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1 # Sex-specific transgenerational effects of diet on offspring life
2 history and physiology

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12 ## Abstract

13 Dietary variation in males and females can shape the expression of offspring life histories
14 and physiology. However, the relative contributions of maternal and paternal dietary
15 variation to phenotypic expression of latter generations is currently unknown. We provided
16 male and female *Drosophila melanogaster* diets differing in sucrose concentration prior to
17 reproduction, and similarly subjected grandoffspring to the same treatments. We then
18 investigated the phenotypic consequences of this dietary variation among grandsons and
19 granddaughters. We demonstrate transgenerational effects of dietary sucrose, mediated
20 through the grandmaternal lineage, which mimic the direct effects of sucrose on lifespan,
21 with opposing patterns across sexes; low sucrose increased female, but decreased male,
22 lifespan. Dietary mismatching of grandoffspring-grandparent diets increased lifespan and
23 reproductive success, and moderated triglyceride levels, of grandoffspring, providing

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24 insights into the physiological underpinnings of the complex transgenerational effects on
25 life histories.

26

27 **Keywords**

28 Transgenerational effects, sucrose, life history, drosophila
29

30 Main

31 From nematodes to primates, parental environments may shape the phenotypes of their
32 offspring through non-genetic mechanisms that are either condition dependent or
33 epigenetic in origin[1–3] [4]. Consequently, when individuals are subjected to environmental
34 heterogeneity prior to reproduction, their exposure to these environments can shape
35 components of fitness in offspring and subsequent generations (transgenerational effects)[5–
36 9]. Recent experiments have shown that variation in environmental factors, such as
37 predation risk and levels of sexual conflict, among parents may catalyse transgenerational
38 effects that differ in magnitude or direction across sexes, and which may also be lineage
39 (genotype) specific[10]. Notwithstanding, currently it remains unclear whether such
40 transgenerational effects are consistently instigated across diverse environmental stresses,
41 whether they generally act to enhance or depress offspring performance, and whether they
42 are transferred primarily through maternal or paternal lineages or hinge on interactions
43 between both.

44 Nutrition is a pervasive and critical source of environmental variation that shapes
45 phenotype. Variation in macronutrient balance or caloric content has been shown to confer
46 direct effects on lifespan, fecundity, and underlying physiology[11–14]. Studies from diverse
47 species have demonstrated that females and males require different diets to maximise their
48 fitness[15–19]. Female fitness is maximised on a higher relative protein concentration because

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49 high protein facilitates egg production, while higher relative carbohydrate content for
50 males provides fuel for attracting and locating a mate[20–24]. Recent studies have also shown
51 dietary-induced intergenerational effects across a variety of species; for example, changes
52 to sugar content of the parental diets in fruit flies[25,26] or dietary fat content in mice[27]
53 induces phenotypic changes in parents that are transmitted to their offspring. Intriguingly,
54 when the sucrose content of both male and female parents are altered, then parental
55 contributions to offspring phenotypes may involve complex dam-by-sire interactions that
56 are non-cumulative and dependent upon the sucrose content of the offspring diet [28]. It is
57 less clear, however, whether these dietary-mediated parental effects are epigenetic in
58 origin, and thus inherited across multiple generations [10,25,29–32], and if so, whether
59 phenotypic consequences for males and females are divergent.

60 Here, we experimentally tested the capacity for dietary sucrose variation among male and
61 female fruit flies (*Drosophila melanogaster*) to precipitate transgenerational effects on
62 components of life-history and physiology in their grandoffspring. Flies were administered
63 one of two diets that varied in the concentration of sucrose (2.5% or 20% sucrose). The diets
64 were administered using a full factorial design: males and females were each assigned to one
65 of the two diets prior to reproduction, and then their grandsons and granddaughters were
66 administered the same dietary treatments. All male-female-grandoffspring dietary
67 combinations were represented, resulting in female-male and grandparent-grandoffspring diet
68 combinations that were either matched or mismatched (Figure 1, panels A and B). This
69 design enabled us to test whether dietary-mediated transgenerational effects exist, to decipher
70 the relative grandmaternal and grandpaternal contributions, and the capacity for interactions
71 between grandparental diets and those of the grandoffspring to shape grandoffspring
72 phenotype, and to determine whether such effects are sex specific.
73

