1 **Impact of mitonuclear interactions on life-history responses to diet**

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3 M. Florencia Camus[1], Michael O'Leary[1], Max Reuter[1] and Nick Lane[1]

4

5 [1] *Research Department of Genetics, Evolution and Environment, University College*

6 *London, Gower Street, London, WC1E 6BT, United Kingdom*

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10

Abstract

12 Mitochondria are central to both energy metabolism and biosynthesis. Mitochondrial function

13 could therefore influence resource allocation. Critically, mitochondrial function depends on

14 interactions between proteins encoded by the mitochondrial and nuclear genomes. Severe

15 incompatibilities between these genomes can have pervasive effects on both fitness and

16 longevity. How milder deficits in mitochondrial function affect life-history trade-offs is less

17 well understood. Here we analyse how mitonuclear interactions affect the trade-off between

18 fecundity and longevity in *Drosophila melanogaster*. We consider a panel of 10 different

19 mitochondrial DNA haplotypes against two contrasting nuclear backgrounds (WE and ZIM)

20 in response to high-protein versus standard diet. We report strikingly different responses

21 between the two nuclear backgrounds. WE females have higher fecundity and decreased

22 longevity on high-protein. ZIM females have much greater fecundity and shorter lifespan

23 than WE flies on standard diet. High-protein doubled their fecundity with no effect on

24 longevity. Mitochondrial haplotype reflected nuclear life-history trade-offs, with a negative

25 correlation between longevity and fecundity in WE flies and no correlation in ZIM flies.

26 Mitonuclear interactions had substantial effects but did not reflect genetic distance between

27 mitochondrial haplotypes. We conclude that mitonuclear interactions can have significant

28 impact on life-history trade-offs, but their effects are not predictable by relatedness.

29

Introduction

Mitochondria are not only hubs of energy metabolism but are also responsible for providing the precursors for biosynthetic pathways, including amino-acid, nucleotide, fatty-acid and sugar biosynthesis (1, 2). This means that mitochondrial function necessarily underpins resource allocation, but little is known about this role. A classic evolutionary example of resource allocation is the trade-off between reproduction and longevity, in which animals live fast and die young or vice versa (3). At a cell physiology level, this trade-off involves a shift in metabolic flux through the Krebs cycle (4-6). Active growth favours a biosynthetic flux pattern (anaplerosis) in which relatively more Krebs-cycle intermediates are fed into biosynthetic pathways (7, 8), whereas extended longevity is linked with more conventional cyclic flux in which a higher proportion of intermediates is directed to oxidative phosphorylation, coupled to autophagy, amplified quality-control and resource recycling (9-11). Importantly, shifts in the balance of Krebs cycle intermediates can signal pervasive changes in gene expression and epigenetic status (12). These alterations in flux balance are best understood in cancer (13, 14), but similar shifts likely underpin resource allocation in life-history trade-offs between fecundity and longevity at the organism level. While Krebs cycle flux unavoidably reflects mitochondrial function (15-18), how far that impacts on life-history traits is often obscured by numerous factors including diet, stress, age and nuclear genotype.

One approach to understanding how mitochondrial function might affect life-history trade-offs is to analyse variations in mitochondrial function produced by different mitochondrial DNA (mtDNA) haplotypes and their interaction with the nuclear genomic background. Crosses between very divergent populations can produce severe incompatibilities between mitochondrial and nuclear genomes, causing hybrid breakdown and arguably speciation (19-23). Much of this evidence comes from the copepod model system (*Tigriopus californicus*), where the mitochondrial divergence between neighbouring populations can exceed 20% (19-23). But less dramatic mitonuclear interactions in populations with lower mitochondrial divergence are also predicted to influence life-history trade-offs, constraining how the two genomes can be selected and coevolve, especially in relation to adaptation to changing environments (24-27). *Drosophila melanogaster* is an invaluable model for 'clean' mitonuclear studies, as balancer chromosomes permit analysis of specific mtDNA haplotypes against an isogenic nuclear background (28). Because mitochondrial function depends on

63 both mitochondrial and nuclear genomes, incompatibilities between these genomes exert a

64 primary effect on metabolic flux. Any alterations in signalling, gene expression, reproductive

65 fitness and longevity then necessarily reflect this primary deficit in mitochondrial function,

66 offering exceptional insight into how mitochondrial function modulates life-history trade-

67 offs. As the variation in mtDNA between different strains of *D. melanogaster* is modest (28,

68 29), it is fairly representative of interbreeding populations, hence any phenotypic effect of

69 mitonuclear mismatches generated in the laboratory should speak directly to real-world life-

70 history trade-offs.

