Evidence of a Paucity of Genes That Interact with the Mitochondrion on the X in Mammals

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Abstract

Mitochondria are essential organelles whose replication, development, and physiology are dependent upon coordinated gene interactions with both the mitochondrial and the nuclear genomes. The evolution of coadapted (CA) nuclear–mitochondrial gene combinations would be facilitated if such nuclear genes were located on the X-chromosome instead of on the autosomes because of the increased probability of cotransmission. Here, we test the prediction of the CA hypothesis by investigating the chromosomal distribution of nuclear genes that interact with mitochondria. Using the online genome database BIOMART, we compared the density of genes that have a mitochondrion cellular component annotation across chromosomes in 16 vertebrates. We find a strong and highly significant genomic pattern against the CA hypothesis: nuclear genes interacting with the mitochondrion are significantly underrepresented on the X-chromosome in mammals but not in birds. We interpret our findings in terms of sexual conflict as a mechanism that may generate the observed pattern. Our finding extends single-gene theory for the evolution of sexually antagonistic genes to nuclear–mitochondrial gene combinations.

Key words: genomic conflict, gene transfer, sex chromosome, coadapted gene complex.

Introduction

Mitochondria are essential organelles whose replication, development, and physiology depend upon coordinated interactions between gene combinations from both the mitochondrial and the nuclear genomes. When genes from both genomes interact, their differing modes of inheritance, bi-parental versus maternal, can result in genomic conflict (Werren 2011). There is direct evidence of the genomic conflict generated by epistatic interactions between the mitochondrial genes and nuclear genes from studies of mitochondria involvement in sperm development (Wang 2004; Rajender et al. 2010; Paoli et al. 2011). Furthermore, introgression of foreign mitochondria into *Drosophila* populations has revealed sex-biased epistatic effects with X-linked nuclear genes (Rand et al. 2001, 2006; Montooth et al. 2010; Aw et al. 2011). When organismal fitness depends on the epistatic interactions between the nuclear and mitochondrial genomes (Rand et al. 2001, 2006; Dowling et al. 2007), the cotransmission of nuclear–mitochondrial gene combinations facilitates epistatic selection (Wade and Goodnight 2006; Brandvain and Wade 2009). Cotransmission is important to epistatic selection because it maintains gene combinations across generations. By increasing the heritability of gene combinations, cotransmission allows epistatic selection to act more effectively on trans-genomic interactions.

The question remains, as to how these genome interactions have shaped the geography of the genome. Theory predicts that the nuclear genome should evolve in a way to maintain nuclear–mitochondrial gene combinations (Wade and Goodnight 2006; Brandvain and Wade 2009). One means of increasing the heritability of nuclear–mitochondrial gene combinations involves changing the physical location of nuclear genes within the nuclear genome as opposed to changing location between the nuclear and mitochondrial genomes. The probability of cotransmission of X-linked genes and mitochondria is significantly higher than that of mitochondria and

autosomal genes (0.67 versus 0.50 in a randomly mating population) (Rand et al. 2001). Therefore, the evolution of coadapted (CA) nuclear–mitochondrial gene combinations would be facilitated if nuclear genes interacting with the mitochondria were located on the X-chromosome instead on the autosomes because of the increased probability of cotransmission (Rand et al. 2001).

The CA nuclear–mitochondrial gene hypothesis posits that selection for beneficial nuclear–mitochondrial gene combinations and against poor ones is facilitated by the location of nuclear genes located on the X chromosome relative to the autosomes. A clear prediction of the CA hypothesis is that nuclear genes which interact with the mitochondrion (N-mt genes), should be overrepresented on the X relative to autosomes. Here, we tested the prediction of the CA by investigating the chromosomal distribution of nuclear genes that interact with the mitochondria.

