Mito-nuclear variation in transgenerational and reproductive effects of amino acid and lipid nutrition

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Abstract

Animals vary genetically in responses to dietary change. Both mitochondrial and nuclear genomes contribute to this variation, but the role of combinatorial "mito-nuclear" genetic variation is understudied. We do not know whether specific nutrients modify patterns of mito-nuclear variation, nor whether putative epigenetic mechanisms play a role. Here, we show that enriching dietary essential amino acids or lipids modifies patterns of mito-nuclear variation in *Drosophila* life-history, including transgenerational effects of lipids. Systematically evaluating alternative statistical models revealed that diet-mito-nuclear interactions were a leading driver of phenotypic variation. Mito-nuclear genotype repeatably predicted phenotypic impacts of nutritional changes, but genotypes bearing naturally co-occurring pairs of mitochondria and nuclei did not necessarily outperform novel pairings, suggesting that nutrition-dependent phenotypes cannot easily be optimised by matching mitochondria to coincident nuclear genotypes. These results enhance understanding of how nutrition and genetics sculpt phenotype, with potential implications for human mitochondrial transfer therapies.

Introduction

Nutrients and a genetic code are the fundaments of cellular life. Consequently, variation in nutrition and genotype are primary determinants of variation in health and biological fitness. Nutritional and genetic variation can also interact, meaning that different genotypes can respond distinctly to the same nutritional change (Dobson et al., 2015; Heymsfield and Wadden, 2017; Jumbo-Lucioni et al., 2010; Liao et al., 2009; Mulvey et al., 2017; Rikke et al., 2010). Biomedically, the interactive effects of diet and human genetic variation have led to interest in personalised nutrition (Ordovas et al., 2018), in which diets would be targeted individually, to treat maladies such as obesity and late-life morbidity (Fontana et al., 2010; Heymsfield and Wadden, 2017; Simpson and Raubenheimer, 2012). Biologically, unravelling genetic variation in how fitness is affected by nutrition will be a major part of bridging the genotype-phenotype gap.

Why do responses to diet vary? Ultimately, cellular metabolic function is the output of the coordinate activity of enzymes encoded by both the mitochondrial and nuclear genome. Both genomes exhibit sequence variation, yet the consequences of mitochondrial genome variation have often been overlooked. Effects of diet do indeed vary amongst mitochondrial haplotypes, altering metabolism and life-history (Aw et al., 2018; Bevers et al., 2019; Camus et al., 2020; Drummond et al., 2019; Mulvey et al., 2017; Nagarajan-Radha et al., 2019; Towarnicki and Ballard, 2018, 2017; Zhu et al., 2014). When both mitochondrial and nuclear genomes vary, we expect “mito-nuclear” variation, in which effects of variants in one can be either

buffered or augmented by variants in the other. In animals, this has been observed for fecundity (Mossman et al., 2016a), fertility (Camus et al., 2015), lifespan (Camus et al., 2015; Vaught et al., 2020), gene expression (Camus et al., 2015; Mossman et al., 2019, 2016b), and epigenomic modifications (Grunau et al., 2018). Novel mito-nuclear pairings can even change the course of nuclear genomic evolution (Healy and Burton, 2020), and lead to reproductive isolation (Hill, 2019; Ma et al., 2016). The intimacy of mito-nuclear coupling leads us to expect coadaptation (Dowling et al., 2008), and consequently to predict that pairs from the same population (sympatric) should function optimally. Some studies have supported this prediction (Baris et al., 2017; Chang et al., 2016; Ellison and Burton, 2006; Ma et al., 2016; Meiklejohn et al., 2013), but others have not (Dowling et al., 2010; Mossman et al., 2016b; Vaught et al., 2020). Despite genetic variation in responses to dietary variation, and the emerging importance of mito-nuclear interactions in overall genetic variation, there has been relatively little work to connect the three, by asking whether the phenotypic impacts of dietary alterations are subject to mito-nuclear interactions. Trailblazing work in *Drosophila melanogaster* has shown diet-mito-nuclear (DMN) variation in development time, lifespan and fecundity (Camus et al., 2020; Montooth et al., 2019; Mossman et al., 2016a; Rand et al., 2018; Zhu et al., 2014), but specific causal nutrients and cellular mechanisms remain to be established.

Perhaps diet's most immediate fitness effect is regulation of reproduction. Parents can gain fitness by increasing offspring quantity, or by promoting offspring quality (Lack, 1947; Smith et al., 1989; Stearns, 1992). Offspring quality and quantity will depend on fertility, fecundity, and development (viability) of those offspring, each of

which are influenced by diet. Equivalent changes in these traits can be elicited in distinct taxa by equivalent dietary changes: for example, in numerous studies of insects and mammals, amino acid nutrition has proven consistently important for fertility, fecundity and offspring development (Bong et al., 2014; Crean and Senior, 2019; Dong et al., 2016; Fanson et al., 2012; Grandison et al., 2009; Jensen et al., 2015; Lee et al., 2008; Leitão-Gonçalves et al., 2017; Liang and Zhang, 2006; Ma et al., 2020; Maklakov et al., 2008; McCracken et al., 2020; Mirth et al., 2018; Skorupa et al., 2008; Solon-Biet et al., 2015; Svajgr et al., 1972; Tufarelli et al., 2015; Winship et al., 2018; Wong et al., 2014a; Zanco et al., 2020); whereas high-lipid or high-sugar diets are obesogenic (Dobson et al., 2019; Hariri and Thibault, 2010; Jang et al., 2018; Rivera et al., 2015; Saud et al., 2015; Wong et al., 2014b; Woodcock et al., 2015; Zhao et al., 2020). However, importantly, reproduction is not only regulated by the individual's current diet: large nutritional fluctuations can induce enduring physiological and molecular changes, which can transmit across generations, influencing quality of offspring and even grand-offspring (Barker and Osmond, 1986; Barker and Thornburg, 2013; Deas et al., 2019; Duque-Guimarães and Ozanne, 2017; Holland et al., 2016; Li et al., 2018; Liang and Zhang, 2006; Öst et al., 2014; Rivera et al., 2015; Stefana et al., 2017; Wei et al., 2014; Winship et al., 2018). Thus, diet can influence reproduction via lasting, programmed effects on offspring quality, which point to epigenetic reprogramming, whilst acute effects of diet on both offspring quality and quantity point to metabolic changes. But do these effects vary amongst genotypes? If so, is variation shaped by mitochondrial genomes, nuclear genomes, or by mito-nuclear genotype?

Understanding health impacts of mito-nuclear interactions is not just a current topic in biology. It is also pertinent biomedically, because dietary intervention is a preeminent treatment for human mitochondrial disease (Gorman et al., 2016), and also following the advent of mitochondrial replacement therapies (Craven et al., 2010; Tachibana et al., 2018; Wolf et al., 2015). These procedures have been licensed in the United Kingdom and a few other countries (Cohen et al., 2020). They aim to treat mitochondrial diseases by placing embryonic nuclei into the cytoplasm, and therefore mitochondrial context, of a disease-free donor. Some argue that the conservation of mitochondrial function and the body of evidence for mito-nuclear interactions suggest that undesired consequences may ensue if the donor mitochondria function poorly with the nuclear genome (Dobler et al., 2018; Dunham-Snary and Ballinger, 2015; Reinhardt et al., 2013); although the UK Human Fertility and Embryology Authority concluded that risks remain “purely theoretical” (HFEA, 2016). To solve this debate it will be important to identify which traits to evaluate in patients, whether costs or benefits may be diet-dependent, and whether transgenerational variation may ensue.

Here, we identify specific nutrients sufficient to drive diet-mito-nuclear variation in *Drosophila* life-history, and show that specific mito-nuclear genotypes can exhibit long-lasting, transgenerational responses to transient dietary changes. We bred a panel of flies with replicated and fully-factorial mito-nuclear variation, and show that essential amino acids and lipids can elicit novel patterns of variation in fecundity and offspring performance (development time). We then show for the first time that the capacity for effects of parental diet on offspring performance are genetically variable,

and mito-nuclear in this case. To elucidate overall variation in fitness, we integrate our analyses of offspring quality and quantity, showing mito-nuclear variation in how diet influences reproductive investment; and also that "mis-matched" pairs of mitochondrial and nuclear genomes do not necessarily perform worse than matched pairs. Finally, we evaluate performance of alternative models of our extensive datasets, in which interactions between diet, mitochondrial and nuclear haplotype were systematically included or excluded. We find that models which include mito-nuclear or diet-mito-nuclear interactions are favoured overwhelmingly over models which assume no interaction, suggesting that models which do not account for these terms are not adequate to explain variance in fitness and health.

Results

We used a set of *D. melanogaster* lines comprising replicated, fully-factorial combinations of varied mitochondrial and nuclear genomes (Figure 1a), derived from heterogeneous populations originally isolated in Australia and Benin (formerly Dahomey). For brevity, we term Australian and Beninese genotypes *A* and *B*, respectively. The populations were introgressed either reciprocally (*AB* = *A* mitochondria / *B* nuclei; *BA* = *B* mitochondria / *A* nuclei), or to themselves to control for introgression and drift (i.e. *AA* = *A* mitochondria / *A* nuclei; *BB* = *B* mitochondria / *B* nuclei). In each generation, a fixed number of females per line were mated to a fixed number of males from the ancestral population. Each combination was replicated in triplicate, generating twelve lines altogether (e.g. *AA1, AA2, AA3, BA1, BA2,* etc). The lines were created to retain equivalent standing nuclear variation from the ancestral populations in each of the two mitochondrial backgrounds. Introgressions had been iterated for 74 generations at the time of our first experiment, and so the percentage of nuclear genome that remained unreplaced by introgression in the *AB* and *BA* genotypes was expected to be negligibly small (~5.3e-21%). Ancestors of all lines were cleared of cytoplasmic *Wolbachia* endosymbionts long prior to these experiments (Vaught et al., 2020), and so we expect cytoplasmic genetic complements to comprise only mitochondria. The *A* and *B* nuclear genomes do not appear to select for differential mitochondrial haplotypes in this design (Vaught et al., 2020), and we expect the rigorous backcrossing regime to minimize differential segregation of nuclear genomic variants over *A* and *B*

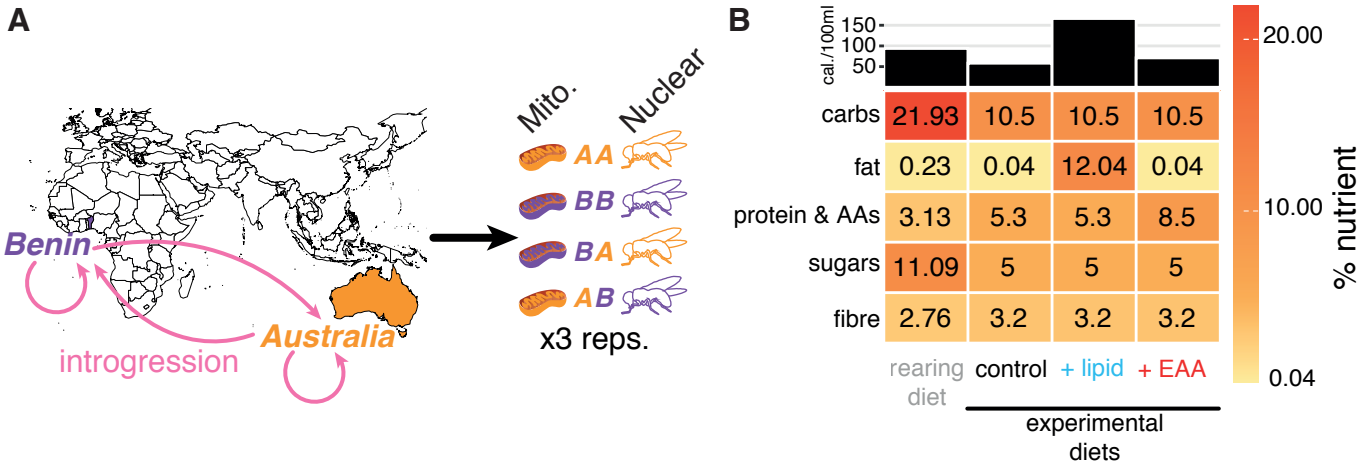
171 mitochondria: this experimental design should therefore produce fully-factorial mito-

172 nuclear variation.

173

174

Figure 1



176 **Figure 1. Study design. A.** Genetically distinct and heterogeneous populations of
177 flies were originally isolated from from Benin (formerly Dahomey), and New South
178 Wales, Australia. Mito-nuclear variation was generated by reciprocally introgressing
179 each line either into its own respective cytoplasmic background, or into the other
180 line's cytoplasmic background. Three biologically independent replicate lines were
181 established per mito-nuclear combination. 74 iterations of introgression were
182 completed before experiments. **B.** Fly lines were cultured on rearing diet and then
183 fed experimental diets in adulthood. The heatmap shows estimated content of
184 different macronutrients, bars at the top indicating caloric content.
185

EAA and lipid nutrition are sufficient to drive diet-mito-nuclear variation in life-history

To identify nutrients which could drive DMN interactions, we added nutrients to a sugar-yeast diet (Bass et al., 2007), as previously (Dobson et al., 2018; Emran et al., 2014; Grandison et al., 2009). To ensure that potential novelty effects were evenly distributed amongst conditions, the baseline diet was distinct from the medium on which the lines were maintained and developed. For experimental diets, we added one of two nutrients to the baseline, selected for their important and conserved health impacts. Essential amino acids (EAAs) are metabolised by mitochondria (Mariño et al., 2014), and their specific enrichment is sufficient to recapitulate fecundity effects of adding yeast (Emran et al., 2014; Grandison et al., 2009). We also manipulated dietary lipid, because mitochondria metabolise fatty acids, and high-fat animal diets can model Western human disease (Hariri and Thibault, 2010; Heymsfield and Wadden, 2017). Lipid source can have large physiological effects (Brankatschk et al., 2018), and flies are largely vegan in nature (Knittelfelder et al., 2020). Therefore, we supplied a plant-based lipid which set in agar (i.e. in contrast to oils), by adding margarine to the diet (15% w/v, after (Woodcock et al., 2015)). Whilst this design did not control calories, it enabled us to test whether specific, ecologically-relevant nutrients can cause diet-mito-nuclear variation. Estimated nutrient contents (Lesperance and Broderick, 2020) are presented in Figure 1b.

Previous work showed that manipulating complex nutrient sources elicits life-history variation in specific mito-nuclear genotypes, including in fecundity (egg laying), and

offspring development (Camus et al., 2020; Montooth et al., 2019; Mossman et al., 2016a; Rand et al., 2018; Zhu et al., 2014). We tested whether EAAs and lipid were sufficient to cause these effects. Starting three days after adult emergence, the control, EAA-enriched and lipid-enriched foods were fed to flies of all lines for seven days. We restricted treatment to adults, to avoid developmental variation (Figure 2a). This experiment was repeated twice. In both experiments, EAAs increased fecundity, and lipids repressed fecundity. Fecundity was correlated between the two experiments, indicating technical repeatability (Figure 2b). Biologically, two important trends were evident. First, genetic replicates consistently grouped together, with all three replicates of each mito-nuclear genotype tending to sit within one standard deviation of each other (Figure S1), despite having been separated for >70 generations of introgression. This suggested a highly stereotyped effect of mito-nuclear genotype on fecundity, which overcame any genetic drift in the fly lines. Therefore, for simplification, we replotted the data without distinguishing experimental or genetic replicates (Figure 2c). This outlined the second important trend, that mito-nuclear variation was shaped by diet, in agreement with a GLMM analysis indicating a three-way interaction ($p=0.009$, Table S1). To visualise pairwise statistical differences between every genotype, we used *post-hoc* estimated marginal means (EMMs (Searle et al., 1980)) analysis. These EMMs represented the GLMM output per experimental condition, and so their correlation to the raw data indicated a good model fit (Figure 2c). Pairwise comparison of EMMs was consistent with our interpretation that overall patterns of among-genotype variation in fecundity were diet-dependent (Table S2). These results revealed distinct patterns of mito-nuclear variation on different diets, and that impacts of consuming a certain diet depend on

mito-nuclear genotype.