71

72 If mitonuclear incompatibilities do indeed force a shift in resource allocation towards either

73 fecundity or longevity, this should produce a negative correlation between these fitness

74 components, with increased fecundity lowering longevity or vice versa. Conversely, if

75 mitonuclear incompatibilities undermine mitochondrial function but resource allocation is

76 unchanged, the null hypothesis is that both fecundity and longevity would be suppressed,

77 generating a positive correlation in which better mitochondrial function increases both

78 fecundity and lifespan. These putative relationships are depicted in **Figure 1**. Our aim is to

79 capture high levels of genetic variation for both fecundity and longevity. For this, we use a

80 panel comprising 10 naturally-occurring mtDNA genomes against two distinct isogenic

81 nuclear backgrounds, w^{1118} (WE) and Zim53 (ZIM) on standard versus high-protein diets.

82 WE and ZIM flies have striking differences in fecundity and longevity (30), with ZIM flies

83 exhibiting some sexual isolation, possibly even incipient speciation (31). High-protein diets

84 tend to promote female fecundity at the expense of longevity, reflecting the high protein

85 requirements of egg production (32-34). We report that mitonuclear interactions do indeed

86 have substantial effects on both fecundity and longevity, but their effect on life-history trade-

87 offs depends on the nuclear background.

88

Materials and Methods

90 *Drosophila* maintenance

91 Twenty mitonuclear genotypes of *Drosophila melanogaster* were used in this experiment.

92 These genotypes were pairwise combinations of two different isogenic nuclear genotypes

93 ZIM53 (35) and WE (w^{1118}, (28)) and ten different mitochondrial haplotypes gathered from

94 sites globally (28): ZIM (Zimbabwe), WE (w^{1118} origin = Ohio, USA), ARM (Armenia),

95 DAH (Dahomey, Benin), BAR (Barcelona, Spain), ORE (Oregon, USA), PUE (Puerto

96   Montt, Chile), MYSO (Mysore, India), MAD (Madang, Papua New Guinea), and ALS

97   (Alstonville, Australia). The w^{1118} nuclear background is derived from the Canton-S strain

98   and harbours the white eye mutation (Bloomington stock number #5905). On the other hand,

99   the ZIM nuclear background was obtained from a field collection in early in the 1990s. The

100   WE mito-nuclear panel was generated over a decade ago, whereas the ZIM panel was

101   generated in 2016. Although the flies have gone through a couple of labs which utilise

102   different recipes for stock maintenance, all flies have been maintained on the

103   molasses/cornmeal/yeast recipe since 2016.

104

105   Lines were propagated by 4-day old parental flies, with approximate densities of 80-100 eggs

106   per vial. Flies were kept at 25°C and 50% humidity, on a 12:12 hour light:dark cycle, and

107   reared on 8mL of cornmeal-molasses-agar medium per vial (see Table S1 for recipe), with *ad*

108   *libitum* live yeast added to each vial to promote female fecundity. All lines had been cleared

109   of potential bacterial endosymbionts, such as *Wolbachia*, through a tetracycline treatment at

110   the time the lines were created. Clearance was verified using *Wolbachia*-specific PCR

111   primers (36).

112

113   Nutritional treatments

114   This study employed two nutritional treatments that were formulated to be roughly isocaloric

115   but differed in their protein-to-carbohydrate ratios. Our control diet (standard – ST) is the

116   molasses-cornmeal-yeast agar media used to propagate the flies (Table S1). We calculated

117   this diet to have an approximate protein-to-carbohydrate ratio of 1:4. Our experimental diet

118   (PRO) was formulated to have increased protein content, by increasing the amount of yeast in

119   the diet (Table S1). The protein-to-carbohydrate ratio for this diet is approximately 1:2. We

120   do acknowledge that there some variation in the nutritional components.

121

122   Longevity assay

123   In the generation prior to the experiment, 4-day old flies were allowed to lay eggs on standard

124   media during a 24-hour period. Ten vials were set up for each mitonuclear line, with egg

125   numbers being immediately culled to 80-100 per vial to avoid overcrowding. Eclosing

126   offspring were collected within a 24-hour period and were placed into separate population

127   cages for each line (20 cages in total). This was to control for confounding environmental

128   sources of variation underpinned by vial-sharing. All population cages were provisioned

129   with standard food media for the flies to feed, thus levelling between-vial variation during

130   development. Flies remained in cages for 48 hours to feed and for mating to occur. Following

131   this period, flies were anesthetised lightly using CO_2 and set up in single-sex groups of 10-15

132   flies in vials containing food of one of the two diet treatments. Ten vials were set up for each

133   combination of genotype, diet and sex, 800 vials in total.

134

135   Flies were transferred to new food vials, of the same dietary treatment, three times a week for

136   the duration of the study. Upon transfer the number of deaths was recorded for each vial.

137   Live flies (identified by twitching or other signs of life) which had become stuck on the

138   media were transferred to the new vial using a scalpel.