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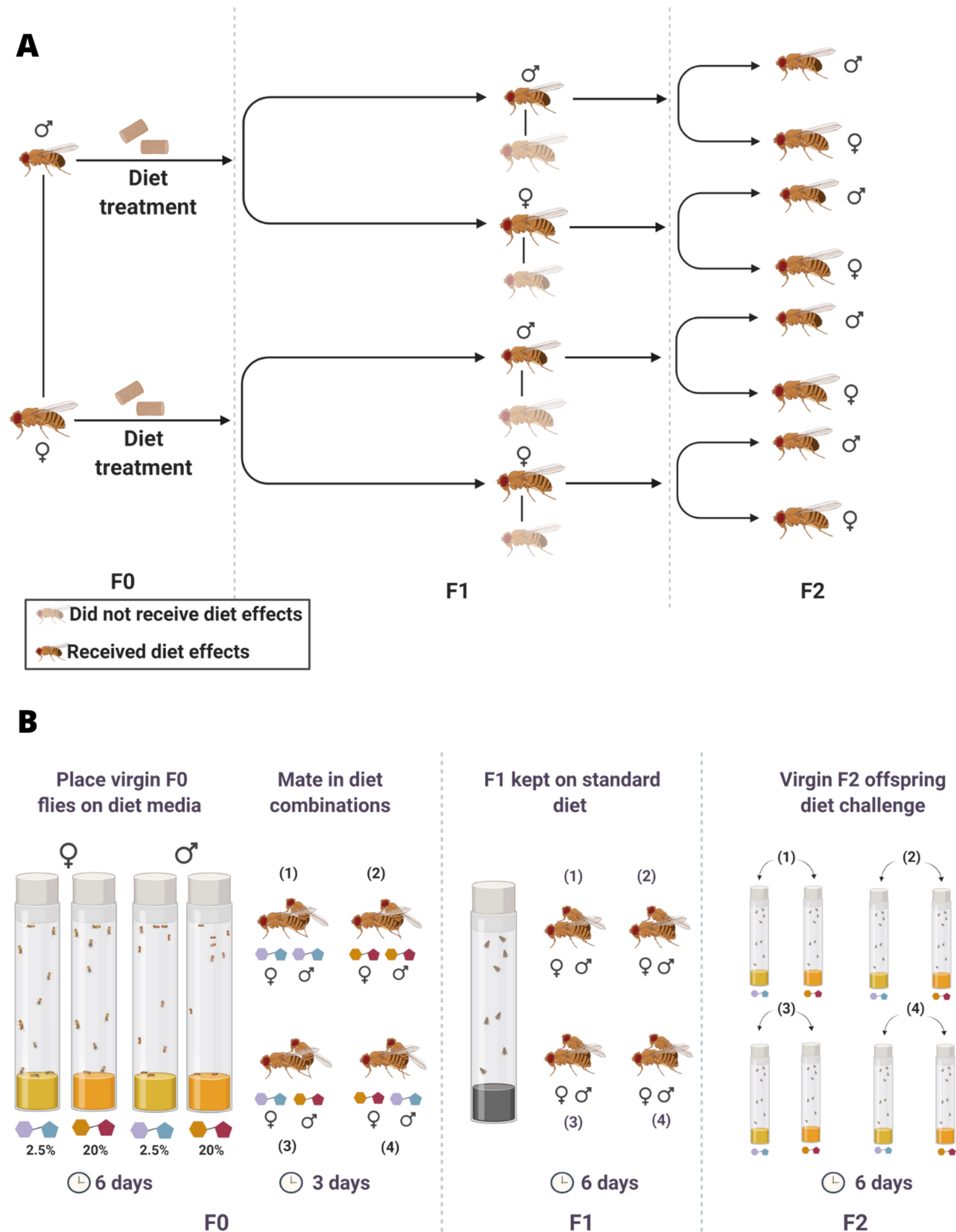

74

75 **Figure 1. A) Diet effects lineage.** Diet treatments were administered to both parents in the

76 F0; and they were mated to create the F1 offspring, and received a standard diet (both

77 males and females for each parent), and F1 offspring were mated with flies outside of the

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78 experiment that received a standard diet. This allowed us to track which F1 sex was
79 passing on the diet effects to the F2 generation. **B) Experimental design.** The F0
80 generation was administered either higher (20% of overall solution) or lower (2.5%)
81 relative sucrose in adulthood, and kept on this diet in sex-specific cohorts for 6 days as
82 virgins before a subsequent three day cohabitation (on common garden media) that
83 allowed mating to occur. Male and female F0 flies were combined in all possible diet
84 combinations. The F1 generation was reared, maintained (6 days again), and cohabited (3
85 days) on common garden media (an intermediate sucrose content of 5%). The F2
86 generation was reared from egg-to-adulthood on common garden media, and then
87 challenged as virgins with either the higher or lower sucrose such that their diet either
88 matched or mismatched one or both of their grandparents (F0).

89

90 Results

91 Direct and indirect effects of dietary sucrose on grandoffspring lifespan are sex-
92 specific

93 The diets of the grandoffspring (F2) flies conferred direct and sex-specific effects on their
94 lifespan ($F_{1,148} = 369.80$, $p < 0.001$, Table S2, Figure 2). Female F2 flies assigned to the
95 low sucrose diet lived longer than females or males assigned to any other treatment, and
96 30% longer than females on the high sucrose diet. Females assigned to a high sucrose diet
97 exhibited the shortest lifespan of any group of flies. In contrast to the large negative effect
98 of high sucrose on female lifespan, high dietary sucrose conferred a moderate increase in
99 male lifespan relative to males assigned to a low sucrose diet (Table S2; Figure 2).