Animal fitness is a function of both quantity and quality of offspring, and DMN variation in offspring development has already been documented. Having shown that EAAs and lipid were sufficient to cause DMN variation in egg laying, we hypothesised that variation in offspring quality may additionally manifest in fertility of those eggs. To test this, after parents had been fed experimental diets for a week as previously, we allowed eggs laid over 24h to develop in the same experimental media. We scored time to adult and pupal emergence, along with sex in adults. We take proportion of eggs emerging as a metric of parental reproductive success, and time to development as a metric of relative offspring fitness. In every mito-nuclear genotype, food enrichment with EAAs or lipid either reduced the proportion developing, extended development time, or both. However, the magnitude of these effects was highly genotype-dependent (Figure 2d). For explicit analysis, we fit Cox mixed-effects (CME) survival models. The DMN interaction was statistically significant for emergence of both males and females to adulthood (each sex $p<0.0005$, Table S3, Figure S2). We did not detect robust sex-specificity (Supplementary Text, Figure S2), and results were broadly repeatable within the triplicates of each fly genotype (Figure S2). To confirm genotype-specific effects, we applied *post-hoc* EMM tests to the CME model. This analysis aggregated time to development and proportion developing into a single quantitative output, because the EMMs were a posterior view of our CME analysis, which integrates time to development and proportion developing. In this framework, high EMMs correspond to faster and/or more frequent development, and vice-versa (Figure 2e). These

EMMs confirmed that EAA or lipid enrichment disadvantaged specific genotypes (Figure 2e). The *AA* flies bore a particular cost of reduced survival on EAA food. The differential ranking of EMMs across genotypes on different diets indicated that the pattern of mito-nuclear genetic variation was dependent on EAA and lipid nutrition. Indeed, as with fecundity, there was little variation within each triplicate per mito-nuclear genotype (except that the detrimental effect of EAAs on development of *AA* flies was pushed to lethality in the *AA3* line, and *BB3* was somewhat more lipid-resistant than *BB1* and *BB2* (Figure S2)), suggesting that the developmental response to nutrition is highly stereotyped by mito-nuclear genotype. A parallel analysis of these same individuals' time to pupation revealed congruent patterns (Figure S3, Figure S4, Table S4). Altogether, these results show that either lipid or EAA enrichment are sufficient to potently modify developmental outputs of mito-nuclear genetic variation.

Figure 2

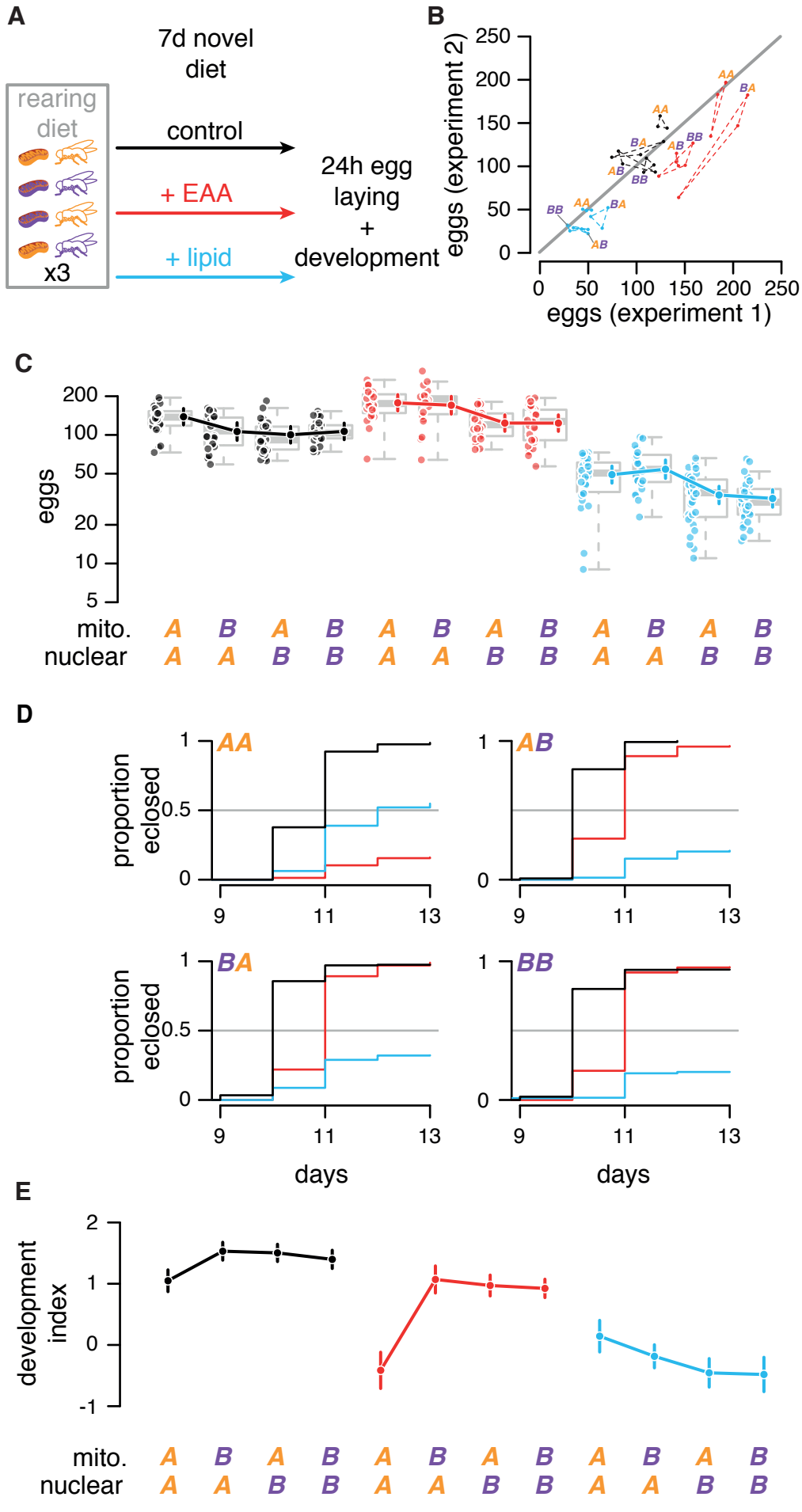


Figure 2. Diet-mito-nuclear interactions modulate *Drosophila* fecundity. A. Experimental design. The 12 mito-nuclear lines were reared from egg to adult on rearing food, and allocated at random to experimental media 6-48h after eclosion, at a density of five of each sex per vial. After seven days, flies laid eggs on fresh food for 24 hours. **B.** Intra-line correlations in impact of diet-mito-nuclear variation on egg laying. Each point shows mean egg laying per line per diet in each of two replicate experiments, with the replicates of each haplotype grouped by dashed lines. Means were correlated between experiments (Pearson's r = 0.87, p = 7.6e-12). **C.** Impact of diet on fecundity is determined by mito-nuclear variation. Data are pooled across the experimental and genetic replicates presented in panel B. Boxplots show medians, first and third quartiles and 5th and 95th percentiles. Connected points to right of each box show *post-hoc* comparisons of means (by estimated marginal means with 95% confidence intervals, calculated from a generalised linear mixed effects model, showing exponent of EMMs in order to fit to original data scale). Translucent points to left of each box show raw data. Complementary statistical analysis presented in Table S1 and Table S2. **D.** Kaplan-Meier plots of development (egg-to-adult) after parents fed and offspring developed on experimental media. Mito-nuclear genotypes are indicated in top-left of each panel. **E.** Estimated marginal means (EMMs) with 95% confidence intervals summarising survival analysis (Cox mixed effects) of data from D. Higher values correspond to faster development and/or increased fertility, lower values correspond to slower development and/or reduced viability. Accompanying statistical analysis presented in Table S3.

297 **Persistent life-history effects of dietary EAAs and lipid in specific mito-nuclear**
298 **genotypes**
299
300 DMN interactions could occur via numerous mechanisms, but no candidate has yet
301 been confirmed. Dietary variation induces not only immediate changes to
302 metabolism, but also long-lasting epigenetic changes (Barker and Osmond, 1986;
303 Barker and Thornburg, 2013; Deas et al., 2019; Dobson et al., 2017; Duque-
304 Guimarães and Ozanne, 2017; Holland et al., 2016; Li et al., 2018; Liang and Zhang,
305 2006; Öst et al., 2014; Rivera et al., 2015; Stefana et al., 2017; Wei et al., 2014;
306 Winship et al., 2018). Mitochondria metabolise substrates for epigenetic
307 modifications (Eisenberg et al., 2014; Mariño et al., 2014; Trefely et al., 2020),
308 suggesting that nutritional regulation of epigenetically-encoded traits may vary
309 amongst mito-nuclear genotypes. We asked whether EAAs and lipid might drive
310 DMN interactions for life-history via long-term reprogramming. Whilst such effects
311 are consistent with epigenetic modifications, we conservatively avoid that term for
312 our data because we did not measure epigenetic marks directly. We tested for long-
313 lasting effects of transient EAA and lipid enrichment, both in the generation that
314 experienced the nutritional manipulation, and in their offspring.
315
316 We conducted a second fecundity experiment, with a week of adult-onset feeding on
317 experimental diets as previously, but followed by a diet switch back to standardised
318 rearing medium. Fecundity was measured at the end of experimental diet feeding,
319 and 24h after switching back to standardised medium, yielding vial-matched egg
320 counts pre- and post- switch (Figure 3a). The overall effect of experimental diets was

equivalent before and after the switch (EAAs elevated fecundity, lipid repressed fecundity), confirming that effects of experimental diets persisted for the 24h feeding on standardised diet (Figure 3b). Before the switch, on experimental diets, a DMN interaction was once again evident (Figure 3c, Table S5, Figure S5a), and pooling these data with preceding data (Figure 2) yielded the same qualitative result (Figure S6, Table S6), confirming that DMN effects were consistent across experiments.

A GLMM analysis confirmed that, after switching back to standardised diet, the main effects of prior EAA or lipid feeding persisted (Figure 3d, Table S7, Figure S5b). However, the DMN interaction did not persist (Figure 3d, Table S7, Figure S5b). These findings suggested that EAA and lipid modify the manifestation of mito-nuclear variation in fecundity via transient effects; on top of longer-term impacts which do not depend on mito-nuclear genotype. If so, recovery of fecundity after diet switch should exhibit a DMN interaction. We estimated recovery rate as the ratio of eggs laid per vial after/before the switch, with greater ratios indicating greater plasticity. Recovery from EAA or lipid feeding was indeed variable amongst mito-nuclear genotypes (Figure 3e; GLMM p=2.91E-09, Table S8; Figure S5c). Altogether, these analyses revealed two layers of variation, with genotype-independent main effects of dietary EAAs and lipid which persist after feeding, and additional DMN variation whilst feeding on the diets, but not after. This suggested that EAAs and lipid can have lasting fecundity effects which generalise amongst mito-nuclear genotypes, but did not exclude the possibility of longer-term, transgenerational diet-mito-nuclear impacts on offspring.

345 Diet can modulate fitness by effects that span one or more generations (Camilleri-
346 Carter et al., 2019; Deas et al., 2019; Öst et al., 2014; Rivera et al., 2015). We are
347 not aware of a prior demonstration of genetic variation in such transgenerational
348 reprogramming, nor of whether it is subject specifically to mito-nuclear variation.
349 Since we had already observed that feeding EAAs and lipid to both parents and
350 offspring modified the manifestation of mito-nuclear genetic variation in development,
351 we speculated that some of that variation could be due to differential legacies of
352 parental diet. If so, we expected that feeding EAAs or lipid to parents alone would be
353 sufficient to modify patterns of mito-nuclear variation in offspring development, even
354 when all offspring developed in a standardized medium.
355
356 We restricted diet manipulations to adults for one week, before egg-laying on
357 standardised developmental medium, and allowing those eggs to develop on that
358 standardised medium. This design differs from the preceding study of developmental
359 effects (Figure 2d-e), because diet was only manipulated in the parental generation.
360 Therefore any diet-mito-nuclear effect (or lower-order diet effect) detected in this
361 experiment is transgenerational. We scored time to adult emergence (along with sex
362 and pupation), and again, results were consistent within each triplicate fly genotype
363 (Figure S2), and so data were plotted after pooling all three replicates per genotype.
364 Plotting these data suggested that, indeed, parental nutrition had genotype-specific
365 effects on development (Figure 3f). This was confirmed by CME analysis (Table S9,
366 males p=0.003, female p=0.03), without strong evidence of sex bias (Supplementary
367 Text, Figure S2). EMM analysis confirmed genotype-specific impacts (Figure 3g):
368 Specifically, *AA* flies developed on average a full day faster when parents were fed

lipid-enriched food (Figure 3f). A parallel analysis of these same individuals' time to pupation revealed congruent patterns (Figure S3, Figure S4, Table S10). Whilst some variation may be due to selection, our experimental design largely excludes this possibility, because the only nutritional variation that individuals under study experienced was *in utero* when mothers fed on experimental diets, before post-embryonic development in standardised medium; and variation in rearing density was also accounted for statistically. Furthermore, since the major effect we observe is an acceleration of development in one condition (*AA* flies fed lipid), selection would have to be otherwise ubiquitous but relaxed specifically for this condition. The results therefore indicate that specific mito-nuclear genotypes are susceptible to transgenerational effects of parental nutrition, in this case lipid.

Notably, the transgenerational effects we observed in development did not correlate patterns when both parents and offspring were fed the diets. Our studies of transgenerational and intergenerational effects of EAAs and lipid were conducted at the same time (Figure 2d-e flies developed from eggs plotted in Figure 3c, Figure 3f-g from eggs in Figure 3d), validating this comparison. Altogether, this discord suggests that nutrients can induce transgenerational effects in specific mito-nuclear genotypes, but this reprogramming in combination with ongoing nutrient enrichment in offspring leads to additional changes to patterns of phenotypic variation.