139

140   <u>Fecundity Measurements</u>

141   Fecundity measurements were collected over 5 time points during the experiment, on days 6,

142   13, 20, 27 and 35. For each time point, female flies were placed on food specific their

143   nutritional treatment for a period of 18-hours. Clutch sizes were determined semi-

144   automatically using the *QuantiFly* software, which counts eggs after a period of user-assisted

145   training (37).

146

147   <u>Statistical Analysis</u>

148   Longevity analyses were performed using the *survival* package in R (38, 39). We used Cox

149   proportional hazard models, with survival as a response variable. Mitochondrial haplotype,

150   nuclear genotype, dietary treatment, sex and all their interactions were entered as fixed

151   effects in the model. We also investigated this dataset in a sex-specific manner, splitting the

152   data by sex and running models for each sex that included mtDNA, nuDNA, diet and their

153   interactions as fixed effects.

154

155   Female fecundity was measured in the format of number of eggs laid per individual fly in

156   each vial. This accounts for differences in fly numbers between vials caused by mortality. We

157   analysed the data in two ways. We first summed the total number of eggs for each vial to get

158   a measure of total fecundity. Generalised linear mixed models (with a quasi-poisson error

159   distribution to accommodate the over-dispersed data) were fitted to total fecundity, with

160   mtDNA, nuDNA and diet and their interactions as fixed terms. The second way of analysing

161   the data was by getting clutch numbers per day, giving us an indication of fecundity changes

162   with time. For this, we used the same method as before; clutch size was a response variable,

163   with mtDNA, nuDNA, diet and day as fixed factors. Models were also fitted using general

164   linear models and quasipoisson distribution.

165

166   To investigate possible trade-offs between egg production and survival, we calculated

167   correlation coefficients between these two traits. Correlation coefficients were assessed using

168   a bootstrapping procedure, in which trait datapoint means were resampled with

169   replacement (10,000 replicates), and 95% confidence intervals were calculated using the

170   Adjusted Percentile (BCa) Confidence interval method (40). Bootstrapped correlation

171   coefficients plus their confidence intervals were calculated using the functions "*boot*" and

172   "*boot.ci*" in the R package *boot* (41).

173

174   Given that sequences are available for the protein coding genes of all mitochondrial

175   haplotypes used in our study (28, 42), we also tested if there was a correlation between the

176   genetic distance between strains and their phenotypic divergence in longevity and fecundity.

177   To this end, we created matrices of genetic and phenotypic distances between strains. Genetic

178   distance was quantified as total number of SNPs difference between lines. Phenotypic

179   matrices were specific to each experimental treatment (performed per sex, nuclear genotype,

180   and diet regime) and phenotypic trait (longevity and fecundity). We used a mantel test for

181   matrix correlation between two dissimilarity matrices. Mantel test were implemented with the

182   "*mantel.rtest*" function from the R package *ade4* (43).

183

Results

<u>Longevity</u>

186   Our results indicate that the nuclear genome plays a large role in determining lifespan, with

187   the WE nuclear genome having a greater lifespan than the ZIM across both sexes ($\chi^2 =$

188   4046.0, p < 0.001, **Table 1, Figure 2**). We also find a significant sex effect ($\chi^2 = 592.28$, p <

189   0.001, **Table 1, Figure 2**), although this effect is contingent on the nuclear genome. Females

190   of the WE nuclear background live longer than males, while this effect is reversed in the ZIM

191   nuclear genome (nuc × sex: $\chi^2 = 1201.95$, p < 0.001, **Table 1**). These patterns are consistent

192   with previous data on these two nuclear backgrounds (30, 44). Diet had a significant effect on

193   longevity ($\chi^2 = 352.94$, p < 0.001, **Table 1, Figure 2**), but again this effect depended heavily

194   on the nuclear background (nuc × diet: $\chi^2 = 738.74$, p < 0.001, **Table 1**). High protein diet

195   caused shorter lifespan in the WE nuclear background but did not have a discernible effect on

196 lifespan in the ZIM nuclear background. Overall, we found significant levels of

197 mitochondrial genetic effects for survival across all treatments ($\chi^2 = 82.12$, p < 0.001, **Table**

198 **1, Figure 2**), but these effects were highly dependent on all other factors (mito × nuc × sex ×

199 diet: $\chi^2 = 19.96$, p = 0.018, **Table 1, Figures S1-S3**).

200

201 <u>Fecundity</u>

202 We found significant fecundity differences between the two nuclear genotypes, with flies

203 carrying the ZIM nuclear background laying more eggs than flies carrying WE (p < 0.001,

204 **Table 2, Figure 3**). High protein diets increased fecundity across all flies (p < 0.001, **Table**

205 **2, Figure 3**) and we found an interaction between nuclear background and diet (nuc × diet: p

206 = 0.044, **Table 2**), reflecting the fact that fecundity increased more on the high protein diet in

207 flies with the ZIM than the WE nuclear background (nuc × diet: p < 0.001, **Table 2, Figure**

208 **4**). We also found that the mtDNA did impact fecundity of the flies (p < 0.001, **Table 2,**

209 **Figure 3**), with this effect being contingent on the nuclear genome (mito × nuc: p < 0.001,

210 **Table 2, Figure 4**), and on diet (mito × diet: p = 0.023, **Table 2, Figure S4**).

