The Potential Role of Sexual Conflict and Sexual Selection in Shaping the Genomic Distribution of Mito-nuclear Genes

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Abstract

Mitochondrial interactions with the nuclear genome represent one of life's most important co-evolved mutualisms. In many organisms, mitochondria are maternally inherited, and in these cases, co-transmission between the mitochondrial and nuclear genes differs across different parts of the nuclear genome, with genes on the X chromosome having two-third probability of co-transmission, compared with one-half for genes on autosomes. These asymmetrical inheritance patterns of mitochondria and different parts of the nuclear genome have the potential to put certain gene combinations in inter-genomic co-adaptation or conflict. Previous work in mammals found strong evidence that the X chromosome has a dearth of genes that interact with the mitochondria (mito-nuclear genes), suggesting that inter-genomic conflict might drive genes off the X onto the autosomes for their male-beneficial effects. Here, we developed this idea to test coadaptation and conflict between mito-nuclear gene combinations across phylogenetically independent sex chromosomes on a far broader scale. We found that, in addition to therian mammals, only *Caenorhabditis elegans* showed an under-representation of mito-nuclear genes on the sex chromosomes. The remaining species studied showed no overall bias in their distribution of mito-nuclear genes. We discuss possible factors other than inter-genomic conflict that might drive the genomic distribution of mito-nuclear genes.

Key words: X chromosome, Z chromosome, sexual conflict, Haldane's sieve, OXPHOS.

Introduction

The eukaryotic cell contains two distinct genomes—the nuclear and the mitochondrial—whose coordinated interactions over billions of years now represent one of life's most important co-evolved mutualisms (Gillham 1994). Many gene products are encoded in the nucleus and exported to the mitochondria, where they interact with other, mitochondrially encoded, genes. Organismal fitness depends upon compatibility between nuclear and mitochondrial gene products (Meiklejohn et al. 2013), and these interactions (hereafter "mito-nuclear") are fundamental to eukaryotic existence and underlie key life history traits, including somatic maintenance, reproductive performance, and aging (Rand et al. 2004; Dowling et al. 2008).

However, because mitochondria are often maternally inherited, selection acting on these mito-nuclear interactions is asymmetrical in males and females. Mutations detrimental to males are not selected against unless they are also detrimental to females, except in some cases involving nonrandom mating, sperm limitation, or paternal mitochondrial transmission (e.g., Rand et al. 2001; Wade and Brandvain 2009;

Unckless and Herren 2009; Hedrick 2011; Zhang et al 2012). In extreme cases, mitochondrial mutations that harm males can even be selected for if they benefit females. This results in a male mutational load, where mutations detrimental to males are not purged from populations and accumulate across generations (Frank and Hurst 1996; Gemmell et al 2004). This male mutational load can be detected in the form of male-biased gene mis-expression (Innocenti et al. 2011), reduction in male lifespan (Camus et al. 2012), and male fertility (Smith et al. 2010; Yee et al. 2013) in individuals that contain mitochondria from different populations.

Maternal inheritance of mitochondria puts mitochondrial genes in contrasting evolutionary dynamics with different parts of the nuclear genome: whereas Y chromosomes have strict paternal transmission, autosomes are equally transmitted through males and females, and X chromosomes spend twice their time in females compared with males. This sexual asymmetry across the genome might set the scene for intergenomic coadaptation or conflict. On the one hand, we expect beneficial gene combinations to be facilitated if genes that interact with the mitochondria are on the X chromosome. The X

chromosomes in mammals and Drosophila have been shown to be feminized for gene expression (Khil et al. 2004; Meisel et al. 2012), and X-linked genes are co-transmitted with mitochondrial genes through the female two-third of the time. Under such a scenario—with inter-genomic co-adaptation driving the distribution of genes that interact with mitochondria—we might expect an over-representation of mito-nuclear genes on the X (Rand et al. 2001; Wade and Goodnight 2006; Brandvain and Wade 2009). On the other hand, the accumulation of mutations that are detrimental to males, referred to as male-biased mitochondrial mutational load, might be ameliorated if genes that interact with the mitochondria move off the X, onto parts of the genome with equal (or even male-biased) transmission. If conflict drives the distribution of mito-nuclear genes, we would expect an under-representation of genes that interact with the mitochondria on the X chromosome (Rice 1984; Werren 2011; Drown et al. 2012).