100

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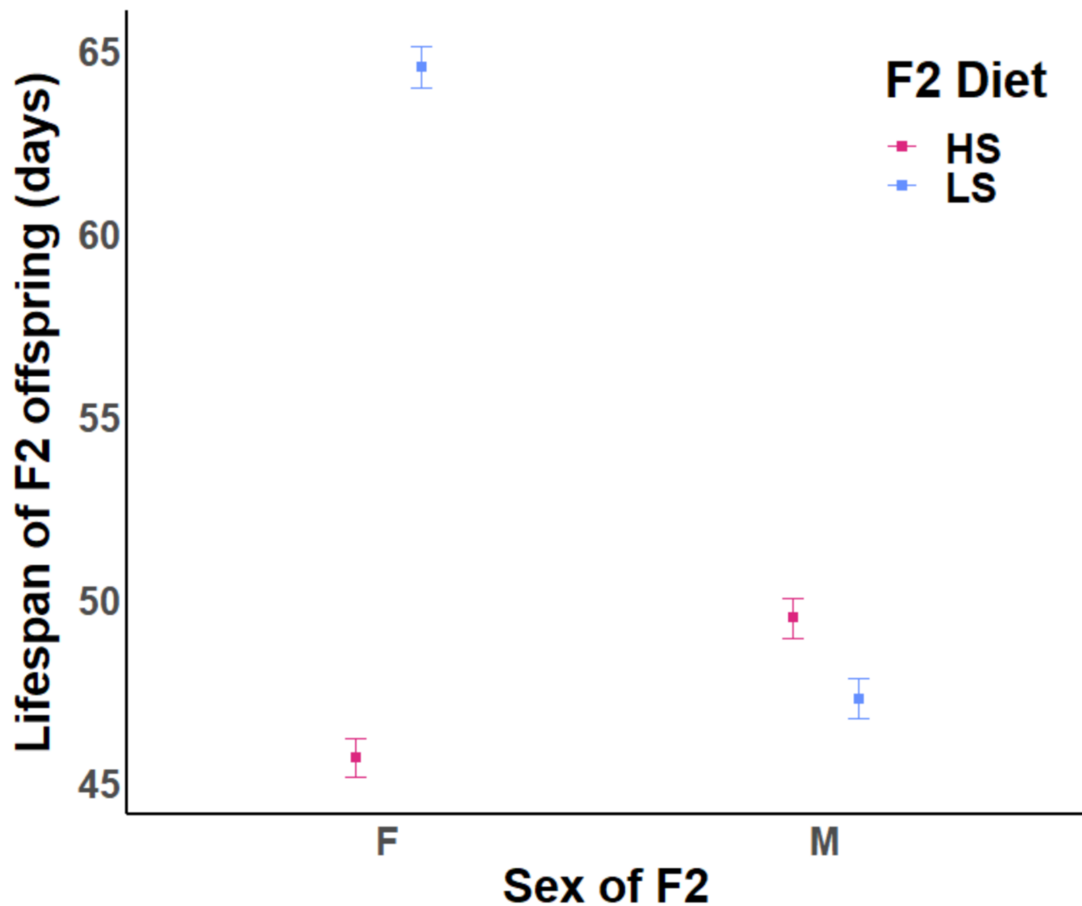

101

102 **Figure 2.** Direct effects of dietary sucrose on the lifespan (± standard error) of F2
103 granddaughters (F) and grandsons (M). HS indicates a high sucrose diet of 20% (P:C ratio
104 1:5.3), LS indicates a low sucrose diet of 2.5% (P:C ratio 1:1.4).

105

106 The lifespan of F2 flies was also in part mediated by the diets of their grandmothers, with
107 the pattern of effects differing across F2 males and females ($F_{1,148} = 9.35$, $p < 0.01$, Table
108 S2, Figure 3, panel A). The transgenerational effects of sucrose concentration mimicked
109 the direction of direct effects described above. That is, F2 females descended from
110 grandmaternal lineages assigned to a low sucrose diet lived longer than those descended
111 from high sucrose lineages, while the opposite pattern was observed in F2 males, whereby
112 those descended from high sucrose grandmaternal lineages outlived those from low
113 sucrose lineages (Figure 3, panel A). Additionally, matching combinations of

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114 grandmaternal-grandoffspring dietary sucrose led to shorter F2 lifespan than mismatched
115 combinations (Figure 3, panel B).

116 In our experimental design, grandparental flies were manipulated, and F2 phenotypes
117 measured. This involved transfer of effects across an intermediate generation – the F1
118 parents. Although the diets of F1 parents were never manipulated (they received a standard
119 diet of 5% sucrose, an intermediate sucrose content), our experimental design ensured the
120 grandparental effects were transferred through either male F1 or female F1 flies (but not both,
121 Figure 1). Thus, we could track whether the sex of the *transferring F1 parents* affected the
122 pattern and direction of the transgenerational effects. Indeed, the interaction between the sex
123 of the F2 flies and the sex of the transferring F1 parents affected F2 lifespan ($F_{1,148} = 4.44$, p
124 <0.05, Table S2); female F2 lived longer if the grandparental dietary treatments were
125 transferred through F1 females, while male F2 lived longer when the effects were transferred
126 through F1 males (Figure 3, panel C). The sex of the transferring F1 parent flies also
127 moderated the direct effects of the F2 diet on F2 lifespan, Table S2, Figure 3, panel D, $F_{1,148}$
128 $= 12.42$, $p < 0.001$). F2 flies assigned directly to a high sugar diet lived longer if grandparental
129 dietary treatments were transferred through F1males rather than through females, while F2
130 flies assigned to a low sugar diet lived longer if grandparental dietary treatments were
131 transferred through F1 females than males.