Methods

Data Collection

Using the online genome database BIOMART (http://www.biomart.org) via their MartView interface (Smedley et al. 2009), we collected genomic information on the following organisms using the available ENSEMBL GENES 63 datasets: *Homo sapiens* (GRCH37.p3) (International Human Genome Sequencing Consortium 2004), *Pan troglodytes* (CHIMP2.1) (Chimpanzee Sequencing and Analysis Consortium 2005), *Gorilla gorilla* (gorGor3) (Scally et al. 2012), *Pongo pygmaeus* (PPYG2) (Locke et al. 2011), *Macaca mulatta* (MMUL_1.0) (Rhesus Macaque Genome Sequencing Analysis Consortium et al. 2007), *Callithrix jacchus* (calJac3) (GenBank Assembly ID GCA_000004665.1), *Mus musculus* (NCBIM37) (Gregory et al. 2002), *Rattus norvegicus* (RGSC3.4) (Gibbs et al. 2004), *Oryctolagus cuniculus* (oryCun2.0) (Lindblad-Toh et al. 2011), *Canis familiaris* (CanFam_2.0) (Lindblad-Toh et al. 2005), *Bos taurus* (Btau_4.0) (Bovine Genome Sequencing Analysis Consortium et al. 2009), *Sus scrofa* (Sscrofa9) (GenBank Assembly ID GCA_000003025.4), *Equus caballus* (EquCab2) (Lindblad-Toh et al. 2011), *Mono-delphis domestica* (monDom5) (Mikkelsen et al. 2007), *Gallus gallus* (WASHUC2) (Hillier et al. 2004), and *Taeniopygia guttata* (taeGut3.2.4) (Warren et al. 2010). We focused on mammalian datasets because they contain the greatest level of annotation, including chromosomal location. Also, we restricted our analysis to this single database so that we could make comparisons between genomes while minimizing data collection artifacts.

For each of the 16 genomes, we collected two gene sets: 1) complete list of all genes with annotated function; 2) including only those nuclear genes with mitochondrion annotation (N-mt genes) as specified by the Gene Ontology ID 0005739. From the database, we obtained for each gene: chromosomal location, ENSEMBLE gene name, and gene ID. Genes without a specific chromosomal location were excluded. We processed this output with scripts written in MATLAB (The Mathworks, Natick, MA) to calculate the counts of genes per chromosome in each genome for the two different datasets.

Statistical Analysis

We compared the density of N-mt genes on the X or Z chromosomes with that of the autosomes relative to the expectation based on overall gene density for 14 vertebrate genomes with male heterogamety and two vertebrate genomes with female heterogamety (the birds, *G. gallus* and *T. guttata*). We performed all of our statistical analysis in MATLAB. To determine if the set of N-mt genes was under- or overrepresented on each chromosome, we compared it with an expected count. For a particular genome, we used the following method to determine the expected number of N-mt genes per chromosome. We calculated the product of the total number of N-mt genes and the fraction of all annotated genes found on each chromosome. This expected gene count per chromosome assumes that the N-mt genes are distributed across the chromosomes in the same pattern as the total set of genes in the genome. The ratio of observed count to expected count will equal one when there is no under- or overrepresentation of N-mt genes on a particular chromosome. When the value is less (greater) than one, then there is an under representation (over representation) of N-mt genes on a chromosome.

We computed the comparisons as described above to limit any potential biases in how the data were collected. In order to create a bias in the density of this particular group of genes, some chromosomes would need to be over- or underannotated for N-mt genes in particular. We are unaware of any feature of the N-mt genes which make them more or less likely to be sequenced and subsequently recognized as genes relative to any other subset of genes. Our method is also robust to differences in annotation level between chromosomes within a genome. If for some reason there was a bias in the annotation level of autosomes compared with the sex chromosome, we would expect more genes to be annotated with the N-mt term as well. Our density of N-mt genes (rather than an absolute count) would not be affected.

We tested the significance of the underrepresentation on the sex chromosome by calculating a confidence interval of the mean over-/underrepresentation for the autosomes based on 10,000 replicate bootstrap samples and then determining if the sex chromosome representation fell below this confidence interval at a Šidák corrected alpha of 0.032, based on an individual alpha of 0.05 and 16 different comparisons (Šidák 1967).