We might also expect converse patterns for Z chromosomes in female-heterogametic (ZW ZZ) species. ZW systems often show reverse patterns for sexual conflict scenarios because the Z is masculinized (Wright et al 2012) while the X is feminized for gene expression. This potentially results in an under-representation of mito-nuclear genes on the Z chromosome because mitochondria are co-transmitted with Z chromosomes only one-third of the time. Alternatively, because the Z and mitochondria can never be transmitted through males, it is possible that there is no expected bias on Z chromosomes with regard to mito-nuclear genes (Drown et al 2012). Finally, it has also been suggested that the Z chromosome might be enriched for mito-nuclear genes due to some types of sexual selection in males (Hill and Johnson 2013).

These predictions for the distribution of mitonuclear genes are predominantly based on probabilities of co-inheritance of mitochondria with different parts of the nuclear genome and do not take into account more complex processes such as linkage patterns of genes interacting with mitochondria. Empirical evidence for mito-sex chromosome interactions is not consistent. Some experimental evidence suggests genes on the X chromosome interact with mitochondrial genomes in Drosophila (Rand et al. 2001), whereas other assessments failed to detect mito-autosomal interactions (Clark 1985; Clark and Lyckegaard 1988). Consistent with the predictions of inter-genomic conflict, a strong under-representation of mitochondrial genes on the X chromosome was found across a range of mammal species (Drown et al. 2012). However, the data set used by Drown et al. (2012) is phylogenetically non-independent, as the X chromosomes in the therian mammals derived from the same common ancestor and show strong conservation of gene content across the clade (Veyrunes et al. 2008). Therefore, the broader generality of the dearth of mitochondrial genes on the X remains largely unexplored.

Here, we test the universality of predictions of mito-nuclear co-adaptation and conflict by exploring the genomic distribution of genes that interact with the mitochondrial genome.

We extend previous studies by exploring these interactions on a broad scale, incorporating multiple examples of male- and female- heterogamety in species with independent origins of their sex chromosomes.

Materials and Methods

Detection and Localization of Genes Interacting with Mitochondria

In order to expand our analysis to species with less complete genome annotations, we modified the protocol from Drown et al. (2012) to compare the chromosomal distribution of genes that interact with the mitochondria across a range of species with phylogenetically independent sex chromosomes. In the first step, we obtained the proteomes for the several therian mammals (*Bos taurus, Pan troglodytes, Canis familiaris, Gorilla gorilla, Homo sapiens, Macaca mulatta, Equus caballus, Oryctolagus cuniculus, Pongo abelii, Rattus norvegicus, Sus scrofa*, and *Monodelphis domestica*), the monotreme *Ornithorhynchus anatinus*, three birds (*Gallus gallus, Meleagris gallopavo,* and *Taeniopygia guttata*), the stickleback fish *Gasterosteus aculeatus, Drosophila melanogaster,* and *Caenorhabditis elegans* from Ensembl v71 (Flicek et al. 2013). In order to increase the number of independently-evolved sex chromosomes, we also obtained the proteomes for *Tribolium castaneum, Bombyx mori,* and *Schistosoma mansoni* from Ensembl Metazoa v18 (Kersey et al. 2012).

Because genome and gene ontology (GO) annotation quality varies across our species, we used a reciprocal best BLAST hit (rBBH) approach to find one-to-one orthologs between the well-annotated *Mus musculus* mito–nuclear genes and the other species using the catalog of genes with mitochondrial annotation (mito-nuclear genes) in the GO (Ashburner et al. 2000) ID 0005739 for *M. musculus* using Biomart (Durinck et al. 2005) from Ensembl v71 (Flicek et al. 2013). This approach relies on the high level of conservation of mitochondrial gene function (Jafari et al. 2013; Lotz et al. 2014). To verify that rBBH is appropriate for mito–nuclear genes, we compared the list of genes obtained through rBBH with the list of mitochondrially annotated genes using GO term GO:0005739 in Biomart for *D. melanogaster* and *C. elegans*—two species with more complete gene annotation. We found that out of the 522 *D. melanogaster* GO:0005739 genes, 66% (345/522) were also identified as mito-nuclear by the rBBH. Of the 251 *C. elegans* GO:0005739 genes, only 7% (18/251) were identified through the rBBH. This suggests that, while rBBH is useful for detecting mito-nuclear orthologs (comparable with computational annotation of GO terms), our approach may miss or incorrectly classify some of the mito-nuclear genes across distantly related species.