211

212 <u>Longevity and fecundity trade-offs</u>

213 We examined the relationship between fecundity and longevity across all our treatments and

214 found a negative correlation across all our samples (r = -0.720, CI = -0.835, -0.570). On

215 closer inspection however, this relationship was dependent on the nuclear genome. Thus,

216 while a survival-fecundity trade-off was present in the WE nuclear background (r = -0.921,

217 CI = -0.959, -0.867, **Figure 4, Figure S5**), we found no significant relationship between the

218 two life-history variables in the ZIM nuclear background (r = 0. 261, CI = -0.262, 0.596,

219 **Figure 4, Figure S5**).

220

221 <u>Matrix correlations</u>

222 We found no significant correlations between the genetic and phenotypic distance matrices in

223 any of the comparisons (**Figure S6**).

224

225 **Discussion**

226 In this study, we set out to examine how interactions between mitochondrial and nuclear

227 genotypes shape resource allocation in relation to the nutritional environment (G×G×E) in

228 *Drosophila melanogaster*. To do so, we measured longevity and fecundity patterns across a

229   panel of 20 fly genotypes comprising two nuclear genotypes coupled with 10 naturally-

230   occurring mitochondrial haplotypes each. We examined this relationship in two different

231   nutritional environments: standard rearing food and a high protein food. Our results reveal

232   complex interactions for both longevity (mito×nuc×diet×sex) and fecundity (mito×nuc×diet).

233   In broad terms, our findings are consistent with previous work on G×G×E in *Drosophila* (24,

234   45, 46) and highlight the difficulty of generalizing about mitonuclear effects without

235   considering a wide sample of mitonuclear variation across populations (47, 48). While

236   nuclear genes largely shape life history traits in response to diet, mitonuclear interactions

237   modulate these nuclear effects in ways that are not easy to predict.

238

239   We show that mitonuclear function is indeed implicated in trade-offs between life history

240   traits. However, the effect of mitonuclear interactions on life-history trade-offs depends on

241   the nuclear genotype (**Figure 4**). Females harbouring the WE nuclear genome exhibit traits

242   that are consistent with a classic trade-off between fecundity and longevity (49), in which

243   mitonuclear interactions produce a negative correlation between mean longevity and the total

244   number of eggs per fly (**Figures 4 and S5**). This corresponds to our hypothesis and shows

245   that mitonuclear interactions do indeed alter resource allocation and life-history trade-offs in

246   flies with the WE nuclear background. In contrast, flies harbouring the ZIM nuclear

247   background behave very differently in terms of resource allocation, showing no correlation

248   between mean longevity and the total number of eggs per fly (**Figures 4 and S5**). This

249   pattern does not correspond to either our mitonuclear resource-allocation hypotheses or the

250   null hypothesis (**Figure 1**). Presumably ZIM flies harbour nuclear genetic variance which

251   benefits fecundity at the cost of longevity regardless of mitochondrial function. On the

252   standard diet, the fecundity of ZIM females was nearly double that of WE, whereas their

253   lifespan was little more than half – shorter even than ZIM males, reversing the normal sexual

254   pattern in *D. melanogaster* (**Figure 2**) (30, 50). The high-protein diet nearly doubled female

255   ZIM fecundity again (**Figure 3**) with no further costs to longevity, suggesting that resource

256   allocation is genuinely resource-limited in ZIM females – females can simply make more

257   eggs on the high-protein than the standard diet. In contrast, the reproductive output of WE

258   females is far more context dependent, based on the allocation between competing life-

259   history traits, rather than limitation on nutrient flow from food to eggs.

260

261   High protein diets are known to increase growth rates through mTOR and insulin-related

262   signalling pathways (8), with well-established costs to longevity (51-53). We predict that

263 these pathways are upregulated in ZIM flies even on standard diet and will examine this

264 possibility in future metabolomic and gene expression work. Curiously, the high-protein diet

265 had no effect on ZIM male longevity either, despite suppressing longevity in WE males to

266 similar degree as in WE females (**Figure 2**). This might imply that ZIM flies have nuclear

267 alleles that benefit reproduction at the cost of longevity in both sexes, with the shorter

268 lifespan in females being caused by the higher costs of egg production compared with sperm

269 production in males (54). However, we did not examine male fertility in this study as the

270 major differences between competitive and non-competitive fitness in males complicate

271 resource-allocation (55, 56). Here we aimed simply to establish how mitochondrial function

272 influences resource allocation and life-history trade-offs in the most clear-cut case, to

273 establish the principle.