Results

Using the online genome database BIOMART (http://www
.biomart.org) via their MartView interface (Smedley et al.
2009), we examined the distribution of genes that have
a mitochondrion cellular component annotation (Gene
Ontology ID 0005739) across chromosomes in 16 vertebrates.
We found a strong and significant underrepresentation of
nuclear genes interacting with the mitochondrion on the sex
chromosomes in all of the mammals (fig. 1) but in neither of
the two birds. In mammals, although the sex chromosome did
not always have the lowest density of N-mt genes relative to
expectation, it was always in the lower end of the distribution
of chromosomes. In both bird species, the sex chromosomes
(Z) had the expected density of N-mt genes based on overall
gene density.

Discussion

We have discovered a striking pattern in the distribution of
genes among chromosomes: nuclear genes which interact
with the mitochondrion are significantly underrepresented
on the X-chromosome in mammals, but not in birds (fig. 1).
These data are inconsistent with the predictions of the
CA nuclear–mitochondrial gene hypothesis which proposes
that due to the increased heritability of gene combinations,
the N-mt genes should be overrepresented on the
X-chromosome. We discuss other hypotheses that may help
to explain the observed pattern of gene distributions.

   We propose that the observed pattern may be explained by
sexual conflict (SC). SC occurs when genes beneficial to the
fitness of one sex are deleterious to the fitness of the other.
The evolution of maternally transmitted organelles can lead to
SC when mitochondrial mutations that interact epistatically
with nuclear genes have favorable effects in females but are
deleterious in males (Rice 1984; Rice et al. 2006; Vicoso and
Charlesworth 2006). With epistatic selection, the effects of
nuclear genes interacting with mitochondria manifest only
on certain mitochondrial backgrounds and not on others
(fig. 2A). Reciprocally, the effects of mitochondrial genes
occur only in specific nuclear backgrounds. Importantly, chan-
ging the mitochondrial background alters the effects of the
interacting nuclear genes without changing the nuclear gene
sequence. Epistatic fitness interactions between nuclear genes
and the mitochondrion can result in sexually antagonistic ef-
fects for nuclear genes.

   With nuclear–mitochondrial epistasis, the force of genomic
conflict (between the nucleus and the mitochondrion) is
aligned with that of intralocus SC (between males and fe-
males). Because mitochondria have nearly exclusive maternal
inheritance, selection can favor those mitochondrial geno-
types that enhance female fitness, even if at the expense of
male fitness (Partridge and Hurst 1998; Rand et al. 2006;
Montooth et al. 2010; but see Wade and Brandvain 2009).

That is, mitochondrial mutations with epistatic effects favor-
able to females will spread owing to maternal inheritance
(fig. 2B). Because the mitochondrial mutations are not se-
lected on the basis of their effect in males, even mutations
which carry unfavorable effects in males will spread as long as
those mutations are favorable in females. As a result, a nuclear
gene interacting with the mitochondria will become more
deleterious to males over time.

   There is direct evidence of sexually antagonistic epistatic
interactions between the mitochondrial and nuclear genes
from studies of the involvement of mitochondria in sperm
development (Wang 2004; Rajender et al. 2010; Paoli et al.
2011). Furthermore, introgression of foreign mitochondria
into Drosophila populations has revealed sex-biased epistatic
effects for X-linked nuclear genes (Rand et al. 2001, 2006;
Montooth et al. 2010; Aw et al. 2011).

   Although SC based on segregating nuclear genes depends
upon the nature of allelic dominance (Rice 1984), selection on
the mitochondrial mutations is independent of the dominance
relationships among alleles of its nuclear partners, requiring
only positive fitness effects on females in some nuclear back-
grounds. Furthermore, the SC process we are proposing does
not depend on the simultaneous polymorphism of both
nuclear and mitochondrial genes or the cotransmission of
nuclear–mitochondrial gene combinations, which was a key
to the alternative CA hypothesis. SC due to the spread of
mitochondrial mutations with epistatic effects with nuclear
loci can occur whether the nuclear partner of the mitochon-
drion is autosomal or X-linked.

