Selfish genetic elements that distort Mendelian segregation to favor their own transmission are common in eukaryotic genomes [1,2]. Segregation distortion can reduce whole organism fitness, resulting in strong counter selection for genes that suppress distorters. Such intragenomic conflicts have the potential to drive recurrent bouts of antagonistic co-evolution [3]. Theory predicts that genetic conflicts should be particularly intense between the sex chromosomes [4,5]. The expectation that sex-linked conflict should be rampant has led to a renewed emphasis on the importance of antagonistic co-evolution for driving genome evolution [6]. However, while numerous examples of genes involved in intragenomic conflict now exist [1], evidence for antagonistic co-evolution between the mammalian X and Y chromosomes has remained elusive.

In this issue of *PLOS Genetics*, Cocquet et al. have demonstrated a genetic basis for X-Y conflict acting during a crucial stage of mouse spermatogenesis [7]. The sex chromosomes are silenced via chromatin remodeling during the initiation of meiosis (meiotic sex chromosome inactivation or MSCI) [8]. Gene silencing persists through the remainder of spermatogenesis (postmeiotic cells and male-biased litters) (Figure 1A). Strikingly, mice deficient for both Sly and Slx/Slxl1 showed a complete rescue of XY expression, male fertility, and sex ratio phenotypes. That is, the genes have antagonistic roles during spermatogenesis: Sly represses XY expression during PMSC and promotes the transmission of the Y, while Slx/Slxl1 activates XY expression and promotes the transmission of the X. The surprising conclusion is that antagonism depends on the relative expression of these genes and not their total abundance.

Several questions remain regarding the mechanistic and genetic bases of distortion. For example, segregation distortion in the Sly-Slx/Slxl1 system appears to be caused by the differential fertilization ability of X- and Y-bearing sperm. Distorter genes often skew transmission through epistatic interactions with one or more responder genes [12]. In this context both Sly and Slx/Slxl1 appear to be distorters acting on one or more responder genes to impair the function of the X- or Y-bearing sperm, respectively [7]. Which raises the question, what are the responders?

Even more interesting are the evolutionary consequences of recurrent sex-linked conflict. If Sly and Slx/Slxl1 were locked in an antagonistic conflict, then we would predict that each would be rapidly evolving on some level. The relevant metric here seems to be gene copy number. Sly and Slx/Slxl1 are recent additions to the mouse genome, appearing within the past 5 million years (Figure 1B).

Why? Is genetic conflict more intense in some species? Or is the antagonistic interaction a consequence of novel functions that have evolved more recently? The *Mus musculus* X is enriched for dozens of other multicycopy gene families expressed primarily in postmeiotic cells, which is thought to be a mechanism for escaping PMSC [14]. This interpretation now appears to be correct, with the added caveat that the entire process may be a side effect of genetic conflict between Sly and Slx/Slxl1. Most of these X-linked amplicons are repressed by Sly during PMSC. Thus, the rapid expansion of Sly—driven by conflict with Slx/Slxl1—may in turn drive compensatory expansion of other sex-linked genes in order to maintain proper expression levels [13].

One important consequence of recurrent sex-linked conflict is its potential to drive speciation [6]. Several of the mice presented in Figure 1B can hybridize, often resulting in hybrid male sterility (HMS). In particular, some reciprocal crosses between *M. m. musculus* and *M. m. domesticus* yield asymmetric HMS; males are only sterile when a *M. m. musculus* female is crossed with an *M. m. domesticus* male. Moreover, sterile males show widespread over-expression of the X, presumably due to a failure of MSCI and/or PMSC [15].