Figure 3

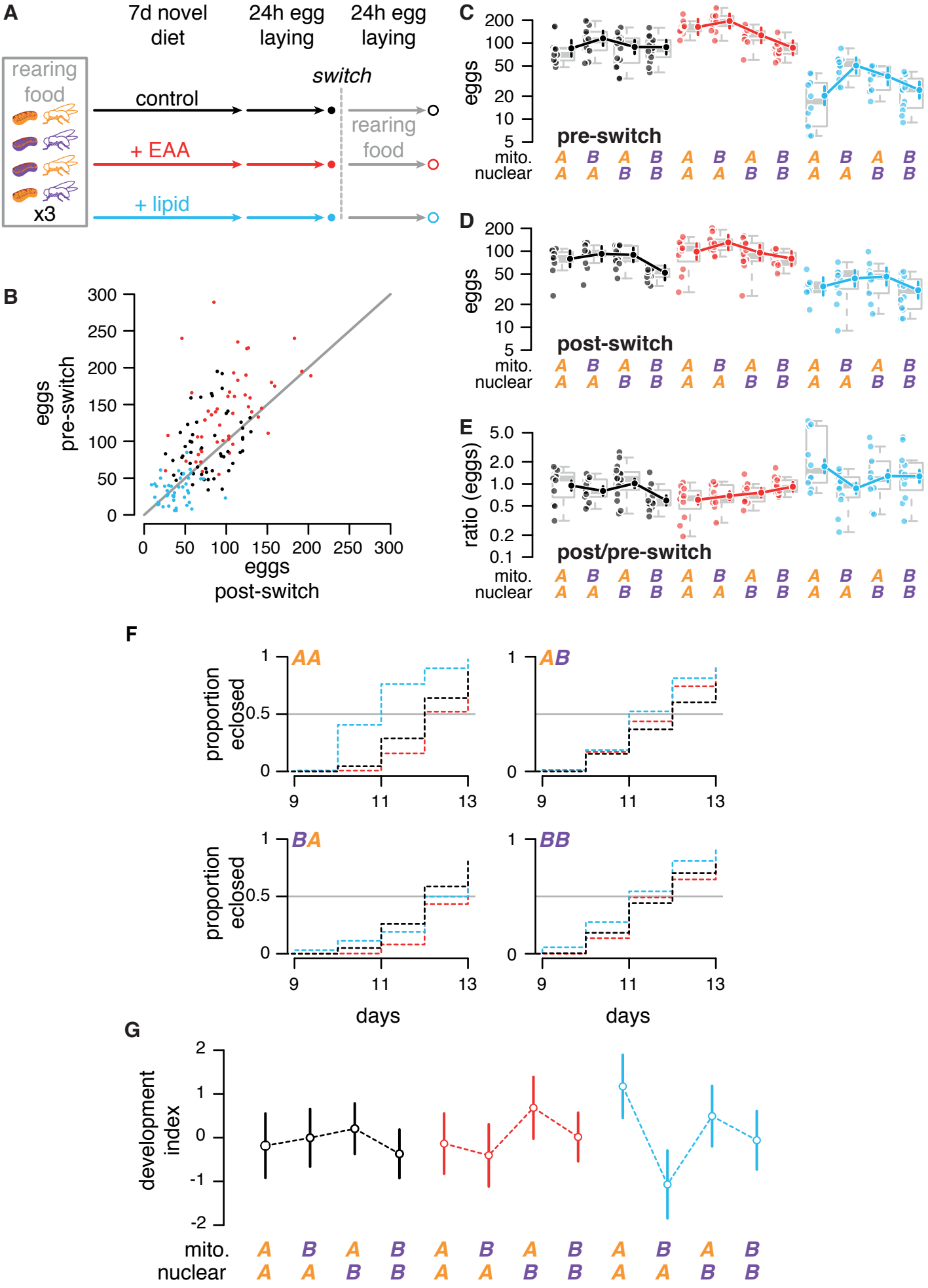


Figure 3. Performance is determined by interplay of mito-nuclear genotype and persistent intragenerational and transgenerational effects of diet. A. Experimental design. 12 Mito-nuclear lines were reared from egg to adult on rearing food, and allocated at random to experimental media 6-48h after eclosion, at a density of five of each sex per vial. After seven days, flies laid eggs on fresh food for 24 hours. Females were then reassigned back to standardised rearing medium, and eggs were laid for a further 24h. **B.** Correspondence between egg laying rate per vial, before and after switch from experimental diets. Egg laying rates before and after switch across all diets were correlated (Pearson's product-moment correlation, $r=0.65$, $p<2.2e-16$). Line of equivalence shown in grey. **C.** Egg laying rate before dietary switch is determined by mito-nuclear variation (*vis* Fig 2C). Boxplots show medians, first and third quartiles and 5th and 95th percentiles. Connected points to right of boxes show *post-hoc* comparisons of means (by estimated marginal means with 95% confidence intervals, calculated from a generalised linear mixed effects model). Translucent points to left of each box show raw data. Complementary statistical analysis presented in Table S5. **D.** Diet-mito-nuclear interactions do not persist 24h after switch from experimental media. Statistical analysis presented in Table S7. **E.** Mito-nuclear interactions govern recovery of fecundity after switch from experimental media. Statistical analysis presented in Table S8. **F.** Kaplan-Meier plots of development to adulthood of eggs laid on developmental media, after parents had previously fed on experimental media. Mito-nuclear genotypes are indicated in top-left of each panel. **G.** Estimated marginal means (EMMs) with 95% confidence intervals summarising survival analysis (Cox mixed effects) of data from F. Higher values correspond to faster development and/or increased fertility, lower values

415 correspond to slower development and/or reduced viability. Accompanying statistical

416 analysis presented in Table S9.

417

418

419 **Mito-nuclear (mis-)matching does not predict differential life-history responses**
420 **to nutrition**

421

422 Together, offspring quantity and quality define fitness (Lack, 1947; Stearns, 1992).
423 Our analyses suggested that both fecundity (quantity) and fertility (quality) are
424 regulated by EAAs and lipid, but specific mito-nuclear genotypes are differentially
425 receptive or resistant to these effects. To date, the widely-held prediction of a
426 disadvantage to "mis-matched" mito-nuclear pairings, has received equivocal
427 support (Baris et al., 2017; Chang et al., 2016; Ellison and Burton, 2006; Ma et al.,
428 2016; Meiklejohn et al., 2013). Our datasets provided an opportunity to ask whether
429 this prediction applied in terms of differential responses to specific nutrients; whilst
430 taking an integrative view of fitness, encompassing both offspring quality and
431 quantity, and putatively epigenetic effects. To this end, we integrated outputs of our
432 previous analyses by plotted EMMs from against one another, indicating effects of
433 diet and mito-nuclear genotype on offspring quality and quantity, and the relationship
434 between them. We did this separately for data from parents and offspring both fed
435 experimental diets (flies in Figure 2e, which developed from eggs in Figure 3c), and
436 from parent-restricted feeding (flies in Figure 3g, which developed from eggs in
437 Figure 3d).

438

439 First, we examined impacts of EAA feeding. Visual inspection indicated that EAA
440 enrichment enhanced offspring quantity, but compromised offspring quality in flies of
441 all genotypes except *BB* (Figure 4a). This was surprising, because we had assumed

that parents should discriminate diets that maximise their own fitness, and therefore that parental egg-laying would correlate offspring development indices. Instead, the apparent tradeoff indicates either that (1) EAAs signal diet quality to parents on normal diets when yeast is the source of most nutrients (including protein), but our manipulations rendered this quality signal misleading (Zanco et al., 2020); or that (2) diet is subject to parent-offspring conflict (Trivers, 1974), with parents maximising their fitness by producing more eggs on high EAA-diets, despite the cost to individual offspring.

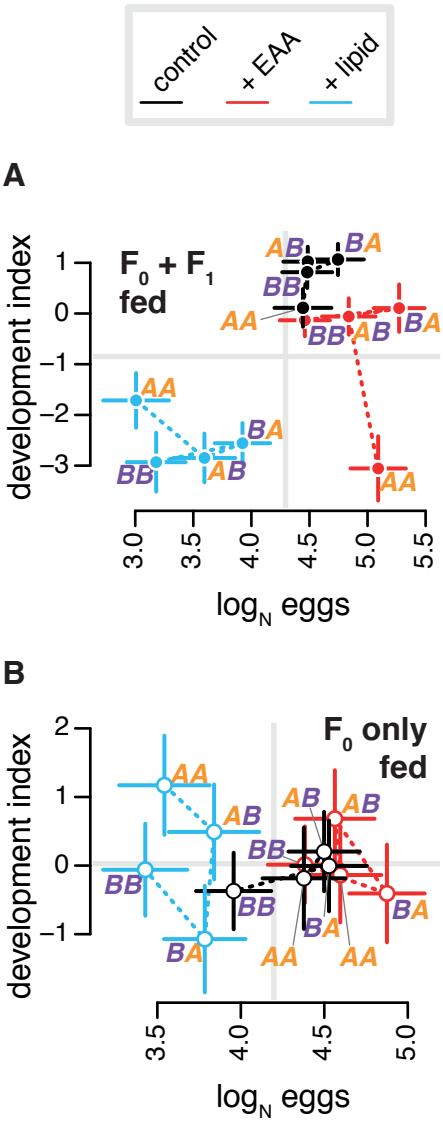
Were costs of EAAs genotype-specific? The quality-quantity tradeoff induced by EAA feeding was exaggerated dramatically in *AA* flies, whose eggs developed exceptionally poorly on EAA-enriched food. However, this cost of EAAs was rescued in both *BA* and *AB* genotypes, indicating that neither *A* mitochondria nor *A* nuclei alone were sufficient for *AA*'s diet-dependent phenotype, and therefore that these effects are mito-nuclear (Figure 4a). This outlined a second surprising finding, in which a sympatric mito-nuclear pairing endured a higher cost of dietary EAAs than allopatric pairings (Figure 4a). Thus, unexpected, costly, and definitively mito-nuclear patterns of variation can emerge on specific diets. However, no clear effects of past EAA feeding on offspring quality or quantity were evident (Figure 4b). Together, these results indicated that EAAs interact with mito-nuclear genotype during feeding, but not thereafter. This suggests that the reproductive DMN variation we observed upon EAA enrichment is an acute effect of diet, and therefore likely metabolic and not epigenetic.

Next we examined the impact of lipid enrichment. Lipid mostly compromised both quality and quantity, but one genotype stood out: *AA* flies were most resistant to the reduction in offspring quality induced by high-lipid diet, and their development index on lipid-enriched food was in fact better than on EAA-enriched food (Figure 4a). By this metric, *AA* flies' performance was substantially better than the *AB* or *BA* flies, confirming that neither *A* mitochondria nor *A* nuclei alone were sufficient to explain *AA*'s response to diet. This again suggested that genotypes which exhibit notable responses to diet do not necessarily bear allopatric combinations of mitochondria and nuclear genomes. Furthermore, the finding that the *AA* genotype was beneficial in one condition (EAA) despite costs in another (lipid) reveals that it was not simply sick as would be expected with a poor-functioning mito-nuclear pairing, but instead exhibits its own genotype-specific pattern of response to diet.

Finally, we evaluated the impact on offspring of parent-restricted lipid enrichment. All genotypes bore costs, but impacts were highly genotype-specific. Again, the *AA* flies stood out. Specifically, *AA* flies bore a cost of parental lipid feeding on offspring quantity, but appeared to enjoy a compensatory benefit from enhanced offspring quality. In fact, these flies exhibited the highest offspring quality (development index) observed in any condition in our study. These benefits were not evident in either the *AB* or *BA* flies, confirming again that neither *A* mitochondria nor *A* nuclei alone are sufficient to explain diet-dependent phenotype: the observed effects are mito-nuclear.

Altogether, these results confirm diet-mito-nuclear shaping of fitness. They show that diet can induce phenotypic change in specific mito-nuclear genotypes, with neither *A* mitochondria nor *A* nuclei accounting for the *AA* flies' differential response to diet. This suggests that mito-nuclear "matching" is not necessarily optimal. In wild populations, the cost of EAA susceptibility may be balanced by lipid resistance, in which case these flies were not sick in all circumstances, but rather exhibit a private pattern of genotype-specific variation, which is evident only on specific diets.

Figure 4



498 **Figure 4. Mito-nuclear interactions dictate dietary modulation of offspring**
499 **quality versus quantity.** Panels show relationships per diet and per genotype (data
500 from Figures 2-3). **A** offspring development index and eggs laid, when both parents
501 and offspring were fed experimental diets, and **B** development index before switch
502 and release of egg laying after diet, when parents were switched from experimental
503 to standard diets. Points show EMMs±95% confidence intervals, and grey lines
504 indicate grand means.
505

506

507 **Diet-mito-nuclear effects cause substantial biological variation**

508

509 Finally, we sought to test the importance of the DMN interactions that we identified
510 for overall variation in our dataset, for two reasons. First, because the DMN patterns
511 that we identified may be of previously-unrecognised importance in nature. Second,
512 because mitochondrial transfer therapy (Craven et al., 2010; Tachibana et al., 2018;
513 Wolf et al., 2015) presents the possibility of a medical procedure giving patients
514 novel combinations of mitochondrial and nuclear genomes, which our results
515 suggest may lead to unpredictable responses to nutritional variation. Therefore, we
516 sought effect sizes of DMN terms, complementing our preceding analyses by
517 outlining whether DMN effects were substantial, or biologically trivial despite
518 statistical significance.

519

520 Since *de facto* effect sizes for interaction terms are not easily interpreted, we used
521 optimization procedures to compare simple models of the data against those
522 including DMN interactions (Akaike, 1974). Calculating Akaike Weights reveals
523 performance amongst a set of models, in terms of the relative probability of being the
524 best description of the data (Wagenmakers and Farrell, 2004). We applied this
525 procedure to all our previously-presented models (Figures 2-3, Figure S5), and
526 simpler alternatives. Cases where this analysis favoured including DMN interactions
527 argued for the importance of this novel tier of variation in health, which mitochondrial
528 transfer will influence in ways that we cannot currently predict.

529

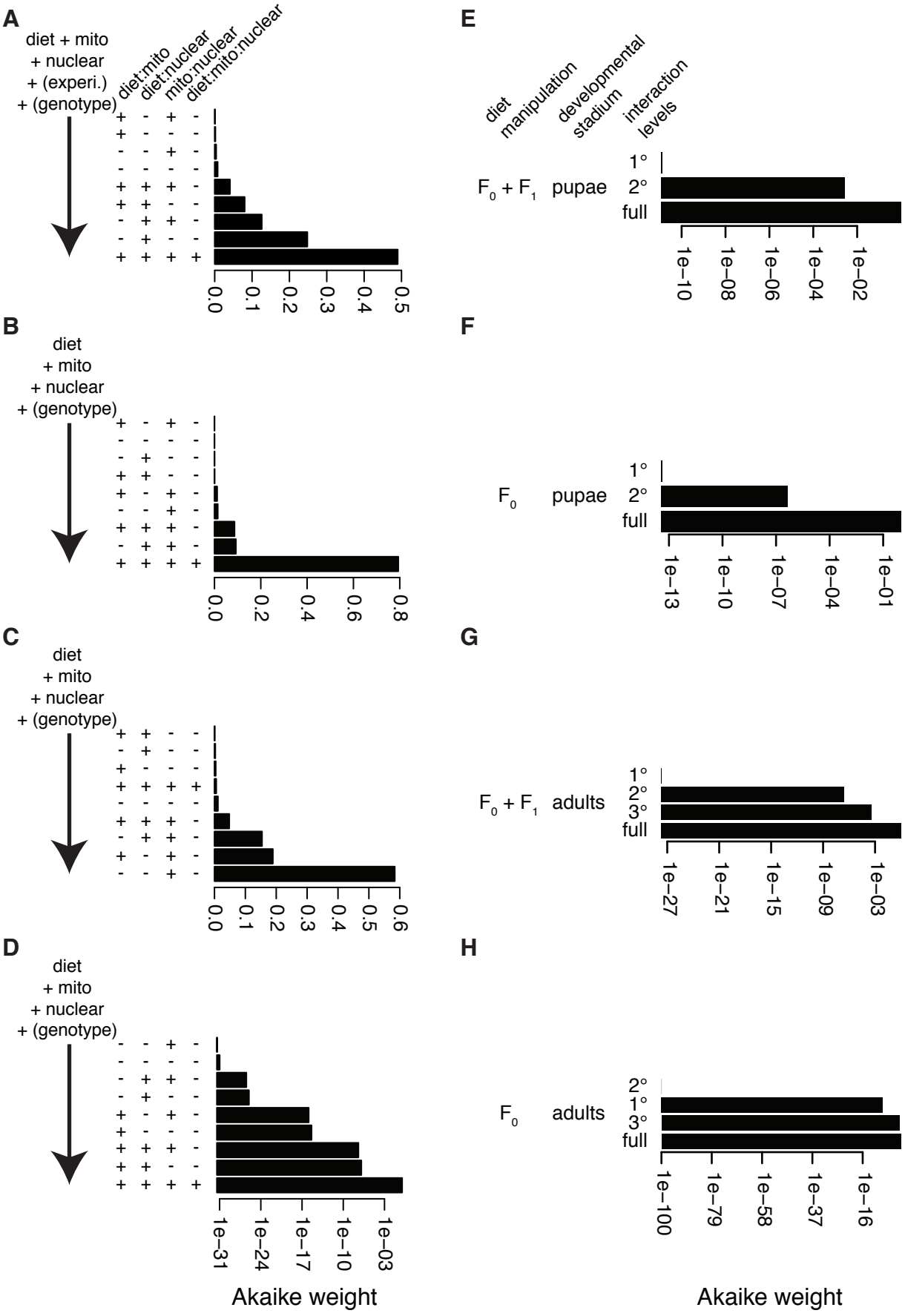
Akaike Weights suggested that models accounting for DMN interactions performed best described all datasets but one, and that purely additive models (assuming no interactions) were deficient by comparison (Figure 5).

For fecundity data (Figures 2-3), we fit a structured series of models, systematically including or eliminating all possible interactions amongst diet, mitochondrial and nuclear genotype. Akaike Weights favoured including DMN interactions (Figure 5a-d): for example, a model of data from Figure 2c including DMN interactions was 61.7 times more likely to be the best model than another with no interactions (Figure 5a); and approximately twice more likely than the next-best, which retained only the diet:nuclear interaction (Figure 5a). Models including DMN terms were also the best descriptor of fecundity before switching from experimental media (data from Figure 3c), and the release of fecundity after switching (data from Figure 3e). The exception to the emergent rule was the case of fecundity after switching from experimental media (Figure 3d), in which the favoured model included a mito-nuclear term, but not a DMN term. This exception demonstrated that the most complex models were not favoured automatically, but therefore encouraging confidence in other cases, suggesting that DMN interactions do indeed explain substantial variation.