In order to account for clade-specific differences, we conducted two further analyses. First, we repeated the rBBH analysis, using Biomart to identify mito-nuclear

GO:0005739 genes for *D. melanogaster* and *C. elegans* in addition to *M. musculus*. Because these are relatively well annotated genomes, we used them as clade-specific reference species in order to reduce taxonomic distance. Therefore, we used 1) *M. musculus* mito-nuclear genes as the reference for other vertebrates (Theria, *O. anatinus*, *G. aculeatus*, and Aves), 2) *D. melanogaster* mito-nuclear genes as the reference set for other insects (*T. castaneum* and *B. mori*), and 3) *C. elegans* mito-nuclear genes for the entozoans (with *S. mansoni*). Second, we also present results using just Biomart GO term annotations for those species where gene products have been annotated.

For the rBBH analysis, we used the longest protein isoform and only considered hits when the BLASTP (Altschul et al. 1997) e-value was below 10^{-7}. In the second rBBH analysis, also using *D. melanogaster* and *C. elegans* as reference points, we used a more stringent e-value threshold of 10^{-10}; hits were then ordered by bitscore, and an rBBH was accepted only when the best hit had a sequence identity larger than 30%. After the rBBH analyses, we determined the chromosomal location for mouse mito-nuclear orthologs in each species. The *S. mansoni* locations are based on Vicoso and Bachtrog (2011), *B. mori* positions were extracted from KAIKObase version 3.2.1 (Shimomura et al. 2009), *T. castaneum* are based on Ensembl Metazoa v18 (Kersey et al. 2012), and all other locations are based on Ensembl v71 (Durinck et al. 2005).

As a result, we created three lists of nuclear genes with mitochondrial annotation and their chromosomal locations: 1) using direct GO annotation (only in *M. musculus*) or based on orthology predictions (all other species), 2) based on direct GO annotation (*M. musculus*, *D. melanogaster* and *C. elegans*) or based on orthology predictions using the closest relative from these three species, and 3) based on direct GO annotation, just for *O. anatinus* and *G. aculeatus* (*S. mansoni*, *T. castaneum* and *B. mori* are not available in Ensembl, and Theria and Aves have previously been reported using this approach by Drown et al. 2012).

Statistical Analysis

In order to avoid problems with phylogenetic non-independence, we combined all species that share the same orthologous sex chromosome into a single data point (i.e., the therian mammals were grouped together, as were the birds). We then compared the density of mito-nuclear genes on the sex chromosomes and the autosomes relative to the expected gene density based on the total number of mitochondrial annotated genes. For *D. melanogaster,* each Muller element (X, 2L, 2R, 3L, 3R, 4) was treated as a separate chromosome. The expected gene count per chromosome was calculated as the total number of mito-nuclear genes multiplied by the proportion of all annotated genes on each chromosome. The bias of mito-nuclear genes was the ratio of the observed number

of mito-nuclear genes on a chromosome to the expected count, where an over-representation is a bias > 1 and an under-representation is a bias < 1. In *G. aculeatus*, we also included the neo-sex chromosome (Kitano et al. 2009; Natri et al. 2013), as well as the *D. melanogaster* ancient-sex chromosome, which displays many properties of an X chromosome (Vicoso and Bachtrog 2013). The only sex-limited sex chromosome with sufficient size and annotation was the *S. mansoni* W, which is also included.

We tested the significance of the over- or under-representation of mitochondrial genes on the sex chromosomes by bootstrapping. To calculate confidence intervals (CIs) for sex chromosome bias, for each species/clade, we sampled with replacement 10,000 times the number of genes on the sex chromosome, summed the number of genes with mitochondrial annotation, calculated bias (as above) and took the 95% CIs of the distribution. To calculate CIs for bias on the autosomes, we sampled with replacement 1,000 times the genes on each of the autosomes (i.e., between 4 and 27 chromosomes, depending upon the clade), calculated bias for each chromosome, calculated the mean bias for each sampling event, and calculated the 95% CIs of the mean (i.e., the CI was calculated from 1,000 samples, and each sample was the mean bias of all chromosomes). For each analysis we corrected for multiple testing for nine different sex chromosomes, at an alpha of 0.05, using Bonferroni correction ($P < 0.0057$). Sex chromosomes had a significant over- or under-representation of mitochondrial genes if the sex chromosome CI did not overlap the CI of the autosomes.