To test for genetic conflict between these genes, Cocquet et al. generated transgenic mice expressing short hairpin RNA (shRNA) that knockdown Sly or Slx/Slxl1 transcript levels without completely knocking out gene function [7]. Both Sly- and Slx/Slxl1-deficient mice showed unpaired spermatogenesis, but Slx/Slxl1 deficiency led to a slight reduction in sex-linked gene expression in postmeiotic cells and male-biased litters (Figure 1A). Strikingly, mice deficient for both Sly and Slx/Slxl1 showed a complete rescue of XY expression, male fertility, and sex ratio phenotypes. That is, the genes have antagonistic roles during spermatogenesis: Sly represses XY expression during PMSC and promotes the transmission of the Y, while Slx/Slxl1 activates XY expression and promotes the transmission of the X. The surprising conclusion is that antagonism depends on the relative expression of these genes and not their total abundance.

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Since that time they have rapidly expanded in some, but not all, lineages [13]. Why? Is genetic conflict more intense in some species? Or is the antagonistic interaction a consequence of novel functions that have evolved more recently? The *Mus musculus* X is enriched for dozens of other multicycopy gene families expressed primarily in postmeiotic cells, which is thought to be a mechanism for escaping PMSC [14]. This interpretation now appears to be correct, with the added caveat that the entire process may be a side effect of genetic conflict between Sly and Slx/Slxl1. Most of these X-linked amplicons are repressed by Sly during PMSC. Thus, the rapid expansion of Sly—driven by conflict with Slx/Slxl1—may in turn drive compensatory expansion of other sex-linked genes in order to maintain proper expression levels [13].

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**Figure 1. The interaction and evolution of Sly and Slx/Slxl1.**

(A) A summary of the results from the various deficiency models generated by Cocquet et al. [7]. X and Y chromosome genotypes are given along the margin with wild-type genotypes in green and deficiency genotypes in red (shSLX and shSLY). Two transgenic constructs were made to target Slx/Slxl1 but are presented together here for clarity. For each genotype, the

| XY Genotype            | Postmeiotic XY Expression       | Male Fertility     | Sex Ratio               |
|------------------------|--------------------------------|--------------------|-------------------------|
| Normal                 | normal (PMSC repression)        | normal             | normal                  |
| S/l deficient           | disrupted (XY over-expression)  | disrupted          | female-biased (8% excess) |
| S/lx deficient          | normal* (minor increase in PMSC repression) | disrupted          | male-biased (11% excess) |
| S/lx / S/ly deficient   | normal (PMSC repression)        | normal             | normal                  |

(B) Evolutionary tree showing the origin of Sly and Slx:

- **M. m. musculus**: 100/80
- **M. m. castaneus**: 60/40
- **M. m. domesticus**: 50/50
- **M. spicilegus**: 9/10
- **M. macedonicus**: ?
- **M. spretus**: 6/40
- **M. caroli**: 30/0
- **M. pahari**: 0/0
- **Rat**: 0/0

The numbers represent the number of sequences with and without the specific gene.
Slxl1 may be the cause of this HMS because copy number differences between the subspecies will yield hybrid males that are Sly deficient [7]. While this model is intriguing, it must be considered in light of recent work showing that HMS between M. m. musculus and M. m. domesticus is genetically complex and not strongly dependent on the origin of Y [16], and that other genetic interactions causing HMS also disrupt XY gene expression [17]. Nonetheless, these data do not exclude an important contribution of Sly/Slxl1 mismatch to HMS in this or any other mouse hybrid crosses. If true, this would provide the first direct evidence that sex-linked genetic conflict can drive mammalian speciation.

Finally, the finding that a few novel genes control epigenetic regulation of a key step in spermatogenesis is quite remarkable. The basic epigenetic processes underlying PMSC appear to be conserved within mammals, yet its genetic regulation has only been elucidated in mice [10]. These insights are exciting, but are tempered by the fact that the key genes regulating PMSC in mice do not exist in the vast majority of mammals. The human X and Y show similar patterns of PMSC repression, including escape from silencing of several single and multicopy genes [18]. However, fewer than 20% of these genes are shared with mouse. Collectively, these findings illustrate the power of evolution to generate novelty in the face of developmental constraint and call into question the notion that research on a few model systems will be sufficient to elucidate the general molecular underpinnings of reproduction. When it comes to the evolution of reproduction and the sex chromosomes, exceptions may prove to be the rule.

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