For development data (Figures 2e, Figure 3f, Figure S4-S6), including terms for offspring sex added an additional order of complexity (i.e. a potential four-way interaction), rendering an assessment of all possible combinations hard to interpret. Therefore, we calculated Akaike Weights for models including either only first-degree terms (a purely additive model, without interaction terms), second-degree terms (all

554 pairwise combinations), third-degree terms (all three-way interactions), or fourth-
555 degree terms (all four-way interactions, but only when sex was known, in models of
556 adult emergence). We did this both for development to pupa (Figure 5e-f) and to
557 adult (Figure 5g-h), and when diet was manipulated either for both parents and
558 offspring (Figures 5e and 5g), or solely for parents (Figures 5f and 5h). In each case,
559 models including all possible interaction terms performed best, often by several
560 orders of magnitude (note logarithmic scale on figures). This analysis indicated that
561 DMN interactions exert a substantial influence on developmental variation.
562
563 In summary, our modelling indicates that key health and fitness metrics - fecundity
564 and development - are subject to significant interactions amongst mito-nuclear
565 genotype and diet, and that those interactions account for significant variation in our
566 data, which reflects the real-world mosaic of diet-mito-nuclear variation.
567

Figure 5



569 **Figure 5. Models including interactions amongst mitochondrial and nuclear**
570 **genotypes and diet treatment are favoured over alternatives.** Barplots indicate
571 Akaike weights analyses of sets of alternative models of data from Figures 2-3.
572 Columns to left of each panel indicate terms in each model. Higher values indicate a
573 better-supported model. The probability that one describes data better than another
574 can be calculated by the ratio of their respective Akaike Weights. Plots compare
575 models of data of fecundity of varied mito-nuclear genotypes fed experimental diets
576 (**A,** data from Figure 2; and **B**, data from Figure 3c), after feeding on experimental
577 diets (**C,** data from Figure 3d), and the ratio of fecundity before and after switching
578 from experimental to standardised diets (**D**, data from Figure 3e). Plots E-H compare
579 models of development time and rate, when models included either no interactions
580 between predictive terms, or various levels of interactions as indicated, for
581 development to pupa when experimental diets were fed to either parents and
582 offspring (**E**, data from Figure S3) or parents alone (**F**, data from Figure S3), and
583 development to adult when experimental diets were fed to either parents and
584 offspring (**G**, data from Figure 2) or parents alone (**H**, data from Figure 3).
585

Discussion

Diet is a major source of biological variation. Evidence is now mounting that mito-nuclear interactions are also a substantial source of variation, with implications in areas as diverse as mitochondrial disease, ageing, evolution of sex, and speciation (Ballard and Melvin, 2010; Đorđević et al., 2017; Gemmell et al., 2004; Gershoni et al., 2009; Havird et al., 2015; Hill, 2017; Innocenti et al., 2011; Latorre-Pellicer et al., 2019; Milot et al., 2017; Reinhardt et al., 2013; Wallace and Chalkia, 2013). Our work complements previous studies showing that diet and mito-nuclear variation interact to produce complex phenotypes, with diet enhancing or reducing the consequences of mito-nuclear interactions. We now reveal specific nutrients which are sufficient to drive these effects. The nutrients we identify are of particular interest, because EAAs are currently attracting a great deal of attention for their extensive regulation of swathes of life-history and health traits, whilst lipid consumption is associated with the pandemic of metabolic disease in humans (Simpson and Raubenheimer, 2012). We now show that responses these nutrients are exaggerated in some mito-nuclear genotypes, and buffered in others. An additional, and perhaps more important novel finding, is that transgenerational effects of these nutrients can emerge in specific mito-nuclear genotypes. These interactions appear to explain substantial variance in reproductive traits and life-history. The Darwinian view that reproduction subjugates all other processes suggests that many further traits may be similarly regulated.

Transient dietary alterations and metabolic disease can drive persistent molecular and phenotypic change, within and across generations (Dobson et al., 2017; Duque-Guimarães and Ozanne, 2017; Holland et al., 2016; Stefana et al., 2017). Our study now shows that specific mito-nuclear genotypes are susceptible to a transgenerational effect of lipid nutrition. To our knowledge, this is the first demonstration of any form of genetic variation in transgenerational effects of diet. Currently, we do not know how this transgenerational effect of lipid is transmitted, but it may be due to altered epigenetic marks, nutrient provision from mother to offspring, or microbiota. It will be interesting in future work to ask whether mito-nuclear genotype shapes further aspects of non-genetic inheritance, and identify mechanisms. Epigenomic profiling will likely prove insightful. Lipids and mito-nuclear genotype may interact via mitochondrial metabolic processes such as beta-oxidation, or by affecting rates of mitochondrial fusion and fission (Senyilmaz et al., 2015). It will also be interesting to ask whether the transgenerational effects we have identified in F_1 descendants extend further, to subsequent generations. These findings indicate that detailed pedigrees containing both mitochondrial and nuclear information may be required to predict transgenerational effects of nutrition.

A significant outcome of our study is that impacts of diet on offspring quality and quantity do not necessarily correlate, and their relationship is subject to mito-nuclear variation. We were surprised that EAA enrichment did not enhance offspring development, because parental preference for laying eggs on this food led us to expect that EAAs would promote offspring anabolism. This discrepancy may indicate that EAAs function as signals of food quality as well as metabolites. This dual role

could drive deleterious outcomes when EAA levels are not representative of the composition of food that would be found in the yeasts which flies are thought to consume in nature. Alternatively, the discrepancy may prove an example of parent-offspring conflict (Trivers, 1974), in which EAAs promote parental fecundity, despite the detriment of reduced investment into individual offspring. How parent-offspring conflict is regulated at the interface of diet and genetic variation in general is not well-studied.

To our knowledge, our study is the first investigation of diet-mito-nuclear interactions to manipulate specific nutrient classes, rather than a complex ingredient such as yeast. Our finding that impacts of dietary lipid depend on mito-nuclear genotype may be relevant to understanding variation in impacts of high-fat human diets. The high-EAA diets that we used have parallels to high-protein anabolic diets used to increase yields of livestock and human muscle mass. Our findings that EAAs generally decrease offspring quality may give pause for thought in use of these diets. Furthermore, the finding of mito-nuclear variation in the response to EAAs enrichment demonstrates that certain individuals may bear particularly strong costs of eating such diets. At present, we do not know the mechanistic basis of the diet-mito-nuclear interactions that we have uncovered, but the transgenerational effects hint at epigenetics, consistent with preceding findings that epigenetic marks respond to genetic variation in the cytoplasm (Bellizzi et al., 2012; Grunau et al., 2018; Vivian et al., 2017; Yan et al., 2011).

Our findings have implications for understanding variation that could emerge when novel mito-nuclear pairings come to be, such as after mitochondrial replacement therapy. Our results do not necessarily support the proposal to "match" mito-nuclear pairs as a positive predictor of therapeutic success (Dowling et al., 2010; Mossman et al., 2016a; Vaught et al., 2020), because a naturally co-occurring mito-nuclear pair (*AA*) responded uniquely to diet, suggesting that costs and benefits are not necessarily straightforward functions of mito-nuclear matching or mis-matching. Thus, comparing performance of matched versus unmatched groups may not be sufficient to evaluate risks of mitochondrial transfer therapy. Our analysis suggests that mitochondrial transfer may be variously deleterious, beneficial, or have unforeseen costs and benefits. Dietary recommendations may promote patient health. Our data also lend kudos to the suggestion of transgenerational effects of mitochondrial replacement therapy, especially when diet varies. A change of nomenclature may ultimately be called for, since "matched" and "mis-matched" carry intrinsic value judgments, but it seems that whether mitochondria and nuclei are of shared origin does not necessarily predict cost or benefit. We suggest that deep knowledge of patient and donor nuclear and mitochondrial genotype will likely be required to predict a healthy match.

Our results have wider implications for the conduct of nutritional and genetic research. Fly nutrition studies have drawn scrutiny for dietary variation between studies, attributed to methodological reporting (Lesperance and Broderick, 2020) and stochastic differences in ingredients (principally yeast) over time (Piper et al., 2014), which may limit repeatability. Similar issues can ensue after inconsistent

681 standardisation of genetic background (Burnett et al., 2011). The mito-nuclear field
682 as a whole suggests that different outcomes are to be expected when mitochondrial
683 and nuclear haplotypes are not carefully controlled by backcrossing, and our results
684 indicate that variation between studies may be amplified when diet is manipulated.
685 Even stochastic variation in diet may interact with mito-nuclear variation, making the
686 case for careful reporting, and perhaps the use of chemically-defined media (Piper et
687 al., 2014). Accounting for these sources of variation will likely improve repeatability.
688
689 In summary, we have shown that diet, mitochondrial and nuclear haplotype have
690 complex interactive effects, each capable of modifying the impact of change in
691 another. Dietary lipid and EAAs are both implicated separately in these interactions,
692 with relevance not just for proximate reproductive output, but also for lasting
693 transgenerational effects of diet which impact relative offspring fitness. These
694 varying, mito-nuclear impacts of diet on offspring quantity and quality appear to be
695 an important determinant of individual fitness and health.
696

Materials & methods

Diets

Development medium contained 1.4% agar and 4.5% brewer's yeast (both Gewürzmühle Brecht, Germany), 10% cornmeal and 11.1% sucrose (both Mühle Milzitz, Germany) (all w/v), 0.45% propionic acid and 3% nipagin (v/v).

Experimental media build on published protocols (Bass et al., 2007; Dobson et al., 2018; Emran et al., 2014). These media contained a final concentration of 10% brewer's yeast, 5% sucrose, 1.5% agar (w/v), 3% nipagin and 0.3% propionic acid (v/v). EAAs were purchased as powder (Sigma), and supplemented by dissolving into a 6.66x solution in ddH_20 pH 4.5 (final media concentrations: L-arginine 0.43 g/l, L-histidine 0.21 g/l, L-isoleucine 0.34 g/l, L-leucine 0.48 g/l, L-lysine 0.52 g/l, L-methionine 0.1 g/l, L-phenylalanine 0.26 g/l, L-threonine 0.37 g/l, L-tryptophan 0.09 g/l, L-valine 0.4 g/l). Margarine (*Ja! Pflanzenmargarine* from Rewe Supermarkets, Germany; 720 kcal / 100g; 80/100g fat from 23/100g saturated fatty acids, 40/100g monounsaturated fatty acids, 17/100g polyunsaturated fatty acids) was briefly melted then mixed thoroughly into the food (15% w/v). Final nutrient contents of rearing and control media were estimated using the *Drosophila* diet content calculator (Lesperance and Broderick, 2020), with additional protein, lipid and caloric content after nutrient supplements calculated according to margarine nutrient content report, and by assuming caloric equity between EAAs and protein at a caloric value of 4 calories/g (USDA). Vials contained ~5ml of food, and were stored at 4^{o}C for up to 1 week before use.

721

722 **Flies**

723 *D. melanogaster* fly lines were established as described in Figure 1 and maintained
724 at 25°C on development medium throughout their history prior to our experiments.
725 The ancestral Australian population was isolated in Coffs Harbour, NSW, Australia
726 (Dowling et al., 2014). The Benin population is the widely-used *Dahomey* population,
727 isolated in the 1970s in Dahomey (now Benin). Ancestral lines had been cured of the
728 cytoplasmic endosymbiont *Wolbachia* 66 generations previously by tetracycline
729 treatment prior to the experiment. For each fly line, 45 females of the desired
730 mitochondrial background were crossed to 45 males of the desired nuclear
731 background per generation. Iterating this process over many generations led to
732 introgression of the desired nuclear background (from males) into each mitochondrial
733 background. For experiments, flies were collected upon eclosion to adulthood and
734 fed fresh developmental medium, before being pooled, split and assigned at random
735 to experimental medium in groups of 5 males and 5 females. Experimental flies were
736 maintained at 25°C, and transferred to fresh media every 48-72h for one week. Flies
737 were transferred to fresh medium 24h before egg laying experiments. For
738 development experiments, eggs were incubated at 25°C and pupation and eclosion
739 were scored daily. Eclosing adults were lightly CO_2-anaesthetised before counting
740 and sexing.

741

742

743 **Analysis**

744 Data were analysed in R 3.6.1. Markdowns of all analyses are provided as
745 supplementary material. Fit of fecundity data to a negative binomial distribution was
746 determined with firdistrplus::descdist and firdistrplus::fitdist. Generalised linear mixed
747 models of the form
748
749 $$y \sim diet * mitochondria * nuclear + (genotype)$$
750
751 were fit with lme4::glmer.nb (egg counts, negative binomial distribution) or
752 lme4::glmer (binomial of egg counts before and after diet switch); in which *diet*
753 (control/EAA/lipid), *mitochondria* (A/B) and *nuclear* (A/B) were fixed factors,
754 genotype was a random factor denoting the full replicated genotype (e.g. *AA1, AA2,*
755 *AA3, AB1, AB2, BA1*, etc). Where relevant (Figure 2), experimental replicate was
756 also included as an additional random factor. Dispersion was assessed with
757 blmeco::dispersion_glmer, and model singularity was tested with lme4::isSingular.
758 Anova tests (type-3) were conducted with car::Anova, and *post-hoc* analyses were
759 applied with the functions emmeans::joint_tests, emmeans::pairs, emmeans::emmip
760 (Searle et al., 1980). AIC was determined with stats::AIC, Akaike weights with
761 MuMIn::dredge. Four vials were excluded from the diet-switching experiment to
762 enable model fitting, on the basis of extreme values (minimum and maximum
763 observations in the experiment) or deviation from a QQ plot before diet switch.
764 These data are retained, with note, in the data associated to this paper.
765
766 Censoring (i.e. failure to develop) was inferred per vial when n. emerging adults was
767 less than n. eggs (noting assignment of zero censors when adult counts were

768 greater than eggs in some vials, due to measurement error). A 50:50 sex ratio was
769 assumed amongst censors. Development to adult was modelled by fitting Cox mixed
770 effects models of the form
771
772 $$y \sim diet * mitocondria * nuclear * sex + eggs + (\frac{genotype}{vial})$$
773
774 with coxme::coxme. *Diet, mitochondria, nuclear* and *genotype* terms were as in
775 models of egg laying, and *vial* was an additional random effect nested in *genotype*.
776 *Eggs* coded number of eggs laid in the vial in which the individual developed, to
777 account for variation in rearing density. Pupal sex was unknown, so the sex term
778 was excluded from models of pupal development. Anova and *post-hoc* EMM tests
779 were conducted as per fecundity analyses, with additional stratification by sex when
780 applying emmeans::joint_tests.
781
782 Figures were assembled in Adobe Illustrator. Mitochondria graphics were recoloured
783 from files freely distributed under an open commons licence. The heatmap of nutrient
784 content was plotted in R with the superheat library.
785
786
787
788

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Declaration of interests

The authors declare no competing interests.