When grouping different species together (the Theria, as well as Aves) or when one species has multiple sex chromosomes (*O. anatinus*), we calculated the CI for sex chromosome bias by summing together all the genes on the sex chromosomes and treating them as one large sex chromosome. When testing the autosomal distribution of the grouped species, sampling with replacement was done from each species such that each species contributed equally to the sampling distribution (i.e., to the 1,000 bootstrapped data points). We tested whether the bias of neo-, ancient-, and sex-limited chromosomes was different from the autosomes by bootstrapping all autosomal genes and excluding the homogametic sex chromosome.

We tested the significance of the overall over- or under-representation of mito-nuclear genes on the sex chromosomes in male- and female-heterogametic systems by bootstrapping 10,000 times the bias for each orthologous sex chromosome (mean bias for those sex chromosomes represented by multiple species) and calculating the 95% CIs for X and Z chromosomes. This slightly different approach to the previous bootstrapping technique enabled each clade to contribute equally to the distribution, irrespective of the size of the sex chromosome.

The significance of over- or under-representations of mito-nuclear genes on the sex chromosomes were also analyzed using χ^2 tests.

Results and Discussion

It has been previously suggested that the paucity of mito-nuclear genes on the therian X chromosome was driven by sexual conflict related to asymmetrical inheritance (Drown et al. 2012). Mito-nuclear genes have been suggested to move off the X onto autosomes due to conflict between the sexes, a process that involves gene duplication, fixation, followed by loss of the sex-chromosome linked parent copy (Gallach et al 2012; Drown et al. 2012). Genes with effects that can ameliorate male-detrimental mitochondrial muta-tions would be selected in males and are more likely to accumulate on autosomes than on female-biased X chro-mosomes. Although some have suggested that there should be a random distribution of mito-nuclear genes on Z chromosomes (Drown et al. 2012), others have pre-dicted an over-representation of mito-nuclear genes on the Z chromosome of female heterogametic species related to sexual selection (Hill and Johnson 2013).

If sexual conflict over asymmetrical inheritance does shape the distribution of mito-nuclear genes, we might expect con-vergent patterns of under-representation across independent X chromosomes (Drown et al. 2012). X chromosomes have in general fewer mito-nuclear genes (i.e., bias < 1) than ex-pected (mean bias = 0.86, CI = 0.72—1.00); however, only two of six independent X chromosomes showed statistically significant under-representations of mito-nuclear genes. The therian mammals exhibit the most extreme distribution of mito-nuclear genes on the X chromosome, with only the *C. elegans* X chromosome showing a significant paucity.

Furthermore, *C. elegans* is a gynodioecios species, with both males and hermaphrodites. The lack of distinct male and female individuals within the species may limit the degree of sexual conflict, as male-harming mutations in mito-nuclear genes would also affect the male function in hermaphrodites. This suggests that sexual conflict may be reduced in this species and may not be the driver of the dis-tribution of mito-nuclear genes. However, it is important to note that gynodioecy is a recently derived trait in the *Caenorhabditis* lineage, and most other species in the genus are fully gonochoristic. This means that any reduction in sexual conflict due to gynodioecy would have been relatively recent.

We also explored the neo-X chromosome in *G. aculeatus* (Kitano et al. 2009; Natri et al. 2013) and the B chromosome in *D. melanogaster*, which has recently been shown to be an ancient sex chromosome that has reverted to an autosome in the *Drosophila* lineage (Vicoso and Bachtrog 2013), in order to test whether recent and past evolutionary history shape cur-rent patterns. Both the *G. aculeatus* X and neo-X showed no significant bias of mito-nuclear genes (tables 1–3). The ancient

X chromosome in *D. melanogaster* also showed no overall bias (tables 1 and 2).

These results across multiple independent X chromosomes suggest that patterns of mito-nuclear gene distribution are not consistently shaped by convergent sexual conflict over asym-metrical inheritance across independent sex chromosome sys-tems. This pattern was consistent across all rBBH approaches (figs. 1 and 2, tables 1 and 2) and species-specific GO anno-tations (fig. 3 and table 3).

