ARTICLE

Mitochondrial genetic effects on reproductive success: signatures of positive intra-sexual, but negative inter-sexual pleiotropy

Running Title: mtDNA effects on reproductive success

Keywords: mitochondria, life-history, sexual conflict, reproduction, maternal inheritance, sexual antagonism

M. Florencia Camus[1,2], Damian K. Dowling[1]

[1] School of Biological Sciences, Monash University, 3800, Australia

[2] Research Department of Genetics, Evolution and Environment, University College London, Gower Street, London, WC1E 6BT, United Kingdom

f.camus@ucl.ac.uk, damian.dowling@monash.edu

Abstract

Theory predicts that maternal inheritance of mitochondria will facilitate the accumulation of mtDNA mutations that are male biased, or even sexually antagonistic, in effect. While there are many reported cases of mtDNA mutations conferring cytoplasmic male sterility in plants, historically it was assumed such mutations would not persist in the streamlined mitochondrial genomes of bilaterian metazoans. Intriguingly, recent cases of mitochondrial variants exerting male-biases in effect have come to light in bilaterians. These cases aside, it remains unknown whether the mitochondrial genetic variation affecting phenotypic expression, and in particular reproductive performance, in bilaterians is routinely comprised of sex-biased or sex-specific variation. If selection consistently favours mtDNA variants that augment female fitness, but at cost to males, this could shape patterns of pleiotropy and lead to negative intersexual correlations across mtDNA haplotypes. Here, we show that genetic variation across naturally occurring mitochondrial haplotypes affects components of reproductive success in both sexes, in the fruit fly *Drosophila melanogaster*. We find that intrasexual correlations across mitochondrial haplotypes, for components of reproductive success, are generally positive, while intersexual correlations are negative. These results accord with theoretical predictions, suggesting that maternal inheritance has led to the fixation of numerous mutations of sexually antagonistic effect.

Introduction

Eukaryotic cells are thought to have arisen from the ancient symbiotic union between two prokaryote cells; one an α-proteobacterium that evolved into the mitochondrion, and the other an archaean-like organism that evolved into the eukaryote [1]. Each of these ancestral entities possessed their own genomes, and their symbiosis kick-started millions of years of inter-genomic coevolution that delineates contemporary eukaryotes from the organisms of other domains [2]. Almost without exception, eukaryotes have retained these two genomes – one mitochondrial (comprised of mtDNA), the other nuclear, and interactions between genes spanning each of these genomes coordinate closely to regulate critical biological processes tied to cellular metabolism via oxidative phosphorylation (OXPHOS) [3-5].

Notwithstanding that large variation exists in both the size and content of the mitochondrial genome across eukaryote taxa (e.g., large, with introns and generally low mutation rates in plants [6]; to streamlined, with high mutation rates in bilaterian metazoans [7]), over the course of evolutionary history most of the genome's protein-coding genes have been translocated to the host nuclear genome. In bilaterians, this process of genome reduction was extreme, with just thirteen protein-coding genes remaining [4]. Given these mitochondrial genes all encode essential subunits of OXPHOS, evolutionary biologists long assumed that purifying selection would generally prevent the accumulation of non-neutral (i.e., phenotype-modifying) genetic variation within the mtDNA sequence. Accordingly, the mitochondrial genome was harnessed as the go-to molecular marker upon which to base evolutionary and population genetic inferences, facilitated by its maternal inheritance, presumed lack of pervasive recombination, and, at least in bilaterians, its high mutation rate [8-12].

Over the past two decades, however, an increasing number of studies has challenged this assumption of neutrality of mtDNA sequence variation, with examples from plants [13, 14] ,

fungi [15, 16] and animals [17-19]. In particular, numerous studies have used multigenerational breeding schemes with the power to partition cytoplasmic genetic from nuclear genetic effects [19]. For example, in plants, cytonuclear interactions (interactions involving polymorphisms within the mitochondrial and/or chloroplast genome and those in the nuclear genome) were shown to affect 23 of 28 phenotypes measured in *Arabidopsis thaliana*, with pervasive effects on traits involved in germination, resource acquisition, phenology, height, fecundity and survival [20], and also on regulation of the metabolome [21]. In bilaterian animals, from flies to mice and humans, genetic polymorphisms that delineate distinct mitochondrial haplotypes have been linked to the expression of traits tied to reproductive success, development, and longevity [3, 22-29].

Maternal inheritance of mitochondrial genomes adds a further layer of complexity to the dynamics of mtDNA evolution, because it means that selection can only act directly on non-neutral mtDNA polymorphisms through the female lineage [30-32]. This hypothesis, which has been called "Mothers Curse" [30, 31], predicts that mutations that are neutral, beneficial or slightly deleterious to females may accumulate in the mtDNA sequence even if these same mutations are harmful in their effects on males ("Mother's Curse mutations") [30, 32-34]. While Mother's Curse effects occur very commonly in plants [35], through mtDNA-mediated Cytoplasmic Male Sterility, it was traditionally thought there was little scope for the streamlined mtDNA sequence of bilaterians to harbour mutations of male-biased effect [31, 32]. Yet, within the past decade, several cases of individual Mother's Curse mutations conferring male-specific fertility effects have been identified in *Drosophila* flies [36-38], mice [39], hares [40] and humans [41]. Furthermore, in humans, emerging evidence suggests that particular candidate mutations in the mtDNA sequence are responsible for male biases in the penetrance of Leber's Hereditary Optical Neuropathy and rates of infant mortality [42]. These examples raise the possibility that sex-specific variation might routinely build up, and be

maintained, within the mitochondrial genome of bilaterians that exhibit strict maternal inheritance of mtDNA.

Indeed, recent studies in *D. melanogaster* have supported this contention by showing that genetic variation across a pool of naturally occurring mtDNA haplotypes is associated with male-biased effects on genome-wide patterns of gene expression [43] and longevity [24, 44]. Nonetheless, the extent to which mitochondrial haplotypes exhibit sex-biases in their effects on the expression of life history phenotypes in metazoans remains generally unclear, because few studies have measured phenotypic effects across sets of naturally occurring mtDNA haplotypes in both males and females, respectively [24-26, 43-48]. The sparsity of studies reporting sex-specificity in effects is particularly evident when it comes to traits tied to reproductive performance [49]. Indeed, only a single study to date has sought to measure the effects associated with natural mtDNA haplotypes on components of reproductive success in both males and females. In that study, Immonen et al. (2016) examined the expression of components tied to reproductive success in each of the sexes across orthogonal combinations of mitochondrial and nuclear genotype sourced from three distinct populations, in the seed beetle, *Callosobruchus maculatus*. The nuclear genomic backgrounds, against which the three different mtDNA haplotypes were placed were not isogenic, but rather represented by large pools of segregating nuclear allelic variance that were sourced from each of three global populations. The authors reported mitochondrial genetic, and mito-nuclear interactions for female fecundity, and male ejaculate weight, and also an effect on female egg size that was traceable to an interaction involving the age and mito-nuclear genotype of the sire. Correlations in the reported mitochondrial, or mito-nuclear, genetic effects across the measured traits were, however, not examined [25].

Currently, little information exists as to the capacity for genetic variants in the mitochondrial genome to exert pleiotropic effects on multiple fitness traits, and whether the directions of

117 pleiotropy might change within and across the sexes. On the one hand, it might reasonably be
118 expected that the sign of mitochondrial genetic correlations for key phenotypic traits will
119 routinely be positive, assuming that mutations that accumulate within the mtDNA sequence
120 are likely to modify the performance of core metabolic processes, with cascading effects on a
121 range of energy-reliant phenotypes. But on the other hand, under the Mother's Curse hypothesis
122 it is plausible that the direction of these correlations will be negative across the sexes.
123 Assuming strict maternal inheritance, female-harming but male-benefiting mtDNA mutations
124 that appear in the mtDNA sequence should be efficiently purged by purifying selection. In
125 contrast, if mtDNA mutations appear that are female-benefiting, but male-harming, they will
126 be under positive selection and potentially increase in frequency [32]. Furthermore, the pool of
127 sexually antagonistic mutations accumulating within the mitochondrial genomes will differ
128 across populations – in terms of the identity of the mutation sites at which they occur, the
129 associated nucleotides, and total number of mutations accrued. Accordingly, we should expect
130 to observe a negative genetic correlation across haplotypes, with haplotypes that harbour
131 numerous female-benefiting but male-harming mutations (or alternatively harbouring a few
132 mtDNA mutations of major sexually antagonistic effect) conferring higher relative female, but
133 lower male, reproductive success. Conversely, those haplotypes harbouring few such mutations
134 (or alternatively mutations of only minor effect) will confer lower female reproductive success
135 relative to other haplotypes, but relatively higher success in males.

