inhibitors has not been possible, perhaps for a combination of reasons, including challenges in function-based screening assays and the intrinsic refractoriness of aquaporins to drug discovery (termed non-druggability). One area of recent progress is in AQP4-based therapeutics for neuromyelitis optica. The idea is to block the binding of pathogenic autoantibodies to astrocyte AQP4, which may provide a targeted, non-immunosuppressive approach to block the disease-initiating event in neuromyelitis optica. One approach has been the engineering of a non-pathogenic, tight-binding anti-AQP4 antibody ('aquaporumab') that competes with pathogenic autoantibodies; another approach has been the identification, by high-throughput screening, of small-molecule blockers of the autoantibody–AQP4 interaction.

What's next? There remain significant gaps in our knowledge about the cellular mechanisms of certain aquaporin functions, such as aquaporin-facilitated cell migration, cell proliferation, and neuroexcitatory phenomena. Though much of the low-hanging fruit in the aquaporin field has been picked and digested, as in areas of aquaporin protein structure and phenotypic analysis of knockout mice, new discoveries and ideas continue to emerge. Compelling recent data warrant further investigation of aquaporins in cancer, immune cell function and obesity. Lastly, notwithstanding the challenges and limited progress in the identification of aquaporin inhibitors, broad opportunities remain in aquaporin-based therapeutics.

Where can I find out more?

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In vivo male fertility is affected by naturally occurring mitochondrial haplotypes

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We harness an experimental design that enables us to completely disentangle mitochondrial from nuclear genetic effects in the fruit fly *Drosophila melanogaster*. Using this design, we directly link male fertility outcomes to the mitochondrial haplotype. Specifically, we show that competitive male fertility, measured *in vivo*, differs across naturally occurring mitochondrial haplotypes. We discuss this result in the context of recent studies that support the evolutionary hypothesis according to which maternal inheritance of mitochondria will facilitate the accumulation of male-harming mutations in the mitochondrial genome, when these same mutations are benign, beneficial or slightly deleterious in their effects on females [1–3]. We predict that at least some of the mitochondrial allelic variance affecting competitive male fertility across the sampled haplotypes will have accumulated under this evolutionary process and be male-specific in its effect on the phenotype. We suggest that the existence of male-harming mitochondrial mutations for male fertility would place strong selection on the interacting nuclear genome to evolve compensatory counter-adaptations that offset the negative effects, and we present support for this idea.

The mitochondrial genome encodes products that are crucial for achieving uncompromised metabolic function in both sexes. Yet, its maternal inheritance suggests that adaptation within the mitochondrial genome will proceed chiefly via natural selection on females. In theory, this could render mitochondrial genomes prone to the accumulation of alleles that — while optimised for female function — are potentially maladaptive for aspects of male function [1–3], particularly those traits exhibiting sexually dimorphic or sex limited expression [2,4]. Candidate traits likely to be prone to the effects of male-specific maladaptive mitochondrial alleles are those integral to male reproductive function, such as the testes and sperm. This hypothesis has received recent experimental support from studies reporting large male biases in the magnitude of mitochondrial genetic effects on patterns of gene and phenotypic expression in *Drosophila melanogaster* [2,5]. Such male-biases are indicative of the existence of mutations in the mitochondrial DNA (mtDNA) that exert male-biased effects. In one study, Innocenti *et al*. [2] found that genetic variance across mitochondrial haplotypes, while having a negligible effect on the female transcriptome, mediated the expression patterns of more than 1000 nuclear genes in males, with manifold effects on gene expression in the male reproductive tissues. Here, we show that these effects on the male transcriptome have ultimate downstream consequences on the outcomes of male fertility, measured under *in vivo* conditions in standard-genotype tester females.

We used six naturally occurring mitochondrial haplotypes, derived from different worldwide *D. melanogaster* populations (Brownsville (Texas, USA), Dahomey (Benin), Israel, Madang (Papua New Guinea), Puerto Montt (Chile), and Zimbabwe), which had been placed alongside a standard nuclear background in *D. melanogaster* [5,6] (Supplemental information), and then assessed the fertility of males harboring each haplotype under competitive (each male competing for fertilizations within a once-mated female) and non-competitive (each male mated once to a virgin female) scenarios. Significant effects of the mitochondrial haplotype were found for competitive male fertility ($F_{5, 677} = 24.14$; $p < 0.0001$; Figure 1A; Supplemental information), but not for non-competitive fertility ($F_{5,7.48} = 1.42$; $p = 0.318$; Figure 1B; Supplemental information). Although the patterns for competitive fertility appeared to be driven by a large effect of the Brownsville mtDNA haplotype (Figure 1A), they remained significant when analysed without this haplotype