274

275 The radically different correlations between life-history traits in ZIM versus WE females

276 emphasize the dangers of generalizing from mitochondrial interactions characterised against

277 a single nuclear background: presumably the nuclear background sets the physiological tone

278 against which the mitochondria must function. However, in both WE and ZIM females, it is

279 striking that the coevolved fly lines (WE_{nu}-WE_{mt} and ZIM_{nu}-ZIM_{mt}) tended to produce fewer

280 eggs than other, *a priori* more mismatched lines, even though their longevity was close to the

281 mean on both standard and high-protein diets (**Figure 4**). Mitonuclear incompatibilities

282 therefore do not have a clear phenotypic cost in either WE or ZIM females, regardless of any

283 life-history trade-off. On the contrary, the coevolved flies appeared to be among the least fit

284 lines in every case. This relationship cannot be ascribed simply to mutations at the level of

285 mtDNA, as the same mtDNA frequently had opposing effects depending on the nuclear

286 background. For example, WE mtDNA was among the least fecund on the coevolved WE

287 nuclear background on both diets, but among the most fecund on the apparently mismatched

288 ZIM nuclear background, again on both diets (**Figure 3**). These findings would imply that

289 mitonuclear incompatibilities contribute little if anything to the partial reproductive isolation

290 (or incipient speciation) of ZIM flies (31, 57, 58). In contrast to the copepod *Tigriopus*

291 *californicus*, in which severe mitonuclear incompatibilities cause hybrid breakdown (19–23),

292 there is much less mitochondrial divergence between ZIM flies and other *D. melanogaster*

293 lines. Far from inducing hybrid breakdown, ostensibly mismatched mtDNA haplotypes seem

294 to enhance rather than disrupt the fitness of ZIM flies.

295

296   Naively, this greater fecundity might seem to be heterosis, but the nuclear background is
297   isogenic in each case, so there cannot be any masking of deleterious alleles by the distinct
298   genes encoded by mtDNA. It is possible there could have been some physiological benefit at
299   the level of metabolic flux: accumulating mutations in inbred laboratory lines could limit
300   mitochondrial function and restrict metabolic flux. Introducing distinct mtDNA haplotypes
301   might open up alternative flux patterns, with benefits for fecundity. Future studies will aim to
302   examine this possibility using a combination of metabolomics and transcriptomics. We note
303   that earlier studies on mice have also implied that there could be benefits to mitonuclear
304   mismatches (59), and that there is little evidence of mitonuclear incompatibility between
305   mtDNA haplotypes (as opposed to nuclear variability producing stochastic mitonuclear
306   incompatibilities between individuals) in humans (60-63).
307
308   Even though the coevolved flies were frequently among the least fit lines on both WE and
309   ZIM nuclear backgrounds, we found no association between genetic distance (number of
310   SNPs difference from the coevolved mtDNA) and differences in either longevity or fecundity
311   (**Figure S6**). This finding implies that there has been limited selection for mitonuclear
312   coadaptation within the two nuclear populations sampled, even though mitonuclear
313   incompatibilities can certainly disrupt function. The maximum genetic divergence between
314   mtDNA haplotypes in our study was around 50 SNPs, which is roughly comparable with
315   differences between human populations and very substantially less than copepods such as
316   *Tigriopus californicus*. In more divergent populations, deleterious mutations in mtDNA are
317   often unmasked through outcrosses that supplant compensating alleles in nuclear DNA (19,
318   64). We did not observe any evidence for nuclear compensation in the present study, insofar
319   as the coevolved strains (which would be expected to exhibit some nuclear compensation for
320   mtDNA mutations) were not among the fittest lines, although our sample size of coevolved
321   genotypes was admittedly limited (N=2). A larger-scale study using multiple mitonuclear
322   genotypes plus wider range of environmental conditions could reveal a stronger signal of
323   coevolution.
324
325   The most likely explanation for the lack of a relationship with genetic distance in this study is
326   that mitonuclear interactions exert significant but inconsistent effects on life-history traits in
327   different contexts. For example, we observe no consistent relationship between mtDNA
328   haplotype and either longevity or fecundity in males or females, on either diet, suggesting
329   that the need to buffer large variations in mitochondrial function renders most mitonuclear

interactions in our study neutral overall (**Figure S1-4**). This might help explain the apparent contradiction between historical claims that most mtDNA mutations are neutral (65, 66), and strong evidence for both purifying and adaptive selection on mitochondrial genes (67-69): mtDNA mutations are not neutral, but selection for any specific mitochondrial trait is offset by the requirement for strong buffering of mitochondrial function in other contexts (different sexes, tissues, diets or temperatures). If so, then adaptation to consistently distinct metabolic environments (specific diets or temperatures) is more likely to reveal consistent mitonuclear coadaptation.