136 Studies that have tested for mitochondrial haplotype effects on multiple traits, and screened for
137 the presence of mitochondrial genetic correlations between the traits, have confirmed that
138 correlations frequently exist, and that they can be either positive or negative in direction. In
139 2009, Dowling *et al.* reported a strong positive association in effects of two mtDNA haplotypes,
140 segregating within a laboratory population (LH_M) of *D. melanogaster*, on two life history traits
141 in females - reproductive performance and longevity [50]. The haplotype conferring higher

142 female reproductive success also conferred higher female lifespan. Rand *et al.* (2001) reported
143 a negative correlation between the sexes for a measure of juvenile viability in *D. melanogaster*
144 (based on a chromosome segregation assay), across two of three mtDNA haplotypes measured
145 (these haplotypes were broadly clustered into 3 groups: Old World 1, Old World 2 and New
146 World). Camus et al (2015) reported that a Single Nucleotide Polymorphism (SNP) within the
147 mtDNA-encoded cytochrome B (Ala-278-Thr in *mt:Cyt-b*) gene of *D. melanogaster*, which is
148 found on a haplotype sourced from Brownsville USA, is associated with low male fertility [38],
149 but high male lifespan and short female lifespan, relative to twelve other haplotypes harbouring
150 other variants of this gene [24]. This SNP is therefore associated with antagonistic pleiotropic
151 effects both within and across the sexes, consistent with the prediction that mtDNA SNPs can
152 accumulate under positive selection in females, even if they are associated with suboptimal
153 male phenotypes [32], leading to sexually antagonistic trajectories of mtDNA evolution [33,
154 51-53].

155 To address patterns of sex-specificity and pleiotropy within the mitochondrial genome, here
156 we measured components of reproductive success in each sex, across a fully replicated panel
157 of thirteen naturally occurring mitochondrial haplotypes in *D. melanogaster*, in which each
158 haplotype is expressed alongside a standard, isogenic nuclear background [44, 54, 55]. Given
159 that the nuclear background of the panel is strictly controlled and isogenic, this experimental
160 approach provides an accurate means to home in on true mitochondrial genetic effects on
161 reproductive trait expression, and test for the magnitude and direction of mitochondrial genetic
162 correlations underpinning these traits. Such an approach provides a powerful proof-of-concept,
163 but also comes with a general caveat. Mitochondrial genetic effects on phenotypic trait
164 expression are likely to be routinely mediated via epistatic interactions between mitochondrial
165 and nuclear genotype [3, 5]. By constraining the number of nuclear backgrounds in our study
166 to just the one isogenic variant, we are unable to assess levels of mito-nuclear epistasis for the

traits under study, nor investigate whether effects or correlations across haplotypes are dependent on the nuclear genetic context. However, while a recent meta-analysis by Dobler et al. (2014) confirmed that effect sizes associated with cyto/mito-nuclear interactions generally exceeded those associated with additive cytoplasmic/mitochondrial genetic effects across plant and animal kingdoms, their analyses nonetheless revealed the additive effects were moderate to strong in magnitude. This therefore suggests that despite the ubiquity of mito-nuclear epistasis, a substantial pool of the genetic polymorphisms maintained within the mitochondrial genome are expressed at least to some degree additively, and will be uncovered using our approach.

Unlike previous screens of mitochondrial variation for longevity that had uncovered strong male-biases in effects [44], we found that both male and female reproductive traits were affected by the mitochondrial genetic variation harboured across our panel of haplotypes. Furthermore, we found signatures of pleiotropy across haplotypes in effects on the reproductive traits. Intriguingly, mitochondrial genetic correlations were generally positive for different reproductive traits measured within a given sex, but negative for traits of the different sexes.

Materials and Methods

Mitochondrial strains

Our experimental design is informed by the evolutionary prediction that nuclear compensatory variants that offset the negative effects of Mother's Curse mutations are likely to routinely arise and be selected for [56]. That is, if surveying natural populations, Mother's Curse mutations should remain cryptic and masked by their rescuing nuclear modifiers. Indeed, this is the scenario we see with Cytoplasmic Male Sterility in plants [35]. Therefore, our strain

construction is based on the premise that in order to detect Mother's Curse effects, we must first unmask them by placing them alongside an evolutionary novel nuclear background. Perhaps the strongest evidence for this premise to date in bilaterians, comes from Yee et al. 2013 [57], who reported that fertility outcomes were higher when mtDNA haplotypes were expressed alongside their putatively coevolved nuclear backgrounds than alongside an evolutionary novel nuclear background. Accordingly, thirteen *Drosophila melanogaster* strains were used, which have been previously described [24, 38, 55]. In brief, the isogenic nuclear background from the w^{1118} strain (Bloomington stock number: 5905) was coupled to mitochondrial haplotypes from thirteen distinct geographic locations using a crossing scheme that is outlined in Clancy (2008). These strains have each been maintained in duplicate since 2007, with the duplicates propagated independently, to enable us to partition mitochondrial genetic effects from cryptic nuclear variance that might have accumulated among the strains, as well as from other sources of environmental variation. Each generation, virgin females are collected from each duplicate of each mitochondrial strain (hereafter *mitochondrial strain duplicate*) and backcrossed to males of the w^{1118} strain, to maintain isogenicity of the nuclear background. Furthermore, w^{1118} is itself propagated by one pair of full-siblings per generation. Thus, if mutations arise in the w^{1118} strain, they will be swiftly fixed and passed to all mitochondrial strain duplicates, thus maintaining the critical requirement of isogenicity of the nuclear genome.

One of the mitochondrial haplotypes (Brownsville) included in our panel incurs complete male sterility in the w^{1118} nuclear background used here, and low male fertility in all other nuclear backgrounds surveyed to date [57, 58], whereas females who harbour this haplotype remain fertile [38]. This strain was therefore excluded from assays of male reproductive success (n=12 haplotypes in these assays), but included in assays of female reproductive success (n=13

haplotypes). All mitochondrial strains and w^{1118} flies were reared at 25˚C, under a 12h: 12h light: dark photoperiod regime, on potato-dextrose-agar food medium and with *ad libitum* access to live yeast. All strains had been cleared of any potential bacterial endosymbionts, such as *Wolbachia*, through tetracycline treatment at the time that the strains were created [59]. Diagnostic PCR with *Wolbachia*-specific primers confirmed all lines are free of *Wolbachia* [60].

Male Reproductive Success

Male reproductive success following exposure to a single female (short-burst offspring production)

This experiment measured offspring produced by a single male after a one-off mating opportunity with a virgin female at 4 days of adult age. This assay measures the ability of a male to convince a virgin female to mate, and then measures the number of offspring produced from sexual interaction with that female, which is likely to be a function of the male ejaculate quality (number and quality of sperm, and content and quality of reproductive proteins, transferred). The assay was run in two blocks, each separated in time by one generation. For three generations leading up to the experiment, each mitochondrial strain duplicate was propagated across 3 vials, with each vial containing 10 pairs of flies of standardised age (4-day old), and at controlled larval densities (approximately 80 eggs per vial). Then, ten virgin males from each mitochondrial strain duplicate (total 20 male flies per haplotype) were collected randomly from the 3 vials that propagate the line, and each stored individually in separate 40 ml vials containing 5mL of food medium. At the same time, virgin females were collected from the isogenic w^{1118} strain to be used as "tester" flies in the experiment. These females were sourced from 10 separate vials, which had been propagated and stored under the same

experimental conditions as described for the mitochondrial strain focal males, and they were stored in groups of 10 females per vial.

When four days old, each focal male was then combined with an equivalently-aged "tester" female, and these flies then cohabited the same vial for a 24 h period. Following this, focal males were removed from the mating vial and discarded. Females were then transferred into fresh vials with food substrate every 24 h over a 4-d period. The total number of offspring eclosing across these four vials was recorded for each focal male.

