingly, Tao et al. [3] found that the suppressor Nmy appears to have originated as a retrotransposed duplication of Dox. A further duplication generated an inverted repeat in Nmy, and deletion of one of these repeats creates a nonsuppressing nmy mutant allele. From these observations, Tao et al. propose that small interfering RNAs (siRNAs) generated from the Nmy hairpin sequence silence Dox via the RNA interference (RNAi) pathway (Figure 1).

These exciting studies beg for a deeper understanding of both the mechanistic basis and evolutionary consequences of sex-ratio. The foremost mechanistic question is how...

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Abbreviations: rasiRNA, repeat-associated small interfering RNA; RNAi, RNA interference; SD, Segregation Distorter; TE, transposable element

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Primers provide a concise introduction into an important aspect of biology highlighted by a current PLoS Biology research article.
**Figure 1.** Mechanistic and Evolutionary Model for sex-ratio Distortion

The X-linked *Dox* gene first evolved to target an unknown component of the Y chromosome, so that Y-bearing sperm fail to develop. This leads to an increased transmission frequency of the *Dox*-bearing X chromosome and a female-biased sex ratio. It remains unclear whether *Dox* is an RNA or protein-coding gene. Later, a transposition of *Dox* to Chromosome 3 created the *Nmy* gene. siRNAs produced from the double-stranded hairpin of *Nmy* target the homologous region of *Dox* for degradation via the RNAi pathway. As a result, Y-bearing sperm develop normally, and X-chromosome meiotic drive is suppressed. The model depicts a pre-meiotic germ cell, but the cellular manifestation of distortion occurs during nuclear condensation and maturation of sperm. Only the sex and third chromosomes are shown.

*Dox* incapacitates Y-bearing sperm. It will then be of great interest to compare the mechanism of *Dox*-mediated sex-ratio with other distortion systems. Tao et al. argue that other systems previously described in *D. simulans* are genetically independent. One of these has been intensively studied by Montchamp-Moreau and colleagues, and the distortion genes have been mapped to high resolution (reviewed in [6]). Hints of additional distortion systems and extensive polymorphism within *D. simulans* that modify sex-ratio are scattered throughout the articles of Tao et al. These findings therefore suggest that cycles of sex-ratio followed by suppression may be quite frequent, and thus may have a marked impact on genome evolution. If so then these reports will certainly reinvigorate long-standing and contentious arguments about whether and how sex-ratio genes contribute to hybrid male sterility and speciation [7–13].

One of the longstanding mysteries of *SD* is the link between mislocalized RanGAP and *Responder*. A previous model has proposed that RanGAP directly binds to *Responder* satellite DNA to disrupt chromatin condensation [14] (Figure 2). However, Tao et al. [3] propose an intriguing alternative hypothesis: that *SD*, like sex-ratio, occurs through an RNAi mechanism (Figure 2). Recent studies have shown that noncoding, highly repetitive DNA sequences called satellites as well as repetitive mobile genetic elements are silenced by the repeat-associated small interfering RNA (rasiRNA; also called Piwi-associated RNA or piRNA) pathway in a number of organisms including *Drosophila* [18–22]. Transcripts made from these typically heterochromatic sequences are processed into 22–30-bp double-stranded RNAs by proteins including Piwi and Aubergine, and are transported into the cytoplasm where they are complexed with other proteins such as Argonaute-3. Current models suggest that these ribonucleoprotein complexes are then shuttled back into the nucleus where they recruit histone methyltransferases.
The 359-bp satellite is needed to induce lethality. The 359-bp satellite, suggesting the possibility that a threshold amount of satellite does not correspond to the 359-bp satellite. In general, lethal genotypes did contain high amounts of satellite repeats on Chromosome 2 during spermatogenesis. Chromatin condensation is disrupted in chromosomes carrying a high number of repeats (Rsp), resulting in developmental failure of sperm bearing these chromosomes [16].