References

Akaike H. 1974. A new look at the statistical model identification. *Ieee T Automat Contr* **19**:716–723. doi:10.1109/tac.1974.1100705

Aw WC, Towarnicki SG, Melvin RG, Youngson NA, Garvin MR, Hu Y, Nielsen S, Thomas T, Pickford R, Bustamante S, Vila-Sanjurjo A, Smyth GK, Ballard JWO. 2018. Genotype to phenotype: Diet-by-mitochondrial DNA haplotype interactions drive metabolic flexibility and organismal fitness. *Plos Genet* **14**:e1007735. doi:10.1371/journal.pgen.1007735

Ballard JWO, Melvin RG. 2010. Linking the mitochondrial genotype to the organismal phenotype. *Mol Ecol* **19**:1523–1539. doi:10.1111/j.1365-294x.2010.04594.x

Baris TZ, Wagner DN, Dayan DI, Du X, Blier PU, Pichaud N, Oleksiak MF, Crawford DL. 2017. Evolved genetic and phenotypic differences due to mitochondrial-nuclear interactions. *Plos Genet* **13**:e1006517. doi:10.1371/journal.pgen.1006517

Barker DJP, Osmond C. 1986. Infant mortality, childhood nutrition, and ischaemic heart disease in England and Wales. *Lancet* **327**:1077–1081. doi:10.1016/s0140-6736(86)91340-1

Barker DJP, Thornburg KL. 2013. The Obstetric Origins of Health for a Lifetime. *Clin Obstet Gynecol* **56**:511–519. doi:10.1097/grf.0b013e31829cb9ca

Bass TM, Grandison RC, Wong R, Martinez P, Partridge L, Piper MDW. 2007. Optimization of Dietary Restriction Protocols in Drosophila. *Journals Gerontology Ser* **62**:1071–1081. doi:10.1093/gerona/62.10.1071

824 Bellizzi D, DAquila P, Giordano M, Montesanto A, Passarino G. 2012. Global DNA
825 methylation levels are modulated by mitochondrial DNA variants. *Epigenomics-uk*
826 **4**:17–27. doi:10.2217/epi.11.109

827 Bevers RPJ, Litovchenko M, Kapopoulou A, Braman VS, Robinson MR, Auwerx J,
828 Hollis B, Deplancke B. 2019. Mitochondrial haplotypes affect metabolic
829 phenotypes in the Drosophila Genetic Reference Panel. *Nat Metabolism* **1**:1226–
830 1242. doi:10.1038/s42255-019-0147-3

831 Bong L-J, Neoh K-B, Lee C-Y, Jaal Z. 2014. Effect of Diet Quality on Survival and
832 Reproduction of Adult Paederus fuscipes (Coleoptera: Staphylinidae). *J Med*
833 *Entomol* **51**:752–759. doi:10.1603/me13145

834 Brankatschk M, Gutmann T, Knittelfelder O, Palladini A, Prince E, Grzybek M,
835 Brankatschk B, Shevchenko A, Coskun Ü, Eaton S. 2018. A Temperature-
836 Dependent Switch in Feeding Preference Improves Drosophila Development and
837 Survival in the Cold. *Developmental Cell* **46**:781-793.e4.
838 doi:10.1016/j.devcel.2018.05.028

839 Burnett C, Valentini S, Cabreiro F, Goss M, Somogyvári M, Piper MD, Hoddinott M,
840 Sutphin GL, Leko V, McElwee JJ, Vazquez-Manrique RP, Orfila A-M, Ackerman
841 D, Au C, Vinti G, Riesen M, Howard K, Neri C, Bedalov A, Kaeberlein M, Sőti C,
842 Partridge L, Gems D. 2011. Absence of effects of Sir2 overexpression on lifespan
843 in C. elegans and Drosophila. *Nature* **477**:482–485. doi:10.1038/nature10296

844 Camilleri-Carter T-L, Dowling DK, Robker RL, Piper MDW. 2019. Transgenerational
845 Obesity and Healthy Aging in Drosophila. *The Journals of Gerontology: Series A*.
846 doi:10.1093/gerona/glz154

847 Camus MF, O'Leary M, Reuter M, Lane N. 2020. Impact of mitonuclear interactions
848 on life-history responses to diet. *Philosophical Transactions Royal Soc B*
849 **375**:20190416. doi:10.1098/rstb.2019.0416

850 Camus MF, Wolf JBW, Morrow EH, Dowling DK. 2015. Single Nucleotides in the
851 mtDNA Sequence Modify Mitochondrial Molecular Function and Are Associated
852 with Sex-Specific Effects on Fertility and Aging. *Curr Biol* **25**:2717–2722.
853 doi:10.1016/j.cub.2015.09.012

854 Chang C-C, Rodriguez J, Ross J. 2016. Mitochondrial–Nuclear Epistasis Impacts
855 Fitness and Mitochondrial Physiology of Interpopulation Caenorhabditis briggsae
856 Hybrids. *G3 Genes Genomes Genetics* **6**:209–219. doi:10.1534/g3.115.022970

857 Cohen IG, Adashi EY, Gerke S, Palacios-González C, Ravitsky V. 2020. The
858 Regulation of Mitochondrial Replacement Techniques Around the World. *Annu*
859 *Rev Genom Hum G* **21**:1–22. doi:10.1146/annurev-genom-111119-101815

860 Craven L, Tuppen HA, Greggains GD, Harbottle SJ, Murphy JL, Cree LM, Murdoch
861 AP, Chinnery PF, Taylor RW, Lightowlers RN, Herbert M, Turnbull DM. 2010.
862 Pronuclear transfer in human embryos to prevent transmission of mitochondrial
863 DNA disease. *Nature* **465**:82–85. doi:10.1038/nature08958

Crean AJ, Senior AM. 2019. High-fat diets reduce male reproductive success in animal models: A systematic review and meta-analysis. *Obesity Reviews* **20**:921–933. doi:10.1111/obr.12827

Deas JB, Blondel L, Extavour CG. 2019. Ancestral and offspring nutrition interact to affect life-history traits in Drosophila melanogaster. *Proceedings of the Royal Society B* **286**:20182778. doi:10.1098/rspb.2018.2778

Dobler R, Dowling DK, Morrow EH, Reinhardt K. 2018. A systematic review and meta-analysis reveals pervasive effects of germline mitochondrial replacement on components of health. *Hum Reprod Update* **24**:519–534. doi:10.1093/humupd/dmy018

Dobson AJ, Boulton-McDonald R, Houchou L, Svermova T, Ren Z, Subrini J, Vazquez-Prada M, Hoti M, Rodriguez-Lopez M, Ibrahim R, Gregoriou A, Gkantiragas A, Bähler J, Ezcurra M, Alic N. 2019. Longevity is determined by ETS transcription factors in multiple tissues and diverse species. *PLOS Genetics* **15**:e1008212. doi:10.1371/journal.pgen.1008212

Dobson AJ, Chaston JM, Newell PD, Donahue L, Hermann SL, Sannino DR, Westmiller S, Wong AC, Clark AG, Lazzaro BP, Douglas AE. 2015. Host genetic determinants of microbiota-dependent nutrition revealed by genome-wide analysis of Drosophila melanogaster. *Nature communications* **6**:6312. doi:10.1038/ncomms7312

884 Dobson AJ, Ezcurra M, Flanagan CE, Summerfield AC, Piper MDW, Gems D, Alic N.
885 2017. Nutritional Programming of Lifespan by FOXO Inhibition on Sugar-Rich
886 Diets. *Cell Reports* **18**:299–306. doi:10.1016/j.celrep.2016.12.029

887 Dobson AJ, He X, Blanc E, Bolukbasi E, Feseha Y, Yang M, Piper MD. 2018.
888 Tissue-specific transcriptome profiling of Drosophila reveals roles for GATA
889 transcription factors in longevity by dietary restriction. *npj Aging and Mechanisms*
890 *of Disease* **4**. doi:10.1038/s41514-018-0024-4

891 Dong H-J, Wu D, Xu S-Y, Li Q, Fang Z-F, Che L-Q, Wu C-M, Xu X-Y, Lin Y. 2016.
892 Effect of dietary supplementation with amino acids on boar sperm quality and
893 fertility. *Anim Reprod Sci* **172**:182–189. doi:10.1016/j.anireprosci.2016.08.003

894 Đorđević M, Stojković B, Savković U, Immonen E, Tucić N, Lazarević J, Arnqvist G.
895 2017. Sex-specific mitonuclear epistasis and the evolution of mitochondrial
896 bioenergetics, ageing, and life history in seed beetles. *Evolution* **71**:274–288.
897 doi:10.1111/evo.13109

898 Dowling DK, Friberg U, Lindell J. 2008. Evolutionary implications of non-neutral
899 mitochondrial genetic variation. *Trends Ecol Evol* **23**:546–554.
900 doi:10.1016/j.tree.2008.05.011

901 Dowling DK, Meerupati T, Arnqvist G. 2010. Cytonuclear Interactions and the
902 Economics of Mating in Seed Beetles. *Am Nat* **176**:131–140. doi:10.1086/653671

903 Dowling DK, Williams BR, Garcia-Gonzalez F. 2014. Maternal sexual interactions
904 affect offspring survival and ageing. *J Evolution Biol* **27**:88–97.
905 doi:10.1111/jeb.12276

906 Drummond E, Short E, Clancy D. 2019. Mitonuclear gene X environment effects on
907 lifespan and health: How common, how big? *Mitochondrion* **49**:12–18.
908 doi:10.1016/j.mito.2019.06.009

909 Dunham-Snary KJ, Ballinger SW. 2015. Mitochondrial-nuclear DNA mismatch
910 matters. *Science* **349**:1449–1450. doi:10.1126/science.aac5271

911 Duque-Guimarães D, Ozanne S. 2017. Early nutrition and ageing: can we intervene?
912 *Biogerontology* **18**:893–900. doi:10.1007/s10522-017-9691-y

913 Eisenberg T, Schroeder S, Andryushkova A, Pendl T, Küttner V, Bhukel A, Mariño G,
914 Pietrocola F, Harger A, Zimmermann A, Moustafa T, Sprenger A, Jany E, Büttner
915 S, Carmona-Gutierrez D, Ruckenstuhl C, Ring J, Reichelt W, Schimmel K, Leeb
916 T, Moser C, Schatz S, Kamolz L-P, Magnes C, Sinner F, Sedej S, Fröhlich K-U,
917 Juhasz G, Pieber TR, Dengjel J, Sigrist SJ, Kroemer G, Madeo F. 2014.
918 Nucleocytosolic Depletion of the Energy Metabolite Acetyl-Coenzyme A
919 Stimulates Autophagy and Prolongs Lifespan. *Cell Metab* **19**:431–444.
920 doi:10.1016/j.cmet.2014.02.010

921 Ellison CK, Burton RS. 2006. Disruption of mitochondrial function in interpopulation
922 hybrids of Tigriopus californicus. *Evolution* **60**:1382–1391. doi:10.1554/06-210.1

923 Emran S, Yang M, He X, Zandveld J, Piper MD. 2014. Target of rapamycin signalling
924 mediates the lifespan-extending effects of dietary restriction by essential amino
925 acid alteration. *Aging* **6**:390–8.

926 Fanson BG, Fanson KV, Taylor PW. 2012. Cost of reproduction in the Queensland
927 fruit fly: Y-model versus lethal protein hypothesis. *Proc Royal Soc B Biological Sci*
928 **279**:4893–4900. doi:10.1098/rspb.2012.2033

929 Fontana L, Partridge L, Longo VD. 2010. Extending Healthy Life Span—From Yeast
930 to Humans. *Science* **328**:321–326. doi:10.1126/science.1172539

931 Gemmell NJ, Metcalf VJ, Allendorf FW. 2004. Mother's curse: the effect of mtDNA
932 on individual fitness and population viability. *Trends Ecol Evol* **19**:238–244.
933 doi:10.1016/j.tree.2004.02.002

934 Gershoni M, Templeton AR, Mishmar D. 2009. Mitochondrial bioenergetics as a
935 major motive force of speciation. *Bioessays* **31**:642–650.
936 doi:10.1002/bies.200800139

937 Gorman GS, Chinnery PF, DiMauro S, Hirano M, Koga Y, McFarland R,
938 Suomalainen A, Thorburn DR, Zeviani M, Turnbull DM. 2016. Mitochondrial
939 diseases. *Nat Rev Dis Primers* **2**:16080. doi:10.1038/nrdp.2016.80

940 Grandison RC, Piper MD, Partridge L. 2009. Amino-acid imbalance explains
941 extension of lifespan by dietary restriction in Drosophila. *Nature* **462**:1061–1064.
942 doi:10.1038/nature08619

943 Grunau C, Voigt S, Dobler R, Dowling DK, Reinhardt K. 2018. The Cytoplasm
944 Affects the Epigenome in Drosophila melanogaster. *Epigenomes* **2**:17.
945 doi:10.3390/epigenomes2030017

946 Hariri N, Thibault L. 2010. High-fat diet-induced obesity in animal models. *Nutr Res*
947 *Rev* **23**:270–99. doi:10.1017/s0954422410000168

948 Havird JC, Hall MD, Dowling DK. 2015. The evolution of sex: A new hypothesis
949 based on mitochondrial mutational erosion. *Bioessays* **37**:951–958.
950 doi:10.1002/bies.201500057

951 Healy TM, Burton RS. 2020. Strong selective effects of mitochondrial DNA on the
952 nuclear genome. *P Natl Acad Sci Usa* **117**:6616–6621.
953 doi:10.1073/pnas.1910141117

954 Heymsfield SB, Wadden TA. 2017. Mechanisms, Pathophysiology, and Management
955 of Obesity. *New Engl J Med* **376**:254–266. doi:10.1056/nejmra1514009

956 Hill GE. 2019. Reconciling the mitonuclear compatibility species concept with
957 rampant mitochondrial introgression. *Integr Comp Biol* **59**:912–924.
958 doi:10.1093/icb/icz019

959 Hill GE. 2017. The mitonuclear compatibility species concept. *Auk* **134**:393–409.
960 doi:10.1642/auk-16-201.1

961 Holland ML, Lowe R, Caton PW, Gemma C, Carbajosa G, Danson AF, Carpenter
962 AA, Loche E, Ozanne SE, Rakyan VK. 2016. Early-life nutrition modulates the

963 epigenetic state of specific rDNA genetic variants in mice. *Science (New York,*
964 *NY)* **353**:495–8. doi:10.1126/science.aaf7040

965 Human Fertility and Embryology Authority. 2016. Scientific review of the safety and
966 efficacy of methods to avoid mitochondrial disease through assisted conception:
967 2016 update.

968 Innocenti P, Morrow EH, Dowling DK. 2011. Experimental Evidence Supports a Sex-
969 Specific Selective Sieve in Mitochondrial Genome Evolution. *Science* **332**:845–
970 848. doi:10.1126/science.1201157

971 Jang C, Hui S, Lu W, Cowan AJ, Morscher RJ, Lee G, Liu W, Tesz GJ, Birnbaum
972 MJ, Rabinowitz JD. 2018. The Small Intestine Converts Dietary Fructose into
973 Glucose and Organic Acids. *Cell Metab* **27**:351-361.e3.
974 doi:10.1016/j.cmet.2017.12.016

975 Jensen K, McClure C, Priest NK, Hunt J. 2015. Sex-specific effects of protein and
976 carbohydrate intake on reproduction but not lifespan in Drosophila melanogaster.
977 *Aging Cell* **14**:605–615. doi:10.1111/acel.12333

978 Jumbo-Lucioni P, Ayroles JF, Chambers M, Jordan KW, Leips J, Mackay TF, Luca
979 M. 2010. Systems genetics analysis of body weight and energy metabolism traits
980 in Drosophila melanogaster. *BMC Genomics* **11**:297. doi:10.1186/1471-2164-11-
981 297

982 Knittelfelder O, Prince E, Sales S, Fritzsche E, Wöhner T, Brankatschk M,
983 Shevchenko A. 2020. Sterols as dietary markers for Drosophila melanogaster.

Biochimica Et Biophysica Acta Bba - Mol Cell Biology Lipids **1865**:158683. doi:10.1016/j.bbalip.2020.158683

Lack D. 1947. The significance of clutch size in birds. *Ibis* 302–352.

Latorre-Pellicer A, Lechuga-Vieco AV, Johnston IG, Hämäläinen RH, Pellico J, Justo-Méndez R, Fernández-Toro JM, Clavería C, Guaras A, Sierra R, Llop J, Torres M, Criado LM, Suomalainen A, Jones NS, Ruíz-Cabello J, Enríquez JA. 2019. Regulation of Mother-to-Offspring Transmission of mtDNA Heteroplasmy. *Cell Metab* **30**:1120-1130.e5. doi:10.1016/j.cmet.2019.09.007

Lee K, Simpson SJ, Clissold FJ, Brooks R, Ballard WJ, Taylor PW, Soran N, Raubenheimer D. 2008. Lifespan and reproduction in Drosophila: New insights from nutritional geometry. *Proceedings of the National Academy of Sciences* **105**:2498–2503. doi:10.1073/pnas.0710787105

Leitão-Gonçalves R, Carvalho-Santos Z, Francisco A, Fioreze G, Anjos M, Baltazar C, Elias A, Itskov PM, Piper MD, Ribeiro C. 2017. Commensal bacteria and essential amino acids control food choice behavior and reproduction. *PLOS Biology* **15**:e2000862. doi:10.1371/journal.pbio.2000862

Lesperance DNA, Broderick NA. 2020. Meta-analysis of Diets Used in Drosophila Microbiome Research and Introduction of the Drosophila Dietary Composition Calculator (DDCC). *G3 Genes Genomes Genetics* **10**:g3.401235.2020. doi:10.1534/g3.120.401235

1004 Li X, Shi X, Hou Y, Cao X, Gong L, Wang H, Li Jiayu, Li Jibin, Wu C, Xiao D, Qi H,
1005 Xiao X. 2018. Paternal hyperglycemia induces transgenerational inheritance of
1006 susceptibility to hepatic steatosis in rats involving altered methylation on Pparα
1007 promoter. *Biochimica Et Biophysica Acta Mol Basis Dis* **1865**:147–160.
1008 doi:10.1016/j.bbadis.2018.10.040

1009 Liang H, Zhang Z. 2006. Food restriction affects reproduction and survival of F1 and
1010 F2 offspring of Rat-like hamster (Cricetulus triton). *Physiol Behav* **87**:607–613.
1011 doi:10.1016/j.physbeh.2005.12.006

1012 Liao C-Y, Rikke BA, Johnson TE, Diaz V, Nelson JF. 2009. Genetic variation in the
1013 murine lifespan response to dietary restriction: from life extension to life
1014 shortening. *Aging Cell* **9**:92–5. doi:10.1111/j.1474-9726.2009.00533.x