Male reproductive success across 8 days (sustained offspring production)

This assay represents a measure of male reproductive stamina (a function of male mating rate across time, and ability to replenish sperm and ejaculate stores). Sustained offspring production was assayed following the method described in Yee et al. (2015). In brief, individual males collected from each mitochondrial strain duplicate were provided with the opportunity to mate with eight different virgin females over eight consecutive 24 h long exposures [61]. To initiate the assay, twenty virgin males were collected from each mitochondrial strain duplicate, and each placed in a separate vial (total of 40 flies per mitochondrial haplotype). Twenty-four hours later, one 4-day-old virgin w^{1118} female was added to each vial, and the focal male and tester female then cohabited for 24 h. Following this 24 h exposure, males were removed and placed with another 4-day-old virgin w^{1118} female for another 24 h period. This process was repeated until day eight of the experiment (8 separate exposures). After each exposure, the w^{1118} females were retained and themselves transferred into fresh vials every 24 h for a total period of 4 consecutive days (including the 24 h cohabitation period), thus providing each female with up to 96 h to oviposit. Thirteen days following the 96 h oviposition period, the number of eclosed adult offspring emerging from each vial was counted.

Female reproductive success

Female components of short-burst offspring production, and short-burst 'egg-to-adult' viability

The first experiment gauged "short-burst" components of success, in which the number of eggs produced per female (fecundity), number of adults (reproductive success) produced, and proportion of eggs that ultimately eclosed into adulthood (an index of short-burst viability) were scored, following a 24 h laying opportunity early in life (4 days of age). The assay was run in five blocks, each separated in time by one generation. Female focal flies from each mitochondrial strain duplicate were collected as virgins, and stored individually. These were collected over numerous 40 mL vials, each of which had been propagated by 10 pairs of age-controlled parents (4 day old), and at controlled larval densities (approximately 80 eggs per vial). When 4 days of age, each female was exposed to one 4 d old tester virgin male, collected from the w^{1118} strain, for a period of 12 hours and then the females transferred to a fresh vial for 24 h to oviposit. Following this 24 hour ovipositioning period, females were discarded. We counted the eggs oviposited per female over this 24 h period (an index of short-burst fecundity), plus the offspring that emerged from these eggs (an index of short-burst offspring production). Furthermore, we calculated the proportion of eggs laid by each female that were converted into adult offspring (short-burst viability).

Female offspring production across 13 days (sustained offspring production)

This experiment measured female reproductive success over a 13-day period, thus representing a measure of reproductive stamina. Forty females from each mitochondrial strain duplicate were collected as virgins, and placed in individual vials. One day later, two 4 d old virgin w^{1118} males were placed into each female vial. Females, and the two males with which each female cohabited, were then transferred into fresh vials every 24 hours, for 13 days. The accompanying males were discarded every fourth day, and two 4 d old virgin males of the w^{1118} strain were

added. This ensured that females were not sperm-limited throughout the duration of the experiment. At the end of day 13, all flies across all vials were discarded, and vials were kept for eggs to develop. Female reproductive success was determined by counting the total number of adult offspring produced by each female, per vial, over the 13-day assay.

Statistical Analysis

General linear mixed models, using a Gaussian distribution, were fitted to the male short-burst offspring production data. Female short-burst fecundity data and female short-burst offspring production were modelled by fitting a generalized linear mixed model, using a Poisson distribution. For data that conformed to a Poisson distribution, we checked for over-dispersion using the function "*dispersion_glmer*" in the package *blmeco* [62]. Short burst viability data was modelled as a binomial vector, composed of the number of adults and number of eggs that failed to hatch (eggs-adults), using a binomial distribution and logit link. For each analysis, mitochondrial strain, the duplicate nested within mitochondrial strain, and the sampling block (for assays of short-burst components, which were assayed over multiple blocks) were modelled as random effects in the *lme4* package [63] in R [64]. Finally, female short-burst offspring production and fecundity had the addition of a random dummy variable to account for over-dispersion. To test for mitochondrial genetic variance for each trait, we used parametric bootstrap analysis to compare a full model to a reduced model which lacked the mitochondrial strain term. The parametric bootstrap was performed using the *PBmodcomp* function implemented in the package *pbkrtest* [65]

For the experiments gauging sustained offspring production, the overall total number of offspring (for both male and female models) was zero-inflated, and the resulting models over-dispersed. We therefore analysed both datasets using a negative binomial distribution [66], in which the zero values are a blend of sampling and structural effects (negative binomial

parameter; variance = $\phi\mu$). These models were performed using the R (v. 3.0.2) package glmmADMB (http://glmmadmb.r-forge.r-project.org/glmmADMB.html). The response variable was total number of offspring produced, with day of sampling being a fixed factor. The random effects in the model were mitochondrial strain, mitochondrial duplicate nested within mitochondrial strain, and the interactions between mitochondrial haplotype with day of sampling. Similar to the previous analyses for components of fitness, we used a model comparison approach whereby we compared the full model with a reduced model that lacked the mitochondrial strain term. Model comparisons were performed using likelihood-ratio tests.

A matrix of mitochondrial genetic correlations (Pearson's correlation coefficients and 95% Confidence Intervals) was created by obtaining mtDNA haplotype-specific means for each reproductive trait across all mitochondrial strains (Table S1). Thus, we had 13 means (mean of all individual datapoints within one haplotype) for each female measure of short burst (including short-burst viability) and sustained offspring production, and 12 means for the male measures (since the Brownsville haplotype was excluded from the male assays). Inter-sexual correlations across haplotypes were thus based on 12 means. Correlation coefficients of all pairwise combinations of traits were then further assessed using a bootstrapping procedure, in which trait means were resampled with replacement (10,000 replicates), and 95% confidence intervals were calculated using the Adjusted Percentile (BCa) Confidence interval method, as recommended by Puth et al. (2015) given its high performance across a broad range of situations [67]. Bootstrapping the confidence intervals appeared appropriate, given that we had captured a representative sample of the total global mtDNA haplotype variation present in *D. melanogaster* (Figure S2) [55], given the modest number (n=12) of data points in each correlation, and given that not all of the underlying distributions for each sampled trait were

Gaussian. Bootstrapped correlation coefficients plus their confidence intervals were calculated using the functions "*boot"* and "*boot.ci"* in the R package *boot* [68].

Results

Male Mitochondrial Reproductive Success Assays

We found statistically significant mitochondrial genetic variance for male short-burst offspring production (parametric bootstrap stat: 2.63, $p < 0.05$, Table 1A). We also uncovered statistically significant mitochondrial variance for male sustained offspring production (haplotype, deviance: 5.53, $p < 0.05$), levels of which were in part contingent on the day of the mating assay (haplotype × day, deviance: 6.54, $p < 0.05$, Table 1B, Figure 1A, Figure S1). Male offspring production tended to increase up to day 4 of adult age, and then incrementally decrease to day 8. However, the magnitude of increase was contingent on the mtDNA haplotype, with only two haplotypes exhibiting a clear peak in reproductive success at day 4 (MYS and ORE, Figure S1). The reaction norms per haplotype crossed-over across the eight days of the experiment, with several haplotypes that exhibited the highest relative reproductive success at the peak of the assay (day 4) generally associated with low reproductive success relative to the other haplotypes at Day 1 and 8 of the experiment (Figure 1A).

Female Mitochondrial Reproductive Success, and Short-burst Viability Assays

We found mitochondrial genetic variance for egg-to-adult viability of a female's clutch (parametric bootstrap stat: 3.51, $p < 0.05$, Table 1C), short-burst offspring production (parametric bootstrap stat: 3.7506, $p < 0.05$, Table 1D), but not short-burst fecundity (parametric bootstrap stat: 0.0132, $p = 1$, Table 1E). We found statistically significant mitochondrial genetic variance for sustained female reproductive success (haplotype, deviance:

6.04, $p < 0.001$), levels of which were again partly contingent on an interaction between mitochondrial strain and day of the mating assay (haplotype × day, deviance: 21.4, $p < 0.001$, Table 1F, Figure 1B, Figure S1). All haplotypes exhibited a similar trend, with reproductive success incrementally increasing up until day 4 of the assay, following which point, reproductive success began to decline albeit with slight upticks at days 8 and 12 that coincided with the addition of fresh tester males to the female vials (Figure S1). Again, however, these patterns were contingent on the mtDNA haplotype, with norms of reaction crossing per haplotype across Days 1, 4 and 8 of the assay (Figure 1B).