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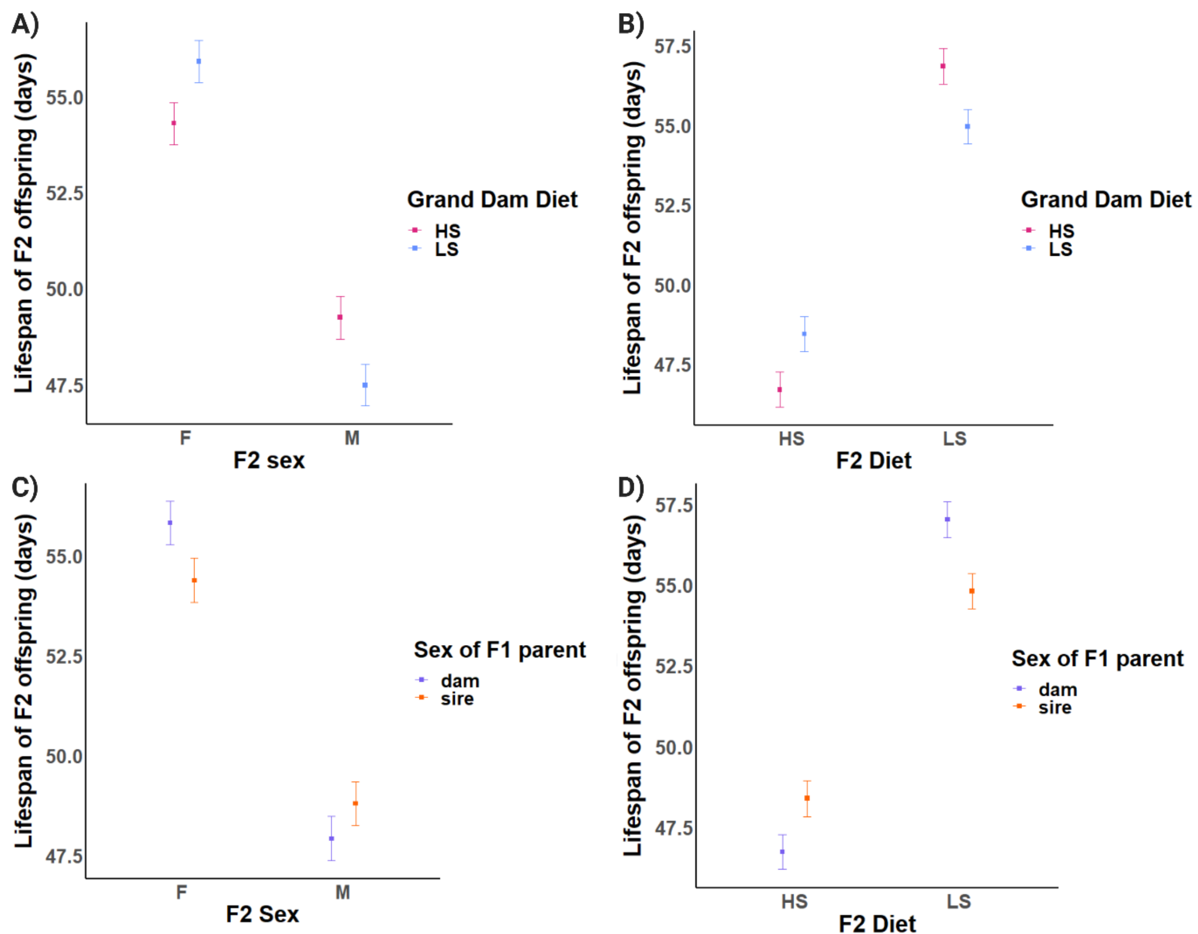

132

133 **Figure 3.**

134 Effects of high sucrose (20% of overall solution) and low sucrose (2.5% of overall
135 solution) on F2 lifespan. Plots show means, and standard error bars. (A) Lifespan of F2
136 flies (y-axis), their grand dam's diet (colour), their sex (x-axis), (interaction: grand dam diet
137 × F2 sex). (B) Lifespan of F2 flies (y-axis), their grand dam's diet (colour), their diet (x-
138 axis), (interaction: grand dam diet × F2 diet). (C) Lifespan of F2 flies (y-axis), the sex of
139 the parental linage that received a diet treatment, (colour), their sex (x-axis), (interaction:
140 F1 sex × F2 sex). (D) Lifespan of F2 flies (y-axis), the sex of the parental linage that
141 received a diet treatment (colour), their diet (x-axis), (interaction: F1 sex × F2 diet).