1015 Ma C, Mirth CK, Hall MD, Piper MDW. 2020. Amino acid quality modifies the
1016 quantitative availability of protein for reproduction in Drosophila melanogaster. *J*
1017 *Insect Physiol* 104050. doi:10.1016/j.jinsphys.2020.104050

1018 Ma H, Gutierrez NM, Morey R, Dyken CV, Kang E, Hayama T, Lee Y, Li Y, Tippner-
1019 Hedges R, Wolf DP, Laurent LC, Mitalipov S. 2016. Incompatibility between
1020 Nuclear and Mitochondrial Genomes Contributes to an Interspecies Reproductive
1021 Barrier. *Cell Metab* **24**:283–94. doi:10.1016/j.cmet.2016.06.012

1022 Maklakov AA, Lummaa V. 2013. Evolution of sex differences in lifespan and aging:
1023 Causes and constraints. *BioEssays* **35**:717–724. doi:10.1002/bies.201300021

1024 Maklakov AA, Simpson SJ, Zajitschek F, Hall MD, Dessmann J, Clissold F,
1025 Raubenheimer D, Bonduriansky R, Brooks RC. 2008. Sex-Specific Fitness Effects
1026 of Nutrient Intake on Reproduction and Lifespan. *Curr Biol* **18**:1062–1066.
1027 doi:10.1016/j.cub.2008.06.059

1028 Mariño G, Pietrocola F, Eisenberg T, Kong Y, Malik SA, Andryushkova A, Schroeder
1029 S, Pendl T, Harger A, Niso-Santano M, Zamzami N, Scoazec M, Durand S, Enot
1030 DP, Fernández ÁF, Martins I, Kepp O, Senovilla L, Bauvy C, Morselli E, Vacchelli
1031 E, Bennetzen M, Magnes C, Sinner F, Pieber T, López-Otín C, Maiuri MC,
1032 Codogno P, Andersen JS, Hill JA, Madeo F, Kroemer G. 2014. Regulation of
1033 Autophagy by Cytosolic Acetyl-Coenzyme A. *Mol Cell* **53**:710–725.
1034 doi:10.1016/j.molcel.2014.01.016

1035 McCracken AW, Adams G, Hartshorne L, Tatar M, Simons MJP. 2020. The hidden
1036 costs of dietary restriction: Implications for its evolutionary and mechanistic
1037 origins. *Sci Adv* **6**:eaay3047. doi:10.1126/sciadv.aay3047

1038 Meiklejohn CD, Holmbeck MA, Siddiq MA, Abt DN, Rand DM, Montooth KL. 2013.
1039 An Incompatibility between a Mitochondrial tRNA and Its Nuclear-Encoded tRNA
1040 Synthetase Compromises Development and Fitness in Drosophila. *Plos Genet*
1041 **9**:e1003238. doi:10.1371/journal.pgen.1003238

1042 Milot E, Moreau C, Gagnon A, Cohen AA, Brais B, Labuda D. 2017. Mother's curse
1043 neutralizes natural selection against a human genetic disease over three
1044 centuries. *Nat Ecol Evol* **1**:1400–1406. doi:10.1038/s41559-017-0276-6

1045 Mirth CK, Alves AN, Piper MD. 2018. Turning Food Into Eggs: insights from
1046 nutritional biology and developmental physiology of Drosophila. *Curr Opin Insect*
1047 *Sci* **31**:49–57. doi:10.1016/j.cois.2018.08.006

1048 Montooth KL, Dhawanjewar AS, Meiklejohn CD. 2019. Temperature-sensitive
1049 reproduction and the physiological and evolutionary potential for Mother's Curse.
1050 *Integr Comp Biol* **59**:890–899. doi:10.1093/icb/icz091

1051 Mossman JA, Biancani LM, Rand DM. 2019. Mitochondrial genomic variation drives
1052 differential nuclear gene expression in discrete regions of Drosophila gene and
1053 protein interaction networks. *Bmc Genomics* **20**:691. doi:10.1186/s12864-019-
1054 6061-y

1055 Mossman JA, Biancani LM, Zhu C-T, Rand DM. 2016a. Mitonuclear Epistasis for
1056 Development Time and Its Modification by Diet in Drosophila. *Genetics* **203**:463–
1057 484. doi:10.1534/genetics.116.187286

1058 Mossman JA, Tross JG, Li N, Wu Z, Rand DM. 2016b. Mitochondrial-Nuclear
1059 Interactions Mediate Sex-Specific Transcriptional Profiles in Drosophila. *Genetics*
1060 **204**:genetics.116.192328. doi:10.1534/genetics.116.192328

1061 Mulvey L, Sands WA, Salin K, Carr AE, Selman C. 2017. Disentangling the effect of
1062 dietary restriction on mitochondrial function using recombinant inbred mice.
1063 *Molecular and cellular endocrinology* **455**:41–53. doi:10.1016/j.mce.2016.09.001

1064 Nagarajan-Radha V, Aitkenhead I, Clancy DJ, Chown SL, Dowling DK. 2020. Sex-
1065 specific effects of mitochondrial haplotype on metabolic rate in Drosophila

melanogaster support predictions of the Mother's Curse hypothesis. *Philosophical Transactions Royal Soc B* **375**:20190178. doi:10.1098/rstb.2019.0178

Nagarajan-Radha V, Rapkin J, Hunt J, Dowling DK. 2019. Interactions Between Mitochondrial Haplotype and Dietary Macronutrient Ratios Confer Sex-Specific Effects on Longevity in Drosophila melanogaster. *The Journals of Gerontology: Series A* 1573–1581. doi:10.1093/gerona/glz104

Ordovas JM, Ferguson LR, Tai ES, Mathers JC. 2018. Personalised nutrition and health. *Bmj Clin Res Ed* **361**:bmj.k2173. doi:10.1136/bmj.k2173

Öst A, Lempradl A, Casas E, Weigert M, Tiko T, Deniz M, Pantano L, Boenisch U, Itskov PM, Stoeckius M, Ruf M, Rajewsky N, Reuter G, Iovino N, Ribeiro C, Alenius M, Heyne S, Vavouri T, Pospisilik AJ. 2014. Paternal Diet Defines Offspring Chromatin State and Intergenerational Obesity. *Cell* **159**. doi:10.1016/j.cell.2014.11.005

Piper MD, Blanc E, Leitão-Gonçalves R, Yang M, He X, Linford NJ, Hoddinott MP, Hopfen C, Soultoukis GA, Niemeyer C, Kerr F, Pletcher SD, Ribeiro C, Partridge L. 2014. A holidic medium for Drosophila melanogaster. *Nature Methods* **11**:100–105. doi:10.1038/nmeth.2731

Rand DM, Mossman JA, Zhu L, Biancani LM, Ge JY. 2018. Mitonuclear epistasis, genotype-by-environment interactions, and personalized genomics of complex traits in Drosophila: Mitonuclear G x G x E. *Iubmb Life* **70**:1275–1288. doi:10.1002/iub.1954

1087 Reinhardt K, Dowling DK, Morrow EH. 2013. Mitochondrial Replacement, Evolution,
1088 and the Clinic. *Science* **341**:1345–1346. doi:10.1126/science.1237146

1089 Rikke BA, Liao C-Y, McQueen MB, Nelson JF, Johnson TE. 2010. Genetic
1090 dissection of dietary restriction in mice supports the metabolic efficiency model of
1091 life extension. *Exp Gerontol* **45**:691–701. doi:10.1016/j.exger.2010.04.008

1092 Rivera HM, Kievit P, Kirigiti MA, Bauman LA, Baquero K, Blundell P, Dean TA,
1093 Valleau JC, Takahashi DL, Frazee T, Douville L, Majer J, Smith MS, Grove KL,
1094 Sullivan EL. 2015. Maternal high-fat diet and obesity impact palatable food intake
1095 and dopamine signaling in nonhuman primate offspring. *Obes Silver Spring Md*
1096 **23**:2157–64. doi:10.1002/oby.21306

1097 Saud S, Summerfield AC, Alic N. 2015. Ablation of insulin-producing cells prevents
1098 obesity but not premature mortality caused by a high-sugar diet in Drosophila.
1099 *Proceedings of the Royal Society of London B: Biological Sciences*
1100 **282**:20141720. doi:10.1098/rspb.2014.1720

1101 Searle SR, Speed FM, Milliken GA. 1980. Population Marginal Means in the Linear
1102 Model: An Alternative to Least Squares Means. *Am Statistician* **34**:216–221.
1103 doi:10.1080/00031305.1980.10483031

1104 Senyilmaz D, Virtue S, Xu X, Tan CY, Griffin JL, Miller AK, Vidal-Puig A, Teleman
1105 AA. 2015. Regulation of mitochondrial morphology and function by stearoylation
1106 of TFR1. *Nature* **525**:124–128. doi:10.1038/nature14601

1107 Simpson SJ, Raubenheimer D. 2012. The Nature of Nutrition.
1108 doi:10.1515/9781400842803

1109 Skorupa DA, Dervisefendic A, Zwiener J, Pletcher SD. 2008. Dietary composition
1110 specifies consumption, obesity, and lifespan in Drosophila melanogaster. *Aging*
1111 *Cell* **7**:478–490. doi:10.1111/j.1474-9726.2008.00400.x

1112 Smith HG, Kallander H, Nilsson J-A. 1989. The Trade-Off Between Offspring
1113 Number and Quality in the Great Tit Parus major. *J Animal Ecol* **58**:383.
1114 doi:10.2307/4837

1115 Solon-Biet SM, Walters KA, Simanainen UK, McMahon AC, Ruohonen K, Ballard
1116 JO, Raubenheimer D, Handelsman DJ, Couteur DG, Simpson SJ. 2015.
1117 Macronutrient balance, reproductive function, and lifespan in aging mice.
1118 *Proceedings of the National Academy of Sciences* **112**:3481–3486.
1119 doi:10.1073/pnas.1422041112

1120 Stearns SC. 1992. The evolution of life histories. Oxford University Press.

1121 Stefana IM, Driscoll PC, Obata F, Pengelly A, Newell CL, MacRae JI, Gould AP.
1122 2017. Developmental diet regulates Drosophila lifespan via lipid autotoxins.
1123 *Nature Communications* **8**:1384. doi:10.1038/s41467-017-01740-9

1124 Svajgr AJ, Hammell DL, DeGeeter MJ, Hays VW, Cromwell GL, Dutt RH. 1972.
1125 Reproductive performance of sows on a protein-restricted diet. *Reproduction*
1126 **30**:455–458. doi:10.1530/jrf.0.0300455

1127 Tachibana M, Kuno T, Yaegashi N. 2018. Mitochondrial replacement therapy and
1128 assisted reproductive technology: A paradigm shift toward treatment of genetic
1129 diseases in gametes or in early embryos. *Reproductive Medicine Biology* **17**:421–
1130 433. doi:10.1002/rmb2.12230

1131 Towarnicki SG, Ballard JWO. 2018. Mitotype Interacts With Diet to Influence
1132 Longevity, Fitness, and Mitochondrial Functions in Adult Female Drosophila.
1133 *Frontiers in Genetics* **9**:593. doi:10.3389/fgene.2018.00593

1134 Towarnicki SG, Ballard JWO. 2017. Drosophila mitotypes determine developmental
1135 time in a diet and temperature dependent manner. *Journal of Insect Physiology*
1136 **100**. doi:10.1016/j.jinsphys.2017.06.002

1137 Trefely S, Lovell CD, Snyder NW, Wellen KE. 2020. Compartmentalized acyl-CoA
1138 metabolism and roles in chromatin regulation. *Mol Metab* **38**:100941.
1139 doi:10.1016/j.molmet.2020.01.005

1140 Trivers RL. 1974. Parent-Offspring Conflict. *Am Zool* **14**:249–264.
1141 doi:10.1093/icb/14.1.249

1142 Tufarelli V, Lacalandra G, Laudadio V. 2015. Reproductive and Metabolic
1143 Responses of Early-lactating Dairy Cows Fed Different Dietary Protein Sources.
1144 *Reprod Domest Anim* **50**:735–739. doi:10.1111/rda.12566

1145 Vaught RC, Voigt S, Dobler R, Clancy DJ, Reinhardt K, Dowling DK. 2020.
1146 Interactions between cytoplasmic and nuclear genomes confer sex-specific

1147 effects on lifespan in Drosophila melanogaster. *J Evolution Biol* **33**:694–713.
1148 doi:10.1111/jeb.13605

1149 Vivian CJ, Brinker AE, Graw S, Koestler DC, Legendre C, Gooden GC, Salhia B,
1150 Welch DR. 2017. Mitochondrial Genomic Backgrounds Affect Nuclear DNA
1151 Methylation and Gene Expression. *Cancer Res* **77**:6202–6214. doi:10.1158/0008-
1152 5472.can-17-1473

1153 Wagenmakers E-J, Farrell S. 2004. AIC model selection using Akaike weights.
1154 *Psychon B Rev* **11**:192–196. doi:10.3758/bf03206482

1155 Wallace DC, Chalkia D. 2013. Mitochondrial DNA Genetics and the Heteroplasmy
1156 Conundrum in Evolution and Disease. *Csh Perspect Biol* **5**:a021220.
1157 doi:10.1101/cshperspect.a021220

1158 Wei Y, Yang C-R, Wei Y-P, Zhao Z-A, Hou Y, Schatten H, Sun Q-Y. 2014. Paternally
1159 induced transgenerational inheritance of susceptibility to diabetes in mammals. *P*
1160 *Natl Acad Sci Usa* **111**:1873–8. doi:10.1073/pnas.1321195111

1161 Winship AL, Gazzard SE, Cullen-McEwen LA, Bertram JF, Hutt KJ. 2018. Maternal
1162 low-protein diet programmes low ovarian reserve in offspring. *Reproduction*
1163 **156**:299–311. doi:10.1530/rep-18-0247

1164 Wolf DP, Mitalipov N, Mitalipov S. 2015. Mitochondrial replacement therapy in
1165 reproductive medicine. *Trends Mol Med* **21**:68–76.
1166 doi:10.1016/j.molmed.2014.12.001

1167 Wong AC-N, Dobson AJ, Douglas AE. 2014a. Gut microbiota dictates the metabolic
1168 response of Drosophila to diet. *J Exp Biology* **217**:1894–1901.
1169 doi:10.1242/jeb.101725

1170 Woodcock KJ, Kierdorf K, Pouchelon CA, Vivancos V, Dionne MS, Geissmann F.
1171 2015. Macrophage-Derived upd3 Cytokine Causes Impaired Glucose
1172 Homeostasis and Reduced Lifespan in Drosophila Fed a Lipid-Rich Diet.
1173 *Immunity* **42**:133–144. doi:10.1016/j.immuni.2014.12.023

1174 Yan H, Yan Z, Ma Q, Jiao F, Huang S, Zeng F, Zeng Y. 2011. Association between
1175 mitochondrial DNA haplotype compatibility and increased efficiency of bovine
1176 intersubspecies cloning. *J Genet Genomics* **38**:21–28.
1177 doi:10.1016/j.jcg.2010.12.003

1178 Zanco B, Mirth CK, Sgrò CM, Piper MDW. 2020. A dietary sterol trade off determines
1179 lifespan responses to dietary restriction in Drosophila melanogaster. *Biorxiv*
1180 2020.08.21.260489. doi:10.1101/2020.08.21.260489

1181 Zhao S, Jang C, Liu J, Uehara K, Gilbert M, Izzo L, Zeng X, Trefely S, Fernandez S,
1182 Carrer A, Miller KD, Schug ZT, Snyder NW, Gade TP, Titchenell PM, Rabinowitz
1183 JD, Wellen KE. 2020. Dietary fructose feeds hepatic lipogenesis via microbiota-
1184 derived acetate. *Nature* **579**:586–591. doi:10.1038/s41586-020-2101-7

1185 Zhu C-T, Ingelmo P, Rand DM. 2014. G×G×E for Lifespan in Drosophila:
1186 Mitochondrial, Nuclear, and Dietary Interactions that Modify Longevity. *PLoS*
1187 *Genetics* **10**. doi:10.1371/journal.pgen.1004354

1188

Table S1. GLMM of fecundity across diets and mito-nuclear haplotypes

Term	Chisq	Df	Pr(>χ^2)
(Intercept)	4342.751	1	<2.20E-16
mito	8.3384	1	0.0039
nuclear	13.6946	1	0.0002
diet	314.9242	2	<2.20E-16
mito:nuclear	6.5883	1	0.0103
mito:diet	10.2151	2	0.0061
nuclear:diet	0.2024	2	0.9036
mito:nuclear:diet	9.3347	2	0.0094