Mitochondrial Genetic Correlations

Intra-sexual correlations between reproductive traits tended to be positive in direction, including a positive correlation between short-burst viability and short-burst offspring production in females, across haplotypes (Figure 3). In contrast, inter-sexual correlations tended to be negative in direction (Figure 3).

Discussion

We explored mitochondrial genetic variance, across distinct and naturally occurring mitochondrial haplotypes, on components of reproductive success in male and female *D. melanogaster*, using an approach that enabled us to unambiguously trace genetic variation to the level of the mtDNA sequence. Notably, genetic polymorphisms located across these haplotypes affected almost all components of reproductive success measured – in females and in males. For measures of sustained reproductive success, we found that the level of mitochondrial genetic variation changed with the age of the focal flies (across the days of the experiment), and such genotype-by-age effects might be one means by which genetic variance within mitochondrial genomes might be maintained within and between populations.

Furthermore, we uncovered a signature of pleiotropy in the reported effects. These patterns of pleiotropy were positive for intra-sexual correlations across haplotypes (e.g. for associations between short-burst and sustained [when calculating means of total reproductive success across all days] components of reproductive success in each of the sexes), but negative for several of the inter-sexual correlations. While individual mutations conferring male sterility are well known in plants [35], and have recently been documented in metazoans [36, 38, 40], the signature of intersexual negative correlations across mtDNA haplotypes detected here, suggests that sexual antagonism might be a pervasive force under which genetic variation in the mitochondrial genome accumulates.

Negative inter-sexual correlations are striking because they indicate that, at the level of whole haplotypes, those haplotypes that confer relatively high reproductive success in one sex, generally confer low success in the other. Furthermore, we note that our estimate of this negative correlation is conservative, because it excluded the Brownsville mtDNA haplotype, which is completely male-sterile in the nuclear background assayed here (w^{1118}), and which we have previously reported to host a sexually antagonistic polymorphism located in the mt:*Cyt-b* gene [24, 58, 69]. The negative correlation between male and female reproductive success is consistent with evolutionary theory first developed by Frank and Hurst (1996), and which is routinely called "Mother's Curse" [31], which proposes that maternal inheritance of the mitochondria will lead to the accumulation of male-biased mutation loads within the mtDNA sequence [43]. Specifically, however, while Frank and Hurst (1996) envisaged that such mutations would accumulate under mutation-selection balance (i.e. the mutations would be largely benign, or slightly deleterious, in their effects on females), our results suggest a role for sexually antagonistic selection [32, 33], with mutations accumulating in the mtDNA sequence that augment female reproductive success, but that come at cost to male reproductive performance.

In our study, we included egg-to-adult viability of the female clutch in our analyses; a measure that lies at the interface between a maternal and an offspring trait [70-75]. It is well established that maternal effects shape this trait in *D. melanogaster* [70-72], in alignment with predictions of classic life-history theory, in which maternal resource provisioning into the ova lies at the heart of the classic evolutionary trade-off between gamete size and number [73]; a trade-off that extends to *Drosophila* [74, 75]. While ultimately it is not possible for us to delineate whether any mitochondrial haplotype effects on short-burst viability are manifested primarily through mothers (as mtDNA-mediated maternal effects) or primarily on the offspring themselves (via the direct effects of mtDNA mutations on survival through juvenile development), it was nonetheless informative to examine patterns of mitochondrial haplotypic variation affecting this trait.

Indeed, we found two intriguing and complementary patterns involving mitochondrial effects on egg-to-adult viability. Firstly, the Brownsville haplotype was associated with high viability, despite its association with male fertility impairment in adult life (Figure 1A&C). The Brownsville haplotype thus harbours a candidate mutation in the *mt:Cyt-b* gene associated with reduced adult male fertility [38, 58], and sexually antagonistic effects on longevity [24], but which is associated with high fitness in the juvenile phase of life. This result is consistent with a recent study, which showed that despite being associated with population suppression via its effects on male fertility impairment, when seeded into large experimental populations of *D. melanogaster* harbouring high levels of segregating nuclear allelic variance, population frequencies of the Brownsville haplotype were stably maintained, and indeed tended to increase across 10 generations of evolution [69]. In combination, these results suggest that this male sterilising mtDNA mutation has been maintained under positive selection on adult female and juvenile fitness. Secondly, the correlations we observed across the other twelve haplotypes further support this contention. The mitochondrial genetic correlation between short-burst

viability and female sustained offspring production was positive, while the correlation between short-burst viability and male short-burst offspring production was negative. These patterns reinforce the case of the Brownsville haplotype, by suggesting that the direction of selection on mitochondrial mutations might not only be routinely antagonistic between adult males and adult female reproductive traits, but also between juvenile components of fitness and components of adult male fitness; thus acting to exacerbate the rate at which male-biased mitochondrial mutation loads could accumulate within populations.

The possibility exists that our results might have been affected by the existence of heteroplasmy across our genetic strains. Heteroplasmy refers to the occurrence of multiple mtDNA haplotypes co-occurring within the same individuals, often brought about following instances of paternal leakage. While such cases have been reported in *Drosophila*, these have typically occurred between interspecific crosses involving individuals of divergent species [76, 77], or intraspecific crosses in species exhibiting much higher levels of divergence across the mtDNA haplotypes than those found in *D. melanogaster* [78]. One study, however, using intraspecific crosses in *D. melanogaster*, reported that as many as 14% of individuals are heteroplasmic, which would suggest the capacity for widespread paternal leakage in this species [79]. Another study indicated higher rates of leakage in males than females [77], but these cases all came from interspecific crosses between distinct species. Clearly, paternal leakage, leading to heteroplasmy, could potentially complicate our inferences, if present across our panel of mitochondrial strains, or if sex-specific in occurrence. The protein-coding sequences of the mtDNA haplotypes of each strain used in this study were originally sequenced by Clancy (2008). Since 2007, we have intermittently confirmed the genotype of each using haplotype-specific diagnostic SNPs, and we have also recently re-sequenced the haplotypes of each strain at high power to detect low frequency heteroplasmies (~1000× coverage). Throughout this time, we have never detected any instances of paternal leakage, or heteroplasmy, within any of

our strain duplicates. We acknowledge that we have only genotyped and sequenced females from each strain to date, leaving open the possibility that heteroplasmy might occur in males among our strains. However, if so, cases of male heteroplasmy should nonetheless be reset each and every generation, given generally strict maternal inheritance of the mtDNA and the predicted rarity of paternal leakage. Thus, we suggest that it is unlikely our results will be influenced by sex differences in levels of mtDNA heteroplasmy in our study.

Our results are based on a panel of 13 mtDNA haplotypes. Given each haplotype is replicated and expressed alongside an isogenic background, this enabled us to unambiguously partition mitochondrial genetic variance underpinning phenotypic trait expression. Furthermore, the panel of haplotypes is large enough to be broadly representative of the total levels of mitochondrial genetic diversity present within the global distribution of *D. melanogaster* [55] (Figure S2), and large enough to overcome the risk of sampling error leading to erroneous inferences that is likely to arise when sampling just a small subset of haplotypes that might have coincidentally similar breeding values. A caveat of our panel, however, is that it remains possible that patterns of mitochondrial genetic variation that we have detected in this study, might be specific to the particular nuclear background in which we have sampled the haplotypes; that is if the mitochondrial genetic variation screened here is only manifested via mito-nuclear interactions [3, 5], and not expressed additively. Indeed, in a broad meta-analysis of the magnitude of cytoplasmic genetic effects, Dobler et al (2014) reported that the effect size associated with cyto-/mito-nuclear interactions across taxa tended to be larger than the additive cytoplasmic/mitochondrial genetic effect size [80]. Importantly, however, the additive mitochondrial genetic effect was nonetheless moderate to large in metazoans, indicating that despite the ubiquity of mito-nuclear epistasis, a substantial pool of the mitochondrial genetic polymorphisms are expressed at least to some degree additively. Furthermore, we note that inferences based on the expression of focal genotypes alongside otherwise isogenic