   As a mitochondrial mutation causing the SC spreads within
the population, there is increasing selective pressure to resolve
the SC. Several mechanisms for the nuclear resolution of the
SC have the potential to alter the genomic distribution of N-mt
genes. An evolutionary means for resolving such SC (among
many) is via *gene movement* off of the X and onto the auto-
somes. This is a process involving several steps, including gene
duplication, fixation and subsequent gene loss, and requiring
significant chromosomal bias at one or all of these steps to
account for the observed pattern (Wu and Yujun Xu 2003).
An alternative process is the creation of duplicate copies on
the autosomes with sex-specific or sex-biased expression
(Connallon and Clark 2011; Gallach and Betrán 2011). Both
proposed mechanisms involve the fixation of nuclear gene
duplications which ameliorate the male detrimental effects
caused by the mitochondrial mutation.

   Theory and empirical evidence suggest that these duplicate
copies accumulate on the autosomes (Connallon and Clark
2011; Gallach and Betrán 2011). The bias in the location of
the duplicate copies creates the overrepresentation on the
autosomes relative to the X chromosome. Female beneficial
(male deleterious) epistatic effects with mitochondrial muta-
tions are not favored equally at the X and autosomal locations
because X-linked loci spend two-thirds of their time in females
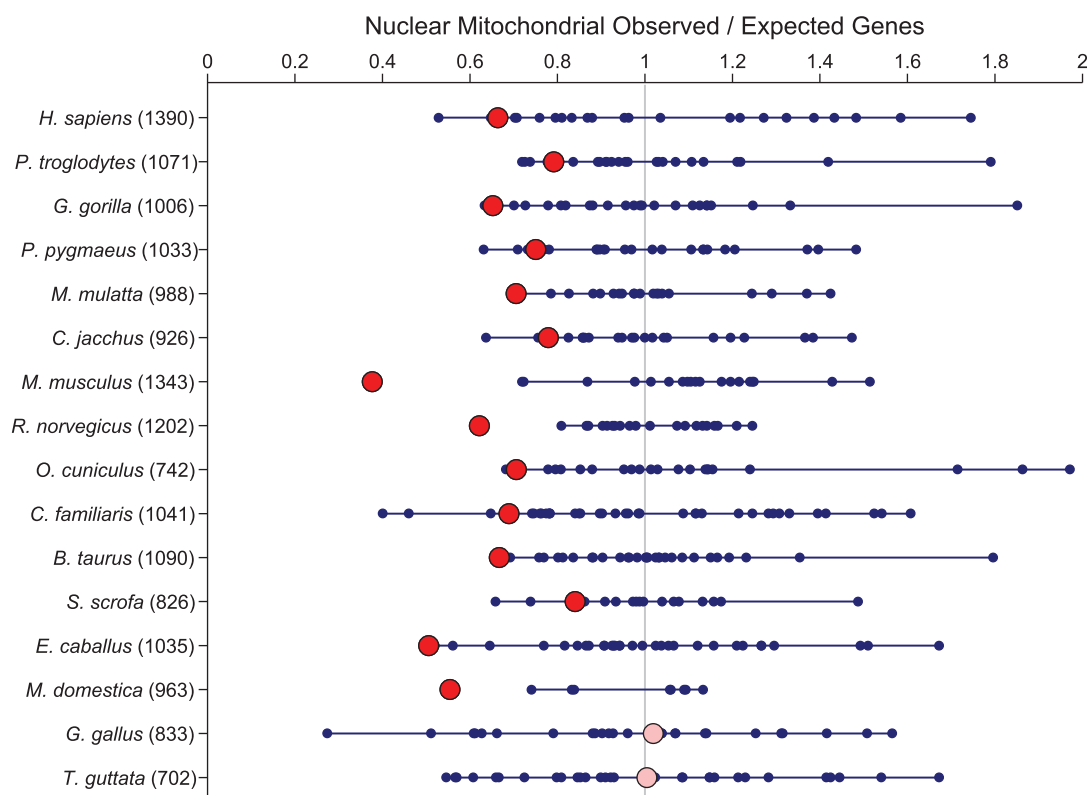and only one-third in males (Rice 1984). Autosomal loci, on