We conclude that mitonuclear interactions can exert substantial, albeit inconsistent, effects on resource allocation and life-history trade-offs in *Drosophila melanogaster*. These effects vary greatly between the two nuclear genotypes we used in our study. WE flies exhibit a negative correlation between female fecundity and longevity, whereas ZIM flies show no correlation at all, likely because the nuclear genotype harbours alleles underpinning a different resource allocation strategy, favouring fast growth, high fecundity and short longevity. Our findings indicate that it is hazardous to generalise from mtDNA interactions with a single nuclear background, and future work will need to examine a wider range of nuclear genotypes, including outbred laboratory lines. In closely related fly populations (where the number of SNPs distance in mtDNA is similar to human populations) mitonuclear interactions do have significant impact on life-history trade-offs, but these effects are not predictable by relatedness.

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530

Figures and Tables

Table 1: Results from the full Cox proportional hazards model. Here we examine the effects of mtDNA, nuDNA, diet and sex on survival.

|	loglik	Chisq	Df	Pr(>Chi)
NULL	-57110			
mito	-57069	82.1177	9	< 0.001
nuc	-55046	4046.0458	1	< 0.001
sex	-54750	592.2899	1	< 0.001
diet	-54573	352.9434	1	< 0.001
mito × nuc	-54525	97.1015	9	< 0.001
mito × sex	-54504	40.5227	9	< 0.001
nuc × sex	-53903	1201.9533	1	< 0.001
mito × diet	-53889	28.3546	9	< 0.001
nuc × diet	-53520	738.7442	1	< 0.001
sex × diet	-53509	21.8285	1	< 0.001
mito × nuc × sex	-53495	28.9026	9	< 0.001
mito × nuc × diet	-53479	31.8569	9	< 0.001
mito × sex × diet	-53456	46.0503	9	< 0.001
nuc × sex × diet	-53453	4.4679	1	0.03453
mito × nuc × sex × diet	-53443	19.9567	9	0.01818

Table 2: Results from full generalised linear model examining the effects on mtDNA, nuDNA and diet on female fecundity.