485 backgrounds is by no means a limitation specific to our study, but a pervasive design feature
486 of many genetic studies, for instance chromosomal substitution studies of the sex chromosomes
487 [81, 82]. Ultimately, while we cannot conclude that the patterns observed here would be evident
488 across the complete pool of nuclear backgrounds in which they are tested, at its root our study
489 provides an important proof of concept that sexually antagonistic fitness variation can be
490 maintained within the mitochondrial genome, thus substantiating predictions of well-
491 established population genetic theory that have previously remained elusive.
492
493 Future studies are, however, now needed to assess the generality of our findings, not only
494 across a broader array of nuclear backgrounds within a species, but across a broad sample of
495 metazoans. Indeed, almost everything we know about metazoan mitochondrial genomes,
496 comes from the bilaterians and their streamlined genomes, but it now clear that non-bilaterian
497 mitochondrial genomes are much different in their gene content and arrangement [83].
498 Assessment of the capacity for sexually antagonistic mitochondrial variation should be
499 extended to these taxa. Furthermore, while our approach assumed that Mother's Curse
500 mutations will routinely lie hidden within natural populations, being offset by co-adapted
501 nuclear modifiers that rescue male fitness [5, 32], this assumption also requires further
502 theoretical and empirical attention. Indeed, a recent population genetic model suggests that
503 even when nuclear genetic variation for compensatory evolution is abundant, the negative
504 impact of Mother's Curse substitutions on male fitness can still be large, particularly in species
505 with intermediate effective population sizes [56]. This suggests that the dynamics of sexually
506 antagonistic mitochondrial evolution will differ across species, providing strong motivation for
507 broadening the emerging platform of research into Mother's Curse effects beyond the few
508 model species currently studied.
509

Acknowledgements: We thank Winston Yee for his help with fly husbandry, and David Clancy for providing *Drosophila melanogaster* mitochondrial populations in 2007.

Funding: The study was funded by the Australian Research Council (grant number DP1092897 and DP170100165 to DKD). During part of the writing/analyses, M.F.C was supported by the European Research Council under the Marie Skłodowska-Curie Actions [#708362]

Data, Code and Materials: Data from this manuscript will be uploaded to Dryad upon acceptance of this manuscript.

Authors' contributions: MFC and DKD conceived the study, analysed the data and wrote the manuscript. MFC conducted the experiments.

Competing interests. The authors declare no competing interests.

References

1. Gray M.W. 1992 The endosymbiont hypothesis revisited. *Int Rev Cytol* **141**, 233-357.

2. Embley T.M., Martin W. 2006 Eukaryotic evolution, changes and challenges. *Nature* **440**(7084), 623-630. (doi:10.1038/nature04546).

3. Wolff J.N., Ladoukakis E.D., Enriquez J.A., Dowling D.K. 2014 Mitonuclear interactions: evolutionary consequences over multiple biological scales. *Philosophical Transactions of the Royal Society of London Series B, Biological Sciences* **369**(1646), 20130443. (doi:10.1098/rstb.2013.0443).

4. Lane N. 2005 *Power, sex, suicide : mitochondria and the meaning of life*. Oxford ; New York, Oxford University Press; xiii, 354 p. p.

5. Rand D.M., Haney R.A., Fry A.J. 2004 Cytonuclear coevolution: the genomics of cooperation. *Trends Ecol Evol* **19**(12), 645-653. (doi:10.1016/j.tree.2004.10.003).

6. Galtier N. 2011 The intriguing evolutionary dynamics of plant mitochondrial DNA. *BMC Biol* **9**, 61. (doi:10.1186/1741-7007-9-61).

7. Boore J.L. 1999 Animal mitochondrial genomes. *Nucleic Acids Res* **27**(8), 1767-1780.

8. Lynch M. 1997 Mutation accumulation in nuclear, organelle, and prokaryotic transfer RNA genes. *Molecular Biology and Evolution* **14**(9), 914-925.

9. Pesole G., Gissi C., Catalano D., Grillo G., Licciulli F., Liuni S., Attimonelli M., Saccone C. 2000 MitoNuc and MitoAln: two related databases of nuclear genes coding for mitochondrial proteins. *Nucleic Acids Res* **28**(1), 163-165. (doi:10.1093/nar/28.1.163).

10. Saccone C., Gissi C., Lanave C., Larizza A., Pesole G., Reyes A. 2000 Evolution of the mitochondrial genetic system: an overview. *Gene* **261**(1), 153-159.

11. Ballard J.W.O., Whitlock M.C. 2004 The incomplete natural history of mitochondria. *Mol Ecol* **13**(4), 729-744. (doi:10.1046/j.1365-294X.2003.02063.x).

12. Galtier N., Nabholz B., Glemin S., Hurst G.D.D. 2009 Mitochondrial DNA as a marker of molecular diversity: a reappraisal. *Mol Ecol* **18**(22), 4541-4550. (doi:10.1111/j.1365-294X.2009.04380.x).

13. Budar F., Roux F. 2011 The role of organelle genomes in plant adaptation: time to get to work! *Plant Signal Behav* **6**(5), 635-639.

14. Barnard-Kubow K.B., So N., Galloway L.F. 2016 Cytonuclear incompatibility contributes to the early stages of speciation. *Evolution* **70**(12), 2752-2766. (doi:10.1111/evo.13075).

15. Albertin W., da Silva T., Rigoulet M., Salin B., Masneuf-Pomarede I., de Vienne D., Sicard D., Bely M., Marullo P. 2013 The Mitochondrial Genome Impacts Respiration but Not Fermentation in Interspecific Saccharomyces Hybrids. *PLOS ONE* **8**(9), e75121. (doi:10.1371/journal.pone.0075121).

16. Zeyl C., Andreson B., Weninck E. 2005 Nuclear-mitochondrial epistasis for fitness in Saccharomyces cerevisiae. *Evolution* **59**(4), 910-914.

17. Ballard J.W., Kreitman M. 1994 Unraveling selection in the mitochondrial genome of Drosophila. *Genetics* **138**(3), 757-772.

18. Rand D.M. 2001 The Units of Selection in Mitochondrial DNA. *Annu Rev Ecol Syst* **32**(1), 415-448. (doi:10.1146/annurev.ecolsys.32.081501.114109).

19. Dowling D.K., Friberg U., Lindell J. 2008 Evolutionary implications of non-neutral mitochondrial genetic variation. *Trends Ecol Evol* **23**(10), 546-554. (doi:10.1016/j.tree.2008.05.011).

20. Roux F., Mary-Huard T., Barillot E., Wenes E., Botran L., Durand S., Villoutreix R., Martin-Magniette M.L., Camilleri C., Budar F. 2016 Cytonuclear interactions affect adaptive traits of the annual plant Arabidopsis thaliana in the field. *Proc Natl Acad Sci U S A* **113**(13), 3687-3692. (doi:10.1073/pnas.1520687113).

21. Joseph B., Corwin J.A., Li B., Atwell S., Kliebenstein D.J. 2013 Cytoplasmic genetic variation and extensive cytonuclear interactions influence natural variation in the metabolome. *Elife* **2**, e00776. (doi:10.7554/eLife.00776).

22. James A.C., Ballard J.W.O. 2003 Mitochondrial genotype affects fitness in *Drosophila simulans*. *Genetics* **164**(1), 187-194.

23. Zhu C.T., Ingelmo P., Rand D.M. 2014 GxGxE for lifespan in Drosophila: mitochondrial, nuclear, and dietary interactions that modify longevity. *PLoS Genet* **10**(5), e1004354. (doi:10.1371/journal.pgen.1004354).

24. Camus M.F., Wolf J.B.W., Morrow E.H., Dowling D.K. 2015 Single Nucleotides in the mtDNA Sequence Modify Mitochondrial Molecular Function and Are Associated with Sex-Specific Effects on Fertility and Aging. *Curr Biol* **25**(20), 2717-2722. (doi:10.1016/j.cub.2015.09.012).