Fig. 1.—Underrepresentation of nuclear–mitochondrial genes on the sex chromosomes of mammals. Expected counts of genes are calculated based on the distribution of all annotated genes in the genome. The fraction of all genes present on a particular chromosome is then multiplied by the total number of N-mt genes in a particular genome to give the expected count. Ratios close to one show no over- or underrepresentation on a chromosome. Each chromosomal value for each genome is represented by a blue (autosomes) or red and pink (sex chromosome [X/Z]) circle. Lines connect the maximum and minimum autosomal ratios. Values in parenthesis after the genome name indicate the number of identified N-mt genes in the genome. Sex chromosomes that are significantly different from the autosomes are highlighted in red and nonsignificant values are pink (*H. sapiens*, $P < 0.0001$; *P. troglodytes*, $P < 0.0001$; *G. gorilla*, $P < 0.0001$; *P. pygmaeus*, $P < 0.0001$; *M. mulatta*, $P < 0.0001$; *C. jacchus*, $P < 0.0001$; *M. musculus*, $P < 0.0001$; *R. norvegicus*, $P < 0.0001$; *O. cuniculus*, $P < 0.0001$; *C. familiaris*, $P < 0.0001$; *B. taurus*, $P < 0.0001$; *S. scrofa*, $P = 0.0001$; *E. caballus*, $P < 0.0001$; *M. domestica*, $P < 0.0001$; *G. gallus*, $P = 0.7621$; and *T. guttata*, $P = 0.5281$).

the other hand, spend half their time in males and such genes with male beneficial (female deleterious) epistatic effects would be expected to fix more easily on autosomes compared with the X chromosome. This autosomal-bias in selection efficiency may be particularly important for male traits involved in reproductive competition, where selection on male fertility can be several times stronger than opposing selection on females (Wade 1979; Shuster and Wade, 2003; Miller et al. 2006). Over time, the difference in selection efficiency would lead to a greater cumulative deterioration of X-linked relative to autosomal nuclear genes as a result of mitochondrial mutation pressure (fig. 2). Empirical support for this mechanism comes from *Drosophila* where Gallach et al. (2010) find that N-mt gene duplications accumulate additional copies on autosomes more readily than the X chromosome. Interestingly, these gene duplicates also show testis-specific expression that the authors suggest is evidence of SC resolution (Gallach et al. 2010).

Other hypotheses regarding the resolution of SC suggest that changes in the sex-specific expression of sexually antagonistic genes, possibly via *cis*-acting expression modifiers, resolve intralocus SC (Rice 1984; Rice and Chippindale 2001; Gibson et al. 2002; Connallon and Clark 2010; Stewart et al. 2010). Our data are based on the genomic distribution of N-mt genes and not on sex differences in gene expression. Thus, although our data in mammals are consistent with resolution of SC via gene movement or duplication, they do not exclude the possibility that SC antagonism may be resolved by alternative means. Our data suggest that mammalian N-mt genes may be useful for elucidating the mechanistic basis by which SC is resolved (Vibranovski et al. 2009; Gallach et al. 2011; Han and Hahn 2012).

