Experimental Evidence Supports a Sex-Specific Selective Sieve in Mitochondrial Genome Evolution

Paolo Innocenti, Edward H. Morrow, Damian K. Dowling

Mitochondria are maternally transmitted; hence, their genome can only make a direct and adaptive response to selection through females, whereas males represent an evolutionary dead end. In theory, this creates a sex-specific selective sieve, enabling deleterious mutations to accumulate in mitochondrial genomes if they exert male-specific effects. We tested this hypothesis, expressing five mitochondrial variants alongside a standard nuclear genome in Drosophila melanogaster, and found striking sexual asymmetry in patterns of nuclear gene expression. Mitochondrial polymorphism had few effects on nuclear gene expression in females but major effects in males, modifying nearly 10% of transcripts. These were mostly male-biased in expression, with enrichment hotspots in the testes and accessory glands. Our results suggest an evolutionary mechanism that results in mitochondrial genomes harboring male-specific mutation loads.

The mitochondrial-eukaryotic union represents one of life’s most important symbioses, equipping the eukaryotic cell with a highly efficient means of energy conversion. The upshot of this union is that contemporary metazoan harbor two obligate genomes. The genome located within the cell nuclei (nuclear genome) contains the vast majority of function-encoding genetic material (about 14,000 genes in Drosophila melanogaster) (1), whereas the mitochondrial genome (mtDNA) is diminutive in size, consisting of just 37 genes (13 protein-coding, 22 tRNA, and 2 ribosomal RNA) (2). Although few in number, mitochondrial genes serve an essential function, with both nuclear- and mtDNA-encoded proteins interacting within the mitochondria to perform cellular respiration. The tight coordination in gene expression necessary for organelle function is achieved by extensive signaling between mitochondrial and nuclear genomes, via anterograde and retrograde regulation (3).

Unlike their nuclear counterparts, mitochondrial genomes generally have solely maternal transmission, resulting in evolutionary dead ends for the mtDNA of males (4). As such, mtDNA should only be able to make a direct evolutionary response to selection through females [but see (5, 6)]. This should pose no problem for traits with iden-

tical function across males and females. However, mutations in mtDNA that contribute to the expression of traits exhibiting different sex-specific optima (i.e., sexually dimorphic or sexually antagonistic traits) will be subject to a sex-specific selective sieve. This means that selection will fail to prevent the accumulation of mtDNA mutations that are deleterious when expressed in males, if the same mutations are only slightly deleterious (4), neutral (7), or beneficial (8, 9) when expressed in females.

If a sex-specific selective sieve is important in mitochondrial genome evolution, then we predict that mitochondrial genomes should generally harbor a mutation load that is more pronounced in males. Also, the effect of the mutation load should increase with the degree of male-biased sexual dimorphism of the trait or tissue in question, because the benefits that males can salvage from relying on female-specific adaptation of mtDNA will diminish as the level of sex-biased expression increases and the intersexual genetic correlation erodes.

We tested these predictions on the basis of the facts that extensive signaling takes place between mitochondrial and nuclear genomes (3) and that polymorphisms in mtDNA typically exert their phenotypic effects through interactions with the nuclear genome (10). We explored genome-wide variation in nuclear gene expression across strains of D. melanogaster that differ only in the origin of their mitochondrial genomes by using GeneChip Drosophila Genome 2.0 microarrays (Affymetrix, Incorporated, Santa Clara, CA). We examined five distinct, naturally occurring mitochondrial genomes from around the globe [Alstonville (New

South Wales, Australia), Brownsville (Texas, USA), Dahomey (Benin), Japan, and Mysore (India)]. An abundance of non synonymous and synonymous polymorphisms exists across the protein-coding sequences of these five genomes [supporting online material (SOM) text]. Each mitochondrial genome was coexpressed with the isogenic nuclear background, w118, in two replicates per strain. Replicates for each mtDNA strain were created as a safeguard to ensure that inferences regarding effects of mtDNA polymorphism were not confounded by cryptic genetic variation that might have accumulated in the nuclear background during the period in which the strains were created and assayed. No differential expression between replicates of any mtDNA strain was found (P > 0.05 after multiple testing corrections in two-tailed t tests for all the transcripts, for each pair of replicate introgressions). Other environmental variables (e.g., food source, temperature, light, age, and mating status) were carefully controlled to minimize sources of variation.