579 25. Immonen E., Collet M., Goenaga J., Arnqvist G. 2016 Direct and indirect genetic effects of
580 sex-specific mitonuclear epistasis on reproductive ageing. *Heredity* **116**(3), 338-347.
581 (doi:10.1038/hdy.2015.112).

582 26. Salminen T.S., Oliveira M.T., Cannino G., Lillsunde P., Jacobs H.T., Kaguni L.S. 2017
583 Mitochondrial genotype modulates mtDNA copy number and organismal phenotype in Drosophila.
584 *Mitochondrion*. (doi:10.1016/j.mito.2017.02.001).

585 27. Latorre-Pellicer A., Moreno-Loshuertos R., Lechuga-Vieco A.V., Sanchez-Cabo F., Torroja
586 C., Acin-Perez R., Calvo E., Aix E., Gonzalez-Guerra A., Logan A., et al. 2016 Mitochondrial and
587 nuclear DNA matching shapes metabolism and healthy ageing. *Nature* **535**(7613), 561-565.
588 (doi:10.1038/nature18618).

589 28. Ruiz-Pesini E., Lapena A.C., Diez-Sanchez C., Perez-Martos A., Montoya J., Alvarez E., Diaz
590 M., Urrieis A., Montoro L., Lopez-Perez M.J., et al. 2000 Human mtDNA haplogroups associated with
591 high or reduced spermatozoa motility. *Am J Hum Genet* **67**(3), 682-696. (doi:Doi 10.1086/303040).

592 29. De Benedictis G., Rose G., Carrieri G., De Luca M., Falcone E., Passarino G., Bonafe M.,
593 Monti D., Baggio G., Bertolini S., et al. 1999 Mitochondrial DNA inherited variants are associated with
594 successful aging and longevity in humans. *FASEB J* **13**(12), 1532-1536.

595 30. Frank S.A., Hurst L.D. 1996 Mitochondria and male disease. *Nature* **383**(6597), 224.
596 (doi:10.1038/383224a0).

597 31. Gemmell N.J., Metcalf V.J., Allendorf F.W. 2004 Mother's curse: the effect of mtDNA on
598 individual fitness and population viability. *Trends Ecol Evol* **19**(5), 238-244.

599 32. Beekman M., Dowling D.K., Aanen D.K. 2014 The costs of being male: are there sex-specific
600 effects of uniparental mitochondrial inheritance? *Philos T R Soc B* **369**(1646). (doi:ARTN 20130440

601 10.1098/rstb.2013.0440).

602 33. Unckless R.L., Herren J.K. 2009 Population genetics of sexually antagonistic mitochondrial
603 mutants under inbreeding. *J Theor Biol* **260**(1), 132-136. (doi:10.1016/j.jtbi.2009.06.004).

604 34. Smith S.R.T., Connallon T. 2017 The contribution of the mitochondrial genome to sex-specific
605 fitness variance. *Evolution* **71**(5), 1417-1424. (doi:10.1111/evo.13238).

606 35. Chase C.D. 2007 Cytoplasmic male sterility: a window to the world of plant mitochondrial-
607 nuclear interactions. *Trends Genet* **23**(2), 81-90. (doi:10.1016/j.tig.2006.12.004).

608 36. Patel M.R., Miriyala G.K., Littleton A.J., Yang H., Trinh K., Young J.M., Kennedy S.R.,
609 Yamashita Y.M., Pallanck L.J., Malik H.S. 2016 A mitochondrial DNA hypomorph of cytochrome
610 oxidase specifically impairs male fertility in Drosophila melanogaster. *Elife* **5**.
611 (doi:10.7554/eLife.16923).

612 37. Xu H., DeLuca S.Z., O'Farrell P.H. 2008 Manipulating the metazoan mitochondrial genome
613 with targeted restriction enzymes. *Science* **321**(5888), 575-577. (doi:10.1126/science.1160226).

614 38. Clancy D.J., Hime G.R., Shirras A.D. 2011 Cytoplasmic male sterility in *Drosophila*
615 *melanogaster* associated with a mitochondrial CYTB variant. *Heredity* **107**(4), 374-376.
616 (doi:10.1038/hdy.2011.12).

617 39. Nakada K., Sato A., Yoshida K., Morita T., Tanaka H., Inoue S., Yonekawa H., Hayashi J.
618 2006 Mitochondria-related male infertility. *Proc Natl Acad Sci U S A* **103**(41), 15148-15153.
619 (doi:10.1073/pnas.0604641103).

620 40. Smith S., Turbill C., Suchentrunk F. 2010 Introducing mother's curse: low male fertility
621 associated with an imported mtDNA haplotype in a captive colony of brown hares. *Mol Ecol* **19**(1), 36-
622 43. (doi:10.1111/j.1365-294X.2009.04444.x).

623 41. Martikainen M.H., Grady J.P., Ng Y.S., Alston C.L., Gorman G.S., Taylor R.W., McFarland
624 R., Turnbull D.M. 2017 Decreased male reproductive success in association with mitochondrial
625 dysfunction. *Eur J Hum Genet* **25**(10), 1162-1164. (doi:10.1038/ejhg.2017.114).

626 42. Milot E., Moreau C., Gagnon A., Cohen A.A., Brais B., Labuda D. 2017 Mother's curse
627 neutralizes natural selection against a human genetic disease over three centuries. *Nat Ecol Evol* **1**(9),
628 1400-1406. (doi:10.1038/s41559-017-0276-6).

629 43. Innocenti P., Morrow E.H., Dowling D.K. 2011 Experimental evidence supports a sex-specific
630 selective sieve in mitochondrial genome evolution. *Science* **332**(6031), 845-848.
631 (doi:10.1126/science.1201157).

632 44. Camus M.F., Clancy D.J., Dowling D.K. 2012 Mitochondria, maternal inheritance and male
633 ageing. *Curr Biol* **22**(18), 1717-1721. (doi:10.1016/j.cub.2012.07.018).

634 45. Jelic M., Arnqvist G., Kurbalija Novicic Z., Kenig B., Tanaskovic M., Andelkovic M.,
635 Stamenkovic-Radak M. 2015 Sex-specific effects of sympatric mitonuclear variation on fitness in
636 *Drosophila subobscura*. *BMC Evol Biol* **15**(1), 135.

637 46. Mossman J.A., Tross J.G., Li N., Wu Z., Rand D.M. 2016 Mitochondrial-Nuclear Interactions
638 Mediate Sex-Specific Transcriptional Profiles in Drosophila. *Genetics* **204**(2), 613-630.
639 (doi:10.1534/genetics.116.192328).

640 47. Mossman J.A., Tross J.G., Jourjine N.A., Li N., Wu Z., Rand D.M. 2017 Mitonuclear
641 Interactions Mediate Transcriptional Responses to Hypoxia in Drosophila. *Mol Biol Evol* **34**(2), 447-
642 466. (doi:10.1093/molbev/msw246).

643 48. Mossman J.A., Biancani L.M., Zhu C.T., Rand D.M. 2016 Mitonuclear Epistasis for
644 Development Time and Its Modification by Diet in Drosophila. *Genetics* **203**(1), 463-484.
645 (doi:10.1534/genetics.116.187286).

646 49. Vaught R., Dowling D.K. 2018 Maternal inheritance of mitochondria: implications for male
647 fertility? *Reproduction*, in press.

648 50. Dowling D.K., Maklakov A.A., Friberg U., Hailer F. 2009 Applying the genetic theories of
649 ageing to the cytoplasm: cytoplasmic genetic covariation for fitness and lifespan. *J Evol Biol* **22**(4),
650 818-827. (doi:10.1111/j.1420-9101.2009.01692.x).

651 51. Dean R., Zimmer F., Mank J.E. 2014 The Potential Role of Sexual Conflict and Sexual
652 Selection in Shaping the Genomic Distribution of Mito-nuclear Genes. *Genome Biol Evol* **6**(5), 1096-
653 1104. (doi:10.1093/gbe/evu063).

654 52. Rogell B., Dean R., Lemos B., Dowling D.K. 2014 Mito-nuclear interactions as drivers of gene
655 movement on and off the X-chromosome. *BMC Genomics* **15**. (doi:Artn 330

656 10.1186/1471-2164-15-330).