We hypothesize that the pattern we observe in mammals differs from that observed in birds, because of one or a combination of differences affecting the proposed process based on mitochondrial mutation pressure and gene movement.
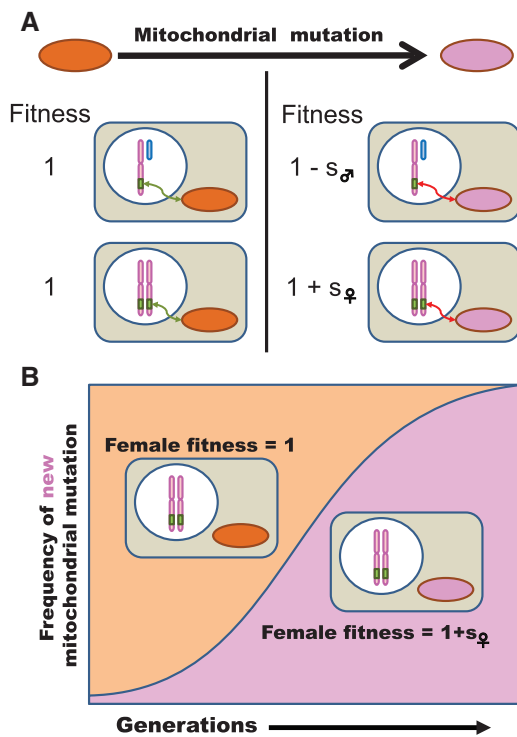
FIG. 2.—Mitochondrial mutations as drivers of SC. A schematic of the hypothesized process generating selection for the movement of N-mt genes off the X chromosome. (*A*) Some nuclear genes interact with the mitochondrion (green box). This interaction produces epistatic fitness effects (green arrow). A mitochondrial mutation occurs (purple circle) with epistatic sexually antagonistic fitness effects (red arrow) on the current nuclear genetic background. (*B*) Adaptive spread of the mitochondrial mutation leads to the eventual fixation of the female beneficial mutant mitochondria despite male deleterious effects.

First, there is reduced mitochondrial mutation pressure in birds (Ogburn et al. 1998; Hickey 2008). Second, there is a near absence of nuclear gene retro-transposition in birds relative to mammals (Haas et al. 2001; Warren et al. 2010) but see (Ellegren 2011). Third, if the fitness effect is caused by epistatic interactions with the mitochondrion, then strict maternal inheritance of the mitochondria results in reduced cotransmission of the mitochondria and Z chromosomes compared autosomes. Additionally, all of the cotransmission occurs from mother to sons at which point that mitochondrial-Z chromosome combination has a zero probability of being passed along to the next generation. The combination these factors suggests that our proposed process will be significantly weaker in birds, relative to mammals and that intralocus SC in birds may be resolved differently than it is in mammals.

We appreciate that our sample of genomes does not meet the criterion of phylogenetic independence. One could argue that we have a single contrast between two types of male heterogametic vertebrates (mammals and marsupials) and one type of female heterogametic vertebrate (birds).

Although other vertebrate genomes are available, none meet the criteria of having sex chromosomes (e.g., *Danio rerio* and *Fugu rubripes*) and the insect gene annotations in this database are not coincident with their annotations in other databases (e.g., drosophilids GO annotation in BIOMART relative to Flybase). It is well recognized that genomes are highly fluid, with frequent translocations among chromosomes, even though a recognizable amount of synteny is present (Hillier et al. 2004; Ross et al. 2005). Thus, despite its taxonomic limitations, our data suggest that the signal of a lack of N-mt genes on the X chromosomes of mammals is present and has been maintained across a diversity of species for tens of millions of years.

Our results significantly extend the single-gene theory for the evolution of sexually antagonistic genes to involve epistatic interactions between nuclear and mitochondrial genes. It also synergistically combines SC theory with results from genomic conflict theory, wherein, mitochondrial genes with deleterious effects on males spread if they are favorable to females owing to uniparental, maternal transmission. The high rate of such mitochondrial mutations and their subsequent adaptive spread leads to the continual accumulation of male deleterious fitness effects on X-linked genes (Rand et al. 2001). Our theory does not preclude patterns of sex-limited gene expression for sex-linked genes with direct (instead of epistatic) fitness effects. Our empirical finding adds to our knowledge of the forces affecting gene clustering and gene expression on sex chromosomes. Our results are consistent with a synergistic interaction between the forces of SC and genomic conflict affecting the distribution of N-mt genes on the sex chromosomes relative to the autosomes in vertebrates.

Acknowledgments

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Associate editor: Esther Betran