Fig. 1. Differentially expressed genes. (A) Venn diagram representing the intersections between the sets of nuclear genes showing a significant effect of mitochondrial strain in males (blue, top left), in females (red, top right), and in the full data set (black, bottom). (B) Expression levels (in glog, scale) of the same sets of genes (blue triangles and dark blue and black dots), in male and female adults of D. melanogaster. Gray dots in the background represent the expression level of non—differentially expressed genes.
When male and female gene expression data were considered together, different mitochondrial strains significantly affected the expression of 1011 nuclear genes [8.0% of tested genes; \( P < 0.05 \) after a multiple testing correction was set as a cut-off for all contrasts (6)]. When male and female gene expression profiles were analyzed separately, the strain effect on males accounted for the differential expression of 1172 genes (9.3%), whereas only 7 genes (~0.06%) were significant for the strain effect on female gene expression. These results suggest that, in general, different mitochondrial strains have a large impact on nuclear gene expression and that this effect extends to a disproportionately larger number of genes in males than in females. The intersection of the three lists of genes (Fig. 1A) also indicates that most of the total variation in gene expression is driven by males (~62% of the genes showing an overall strain effect also show a significant strain effect in male gene expression). In total, 1554 nuclear genes were differentially expressed in at least one sex as a consequence of their coexpression with mitochondrial genomes of different origins.

We then examined the relationship between the significant genes and the levels of sexual dimorphism of the transcriptome. The nuclear genes with expression affected by the mitochondrial genomes were significantly more male-biased in expression than expected [two-tailed Fisher’s exact test: odds ratio = 1.13, \( P = 0.028 \), mean-rank gene set enrichment test (11), MR-GSE, \( P = 3.3 \times 10^{-12} \) (Fig. 1B)], and this effect was magnified when we considered only the transcripts that were differentially expressed in the male-only data set (Fisher’s exact test: odds ratio = 1.56, \( P = 1.1 \times 10^{-12} \), MR-GSE test, \( P = 1.8 \times 10^{-35} \)). To evaluate the likely impact of the mitochondrially affected genes on fitness, we tested their association with transcripts known to be associated with expression affected by the mitochondrial genomes of different origins.

Main transcriptional modules. See (6) for details on the tests performed. Size indicates number of genes; Chr. dist., chromosomal distribution bias; GO, Gene Ontology; Associated with, module is over-represented in lists of candidate male-female fitness-associated genes, published in (22); dash entries, not significant.

<table>
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<tr>
<th>Module</th>
<th>Size</th>
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Fig. 2. Transcriptional modules. Level plot representing pairwise correlations between the 1554 differentially expressed genes. The correlation matrix has been used to compute modules of correlated expression. Modules are separated by gray lines (very small modules can be masked by overlapping dividers). The 18 modules with size greater than 10 are labeled.

Table 1. Main transcriptional modules. See (6) for details on the tests performed. Size indicates number of genes; Chr. dist., chromosomal distribution bias; GO, Gene Ontology; Associated with, module is over-represented in lists of candidate male-female fitness-associated genes, published in (22); dash entries, not significant.
and is also mostly male-limited [$\chi^2 = 9.6, \text{SD} = 1.8, x_1^2 = 6.8, \text{SD} = 1.2$, Wilcoxon test: $W = 31608$, $P = 2.8 \times 10^{-46}$] across the five mitochondrial haplotypes. Red and blue lines represent the within-line module average. (F) across the five mitochondrial haplotypes. Red and blue lines represent (Fig. 3, E and F, and fig. S16B), with expression primarily in the accessory gland (Fisher’s exact test: odds ratio = 72, $P = 5 \times 10^{-11}$) and ejaculatory duct (Fisher’s exact test: odds ratio = 7.8, $P = 1 \times 10^{-3}$) and relatively low or absent in the other tissues (Fig. 3H and fig. S16F).