657 53. Drown D.M., Preuss K.M., Wade M.J. 2012 Evidence of a Paucity of Genes That Interact with
658 the Mitochondrion on the X in Mammals. *Genome Biol Evol* **4**(8), 875-880. (doi:10.1093/gbe/evs064).

659 54. Clancy D.J. 2008 Variation in mitochondrial genotype has substantial lifespan effects which
660 may be modulated by nuclear background. *Aging Cell* **7**(6), 795-804. (doi:10.1111/j.1474-
661 9726.2008.00428.x).

662 55. Wolff J.N., Camus M.F., Clancy D.J., Dowling D.K. 2016 Complete mitochondrial genome
663 sequences of thirteen globally sourced strains of fruit fly (*Drosophila melanogaster*) form a powerful
664 model for mitochondrial research. *Mitochondrial DNA A* **27**(6), 4672-4674.
665 (doi:10.3109/19401736.2015.1106496).

666 56. Connallon T., Camus M.F., Morrow E.H., Dowling D.K. 2018 Coadaptation of mitochondrial
667 and nuclear genes, and the cost of mother's curse. *Proceedings of the Royal Society B: Biological*
668 *Sciences* **285**(1871). (doi:10.1098/rspb.2017.2257).

669 57. Yee W.K.W., Sutton K.L., Dowling D.K. 2013 In vivo male fertility is affected by naturally
670 occurring mitochondrial haplotypes. *Curr Biol* **23**(2), R55-R56.
671 (doi:http://dx.doi.org/10.1016/j.cub.2012.12.002).

672 58. Wolff J.N., Tompkins D.M., Gemmell N.J., Dowling D.K. 2016 Mitonuclear interactions,
673 mtDNA-mediated thermal plasticity, and implications for the Trojan Female Technique for pest control.
674 *Sci Rep* **6**, 30016. (doi:10.1038/srep30016).

675 59. Clancy D.J., Hoffmann A.A. 1998 Environmental effects on cytoplasmic incompatibility and
676 bacterial load in Wolbachia-infected *Drosophila simulans*. *Entomol Exp Appl* **86**(1), 13-24.

677 60. ONeill S.L., Giordano R., Colbert A.M.E., Karr T.L., Robertson H.M. 1992 16s ribosomal-
678 RNA phylogenetic analysis of the bacterial endosymbionts associated with cytoplasmic incompatibility
679 in insects. *Proc Natl Acad Sci U S A* **89**(7), 2699-2702.

680 61. Yee W.K.W., Rogell B., Lemos B., Dowling D.K. 2015 Intergenomic interactions between
681 mitochondrial and Y-linked genes shape male mating patterns and fertility in *Drosophila melanogaster*.
682 *Evolution*, n/a-n/a. (doi:10.1111/evo.12788).

683 62. Korner-Nievergelt F., Roth T., Felten; S.v., Guelat J., Almasi B., Korner-Nievergelt P. 2005
684 *Bayesian Data Analysis in Ecology using Linear Models with*

685 *R, BUGS and Stan*, Elsevier.

686 63. Bates D., Maechler, M., Bolkler, B. 2012 *lme4*: Linear mixed-effects models using S4 classes.
687 *R package version 0999999-0* http://cranr-projectorg/package=lme4.

688 64. Fox J. 2002 *An R and S-Plus companion to applied regression*. Thousand Oaks, Calif., Sage
689 Publications; xvi, 312 p. p.

690 65. Halekoh U., Højsgaard S. 2014 A Kenward-Roger approximation and parametric bootstrap
691 methods for tests in linear mixed models: The R package pbkrtest. *Journal of Statistical Software* **59**(9),
692 32. (doi:10.18637/jss.v059.i09).

693 66. Mullahy J. 1986 Specification and Testing of Some Modified Count Data Models. *Journal of*
694 *Econometrics* **33**(3), 341-365. (doi:Doi 10.1016/0304-4076(86)90002-3).

695 67. Puth M.T., Neuhauser M., Ruxton G.D. 2015 On the variety of methods for calculating
696 confidence intervals by bootstrapping. *J Anim Ecol* **84**(4), 892-897. (doi:10.1111/1365-2656.12382).

697 68. Davison A.C., Hinkley D.V. 1997 *Bootstrap Methods and Their Applications*, Cambridge
698 University Press.

699 69. Wolff J.N., Gemmell N.J., Tompkins D.M., Dowling D.K. 2017 Introduction of a male-
700 harming mitochondrial haplotype via 'Trojan Females' achieves population suppression in fruit flies.
701 *Elife* **6**. (doi:10.7554/eLife.23551).

702 70. Hercus M.J., Hoffmann A.A. 2000 Maternal and grandmaternal age influence offspring fitness
703 in Drosophila. *Proc R Soc Biol Sci Ser B* **267**(1457), 2105-2110. (doi:10.1098/rspb.2000.1256).

704 71. Kern S., Ackermann M., Stearns S.C., Kawecki T.J. 2001 Decline in offspring viability as a
705 manifestation of aging in *Drosophila melianogaster*. *Evolution* **55**(9), 1822-1831.

706 72. Vijendravarma R.K., Kawecki T.J. 2015 Idiosyncratic evolution of maternal effects in response
707 to juvenile malnutrition in Drosophila. *J Evol Biol* **28**(4), 876-884. (doi:10.1111/jeb.12611).

708 73. Stearns S.C. 1992 *The Evolution of Life Histories*, OUP Oxford.

709 74. Montague J.R., Mangan R.L., Starmer W.T. 1981 Reproductive Allocation in the Hawaiian
710 Drosophilidae - Egg Size and Number. *Am Nat* **118**(6), 865-871. (doi:Doi 10.1086/283877).

711 75. Berrigan D. 1991 The Allometry of Egg Size and Number in Insects. *Oikos* **60**(3), 313-321.
712 (doi:Doi 10.2307/3545073).

713 76. Kondo R., Matsuura E.T., Chigusa S.I. 1992 Further observation of paternal transmission of
714 Drosophila mitochondrial DNA by PCR selective amplification method. *Genet Res* **59**(2), 81-84.

715 77. Dokianakis E., Ladoukakis E.D. 2014 Different degree of paternal mtDNA leakage between
716 male and female progeny in interspecific Drosophila crosses. *Ecology and evolution* **4**(13), 2633-2641.
717 (doi:10.1002/ece3.1069).

718 78. Wolff J.N., Nafisinia M., Sutovsky P., Ballard J.W. 2013 Paternal transmission of
719 mitochondrial DNA as an integral part of mitochondrial inheritance in metapopulations of Drosophila
720 simulans. *Heredity (Edinb)* **110**(1), 57-62. (doi:10.1038/hdy.2012.60).

721 79. Nunes M.D., Dolezal M., Schlotterer C. 2013 Extensive paternal mtDNA leakage in natural
722 populations of Drosophila melanogaster. *Mol Ecol* **22**(8), 2106-2117. (doi:10.1111/mec.12256).

80. Dobler R., Rogell B., Budar F., Dowling D.K. 2014 A meta-analysis of the strength and nature of cytoplasmic genetic effects. *J Evol Biol* **27**(10), 2021-2034.

81. Lemos B., Araripe L.O., Hartl D.L. 2008 Polymorphic Y chromosomes harbor cryptic variation with manifold functional consequences. *Science* **319**(5859), 91-93. (doi:10.1126/science.1148861).

82. Graze R.M., McIntyre L.M., Morse A.M., Boyd B.M., Nuzhdin S.V., Wayne M.L. 2014 What the X has to do with it: differences in regulatory variability between the sexes in Drosophila simulans. *Genome Biol Evol* **6**(4), 818-829. (doi:10.1093/gbe/evu060).