|	Df	Deviance	Resid. Df	Resid. Dev	Pr(>Chi)
NULL			399	2411.2	
mito	9	32.12	390	2379.08	< 0.001
nuc	1	1011.18	389	1367.9	< 0.001
diet	1	874	388	493.9	< 0.001
mito × nuc	9	45.61	379	448.29	< 0.001
mito × diet	9	21.6	370	426.69	0.02329
nuc × diet	1	4.52	369	422.16	0.044769
mito × nuc × diet	9	13.47	360	408.69	0.213812
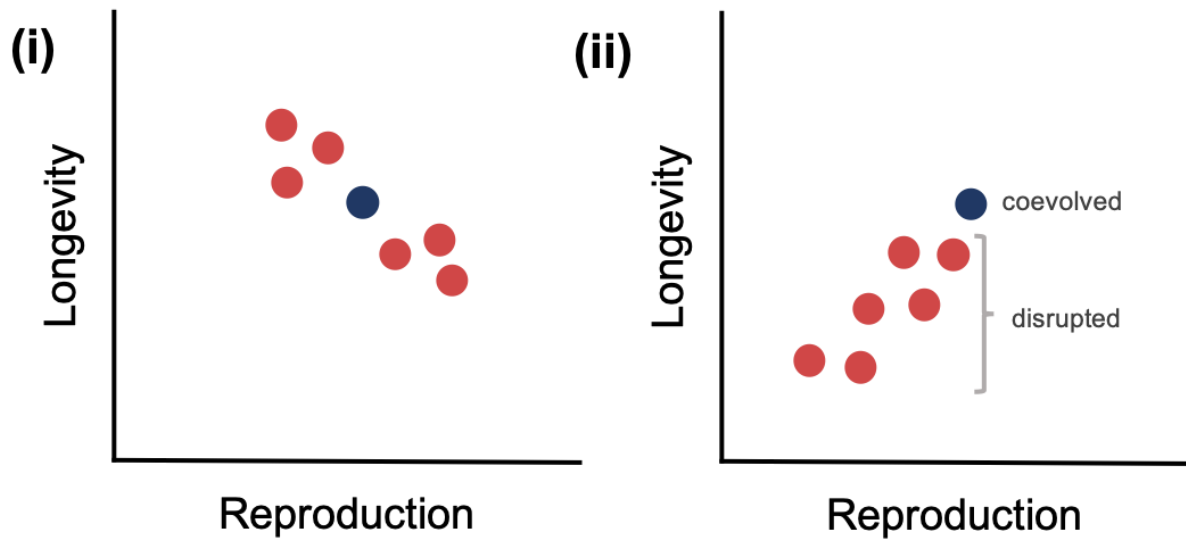
541
Figure 1: Schematic showing how mitochondrial function might affect resource allocation:
(i) mitonuclear incompatibilities force a shift in resource allocation towards either fecundity
or longevity, giving a negative correlation between these fitness components; or (ii) the null
hypothesis: if mitonuclear incompatibilities undermine mitochondrial function but resource
allocation is unchanged, then both fecundity and longevity would be suppressed, generating a