142

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143 **Grandoffspring fecundity, viability, and triglycerides are mediated by grand**
144 **maternal and grand paternal diets**

145 *Fecundity & viability*

146 Direct dietary effects were observed in the F2 generation; F2 Females had higher fecundity
147 when ingesting the low sucrose than high sucrose diet. These direct effects of diet were,
148 however, shaped by the grand paternal, but not grand maternal diet (Table S3, $F_1 = 5.49$, p
149 <0.05). Mismatched combinations of grandpaternal-F2 female diet resulted in F2
150 granddaughters producing more eggs than matched combinations (Figure 4, panel A).
151 Female F2 fecundity was also shaped by an interaction between the grand maternal and
152 grand paternal diets (Table S3, Figure 4, panel B, $F_1 = 14.77$, $p <0.05$); F2 females that
153 descended from matched grandmaternal-grandpaternal combinations tended to have lower
154 fecundity than those arising from mismatched combinations, and in particular F2 females
155 descended from grandparents that were each assigned to low sucrose diets exhibited lowest
156 fecundity (Figure 4, panel B). The reproductive success (as gauged by the number of adult
157 offspring produced) of the F2 females was also shaped by a similar interaction between
158 grandmaternal and grandpaternal diet, in which the clutch size was lower for F2 females
159 descended from matched, relative to mismatched, combinations of grandmaternal-
160 grandpaternal diet (Table S4, Figure 4, panel C, $F_1 = 5.25$, $p <0.05$).

161

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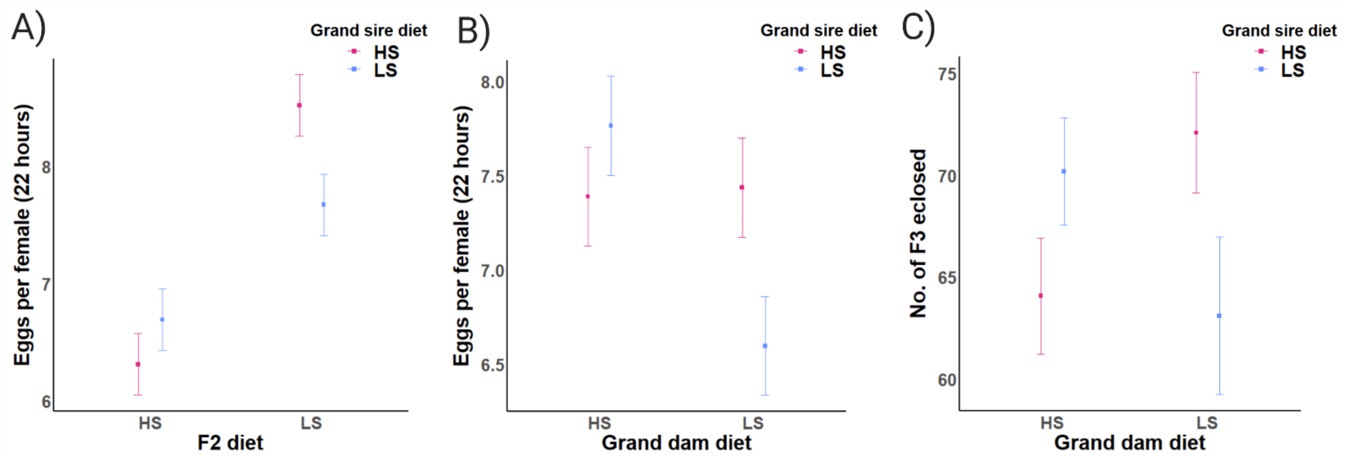
162 

163

164 **Figure 4**

165 Effects of high sucrose (20% of overall solution) and low sucrose (2.5% of overall
166 solution) on female F2 reproductive output. Plots show means, and standard error bars. (A)
167 Number of eggs laid by F2 flies (y-axis), their grand sire's diet (colour), their diet (x-axis),
168 (interaction: grand sire diet × F2 diet). (B) Number of eggs laid by F2 flies (y-axis), their
169 grand sire's diet (colour), their grand dam's diet (x-axis), (interaction: grand sire diet ×
170 grand dam diet). (C) Number of F3 flies eclosed per vial (y-axis), their grand sire's diet
171 (colour), their grand dam's diet (x-axis), (interaction: grand sire diet × grand dam diet).

172 *Triglyceride levels*

173 An interaction between the diet of F2 offspring and the grandmaternal diet affected the
174 triglyceride level of the F2 flies (Table S5, Figure 5, panel A, $F_{1,98}$= 8.56, p <0.01). F2 flies
175 fed high sucrose diets that descended from grandmothers assigned to high sucrose,
176 exhibited much higher triglyceride levels than F2 flies from any other combination of
177 grandmaternal-F2 offspring diet (Figure 5, panel A). Similarly, the interaction between F2
178 diet and grandpaternal diet shaped triglyceride level; however in this case, F2 offspring
179 assigned to a high sucrose diet and descended from grandfathers assigned to low sucrose,