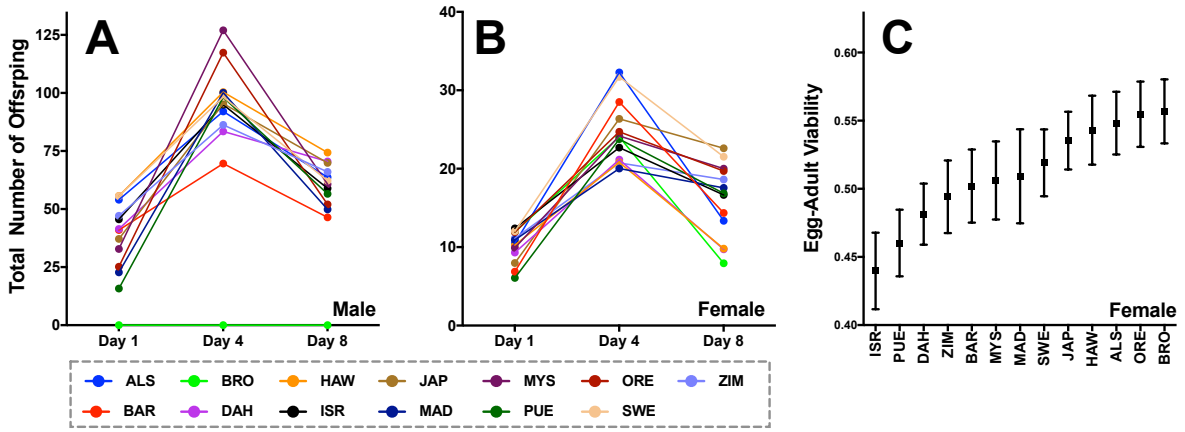
83. Lavrov D.V., Pett W. 2016 Animal Mitochondrial DNA as We Do Not Know It: mt-Genome Organization and Evolution in Nonbilaterian Lineages. *Genome Biol Evol* **8**(9), 2896-2913. (doi:10.1093/gbe/evw195).

Tables and Figures

Table 1: Mitochondrial genetic variance for male (**A**) short-burst offspring production and (**B**) sustained offspring production, and female (**C**) short-burst viability, (**D**) short-burst offspring production, (**E**) short-burst fecundity, and (**F**) sustained offspring production. Haplotype denotes the effect of mitochondrial strain (hence mtDNA haplotype), and Duplicate[Haplotype] denotes the mitochondrial strain duplicate. In the short-burst assays, each experiment was conducted over consecutive sampling blocks (Block), and required a dummy variable to account for overdispersion. In the sustained offspring production assays, each experiment was conducted over a number of consecutive days (Day; 8 in males, 13 in females). For all models, statistical significance of levels of mitochondrial genetic variance is based either on a parametric bootstrap model comparison (for the short burst traits), or Likelihood-ratio test (for the sustained traits). We also present variance (Var) and standard deviation (SD) for random effects.

A) Male short-burst offspring production			
	PB stat	**df**	**P**
Haplotype	2.625	1	0.044
	var		
duplicate[Haplotype]	8.795		
Haplotype	23.125		
Block	0		
Residual	464.21		
B) Male sustained offspring production			
		deviance	**P**
Haplotype		5.53	0.038
Haplotype × day		6.54	0.010
	var		
Haplotype	1.13E-07		
duplicate[Haplotype]	0.066		

Haplotype x day	0.01995	0.1412	
C) Female short-burst viability			
	PB stat	**df**	**P**
Haplotype	3.518	1	0.047
	var		
duplicate[Haplotype]	0.013		
Haplotype	0.017		
Block	0.122		
D) Female short-burst offspring production			
	PB stat	**df**	**P**
Haplotype	3.751	1	0.012
	var		
dummy	0.301		
duplicate[Haplotype]	0		
Haplotype	0.006		
Block	0.016		
E) Female short-burst fecundity			
	PB stat	**df**	**P**
Haplotype	0.0132	1	1
	var		
dummy	0.190		
duplicate[Haplotype]	0.001		
Haplotype	0		
Block	0.035		
F) Female sustained offspring production			
		deviance	**P**
Haplotype		6.04	<0.001
Haplotype × day		21.4	<0.001
	var		
Haplotype	0.005		
duplicate[Haplotype]	0.021		
Haplotype x day	0.004		



750 **Figure 1:** Mean number of offspring produced (reproductive success) for (**A**) males and (**B**) females across the mitochondrial strains, at 3 different age points of the sustained offspring production experiment. **C**) Female egg-adult viability (mean ± SE) (short-burst viability) across all mitochondrial lines.

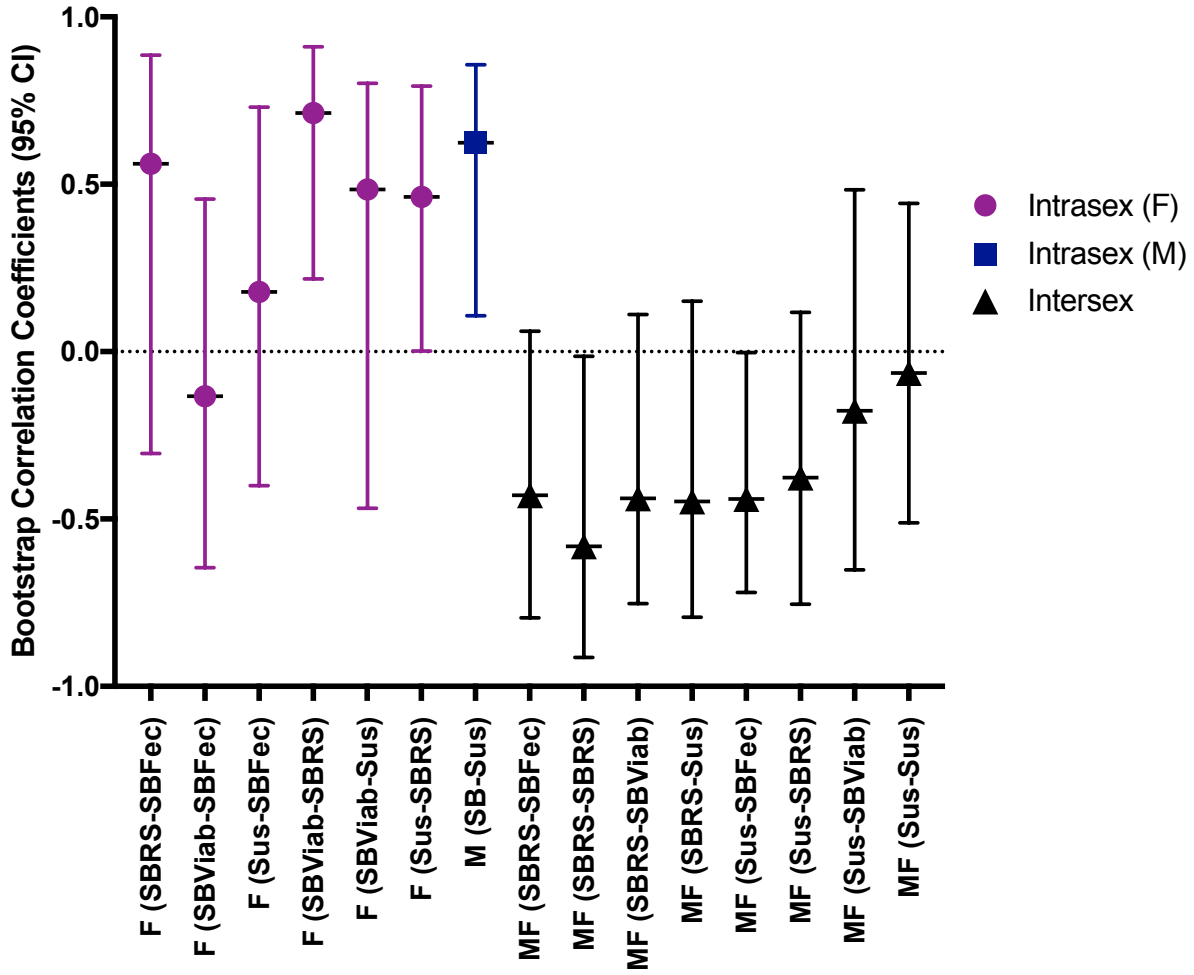


Figure 2: Bootstrapped correlation coefficients (± 95% confidence intervals) estimates of intra- and inter-sexual genetic correlations for male and female reproductive traits across mitochondrial haplotypes. Acronyms refer to: SBRS (short-burst reproductive success), SBFec (short-burst fecundity), SBViab (short-burst viability), Sus (sustained reproductive success). For instance, F(SBRS-SBFec) denotes the mitochondrial correlation between short-burst reproduction success and short-burst fecundity in females.