Table S2. Pairwise comparisons of fecundity across diets and mito-nuclear haplotypes

diet	contrast	estimate	SE	Z ratio	P value
sya	AA vs BA	0.264859	0.0917	2.888	0.0203
	AA vs AB	0.32267	0.0872	3.701	0.0012
	AA vs BB	0.262022	0.0871	3.008	0.014
	BA vs AB	0.057811	0.0922	0.627	0.9233
	BA vs BB	-0.002838	0.092	-0.031	1
	AB vs BB	-0.060649	0.0876	-0.692	0.9001
EAA	AA vs BA	0.04355	0.0918	0.474	0.9648
	AA vs AB	0.362769	0.0865	4.193	0.0002
	AA vs BB	0.363417	0.0866	4.199	0.0002

	BA vs AB	0.319219	0.0922	3.464	0.003
	BA vs BB	0.319866	0.092	3.475	0.0029
	AB vs BB	0.000647	0.087	0.007	1
mar	AA vs BA	-0.09705	0.097	-1.001	0.7491
	AA vs AB	0.365225	0.0932	3.918	0.0005
	AA vs BB	0.42652	0.0935	4.56	<.0001
	BA vs AB	0.462275	0.0982	4.706	<.0001
	BA vs BB	0.523571	0.0986	5.309	<.0001
	AB vs BB	0.061296	0.0951	0.645	0.9174

Table S3. Development on experimental diets: Cox Mixed-Effects model of adult emergence, sex-stratified posterior analysis by Estimated Marginal Means

Sex	Term	Df	F ratio	P
female	mito	1	15.517	0.0001
	nuclear	1	13.541	0.0002
	diet	2	322.17	<.0001
	mito:nuclear	1	20.686	<.0001
	mito:diet	2	25.789	<.0001
	nuclear:diet	2	16.855	<.0001
	mito:nuclear:diet	2	25.733	<.0001
male	mito	1	10.307	0.0013
	nuclear	1	0.223	0.6371

diet	2	226.121	<.0001
mito:nuclear	1	16.511	<.0001
mito:diet	2	8.529	0.0002
nuclear:diet	2	15.201	<.0001
mito:nuclear:diet	2	7.778	0.0004

Table S4. Development on experimental diets: Cox Mixed-Effects model of egg-pupa survival

Term	Chisq	Df	Pr(>χ^2)
mito	1	129.732	<2.20E-16
nuclear	1	148.282	<2.20E-16
diet	2	146.228	<2.20E-16
eggs	1	12.377	0.0004
mito:nuclear	1	66.987	2.73E-16
mito:diet	2	74.55	<2.20E-16
nuclear:diet	2	90.713	<2.20E-16
mito:nuclear:diet	2	35.714	1.76E-08

Table S5. Fecundity before switch from experimental diets: GLMM of egg laying across mito-nuclear haplotypes, after feeding on experimental diets

Term	Chisq	Df	Pr(>χ^2)
(Intercept)	1748.5684	1	<2.20E-16
mito	1.1888	1	0.2756
nuclear	2.2325	1	0.1351

diet	127.1386	2	<2.20E-16
mito:nuclear	5.845	1	0.0156
mito:diet	9.7233	2	0.0077
nuclear:diet	10.8029	2	0.0045
mito:nuclear:diet	9.1987	2	0.0101

Table S6. GLMM of fecundity across diets and mito-nuclear haplotypes – data pooled from Figure 2 and Figure 3c

Term	χ^2	Df	Pr(>χ^2)
(Intercept)	4338.6592	1	<2.20E-16
mito	1.7911	1	0.1808
nuclear	9.8394	1	0.0017
diet	500.2988	2	<2.20E-16
mito:nuclear	1.8161	1	0.1779
mito:diet	14.5664	2	0.0006
nuclear:diet	2.829	2	0.2430
mito:nuclear:diet	17.8267	2	0.0001

Table S7. Fecundity after switch from experimental diets: GLMM of egg laying across mito-nuclear haplotypes, after feeding on experimental diets

Term	χ^2	Df	Pr(>χ^2)
(Intercept)	1341.3688	1	<2.20E-16
mito	2.6895	1	0.1010
nuclear	0.0347	1	0.8522

diet	36.59	2	1.13E-08
mito:nuclear	3.7278	1	0.0535
mito:diet	0.3195	2	0.8523
nuclear:diet	1.7084	2	0.4256
mito:nuclear:diet	0.5948	2	0.7427

Table S8. Rescue of fecundity after switch from experimental diets: GLMM of egg laying across mito-nuclear haplotypes: after feeding, relative to during feeding (binomial)

Term	χ^2	Df	Pr(>χ^2)
(Intercept)	44.7143	1	2.28E-11
mito	1.4802	1	0.2237
nuclear	4.4348	1	0.0352
diet	139.1165	2	<2.20E-16
mito:nuclear	0.1714	1	0.6788
mito:diet	51.4892	2	6.60E-12
nuclear:diet	17.9358	2	0.0001
mito:nuclear:diet	39.3128	2	2.91E-09

Table S9. Fertility & development after parental feeding on experimental diets: sex-stratified posterior (EMM) analysis of adult emergence

Sex	Term	Df	F ratio	P
female	mito	1	10.243	0.0014
	nuclear	1	1.313	0.2518

	diet	2	0.345	0.7083
	mito:nuclear	1	0.218	0.6407
	mito:diet	2	4.961	0.007
	nuclear:diet	2	0.777	0.4599
	mito:nuclear:diet	2	3.477	0.0309
male	mito	1	9.262	0.0023
	nuclear	1	1.331	0.2486
	diet	2	0.396	0.6731
	mito:nuclear	1	0.07	0.7913
	mito:diet	2	3.454	0.0316
	nuclear:diet	2	1.545	0.2133
	mito:nuclear:diet	2	5.862	0.0028

Table S10. Development after parental feeding on experimental diets: Cox Mixed-Effects model of egg-pupa survival

Term	Chisq	Df	Pr(>χ^2)
mito	1	3.8079	0.0510
nuclear	1	13.3143	0.0003
diet	2	10.8094	0.0045
eggs	1	31.5515	1.94E-08
mito:nuclear	1	3.8928	0.0485

mito:diet	2	5.5291	0.0630
nuclear:diet	2	8.4317	0.0148
mito:nuclear:diet	2	1.717	0.4238

Table S11. Fertility & development on experimental diets: Cox Mixed-Effects model of adult emergence

Term	Chisq	Df	Pr(>χ^2)
mito	1	75.1042	<2.20E-16
nuclear	1	65.8859	4.78E-16
diet	2	86.1542	<2.20E-16
sex	1	12.9677	0.0003
eggs	1	1.7022	0.1920
mito:nuclear	1	55.7929	8.05E-14
mito:diet	2	86.1953	<2.20E-16
nuclear:diet	2	68.6659	1.23E-15
mito:sex	1	14.1785	0.0002
nuclear:sex	1	14.9357	0.0001
diet:sex	2	12.8281	0.0016
mito:nuclear:diet	2	51.4656	6.67E-12
mito:nuclear:sex	1	13.8949	0.0002
mito:diet:sex	2	25.9358	2.33E-06
nuclear:diet:sex	2	11.5055	0.0032
mito:nuclear:diet:sex	2	16.6248	0.0002

Table S12. Fertility & development after parental feeding on experimental diets: Cox Mixed-Effects model of adult emergence

Term	Chisq	Df	Pr(>χ^2)
mito	1	0.3711	0.5423
nuclear	1	1.9887	0.1585
diet	2	10.3938	0.0055
sex	1	0.1665	0.6832
eggs	1	7.2316	0.0072
mito:nuclear	1	0.2117	0.6454
mito:diet	2	14.3708	0.0008
nuclear:diet	2	6.0394	0.0488
mito:sex	1	1.517	0.2181
nuclear:sex	1	1.4268	0.2323
diet:sex	2	2.9565	0.2280
mito:nuclear:diet	2	6.9531	0.0309
mito:nuclear:sex	1	0.565	0.4522
mito:diet:sex	2	0.2748	0.8716
nuclear:diet:sex	2	1.0484	0.5920
mito:nuclear:diet:sex	2	6.6992	0.0351

Figure S1

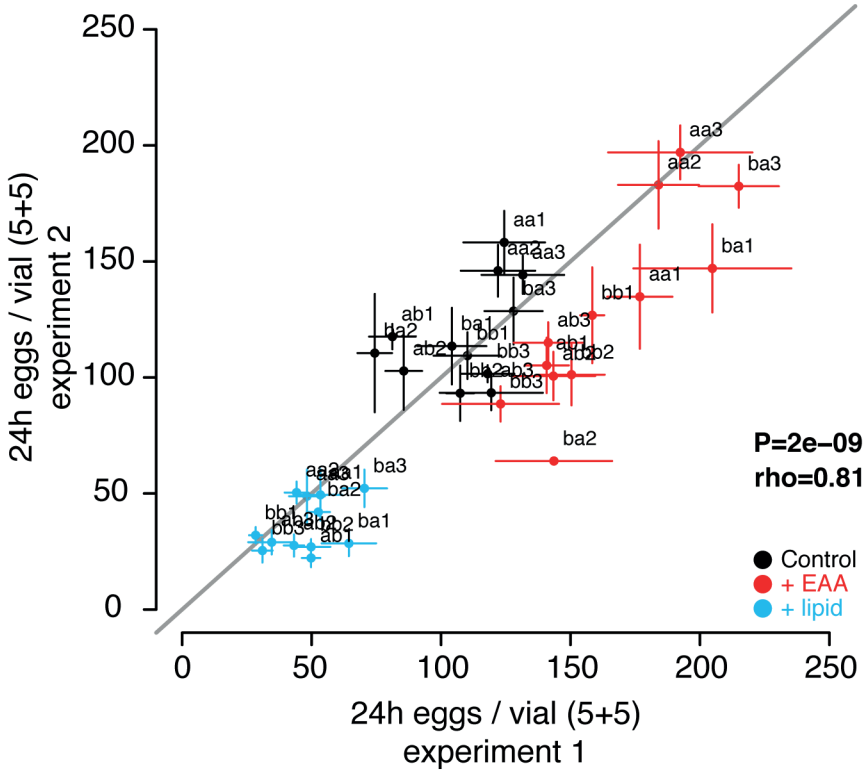
24h eggs / vial (5+5)
experiment 2
24h eggs / vial (5+5)
experiment 1
0
50
100
150
200
250
P=2e−09
rho=0.81
Control
+ EAA
+ lipid

Figure S1. Diet-mito-nuclear interactions modulate *Drosophila* fecundity.

Genetic correlations in impact of mito-nuclear variation on egg laying, and impact of diet on egg laying. Each point shows mean egg laying per line per diet in each of two replicate experiments. Bars show standard error. Accompanying statistical analysis presented in Table S1 and Table S2.

Figure S2

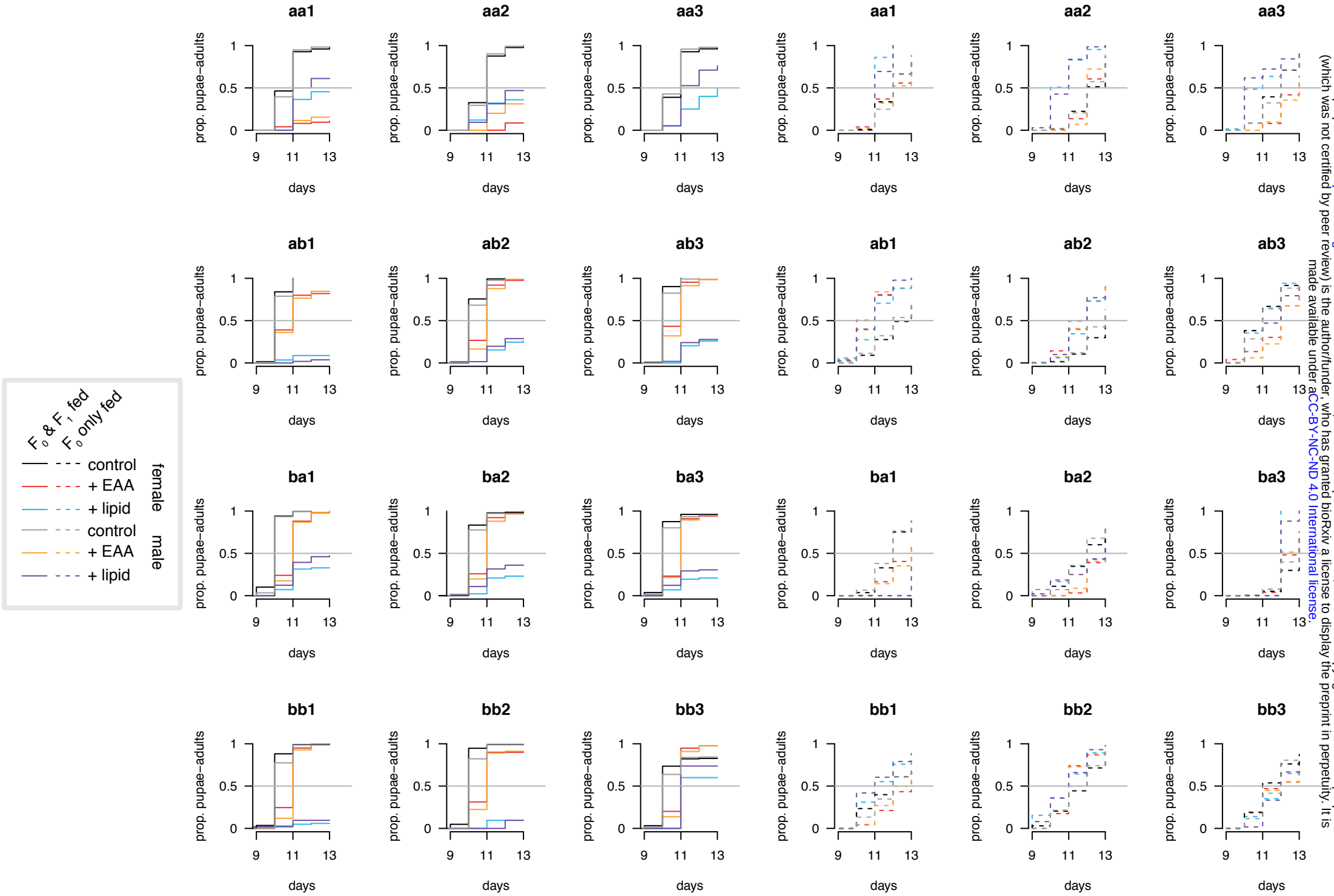

Figure S2. Mito-nuclear variation in dietary regulation of adult development: impacts per genetic replicate. Kaplan-Meier plots of development after feeding on experimental diets as specified in key. Mito-nuclear genotypes are indicated per panel. Data are presented pooled across each replicate genotype in Figure 4. Accompanying statistical analysis presented in Table S3.