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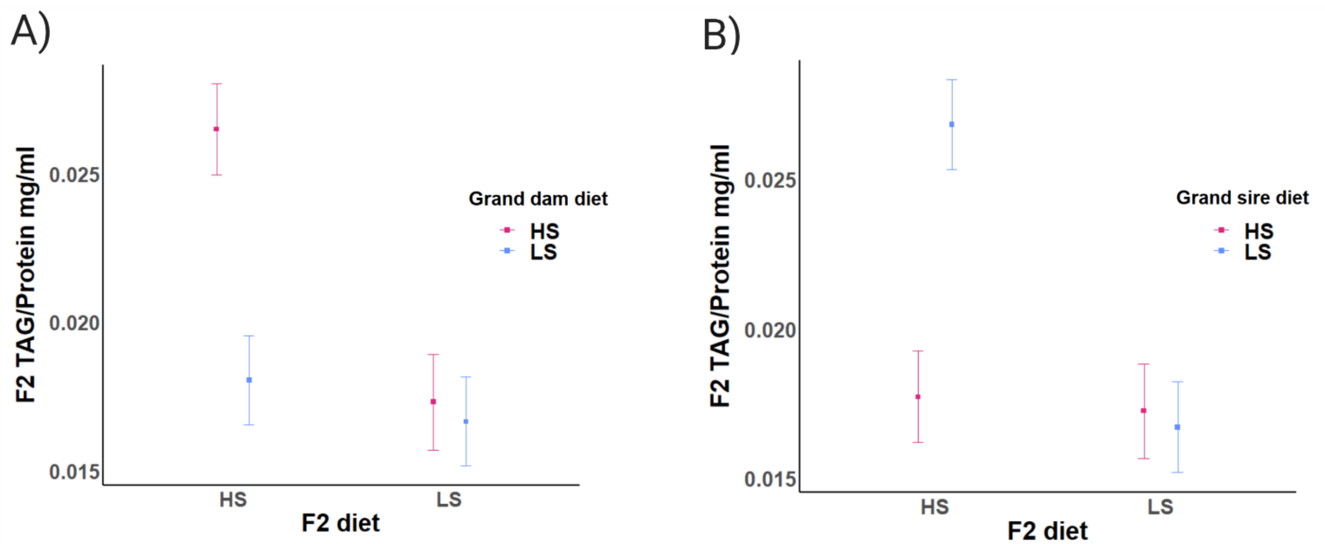
180 exhibited much higher triglyceride levels than any other combination of grandpaternal-F2

181 diet (Table S5, Figure 5, panel B, $F_{1,98}$= 12.75, p <0.001).

182



183 **Figure 5.**

184 Effects of high sucrose (20% of overall solution) and low sucrose (2.5% of overall

185 solution) on F2 whole body triglyceride (TAG) levels divided by their whole body protein

186 levels, per fly. Plots show means, and standard error bars. (A) F2 TAG per fly (y-axis),

187 their grand dam's diet (colour), their diet (x-axis), (interaction: grand dam diet × F2 diet).

188 (B) F2 TAG per fly (y-axis), their grand sire's diet (colour), their diet (x-axis), (interaction:

189 grand sire diet × F2 diet).

190 **No direct effect of dietary sucrose on female F0 fecundity**

191 Neither the male, nor female diet, affected egg output of the F0 females (Table S1).

192

193 ## Discussion

194 Here, we show opposing effects of dietary sucrose on the lifespan of each sex, in *D.*

195 *melanogaster*—low sucrose enhances female lifespan, but decreases male lifespan relative

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196 to high sucrose. Notably, these effects were observed in both direct and indirect (i.e.
197 transgenerational) contexts. Moreover, the dietary-mediated transgenerational effects on
198 lifespan mimicked the observed direct effects for each sex: a low sucrose grandmaternal
199 diet conferred elevated F2 female lifespan, but decreased male F2 lifespan, relative to a
200 high sucrose grandmaternal diet. We also revealed strong effects of specific combinations
201 of grandparental and grandoffspring diet, and between grandmaternal and grandpaternal
202 diets, in shaping the measured traits; all of which exhibited a similar pattern—a mismatch
203 in diet enhanced trait expression in subsequent generations. We highlight inherent
204 complexity in the nature of the transgenerational effects. The effects are generally sex-
205 specific, and unexpectedly, are affected by the sex of the transferring F1 parent. Finally,
206 we note that interactions between grandparental diet and F2 diet affected triglyceride levels
207 of F2 flies, suggesting that dietary-mediated modifications of triglyceride levels, across
208 generations, may contribute to the observed transgenerational effects on life history
209 phenotypes.

210 Studies investigating sex-specificity of transgenerational effects across a range of taxa
211 have observed instances in which environmental modification such as dietary challenges,
212 presence of predators, or behaviour-modifying drugs of the grandparental environment
213 triggered sex-specific effects on grandoffspring phenotype. Intriguingly, in these cases,
214 transgenerational effects tend to manifest in the opposite sex to that subjected to the
215 grandparental treatment; that is, modification of the grandmaternal environment may
216 enhance or inhibit trait expression among grandsons, or conversely, modification to the
217 grandpaternal environment may enhance or inhibit trait expression among granddaughters
218 [10,25,30,32,33]. Our findings are consistent with previous research, revealing opposing
219 directions of sucrose-mediated grandmaternal effects in each of the sexes. Notably, we
220 have uncovered further levels of complexity in the nature of the transgenerational effects.

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221 First, we revealed sex differences in the magnitude of transgenerational effect (the effect
222 transmitted from dietary-treated F0 flies to F2 flies) are dependent on the sex of the
223 transferring F1 parent. Second, we observed that the outcomes of transgenerational effects
224 depend on interactions between the diets of the grandparents and those of the
225 grandoffspring; dietary mismatching across generations tends to enhance lifespan
226 (mediated by a grandmaternal-by-grandoffspring interaction) and fecundity (mediated by a
227 grandpaternal by granddaughter diet interaction). Whether or not these effects are mediated
228 by underlying triglyceride levels of the experimental flies remains unclear; yet one pattern
229 was notable, suggestive of a possible transgenerational link between physiology and
230 lifespan. F2 offspring assigned to a high sucrose treatment, and descended from high
231 sucrose grandmaternal lineages exhibited the highest triglyceride levels and the shortest
232 lifespans.