Many patterns driven by sexual conflict on X chromosomes are predicted to display converse patterns on Z chromosomes (Rice 1984), and this has been true for genomic characters including the sexualization of gene expression (Dean and Mank 2014). We might therefore also expect convergent over-representation of mito-nuclear genes on Z chromo-somes, although the low co-transmission between the mito-chondria and the Z chromosome may ameliorate this prediction (Drown et al. 2012). Our results indicate that Z chromosomes overall have slightly more mito-nuclear genes (i.e., bias > 1) than expected (mean bias = 1.06, CI = 1.02—1.11), but there was no taxon-specific case where a Z chromosome carried a significantly greater proportion of mito-nuclear genes than expected based on its relative size.

The W chromosome and mitochondria are in complete linkage, being co-transmitted each generation. Consequently, we may expect an over-representation of co-adapted, female-benefitting mito-nuclear genes on the W. Although we do observe some W-linked mito-nuclear genes in *S. mansoni*, suggesting that some genes have sex-specific expression, there is no significant over-representation of these genes on the W chromosome (tables 1 and 2). The lack of bias of mito-nuclear genes on W could be due to lack of selection for gene movement in the female—the mitochondria is already opti-mized for females and so no advantage for the female is gained by movement of Z or autosomal genes onto the W.

It is possible that the genomic distribution of mito-nuclear genes is somewhat confounded by other genomic phenom-ena. First, mitochondrial mutation rate differs substantially across species; for example, mammals tend to have high rates and *Drosophila* have low rates (Montooth and Rand 2008). Mitochondrial mutation rate will affect the extent to which mitochondria can evolve female-beneficial mutations. Second, the relative rate of evolution of sex chromosomes to autosomes (the Faster-X Effect, Charlesworth et al. 1987) varies across species and depends on the relative effective population size of the X compared with the autosomes (Mank et al. 2010). The relative effective population size of different X chromosomes to autosomes varies substantially (Mank et al. 2010 and references therein); however, this does not necessarily explain our data, as, for example, *E. caballus* and *D. melanogaster* both have high relative effec-tive population sizes of the X chromosome (Andolfatto 2001; Connallon 2007; Singh et al. 2007; Lau et al. 2009), and yet *D. melanogaster* shows no overall bias, while *E. caballus*

Table 1

Mean Bias and 95% CIs of Mito-nuclear Genes on the Sex Chromosomes and Autosomes

Species or Clade	Over-/Under-representation of Mito-nuclear Genes on Sex Chromosome (Bias)	95% Bonferroni-Corrected CI of the Sex Chromosome	95% Bonferonni-Corrected CI of the Autosomes	χ^2 Test and P Value
Male heterogamety	0.86	0.72–1.00		
Therian mammals	**Under (mean = 0.64)**	**0.55–0.72**	**0.90–1.13**	**89.5, P < 0.0001**
H. sapiens	0.63			
P. troglodytes	0.69			
G. gorilla	0.62			
P. abelii	0.60			
M. mulatta	0.65			
E. caballus	0.59			
B. taurus	0.64			
S. scrofa	0.77			
O. cuniculus	0.63			
R. norvegicus	0.60			
M. musculus	0.69			
M. domestica	0.44			
O. anatinus	Under (mean = 0.85)	0.45–1.26	0.64–1.27	0.92, P = 0.34
G. aculeatus	Under (0.88)	0.57–1.20	0.92–1.09	0.93, P = 0.33
D. melanogaster	Over (1.11)	0.89–1.33	0.77–1.23	2.17, P = 0.14
T. castaneum	Over (1.06)	0.69–1.42	0.91–1.11	0.18, P = 0.67
C. elegans	**Under (0.72)**	**0.51–0.92**	**0.98–1.18**	**12.06, P = 0.0005**
Female heterogamety	1.06	1.02–1.11		
Aves	Over (mean = 1.07)	0.86–1.28	0.86–1.09	0.92, P = 0.34
G. gallus	1.10			
M. gallopavo	0.97			
T. guttata	1.12			
B. mori	Over (1.02)	0.61–1.43	0.86–1.04	0.01, P = 0.90
S. mansoni	Over (1.11)	0.61–1.60	0.87–1.17	0.41, P = 0.52
Sex-limited/neo/ancient				
G. aculeatus neo-X	Under (0.92)	0.57–1.19	0.92–1.09	0.47, P = 0.59
D. melanogaster ancient-X (chromosome 4)	1.00	−0.08–2.08	0.91–1.09	0.00, P = 0.97
S. mansoni W	Under (0.90)	0.63–1.16	0.85–1.18	1.00, P = 0.32