Figure S3

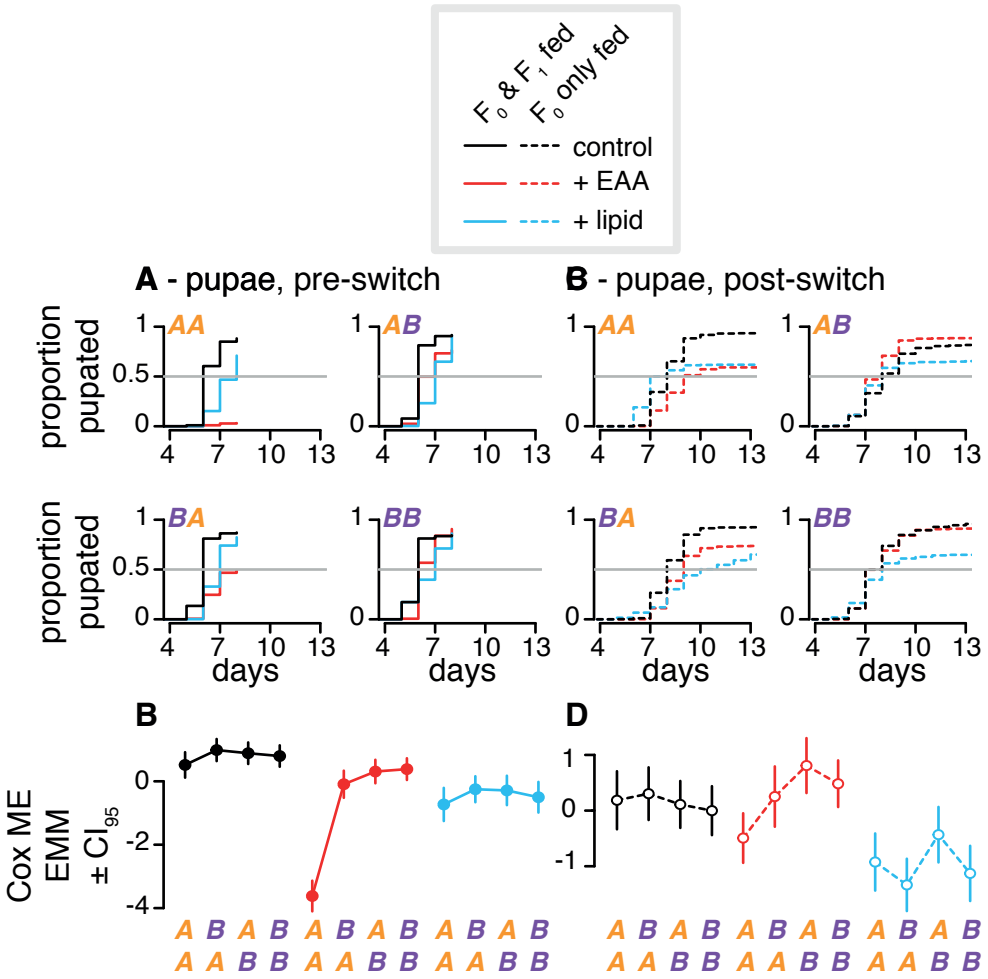

Figure S3. Mito-nuclear variation in dietary regulation of pupation. (**A**) Kaplan-Meier plots of pupation after feeding on experimental diets as specified in key. Mito-nuclear genotypes are indicated in top-left of each panel. (**B**). Estimated marginal means (EMMs) with 95% confidence intervals summarising survival analysis (Cox mixed effects) of data from A. Higher values correspond to faster development and/or increased fertility, lower values correspond to slower development and/or reduced viability. Data are presented per genetic replicate in Figure S4. Accompanying statistical analysis presented in Table S4.

Figure S4

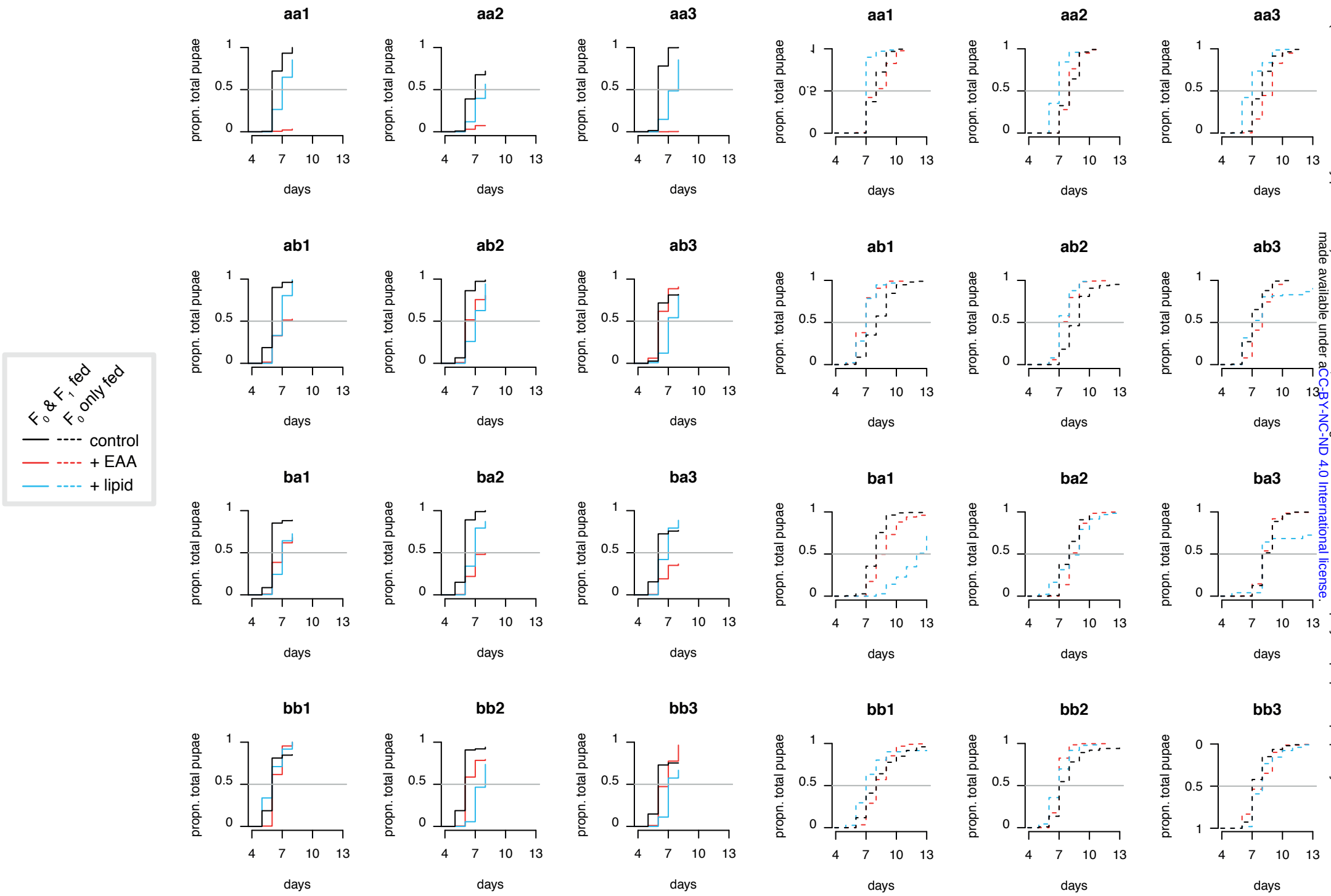

Figure S4. Mito-nuclear variation in dietary regulation of pupation: impacts per genetic replicate. Kaplan-Meier plots of pupation after feeding on experimental diets as specified in key. Mito-nuclear genotypes are indicated per panel. Data are presented pooled across each replicate genotype in Figure S5. Accompanying statistical analysis presented in Table S4 and Table S10

Figure S5

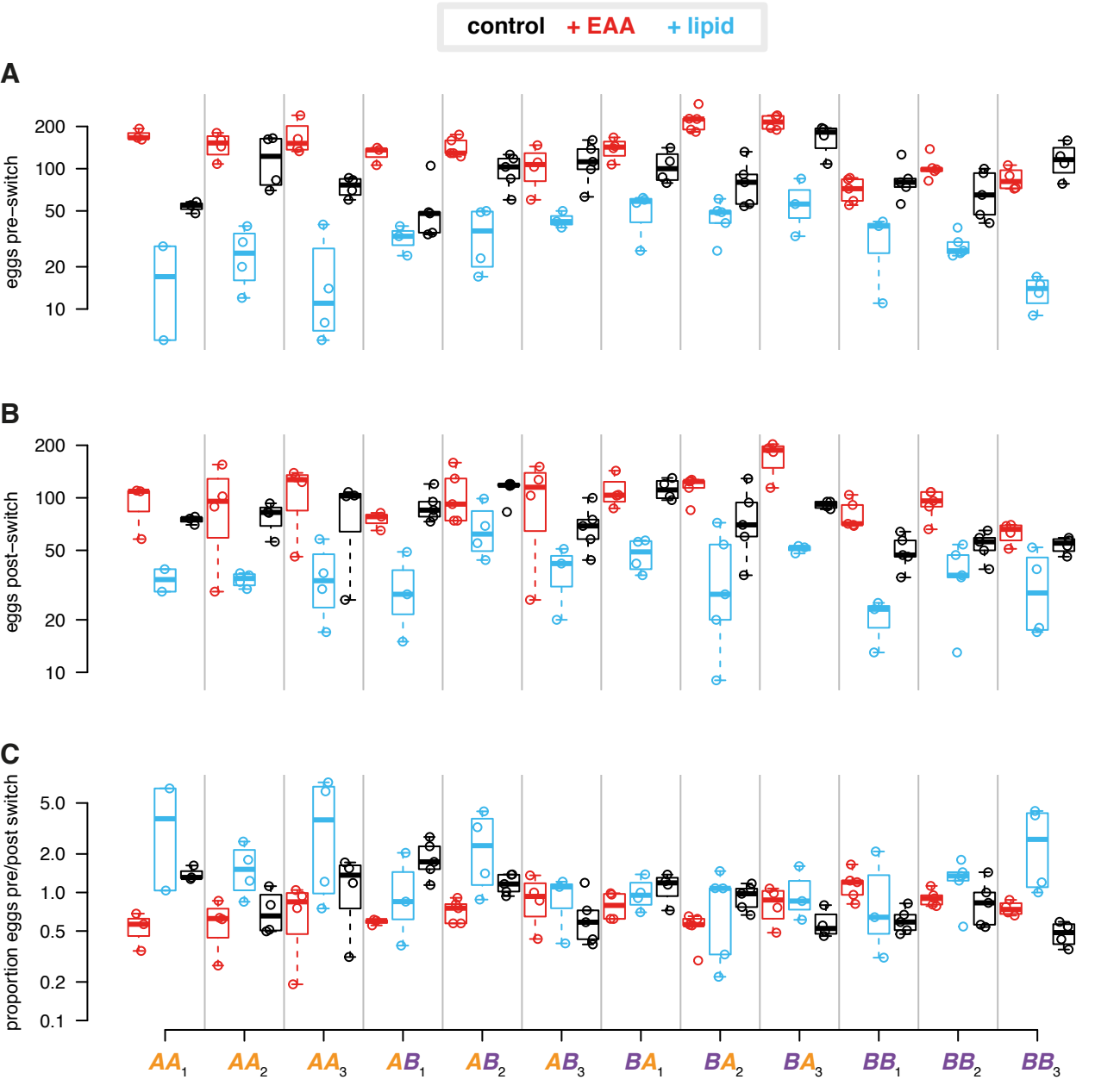
control
+ EAA
+ lipid
A
eggs pre–switch
200
100
50
20
10
B
eggs post–switch
200
100
50
20
10
C
proportion eggs pre/post switch
5.0
2.0
1.0
0.5
0.2
0.1
AA1
AA2
AA3
AB1
AB2
AB3
BA1
BA2
BA3
BB1
BB2
BB3

Figure S5. Mito-nuclear variation in fecundity response to switching from experimental diets to a standardised diet: impacts per genetic replicate.

Fecundity measures are shown per genotype as specified on bottom x axis. Boxplots show medians, first and third quartiles and 5th and 95th percentiles. Points show individual data. **A**. Fecundity before diet switch. **B.** Fecundity after diet switch. **C.** Ratio of fecundity before and after diet switch. Data are presented pooled across each replicate genotype in Figure 3. Accompanying statistical analysis presented in Tables S5, S7 and S8.

Figure S6

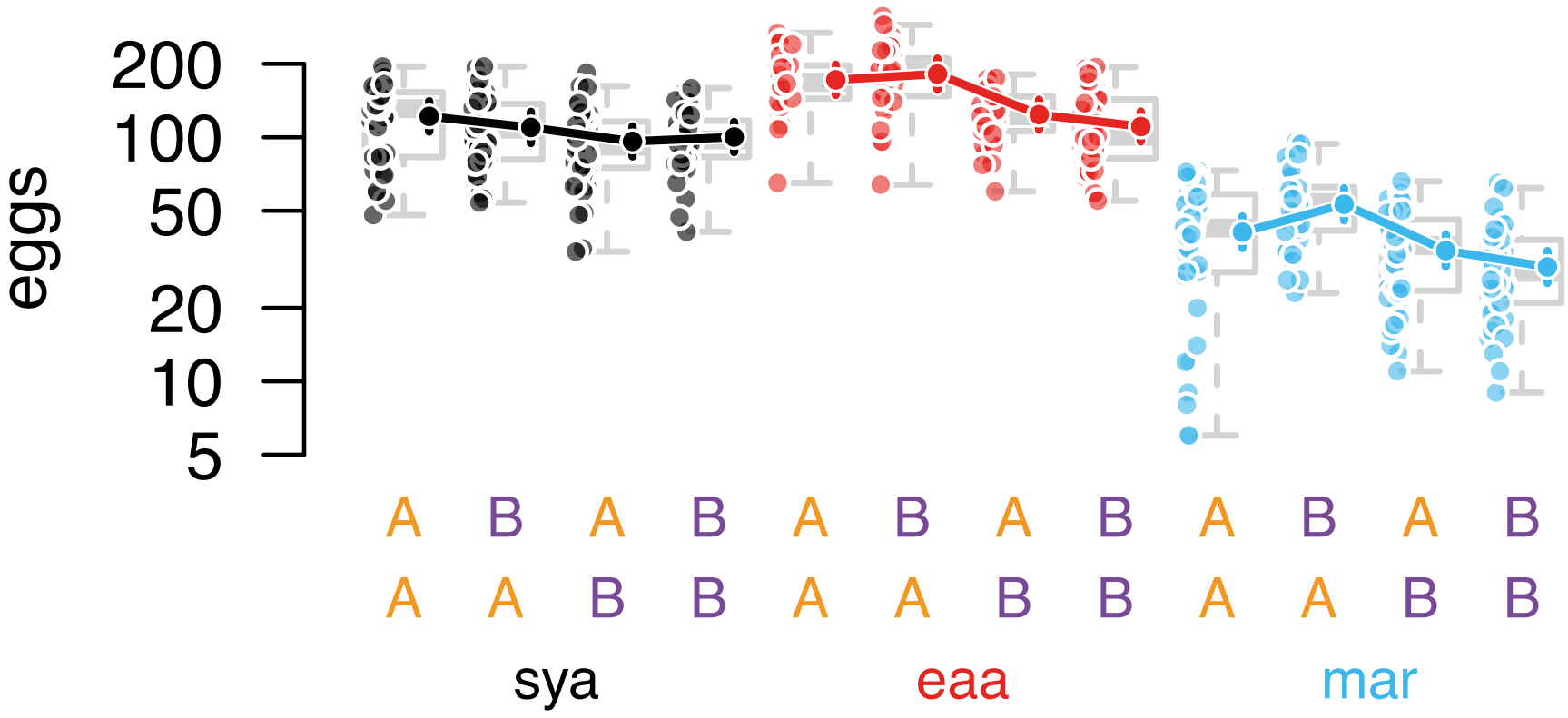
eggs
200
100
50
20
10
5
A B A B A B A B A B A B
A A B B A A B B A A B B
sya
eaa
mar

Figure S6. Diet-mito-nuclear interactions modulate *Drosophila* fecundity - emergent pattern from three replicate experiments. Fecundity is shown per genotype as specified on bottom x axis. Boxplots show medians, first and third quartiles and 5th and 95th percentiles. Connected points to right of each box show *post-hoc* comparisons of means (by estimated marginal means with 95% confidence intervals, calculated from a generalised linear mixed effects model, showing exponent of EMMs in order to fit to original data scale). Translucent points to left of each box show raw data. Accompanying statistical analysis presented in Table S6.

Supplementary Text

Much recent research in *Drosophila* and other organisms has been directed to characterising the consequences transmission of mitochondria from mothers only, which restricts mito-nuclear coevolution to females (Gemmell et al., 2004; Maklakov and Lummaa, 2013; Montooth et al., 2019; Nagarajan-Radha et al., 2020). This is thought to cause a "mother's curse" (Gemmell et al., 2004), in which males bear their mothers' mitochondria despite potential mismatch to variants inherited from fathers. In our study of development we manipulated diet and mito-nuclear genotype, and recorded sex of emerging adult offspring, giving four potential predictive factors. Our main analyses are presented stratified by sex. ANOVA tests of the complex diet:mito:nuclear:sex interaction were nominally supportive of sex biases in DMN interactions, but did not point solely to males. We detected significant sex:diet:mito:nuclear interactions, but even with our large sample sizes we are cautious in interpreting such complex interaction terms. F-ratios for DMN interactions were greater in males when only parents were fed experimental diets (Table S12), but were greater in females when both F0+F1 were fed experimental diets (Table S11), suggesting that sex dimorphisms manifest as different magnitudes of the same interaction. Visual inspection of the data led to a similar conclusion (Figure S2). We therefore interpret that any statistical differences reflected relatively modest differences in the present data, suggesting that mothers curse may exert a relatively benign influence in this case.