Battle of the Xs

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Summary

Females and males often exhibit conspicuous morphological, physiological and behavioral differences. Similarly, gene expression profiles indicate that a large portion of the genome is sex-differentially deployed, particularly in the germ line. Because males and females are so fundamentally different, each sex is likely to have a different optimal gene expression profile that is never fully achieved in either sex because of antagonistic selection in females versus males. Males are hemizygous for the X chromosome, which means that recessive male-favorable de novo mutations on the X chromosome are subject to immediate selection. In females, a recessive female-favorable mutation on one of two X chromosomes is not available for selection until it becomes frequent enough in the local population to result in homozygous individuals. Given that most mutations are recessive, one would expect that genes or alleles favoring males should accumulate on the X chromosome. Recent microarray work in *Drosophila* and *C. elegans* clearly shows the opposite. Why is the X chromosome a highly disfavored location for genes with male-biased expression in these animals? *BioEssays* 26:543–548, 2004.

Sexually antagonistic selection

Females and males share nearly identical genetic information, but differ in the utilization of that information. Thus, sexual differentiation is much like any other developmental event except that it results in distinct whole organisms. A gene expression network is a central underlying component of essentially all differentiation events[1] and, while it is not yet clear how many different configurations are possible, it is logical to assume that all these configurations are suboptimal for the desired output. For example, a gene used in both liver and brain development is something of a compromise, as the optimal amino acid sequence for liver is unlikely to be optimal for the brain. When applied to sexual dimorphism, this idea of a compromise genome has profound implications for evolution and has led to the concept and experimental verification of sexually antagonistic selection by Rice and colleagues.[2,3] Briefly, in the pool of alleles in a population, some will favor females and some males. In a population with a sex ratio of one, selection in females and males is a balanced tug-of-war resulting in suboptimal genomes for both sexes. Escape from this stalemate is sex-specific function driven by changes in the regulation of the gene in question. For example, a gene with female-specific function is selected for in females and neutral in males, while a gene expressed to some degree in both is available for selection in both.

Testing multiple genotypes for survival and reproductive success has shown that there is extensive sexual antagonism in adult *Drosophila*. Genomes associated with good female fitness are usually associated with poor male fitness and vice versa.[4] While the effect is large, it is not clear how many genes contribute to this antagonism. Recent global gene expression studies suggest that there may be many contributing loci. Transcription is a good metric for sex-biased function, and recent gene expression profiles of the sexes using ESTs[5,6] and microarrays show that there are substantial numbers of genes with sex-biased expression in *Drosophila*[5,7–11] and *C. elegans*.[12–14] How many genes show sex-biased expression? While this is an elementary question, directly comparing studies from different laboratories is more daunting than might be imagined. For example, the elements on the array introduce design-bias, as some array platforms are based on spotted cDNAs none of which are derived from testis[7,8,10,11] or all of which are derived from testis.[5] Other arrays are based on gene predictions[9] but the assumption that gene prediction programs are equally good at finding genes expressed preferentially in females or males is unproved. Somewhat remarkably, despite these and other difficulties in distilling various array studies, conservative estimates from global gene profile studies indicate that ~15% of the genome shows sex-biased expression in adults based on a two-fold magnitude of effect, regardless of array platform or organism. Despite the apparent consensus, this is likely to be an underestimate. For example, dissection of gonads enriches for many of the genes with sex-biased expression and raised the *Drosophila* sex-biased number from ~15 to ~35% of the genome in one study.[9] When significant differences (including those with a lesser magnitude) are included, up to ~50% of the genome may show sex-biased transcript levels.[7,8,10]

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Selection acts on existing genetic variation within a population, and there is clear evidence that much of the sex-biased expression in *Drosophila* is polymorphic. Males within and between *Drosophila* species appear to be especially polymorphic in terms of male-biased gene expression.[7,8] In hybrids, many of the genes with male-biased expression are mis-regulated (being usually downregulated), indicating that both the genes per se and the regulatory network that controls them in males evolve rapidly.[15] Thus, there is great deal of sex-biased expression and a great variety of sex-biased expression patterns.