NOTE.—Significant under or over-representations are in bold. CIs calculated by bootstrapping. χ^2 statistics are also presented. One-to-one orthologs were identified using *M. musculus* as the reference.

shows an under-representation (tables 1 and 2). Third, we may expect variation in the magnitude of the male-biased mutation rate, for example, due to species differences in generation time and in the strength of sexual selection and associated intensity of sperm competition (Ellegren 2007). However, it is difficult to see how the patterns we observe are driven by variation in male-biased mutation. Finally, levels of gene transfer and genome rearrangement are lineage-specific (Rand et al. 2001), where low levels of movement will restrict the ability of different parts of the genome to respond to inter-genomic coadaptation and conflict. This may explain many of the non-significant associations.

Alternatively, interactions between the mitochondrial genome and the X and Z chromosome have been suggested to play a role in sexual selection and might be enriched for mito-nuclear genes that play a role in coloration, such as those involving carotenoids (Hill and Johnson 2013). We did not observe this predicted over-representation on any Z chromosomes, and it is difficult to see how differences among our study species in the degree and type of sexual selection explain the variance in the distribution of mitochondrial genes.

A further possibility is that the genomic distribution of mito-nuclear genes is driven by gametic function. Although mitochondrial activity is generally not crucial for non-motile egg function (de Paula et al. 2013), it is integral to sperm energy production and motility (Cummins 2009). Although many genes are functionally diploid in sperm (Braun et al. 1989), there is evidence that many genes are expressed within the spermatid and are subject to haploid selection (Joseph and Kirkpatrick 2004). Because any single spermatozoon will only carry either an X or Y chromosome, expression of mito-nuclear genes within the sperm would lead to selection against sex-linkage as half of the male gametes would lack

Table 2

Mean Bias and 95% CIs of Mito-nuclear Genes on the Sex Chromosomes and Autosomes

Species or Clade	Over-/Under-representation of Mito-nuclear Genes on Sex Chromosome (Bias)	95% Bonferroni-Corrected CI of the Sex Chromosome	95% Bonferonni-Corrected CI of the Autosomes	χ^2 Test and P Value
Male heterogamety				
Therian mammals	**Under (mean = 0.71)**	**0.61–0.79**	**0.90–1.13**	**62.8, $P < 0.0001$**
H. sapiens	0.73			
P. troglodytes	0.69			
G. gorilla	0.72			
P. abelii	0.69			
M. mulatta	0.72			
E. caballus	0.64			
B. taurus	0.71			
S. scrofa	0.87			
O. cuniculus	0.77			
R. norvegicus	0.65			
M. musculus	0.68			
M. domestica	0.48			
O. anatinus	Under (mean = 0.83)	0.43–1.22	0.69–1.29	1.38, $P = 0.24$
G. aculeatus	Under (0.92)	0.60–1.23	0.93–1.09	0.47, $P = 0.49$
D. melanogaster	No bias (1.00)	0.70–1.30	0.86–1.13	0.00, $P = 0.99$
T. castaneum	Under (0.96)	0.37–1.55	0.84–1.14	0.03, $P = 0.86$
C. elegans	**Under (0.23)**	**0.0–0.46**	**0.91–1.28**	**23.8, $P < 0.0001$**
Female heterogamety				
Aves	Over (mean = 1.02)	0.83–1.22	0.86–1.09	0.10, $P = 0.75$
G. gallus	1.06			
M. gallopavo	0.89			
T. guttata	1.10			
B. Mori	Under (0.84)	0.22–1.45	0.83–1.12	0.47, $P = 0.49$
S. Mansoni	Under (0.52)	−0.50–1.54	0.64–1.69	0.95, $p = 0.33$
Sex-limited/neo/ancient				
G. aculeatus neo-X	Under (0.84)	0.54–1.13	0.92–1.09	1.96, $P = 0.16$
D. melanogaster ancient-X (chromosome 4)	Under (0.99)	−0.58–2.55	0.86–1.13	0.00, $P = 0.99$
S. mansoni W	Under (1.04)	0.18–1.90	0.61–1.77	0.00, $P = 0.97$

NOTE.—Significant under or over-representations are in bold. CIs calculated by bootstrapping. Mito-nuclear genes detected by the rBBH analysis using *M. musculus*, *D. melanogaster*, and *C. elegans* to find orthologs.