233 Investigations into life history traits are imperative in assessing the adaptive significance of
234 transgenerational effects on offspring, given the close link between these traits and lifetime
235 fitness[5]. Our experiments, across three generations, with the diet challenge also given to
236 the F2 generation had the requisite power to address these previous knowledge gaps. Our
237 finding that dietary mismatching (between both grandparents and between grandparents
238 and grandoffspring) tends to enhance trait expression adds new insight to studies
239 investigating transgenerational effects of diet, and of transgenerational effects of
240 environmental change more generally. Previous studies of dietary-mediated
241 transgenerational effects have tended to focus on changes in metabolite profiles and
242 physiology across generations, rather than on changes to expression of life history
243 traits[25,26]. Moreover, these designs typically do not have the requisite power to partition
244 relative influences of (grand)maternal and (grand)paternal effects on transgenerational

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245 phenotypes, nor the factorial design required to determine whether transgenerational
246 mismatches enhance or depress performance [9].

247 The prevailing prediction is that a matching of environment between grandparents and
248 grandoffspring may augment offspring fitness-related traits, because the matching
249 environments may allow parents to prime offspring to cope with environments that their
250 parents faced (anticipatory effects). The evidence for anticipatory effects across contexts
251 and taxa is, however, mixed and weak[9,29], and many studies that have leveraged
252 experimental designs with the power to test for these effects have primarily focused on
253 intergenerational effects (from F0-F1[29]), with very few studies classified as
254 transgenerational where grandoffspring should have no direct experience of the
255 grandparental environment[34]. Our study generally revealed patterns that were contrary to
256 the predicted pattern – dietary mismatching, rather than matching, between grandparents
257 and F2 offspring tended to augment offspring performance. This begs the question of
258 whether cross-generational dietary mismatching may be a general phenomenon that
259 extends across the diets used in our study.

260 Two recent studies shed some light on this question. Deas et al. (2019) manipulated dietary
261 quality across three generations (F0 to F2) in *D. melanogaster*, providing flies of each
262 generation with a 'rich' diet (rich in calories and supplemented with yeast) or a poor diet
263 (calorie diluted, with no yeast supplementation), in all combinations, and then measuring
264 phenotypic expression in the grandoffspring (F2). They reported that a mismatch between
265 the diet quality ("poor vs "good" diet) of granddams and granddaughters led to a faster
266 development time in the pupal stage of the granddaughters, but this effect did not hold for
267 the entire development time[35]. This study also focused on females, and therefore was not
268 able to capture sex specificity in any generation. On the other hand, Camilleri et al. (2022)
269 tested effects of dietary mismatching of F0 flies and their F1 offspring, manipulating the

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270 diets of parents of each sex and their offspring, and utilising the same sucrose diets used in
271 the current study. We found that dietary mismatching between parents and F1 offspring led
272 to an increase in lifespan, and fecundity of the offspring[28]. Here, we advance these
273 findings by demonstrating that these effects of dietary mismatch are carried over for
274 multiple generations, are also dependant on the sex of F1 lineage. Because the effects are
275 unambiguously transgenerational (extending from F0 to F2), they are less likely to result
276 from differences in condition of the grandparents, suggesting instead possible epigenetic
277 mechanisms regulating the effects.

278 In sum, our work uncovers dietary-mediated transgenerational effects that are on the one
279 hand remarkably consistent across generations – transgenerational effects of sucrose tend
280 to mimic the direct effects. We have also extended previous work to demonstrate that
281 dietary mismatching across generations tends to augment phenotype in a manner that is
282 unlikely to be directly linked to condition-dependence. Future work should focus on
283 uncovering the ecological and evolutionary significance of these results, and the
284 underpinning mechanistic drivers. We suggest that a process in which transgenerational
285 dietary mismatching promotes fitness of future generations could buffer populations from
286 future changes in environment, and be particularly adaptive for species that live and
287 depend on ephemeral resources for their source of nutrients. If this is the case, then
288 populations evolving in fluctuating environments may be more likely to evolve
289 mechanisms that promote the fitness of offspring encountering novel environments.

290

291

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293 # Methods

294 ## Study species and generating experimental flies

295 We sourced flies from Dahomey, a large laboratory population of *D. melanogaster*,
296 originally sourced from Benin West Africa[36]. The flies have been maintained in large
297 population cages, with overlapping generations in the Piper laboratory, Monash
298 University, Australia, since 2017, and prior to that in the Partridge laboratory, University
299 College London [37]. Prior to the beginning of the experiment, we collected ~3000 eggs from
300 the cages, and distributed them into 250mL bottles containing 70mL of food. Food
301 comprised 5% sucrose (50 grams sucrose, 100 grams yeast, 10 grams agar per 1 litre
302 solution with an estimated protein to carbohydrate [P:C] ratio of 1:1.9, and 480.9 kcal per
303 litre (see Supplementary Material Figure S4 for further diet details). Every generation (for
304 7 generations), adult flies eclosing from multiple bottles were admixed prior to
305 redistributing the flies across new bottles. To control for potential sources of variation in
306 their environment, during these 7 generations we strictly controlled both the age of flies at
307 the time of ovipositioning—all flies were within 24 h of eclosion into adulthood when
308 producing the eggs that propagated the subsequent generation, and their population density
309 was 300-320 adult flies within each bottle in each generation.