The nature of the sexually antagonistic selection model suggests different outcomes for sex chromosomes and autosomes.[2–4,16,17] The Y chromosome passes from fathers to sons and is thus subject to selection only in males. Y chromosomes tend to lose genes and maintain a few genes required only in males. This is well illustrated by the recent sequencing of the human Y chromosome.[18] The X chromosome is less well studied, but should also be different from an autosome.[2,3,16] Whether an X chromosome loses genes favorable to males or gains them depends on whether those genes are dominant or recessive. A dominant gene will be exposed to selection immediately in both males and females, but will also be subjected to more rounds of selection in females because two thirds of X chromosomes are in females. This would result in decreased numbers of genes with male-biased function on the X chromosome. Most mutant alleles are recessive. Because new alleles are initially quite rare in a large population, a recessive female-favorable allele, detrimental to males, rarely becomes common enough to be selected. In contrast, X chromosome alleles detrimental to females and advantageous to males can accumulate because of the immediate availability of hemizygous alleles for selection. Thus the expectation has been that X chromosomes should accumulate genes with male-biased function.

Gene expression profiles generated by EST and microarray studies are particularly well suited to seeing how different the X chromosome might really be. Mammalian spermatocytes show the expected enrichment of X-chromosome genes highly expressed in those cells (in other words a masculinized X chromosome).[19] Surprisingly in two species of *Drosophila* and in *C. elegans*, rather than being masculinized (as is expected based on immediate selection in hemizygous males), there are fewer than expected numbers of X chromosome genes with male-biased expression (which we have termed demasculinized) and evidence for some degree of X-chromosome enrichment for genes preferentially expressed in females (feminization). After first outlining the unexpected observations in the invertebrates (also reviewed by Rogers et al[20]), we discuss how the superficially contradictory results from mammals and invertebrates might fit into current theory. The nature of sex chromosome evolution, a higher degree of dominance among sexually antagonistic alleles, and X-inaction in the male germline may all figure in the equation. The observations in mammals and invertebrates need not be due to a different balance of power in the battle of the Xs, although that is certainly one possibility.

Genes with male-biased expression are under-represented on the X chromosome

Sex chromosomes are phylogenetically widespread and are believed to be diverged from an ancestral autosome.[21] In diploid *Drosophila* or *C. elegans*, zygotes with two X chromosomes are female or hermaphrodite (essentially females that produce a few sperm), while those with a single X chromosome are male (reviewed by Cline and Meyer[22]). Y chromosomes of *Drosophila* have only a few genes, and *C. elegans* is Y-less. The complete loss of the Y chromosome can be interpreted as the ultimate divergence, and might suggest that the *C. elegans* X chromosome is especially ancient. X-chromosome dosage compensation occurs in the soma of both organisms to equilibrate X-chromosome and autosome transcript levels, but compensation mechanisms are different in the species. *C. elegans* hermaphrodites downregulate expression of both X chromosomes, while *Drosophila* males upregulate the single X chromosome. Although none of the known dosage compensation genes in either organism regulate dosage compensation in the germline, direct evidence from gene expression profiles suggests that *Drosophila* germ cells do show dosage compensation.[9] The mechanism of germline dosage compensation is unknown.

The evolution of X chromosomes is not as well studied as that of the Y chromosomes, but the X clearly differs from autosomes. There is experimental evidence indicating that most of the genetic variation for sexually antagonistic function in *Drosophila* is on the X chromosome,[16] despite the fact that the over-all sequence polymorphism is lower on this chromosome.[23] This, in turn, suggests that the sequence polymorphism of a subset of the genes is quite large or that there are *trans*-effects. Where are the genes with sex-biased expression? Are these the same as those showing fitness variation? There are a number of studies suggesting that genes with a function in reproduction are enriched on the X chromosome, although these studies have not been systematic.[24,25] Global expression studies and some retrospective genetic analysis contradict the idea that male reproductive function are enriched on the X.

There is very clear microarray evidence from two species of *Drosophila* [7–9] and *C. elegans,* [12–14] as well as EST data for *Drosophila*[5,8,9] showing that the X chromosome has a significantly reduced number of genes with male-biased expression. Only a cDNA study of mammalian primary spermatocytes shows the expected enrichment for genes with male-biased expression on the X chromosome,[19] and this appears to be explained by transcription in advance of precocious X inactivation in the male germline.[26] Interest-

ingly, there is some indication that there are significantly more than the expected number of genes with female-biased expression,[7,8,27] suggesting that genes with female-biased expression might be enriched on the X chromosome (Fig. 1). The depletion of genes with male-biased expression is independent of tissue in *Drosophila*.[5,6,9]

Expression is not function. However, there is genetic evidence that male-biased functions are under-represented on the X chromosome in *Drosophila*. Male-sterile mutations on the X chromosome are more likely to also lower viability and female fertility, suggesting that there are few strictly male-functioning genes on the X.[28] Similarly, the reduction in the number genes with male-biased expression on the ancient X chromosome of *C. elegans* is especially pronounced[13,14] and RNAi functional studies point to a strong depletion of vital functions from that chromosome.[29] Because it is quite reasonable to use expression as a metric for probing sex-biased function, the results suggest that the demasculinization and/or feminization of the X chromosome is not strictly a consequence of some of the unusual tissue-specific features of the germline (e.g. different dosage compensation mechanisms and male X-inactivation).