positive correlation in which better mitochondrial function increases both fecundity and
lifespan.
549
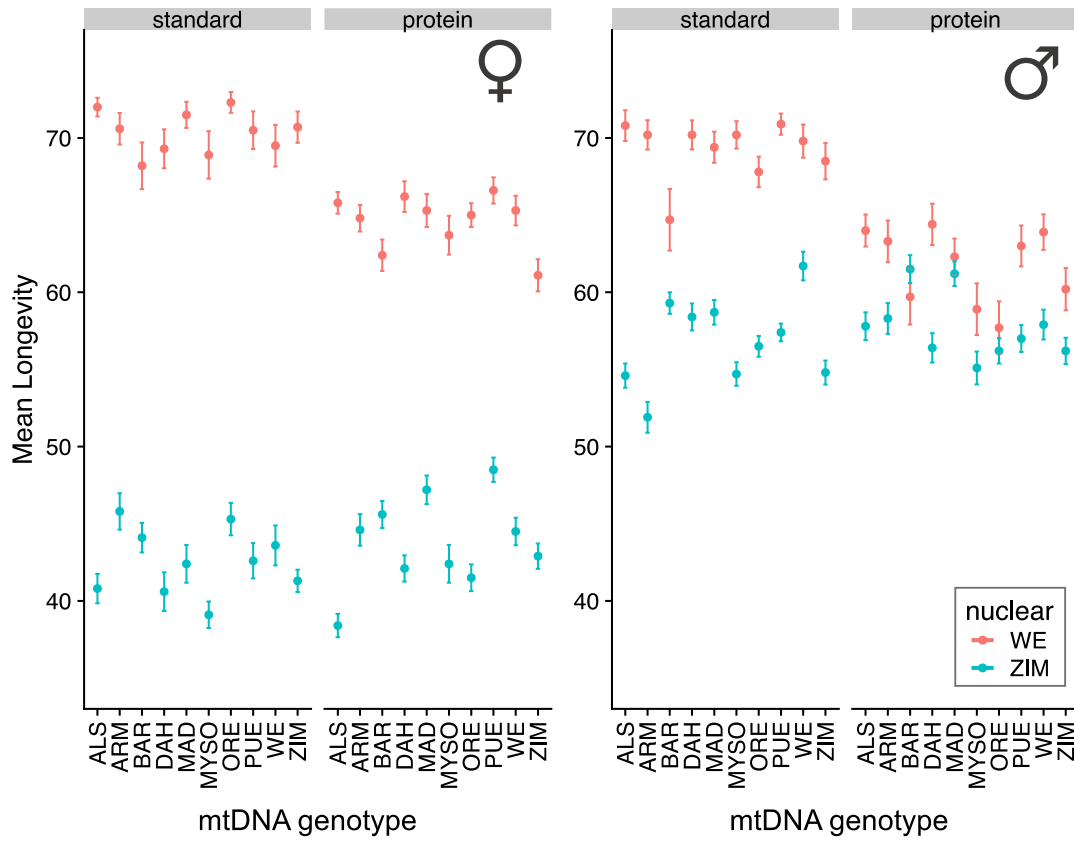
Figure 2: Mean longevity estimates across all experimental treatments. Female estimates are on the left, and male survival estimates are on the right. Within each sex we show mean longevity for the ZIM (blue) and WE (red) nuclear background for both diet treatments.
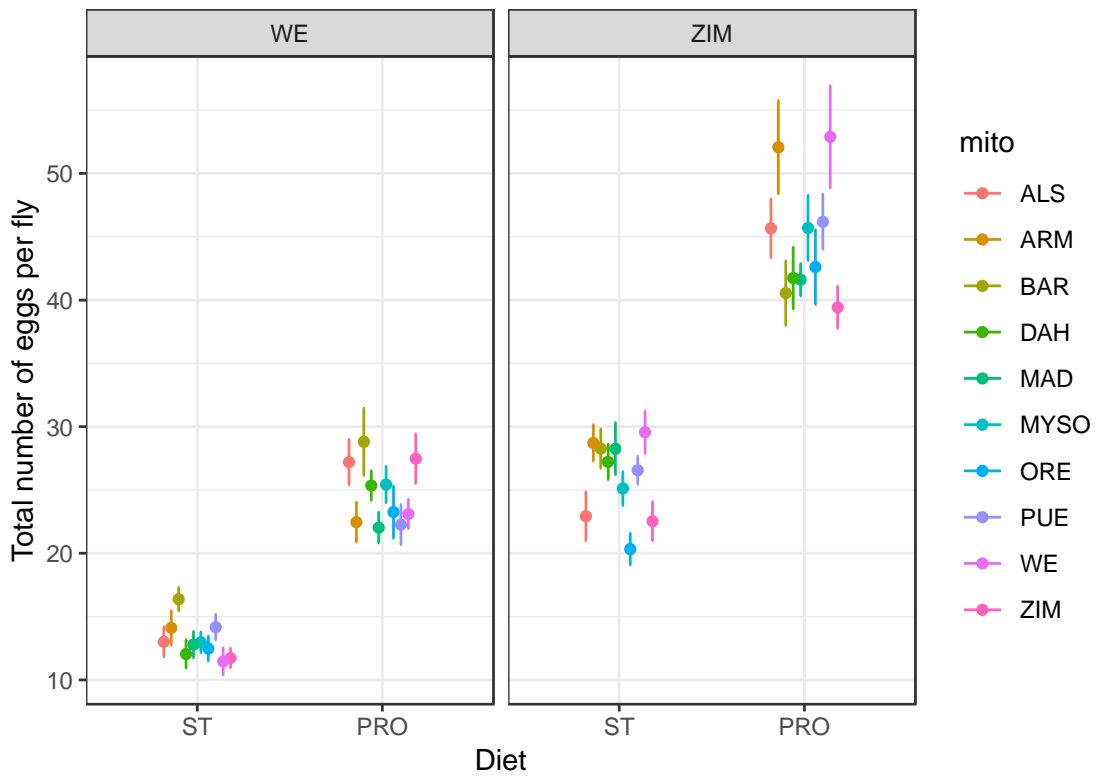
Figure 3: Total number of eggs produced per fly over 5 timepoints. ST = standard diet; PRO = high-protein diet
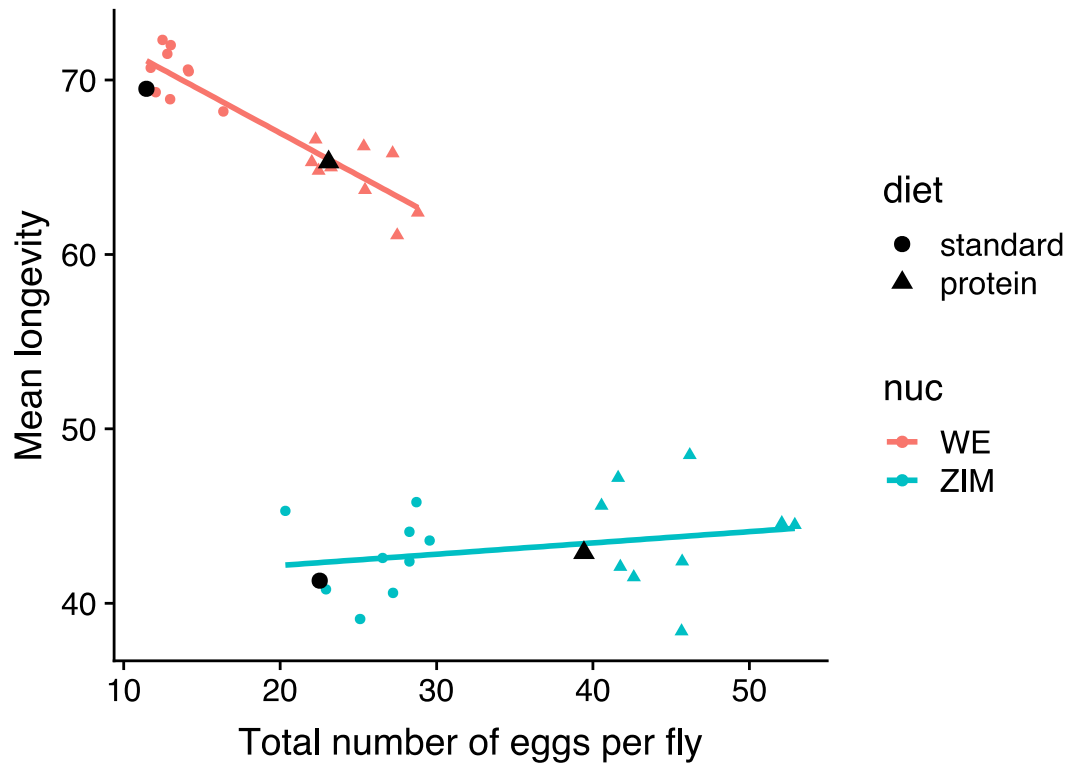
569
570 **Figure 4:** Longevity versus fecundity relationship across all treatments. Correlations within
571 each nuclear genotype are highlighted with each datapoint being a mito-nuclear genotype.
572 Black datapoints denote genotypes that are coevolved (WE-WE, ZIM-ZIM).
573