Table 3

Mean Bias and 95% CIs of Mito-nuclear Genes on the Sex Chromosomes and Autosomes

| Species or Clade	Over/underrepresentation of Mitonuclear Genes on Sex Chromosome (Bias)	95% Bonferroni-Corrected CI of the Sex Chromosome	95% Bonferonni-Corrected CI of the Autosomes	χ^2 Test and P Value
Male heterogamety				
O. Anatinus	Under (mean = 0.87)	0.41–1.33	0.36–1.35	0.60, $P = 0.44$
G. Aculeatus	Under (0.34)	−0.58–1.23	0.66–1.44	1.46, $P = 0.23$
Sex-limited/neo/ancient				
G. aculeatus neo-X	1.00	−0.61–2.60	0.63–1.44	0.00, $P = 0.95$

NOTE.—Mitonuclear genes identified using GO terms in Biomart.

a functional copy. Conversely, all sperm in female heterogametic species contain a Z chromosome, and there would be no expected selection against Z-linkage of mito-nuclear genes.

Furthermore, differences among taxa in sperm biology could explain some of the patterns we observe among male heterogametic taxa. For example, species differ in the presence or absence of sperm hyper-activation, which requires
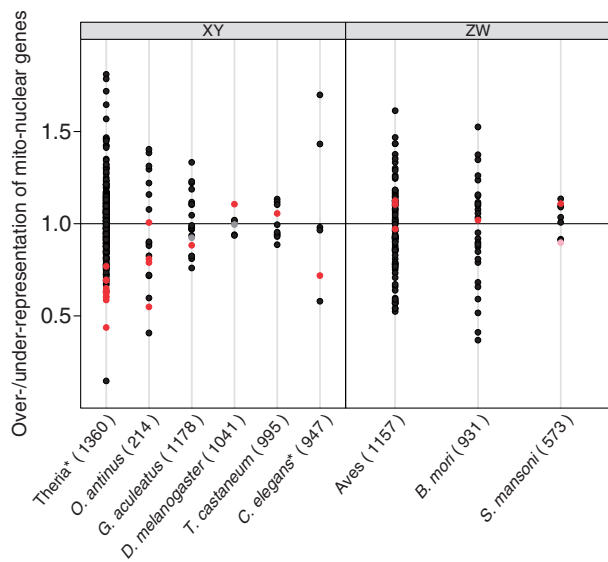
Fig. 1.—Bias of nuclear–mitochondrial genes on the sex chromosomes across species with independent sex chromosomes. Values for each autosome are in black, major sex chromosomes (X or Z) in red, old (i.e., *D. melanogaster* fourth) and neo (i.e., *G. aculeatus* chromosome 9) in gray, and the *S. mansoni* W chromosome in pink. Values in parenthesis after species names indicate the total number of mito-nuclear genes in the genome detected by the rBBH analysis with *M. musculus*. Species marked by * have a significant under-representation of nuclear–mitochondrial genes on the X chromosome. Note: Some of *D. melanogaster* autosomal points overlap.
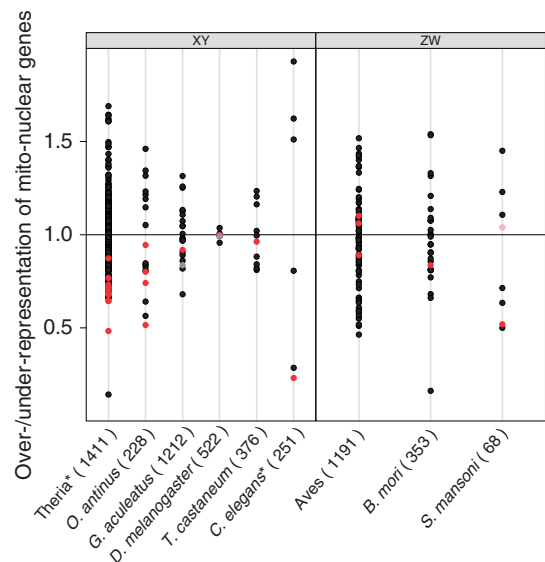
Fig. 2.—Bias of nuclear–mitochondrial genes on the sex chromosomes across species with independent sex chromosomes. Values for each autosome are in black, major sex chromosomes (X or Z) in red, old (i.e., *D. melanogaster* fourth) and neo (i.e., *G. aculeatus* chromosome 9) in gray, and the *S. mansoni* W chromosome in pink. Values in parenthesis after species names indicate the total number of mitonuclear genes in the genome detected by the rBBH analysis using *M. musculus*, *D. melanogaster*, and *C. elegans* to find orthologs. Species marked by * have a significant underrepresentation of nuclear–mitochondrial genes on the X chromosome. Note: Some of *D. melanogaster* autosomal points overlap.