It appears that the reduced number of X chromosome genes with male-biased expression is due to loss over time. It has been evident for many years that primary and secondary sexual characteristics evolve rapidly.[25] Part of this change over time is due to alteration in coding sequences and some is due to changes in gene expression. More than 200 million years separate *Drosophila* and *Anopheles* and is a strong negative correlation between male-biased expression in *Drosophila* germ cells and gene conservation, especially for X chromosome genes.[9] Morphological divergence does not require such a dramatic genome change (as illustrated by the high genomic similarity of humans and chimpanzees). In contrast, there are very impressive gene expression differences even between species that are quite similar in appearance and between strains of the same species. X-chromosome genes with male bias also show accelerated expression-level changes within these short time frames.[7,8] Thus, the more dynamic changes in male-biased expression from the X chromosome is visible by back extrapolation and in extant populations.

Briefly, demasculinization of the X chromosome is tissue independent and appears to be due to a net loss of these gene sequences from the X-chromosome, or a change in their expression bias. Different types of gene-expression assays and genetic analysis in at least three species (*C. elegans*, *Drosophila melanogaster* and *Drosophila simulans*) support this idea. Understanding what these observations mean will require some re-thinking and further experimentation.[20] In that spirit, we present a few ideas and associated problems in the following pages.

Dose, dosage compensation and inactivation

Demasculinization could be a simple dosage effect where expression of genes with male-biased expression is half of the level of similar autosomal genes with male-biased expression because of hemizygosity. This is highly unlikely because of dosage compensation (reviewed by Cline and Meyer[22]). Direct analysis of global gene expression in *Drosophila* shows

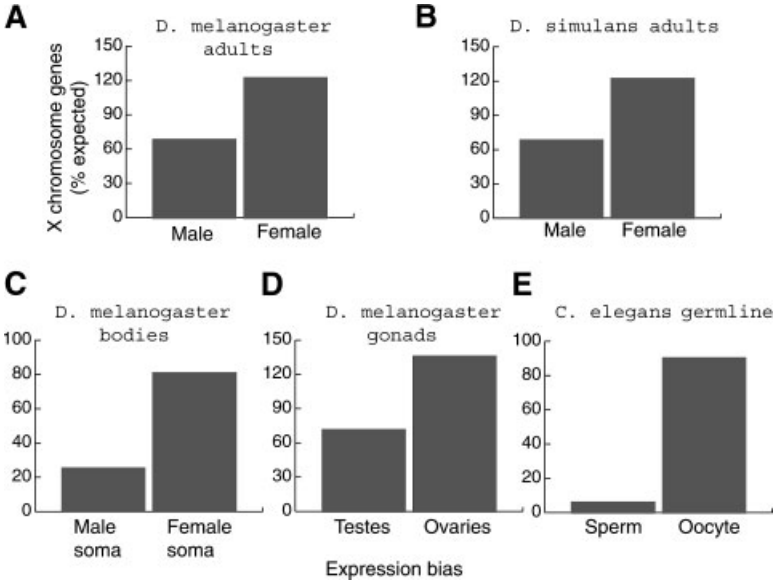

Figure 1. X-chromosome genes with male-biased expression are found at lower than expected frequencies in multiple microarray experiments. Graphs show the percentage of genes found against expected based on a random distribution among the chromosomes. Columns show the sex-biased class. These are examples. See text for a more extensive treatise. **A:** *D. melanogaster* adults. **B:** *D. simulans* adults. A,B: Assayed on printed cDNA platform.[8] **C,D:** *D. melanogaster* adult carcasses (bodies with gonads removed).[9] **D:** *D. melanogaster* adult gonads.[9] **E:** *elegans* genes with sperm- or oocyte-biased (from animals with genetically deleted germlines.[13]

no evidence for large-scale escape from dosage compensation in either the soma or germline.[9] Additionally, there are also fewer numbers of X chromosome genes with male-biased expression when XX females genetically transformed into males are compared to normal XX females, indicating that simply decreasing the dosage of X chromosomes does not result in an artifactual decrease in the number of genes with male-biased expression (M.P. and B.O., unpublished).