Remarkably, the correlation in expression between these two clusters of genes in their respective male-limited tissues and other tissues is on average zero or even negative (Fig. 3, C and G), in stark contrast to the high levels of correlation typically observed among tissues for the whole transcriptome and subsets thereof (see, for example, figs. S7D, S8D, and S9D). Taken together, these data support the hypothesis that a large proportion of the observed differential effects attributable to the mitochondrial variants are driven by the accumulation of mutations in mtDNA that exert male-specific effects. That is, selection on mtDNA in females will presumably fail to screen genetic polymorphisms that are strongly male-biased, active only in male-limited tissues, and uncorrelated in their expression with other “shared” tissues.

A mitochondrial mutation load in the male reproductive tissues is expected to lead to a sharp decline in male fertility. This has been termed “mother’s curse” (13) and has gained empirical support from studies in humans (14), hares (15), and beetles (16), which have documented associations between particular mitochondrial genetic variants and components of male fertility and reproductive fitness (13, 15). Genetic polymorphisms within the cytoplasm leading to reductions or elimination of male fertility, via cytoplasmic male sterility in plants (17, 18) and yeast (19) and via cytoplasmic incompatibility induced by intracellular parasites (e.g., Wolbachia) in arthropods (20, 21), have long been documented. We purified the studied Drosophila lines of bacterial infection by using antibiotics and can conclude that the differential gene expression witnessed in our study is most likely driven by mtDNA polymorphisms (6). Indeed, males carrying one of the five mitochondrial haplotypes (Brownsville) used here are sterile when that haplotype is expressed alongside the $w^{118}$ isogenic background (22) but are fertile when the same haplotype is expressed alongside the coevolved maternal background (6). The findings involving mtDNA polymorphism, and cytoplasmic genomes in general, indicate the profound scope by which cytoplasmic genomes affect male fertility. Existence of a sex-specific selective sieve in mtDNA is likely to exert strong selection on counteradaptations in the nuclear genome that restore lost male function (10, 23, 24).

Among the other clusters, we identified two broad groups whose characteristics suggest that they are involved in retrograde regulation of nuclear DNA transcription (3): genes in modules 6, 7, 10, and 11 (and to some extent 8 and 9) that are generally moderately expressed in every tissue but that show a peak in the ovary, suggesting a weak female bias in expression, and genes with a tendency to be highly correlated across tissues (figs. S6 to S11). Furthermore, Gene Ontology (www.geneontology.org)—enriched molecular functions mostly refer to proteins with enzymatic, adenosine triphosphate (ATP)–binding and trans-membrane transporter activity (tables S6 to S11), involved in metabolic and biosynthetic processes, macromolecule localization, and post-transcriptional modifications. The second group is characterized by transcripts specifically involved in mitochondrial functions (modules 1, 12, 14, and 17; see tables S1, S12, S14, and S17). Enriched gene ontologies for these modules explicitly refer to mitochondrial cellular structures (inner membrane, intermembrane space, matrix and ATP respiratory chain complex) and molec-

![Fig. 3](www.sciencemag.org/). Module 12 and 16. (A and E) Scatter plot representing sex bias in expression of the 275 genes in module 12 (A) and the 185 genes in module 16 (E). (B and F) Sex-specific expression of genes in module 12 (B) and module 16 (F) across the five mitochondrial haplotypes. Red and blue lines represent the within-line module average. (C and G) Correlation of the genes in module 12 (C) and module 16 (G) across the major tissues of D. melanogaster. (D and H) Average levels of tissue-specificity in expression for genes in module 12 (D) and 16 (H). The range of the data set is delimited by the center (minimum) and the external perimeter (maximum). The circular black line indicates the average expression in the whole body. The red line represents the expression in each tissue. Data on tissue-specific expression were obtained from FlyAtlas (30).
ular processes (oxidative phosphorylation, respiratory electron transport chain, ATP synthesis).