high mitochondrial activity (Cummins 2009). Also, the degree to which oxidative metabolism is required for sperm motility differs, and both human and mouse sperm do not need mitochondrial activity for motility (Cummins 2009). Factors such as this may affect the degree of haploid expression of mito-nuclear genes in sperm and therefore the distribution of mito-nuclear genes on X chromosomes. However, we hasten to point out that none of these explanations alone fully account for why Theria and *C. elegans* have an under-representation of mito-nuclear genes on their X chromosomes. More complex theory, taking into account patterns of gene duplication and gene movement, may be required to make sense of these patterns.

The need to maximize the number of independent sex chromosomes in our analyses means that we had to include some genomes with incomplete functional annotation. To solve this, we employed an rBBH approach in order to detect orthologs of mitochondrial interacting genes that are annotated in model organisms like *M. musculus*, *D. melanogaster,* and *C. elegans*. However, this approach could be influenced by taxon-specific mito-nuclear genes and difficulties in orthology identification across large evolutionary distances. Although this does limit the number of genes we identify through strict orthology identification in some taxa, we do

not believe that it has unduly biased our results for several reasons. First, nuclear genes that interact with the mitochondria are conserved across broad taxonomic groups (Porcelli et al. 2007; Lotz et al. 2014), suggesting that rBBH is broadly applicable. The convergence between our results using *M. musculus* as the reference for all rBBH with results using *D. melanogaster* and *C. elegans* as reference suggests that conservation predominates over clade- or species-specific patterns. We also detected similar patterns using species-specific GO annotations.

In conclusion, our results are not universally consistent with either sexual conflict (Drown et al. 2012) or sexual selection (Hill 2013; Hill and Johnson 2013), driving the general distribution of mito-nuclear genes on all sex chromosomes. We observed significant under-representation of mito-nuclear genes in just two of six analyzed X chromosomes, and no patterns of non-random distribution on any analyzed Z chromosome. The results suggest that other genomic phenomena may limit the extent to which inter-genomic conflict (Drown et al. 2012) or sexual selection (Hill and Johnson 2013) affect mito-nuclear distributions and confirm the importance of broad, phylogenetically independent analysis.
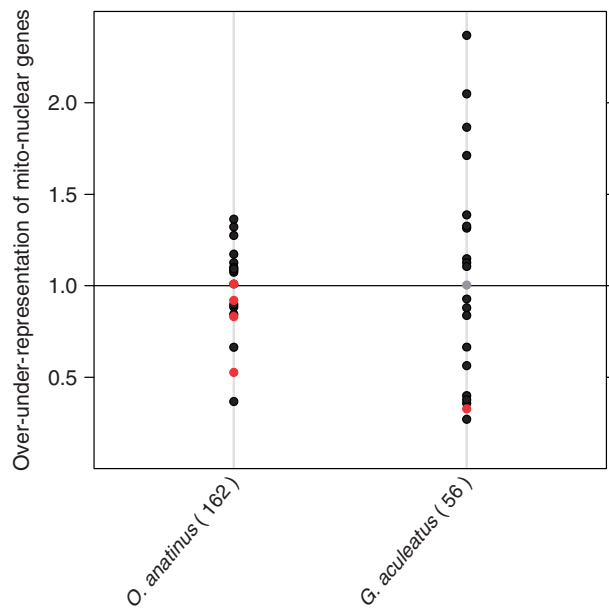
Fig. 3.—Bias of nuclear–mitochondrial genes on the sex chromosomes across *G. aculeatus* and *O. anatinus*. Values for each autosome are in black, X chromosomes in red, and neo (i.e. *G. aculeatus* chromosome 9) in gray. Values in parenthesis after species names indicate the total number of mitonuclear genes in the genome detected using GO:0005739 to identify genes that interact with the mitochondria.

Acknowledgments

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