However, there are genes on the X chromosome that escape dosage compensation and a new gene on the X might not be immediately dosage compensated.[30,31] This initial disadvantage might result in a loss of male-specific functions. For example, male-biased genes on a translocation to the X from an autosome would initially be functionally haploid and would be likely to be under-represented in a gene expression profile. In this situation, autosomal genes encoding overlapping functions might gradually usurp the male-biased functions originally encoded on the new segment of the X chromosome. This would ultimately result in loss of genes with male-biased function from the X. If demasculinization depends on these initial conditions, then the process would be expected to be slow in mature X chromosomes; however, male-biased gene expression is highly polymorphic within *Drosophila melanogaster*, suggesting ongoing rapid evolution of the X chromosome.

A mechanistic focus for the observed under-representation of X-chromosome genes with male-biased expression in *C. elegans* has concentrated on X inactivation in the male germline. X-chromosome condensation occurs during male gametogenesis in many species (reviewed by Wu and Xu[28]). Certainly, the precocious inactivation of the X chromosome could account for the paucity of spermatocyte transcripts from the germline X chromosome. Histone modifications specific to the X chromosome in the germline are consistent with shutting down the X.[12,32] Additionally, a systematic evaluation of gene function by RNAi shows that genes on the X chromosome are less likely than autosomal genes to have essential functions, suggesting the X and autosomes of *C. elegans* have evolved a segregation of genes by class, perhaps to escape cell lethal consequences of male X inactivation in the germline.[29] Indeed, X inactivation might even explain the apparent contradiction between the gene expression profile data from *Drosophila* and *C. elegans* and a study of 25 genes specifically expressed in the primary spermatocytes of mammals, where it was found that the X chromosome accumulates male-biased genes rather than shedding them.[33] Wu and Xu[28] have suggested that X inactivation promotes the precocious expression of X chromosome genes required in spermatogenesis so that those transcripts will be available when they are needed later, after the X chromosome is silenced. In support of this argument, none of 26 genes acting in late mammalian spermatogenesis are found on the X-chromosome.[34] Additionally, *Drosophila* genetics supports both of these observations, as 60% of X-chromosome versus 21% of autosomal male-sterile alleles affect early (premeiotic) stages of spermatogenesis.[28] Thus, even though there is net demasculinization in *Drosophila*, it appears that the X-chromosome is relatively rich in genes encoding early spermatogenesis functions, as in mammals. Because there are so many more late differentiation functions net demasculinization could be explained by loss of late spermatogenesis genes from the X chromosome.

As attractive as the X-inactivation model is, it has some serious problems. Most prominently, X inactivation fails to explain the dramatic reduction in the number of X chromosome genes showing male-biased expression in the *Drosophila* soma, where X inactivation does not occur.[6,9] It will be interesting to see if the reduction in male-biased expression of X chromosome genes in somatic tissues extends to other species. The X-inactivation model is a variant of the chicken or the egg problem—is the pressure to inactivate the X chromosomes strong enough to account for demasculinization, or does pre-clearing of genes with male-biased expression from the X-chromosome allow X inactivation? X inactivation would tend to accelerate the demasculinization process for male-biased genes in the germline and demasculinization would facilitate inactivation. They could be reinforcing pressures.

Sexually antagonistic selection

Sexually antagonistic selection predicts that genes with male-biased function should accumulate on the X chromosome based on two assumptions.[3] First, that there is immediate selection in males because of hemizygosity; second that most mutations subject to selection are recessive. Questioning these assumptions, rather than the theory, leads to another set of models for demasculinization of the X chromosome.

It is widely believed that the X and Y chromosomes are derived from an autosome pair that diverged due to the presence of a dominant sex-determining gene (reviewed by Charlesworth[21]). If this is the case, then individual genes on the X chromosome had a corresponding Y chromosome allele at some point in their history and were therefore not hemizygous. Wu and Xu[28] recently outlined some of the very interesting consequences of the pseudoautosomal nature of a young set of sex chromosomes. The exclusive father-to-son transmission of the Y strongly favors alleles benefiting males. Therefore, a mutation destroying the function of the X-chromosome copy is likely to be more than simply masked by the functional Y chromosome allele. If there is an allele of this X chromosome locus that benefits females, it can therefore move towards fixation even if it is detrimental to males, because two-thirds of the X chromosomes reside in females and the males have a Y-linked copy. It is far more likely that a de novo mutation will destroy a gene than make an improvement, so this model is consistent with the strong demasculinization and weaker feminization of the X chromo-

some observed in *Drosophila* and *C. elegans*. Implicit in this model, is a declining loss-rate of male-biased genes from the X chromosome as the Y-chromosome loses genes. This might be testable in species with young versus ancient sex chromosomes. However, different strains of *Drosophila melanogaster* show no evidence of an evolutionary quiescent X chromosome,[8] consistent with an ongoing process. Pseudoautosomal sex chromosomes cannot be the full answer.