Population genetic models (4) suggest that mtDNA mutations that are slightly deleterious to females can be maintained at low frequencies under mutation-selection balance even when highly deleterious to males. Genetic drift will fix some of these mutations over time, as well as male-harming mutations that are totally neutral in females. This is because the efficiency of selection acting on mitochondrial genomesshould be dampened, owing to their haploidy, maternal transmission, and a lack of recombination (10, 25). In this sense, they are destined to accrue mutations in the same way that asexual organisms will via Muller’s ratchet (26).

Sexually antagonistic selection may also contribute to this mutation load, given that mutations of even slight benefit to female fitness will be selectively favored even if of catastrophic consequence to males. Under this scenario, the signal in nuclear gene expression induced by these mutations might simply be too subtle to detect within the nuclear transcriptome of females, if the phenotypic effects tied to the different mtDNA strains are much larger in males. Alternatively, the nuclear transcriptome might house modifiers (17, 27), expressed within the male reproductive tissues, that have evolved to counter the negative effects of mtDNA mutations and that responded to the variance in mitochondrial genomes in our study. Although we cannot confidently ascribe which of the above processes is the major contributor to the male-specific mutation load that we have detected, they are all likely to have played some role. Lastly, we raise the possibility that the existence of large male-specific differences in nuclear gene expression across our experimental lines might occur if modifications to the expression of these nuclear transcripts are neutral to selection. However, this possibility seems unlikely given that (i) the differentially expressed transcripts are associated with male (not female) fitness, (ii) one experimental mtDNA haplotype renders males sterile when expressed in the w1118 genetic background, whereas it does not have this effect in the nuclear background of origin, and (iii) mitochondrial genetic variation for male fertility has been previously documented (14–16). Moreover, if widespread differences in gene expression are neutral to selection, this phenomenon should also have been observed in females, short of assuming a generalized sex-specific difference in sensitivity to transcriptional levels.

In sum, mitochondrial genetic effects on male gene expression within the nuclear transcriptome were strong, affecting about 10% of all genes tested. Notably, these affected transcripts stretched well beyond those involved directly in bioenergetic processes of the mitochondria (oxidative phosphorylation and ATP production), demonstrating that retrograde signaling between the genomes is much more pervasive than previously considered. In this sense, there are parallels with the Y chromosome (28), which also houses a paucity of protein-coding genes and shows asymmetric transmission, but is nonetheless entwined in widespread regulatory control of the nuclear transcriptome (28, 29). In contrast to the effects on males, mitochondrial effects on female-specific expression of the transcriptome were weak in magnitude and negligible in number. This discrepancy between the sexes is entirely consistent with the idea that the mitochondrial genetic polymorphisms involved have accrued under a sex-specific selective sieve.

References and Notes
6. Materials and methods are available as supporting material on Science Online.

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Supporting Online Material
www.sciencemag.org/cgi/content/full/332/6031/845/DC1 Materials and Methods SOM Text Tables S1 to S18 References

Role for piRNAs and Noncoding RNA in de Novo DNA Methylation of the Imprinted Mouse Rasgrf1 Locus

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Genomic imprinting causes parental origin–specific monoallelic gene expression through differential DNA methylation established in the parental germline. However, the mechanisms underlying how specific sequences are selectively methylated are not fully understood. We have found that the components of the PIWI-interacting RNA (piRNA) pathway are required for de novo methylation of the differentially methylated region (DMR) of the imprinted mouse Rasgrf1 locus, but not other paternally imprinted loci. A retrotransposon sequence within a noncoding RNA spanning the DMR was targeted by piRNAs generated from a different locus. A direct repeat in the DMR, which is required for the methylation and imprinting of Rasgrf1, served as a promoter for this RNA. We propose a model in which piRNAs and a target RNA direct the sequence-specific methylation of Rasgrf1.

Imprinted genes show parental origin–specific monoallelic expression, which is controlled by DNA methylation in the differentially methylated regions (DMRs) (1–5). Differential methylation of the DMRs is established in the parental germline, passed to the zygote, and maintained in the somatic cells, which eventually leads to monoallelic expression. During fetal development, the DMRs are demethylated (imprint erasure) in primordial germ cells at 10.5 to 12.5