After examining multiple 

SD

(Responder) in 

SD

Segregation Distortion

(A) In a previous model [14], nuclear RanGAP binds abnormally to Rsp satellite repeats on Chromosome 2 during spermatogenesis. Chromatin condensation is disrupted in chromosomes carrying a high number of repeats (Rsp), resulting in developmental failure of sperm bearing these chromosomes [16]. (B) Alternatively, SD is caused by disruption of an RNAi-dependent silencing process as suggested by Tao et al. [3]. Mislocalized RanGAP disrupts proper nuclear transport of small Rsp-derived RNAs and ribonucleoprotein (RNP) complexes that are required to repress the Rsp satellites. As a result, proper heterochromatic repression of Rsp is disrupted, causing defects in chromatin condensation and loss of Rsp-bearing sperm.

We suggest that a similar failure in RNAi-mediated silencing of pericentromeric satellite DNA, which is adjacent to the centromere, may cause hybrid lethality in Drosophila. Hybrid daughters of D. simulans females crossed to D. melanogaster males die as embryos. Sawamura et al. [23] discovered that lethality depends on the D. melanogaster X-chromosomal locus Zygotic hybrid rescue (Zhr). Sawamura et al. [24] proposed that Zhr may correspond to a 359-bp repeat belonging to the 1.688-g/cm^3 family of satellites, which is located on the D. melanogaster X chromosome. After examining multiple Zhr^+ and Zhr^- chromosomes, they observed that the amount of satellite did not perfectly correlate with hybrid lethality and concluded that Zhr does not correspond to the 359-bp satellite. In general, however, lethal genotypes did contain high amounts of the satellite, suggesting the possibility that a threshold amount of satellites is needed to induce lethality. The 359-bp satellite was recently shown to be silenced in the ovary by the RNAi machinery [25]. We propose that maternally contributed small RNAs deriving from the 359-bp repeat are required to silence paternally inherited copies of the repeat in the developing embryo. These maternal RNAs will be particularly critical during early embryonic cell cycles, before zygotic transcription begins. D. simulans does not appear to contain the 359-bp repeat found on the D. melanogaster X chromosome [26]. Therefore, the 359-bp satellite on the paternally inherited D. melanogaster X chromosome will be de-repressed in early hybrid female embryos (Figure 3). This hypothesis explains why lethality is female-specific, because hybrid sons carry only the D. simulans X chromosome.

Other factors may contribute to this embryonic hybrid lethality, and our hypothesis raises further questions of whether other repeat classes that are specific to either D. melanogaster or D. simulans are de-repressed in their hybrids. Similar RNAi-based mechanisms have also been suggested to maintain silencing of transposable elements (TEs). Hybrid dysgenesis occurs when females lacking a TE family mate with males from a different strain containing the TE. The TEs become active in the hybrid progeny, and their mobilization can lead to increased mutation rates, chromosomal rearrangements, and sterility (for an early review see [27]). A striking example of the maternal specificity comes from a study of Arabidopsis, where molecular polymorphisms distinguishing different families of the ATHILA retrotransposon were used to demonstrate that only paternally inherited copies are de-repressed in interspecific hybrids [28]. A link to rasiRNA production was suggested in a D. virilis hybrid dysgenesis system (marked by abnormal gonadal development), where maternal expression of small
RNAs derived from the Penelope retrotransposon correlates with suppression of dysgenesis [29]. Extensive sequencing of rasiRNAs in D. melanogaster has recently led to the suggestion that RNAi-mediated silencing may regulate many different TE families [22]. The aforementioned Arabidopsis study reported additional defects in imprinting in hybrids, and the authors suggest that misregulation of chromatin states may be a general cause of hybrid lethality [28]. Mechanistic details of how RNAi contributes to gene silencing and heterochromatin establishment and maintenance are currently the topic of much exciting research (reviewed in [30,31]). This first mechanistic glimpse of a sex-ratio distortion system provided by Tao and colleagues suggests that RNAi-mediated regulation, and its failure, may have important implications for understanding fundamental problems in evolutionary genetics and speciation.

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