However, there are other ways for males to be functionally diploid for X-chromosome genes. Both mammals and *Drosophila* show preferential movement of X chromosome genes to autosomes via retrotransposition.[28,34] Indeed, retrotransposition from the X chromosome is twice as frequent as expected.[34] Additionally, in both mammals and *Drosophila* expression of most of the autosomal copies occurs in the testis (91% of them in *Drosophila*), while the X-chromosome copies are not expressed preferentially in males. The transposed copy would be good fodder for positive selection in males, due to the high expression in the testis, and again would mask the recessive X-chromosome gene in otherwise hemizygous males. The fact that two-thirds of X chromosomes are in females would promote demasculinization or feminization of the original X chromosome copy. This model has a great deal of appeal because we can see it happening today. It would be quite interesting to see if X-linked versions of retrotransposed genes also show higher polymorphic expression.

As outlined by Rice,[3] whether or not a sexually antagonistic allele spreads through a population depends dramatically on the degree of dominance. A fully recessive male-advantage allele can spread in the population even if it is lethal to homozygous females, but it is also true that a fully dominant female-advantage allele can spread rapidly. Differences of as little as 10% in the index of dominance are consequential. Alleles available for selection in a population start as unique de novo mutations. Most de novo mutations in a gene that alter the coding sequence are deleterious loss-of-function alleles (having no function or having reduced function). Most of these are recessive. For example, in a full genome screen of *Drosophila*, employing aneuploid strains, only a handful of genes were found to be required in two copies.[35] Therefore, in a reasonably large out-bred population, new alleles on paired X chromosomes in females and on autosomes in both sexes, must undergo expansion within the population before they become frequent enough to become available for selection as homozygotes (assuming that it does not become extinct due to random drift).

To what degree are sex-biased genes dominant? Even though most individual mutant alleles appear to be completely recessive, it is also true that heterozygous deletions of multiple genes (>a few percent of the genome) are invariably lethal.[35] Therefore, it seems reasonable to assume that many mutations have a subtle dominant phenotype—indeed this is the basis for many very successful screens for genetic modifiers in *Drosophila*.[36] Leaky expression of genes with male-biased expression might result in a deleterious effect on females and could therefore be under strong selection for reduced expression. Loss-of-function alleles favoring females due to haploinsufficiency would be demasculinizing.

Sexual antagonism can be resolved by divergence of new alleles, or genes, such that they function exclusively in the sex that they benefit (reviewed by Rice and Chippindale[2]). If demasculinization is due to female selection against genes with male-biased expression, then a gene that is outright male-specific should be immune. It is therefore quite surprising that genes with a high degree of male-bias appear to be under the most intense pressure.[7–9] Perhaps truly sex-specific gene expression is very rare. It has been suggested that generating a absolute off-state for any gene is quite difficult[37] and, in our array work, we almost never encounter genes highly expressed in one sex and not at all in the other.[9] Leaky expression might set-up an interesting, but ultimately unbalanced set of selection pressures. For example, if a partial loss of gene function occurred due to antagonistic selection in females, then counter-selection in males may be responsible for boosting the expression of that partially functional allele and simultaneously boosting the negative selection pressure in females. It is clear that changes in sex-biased expression occur more readily than coding sequence change and over very short timeframes.[7,8] Compensation for poor function via increased levels would be immediately selected for in males. Increasing expression of genes with male-biased expression might be a rapid, but ultimately a temporary and futile male response to negative selection in females. Selection for increased expression of such dominant advantage genes for males on autosomes would be less susceptible to counter selection in females due to balanced selection. If this idea is valid, then X chromosome genes showing highly male-biased expression should encode proteins with lower specific activity. This might be detectable among strains of *Drosophila* showing polymorphic expression of these genes.

Conclusions

We are still in the initial phase of the global study of sexual dimorphism, but already we have gained some important insights. Expression analysis in invertebrates has uncovered a surprising difference between the X chromosome and the autosomes, which is best summarized by the concept of demasculinization. Peculiar features of dosage compensation, X inactivation in males and sexually antagonistic selection are possible culprits, but there is much work to be done. It will be interesting to see if testing various hypotheses uncovers which, if any, of these mechanisms is a root cause. It will be particularly interesting to see what the global expression patterns tell us about the locations of mammalian genes with sex-biased expression, as there are lingering questions about the universality of X chromosome demasculinization.

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