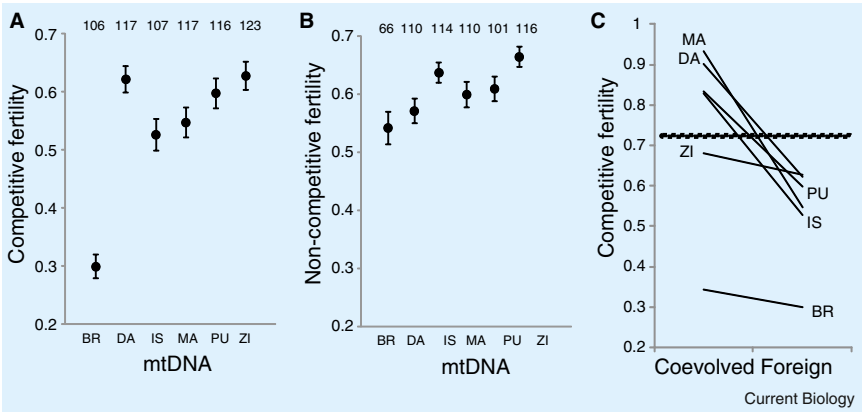

Figure 1. Mitochondrial effects on male fertility.
(A) Competitive male fertility. (B) Non-competitive male fertility. (C) Evidence for nuclear compensation. In (A) and (B), data points denote means ± 1 SE of the six mitochondrial haplotypes when expressed alongside standard nuclear backgrounds. The standardized nuclear background was w^{1118}: ATHENS for (A) competitive, and w^{1118}: LH_M for (B) non-competitive male fertility. In (C), mean competitive male fertility is plotted for each mitochondrial haplotype when expressed alongside a coevolved haploid nuclear genome and alongside the foreign standardized nuclear counterpart (w^{1118}: ATHENS). The mean value associated with the control genotype (w^{1118} mtDNA expressed alongside a w^{1118}: ATHENS nuclear background) is denoted by the black hashed line that extends horizontally across the panel (X = 0.72). MA denotes the Madang mtDNA haplotype, DA Dahomey, PU Puerto Montt, IS Israel, ZI Zimbabwe and BR Brownsville.

($F_{4, 572}$ = 3.20; p= 0.013). The measure of competitive male fertility used here is arguably the more evolutionarily relevant, given the polyandrous mating system of this species. There was no general association between competitive and non-competitive fertility across the six mitochondrial haplotypes (with Brownsville: r = 0.639; p = 0.172, without: r = -0.047; p = 0.941).

Particular point mutations and deletions in the mtDNA have previously been tied to aspects of sperm performance and function [7,8]. Here, we have provided a new advance by demonstrating that the naturally occurring genetic variation that exists across distinct mitochondrial haplotypes has direct effects on the outcomes of male reproduction *in vivo*. When reconciled with recent findings showing strong male biases in mitochondrial genetic effects on phenotypic expression [2,5], it seems plausible to suggest that at least part of the mitochondrial allelic variance for male fertility will consist of deleterious and male-specific mitochondrial mutation loads that accumulated under the maternal inheritance of the mtDNA. Under this scenario, we hypothesize that the evolutionary means enabling males to overcome the effects of these mutations will lie in the capacity of the nuclear genome to come up with counter-adaptations that restore losses of function [9,10]. We present preliminary evidence consistent with this hypothesis.

In all six cases in our study, mitochondrial haplotypes conferred higher competitive male fertility when expressed alongside a co-evolved haploid nuclear background (mean = 0.75 ± 0.08) than alongside a standard foreign haploid background (mean = 0.54 ± 0.05; Wilcoxon signed-rank test: z = -2.201; p = 0.028; Figure 1C). Furthermore, competitive fertility associated with a separate control nuclear background, which consisted of the standard foreign nuclear background expressed alongside its own coevolved mtDNA haplotype, was significantly (on average 33%) higher (mean = 0.72 ± 0.02; n = 112, and typical of that of the other coevolved mitochondrial-nuclear combinations) than when hosting any of the six focal mtDNA haplotypes in the disrupted mitochondrial-nuclear state (mean = 0.54 ± 0.05; two sample t-tests, one tailed, sequential Bonferroni corrections applied). Given the high fertility of the control line, it would seem unlikely that this result is simply attributable to a low additive breeding value of the standard foreign nuclear background relative to the coevolved nuclear backgrounds. These results are consistent with the hypothesis that the coevolved nuclear genomes harbor counter-adaptations that offset mitochondrial mutation loads for male fertility (Supplemental information). This emerging hypothesis warrants further attention to confirm the results are not an artefact of the particular lines and sampling approach used in this study, to ascertain their generality across populations and species, and to investigate the extent to which mitochondrial-nuclear coadaptation in natural populations is fuelled by mitochondrial mutations that are male-specific in effect.

Supplemental Information

Supplemental Information including experimental procedures, one figure and one table can be found with this article online at http://dx.doi.org/10.1016/j.cub.2012.12.002.

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