310

311 ## Dietary treatments

312

313 The diet media we used consists of sucrose, autolysed brewer's yeast powder (sourced
314 from MP Biomedicals SKU 02903312-CF), and agar (grade J3 from Gelita Australia), as
315 well as preservatives—propionic acid, and nipagin. We prepared two dietary treatments,
316 differing in relative sucrose concentration; 2.5% sucrose (that we refer to as a lower

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317 sucrose treatment relative to the 5% concentration usually provided to the population of
318 flies used in this experiment), and 20% sucrose (that we refer to as a higher sucrose
319 treatment) of overall food solution. The 2.5% sucrose diet contains 25 grams of sucrose,
320 100 grams of yeast and 10 grams of agar per litre of food prepared, with an estimated P:C
321 ratio of 1:1.4 and 380.9kcal per litre of food. The 20% sucrose treatment contains 200
322 grams of sucrose, 100 grams of yeast, and 10 grams of agar per litre of food prepared, with
323 an estimated P:C ratio of 1:5.3 and 1080.9kcal per litre of food. The diets thus differed not
324 only in sucrose concentration, but overall macronutrient balance and their total caloric
325 content, resembling differences typically observed between obesogenic and healthy diets in
326 humans. The higher sucrose concentration was selected based on preliminary experiments
327 that we conducted, and which elicited an obese-like phenotype in the flies, consistent with
328 results from previous work in *D. melanogaster* [11,25,38]. All diets contained 3ml/l of
329 propionic acid and 30ml/l of a Nipagin solution (100g/l methyl 4-hydroxybenzoate in 95%
330 ethanol) and were cooked according to the protocol described in Bass *et al.* (2007) [39].
331 Each vial is 40mL in volume, and contained 7mL of food.

332 **Experimental design**

333 Male and female virgin flies were assigned to one of two of the dietary treatments prior to
334 mating (we refer to this generation of flies as F0), and then the grandoffspring produced (F2
335 generation) were also assigned to one of the two treatments. All possible combinations of
336 grand dam × grand sire × grandoffspring diet treatment were represented (= 2 × 2 × 2 = 8
337 combinations). Specifically, we collected 1280 flies of the F0 generation as virgins and
338 placed them onto either the high sucrose (20%) or the low sucrose (2.5%) diets for the first 6
339 days of their adult life. They were in vials of 10 flies across 64 vial replicates per treatment,
340 and per sex (High sucrose: 32 vials of males and 32 vials of females; low sucrose, 32 vials of
341 males and 32 vials of females, 128 vials in total;1280 flies, 640 of each sex). They were kept

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342 in their respective sexes. We transferred flies to vials containing fresh food of the designated
343 diet every 48 hours during this 6 day period.
344
345 At day 6, we randomly sampled six vials from each treatment, and snap froze (using liquid
346 nitrogen) the flies of these vials, storing them at -80°C for subsequent measures of
347 triglyceride levels. Cohorts of flies in the remaining vials then entered a cohabitation phase to
348 enable female and male F0 flies to mate. Cohorts of males and female flies were combined, in
349 vials of 10 pairs, in each of all four possible diet combinations: lower sucrose females ×
350 lower sucrose males; higher sucrose females × higher sucrose males; lower sucrose females ×
351 higher sucrose males; higher sucrose females × lower sucrose males. During this phase, flies
352 cohabited for 96 hours. They were transferred to a new vial with fresh food of standard 5%
353 sucrose diet every 24 hours during this time.
354
355 The vials from the 6 day old F0 flies (i.e., the vials from Day 1 of the 96 h cohabitation
356 phase) were retained, and the eggs that had been laid by females of the respective vials were
357 trimmed to 80 per vial by removing excess eggs with a spatula. The remaining eggs were left
358 to develop into adult offspring over 10 days at 25°C (on a 12:12 light/dark cycle in a
359 temperature-controlled cabinet; Panasonic MLR-352H-PE incubator). These adult flies
360 constituted the F1 offspring in the experiment, and F1 flies developed on standard 5%
361 sucrose media. We collected 2080 virgin F1 flies from each of the four combinations of
362 parental diet treatments, and placed them in sex-specific cohorts of 10 individuals per vial, on
363 standard 5% sucrose media for 6 days. We then allowed these F1 males and F1 females to
364 cohabit and mate with male or female *tester* flies (creating 10 pairs per vial) that had been
365 collected from the same Dahomey stock population (but not subjected to a dietary sucrose
366 treatment) to create the F2 generation. The diet treatments applied to the F0 flies were thus

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367 transferred to the F2 generation via either F1 males or F2 females, but never through both
368 sexes. The F1 flies were 6 days of adult age when laying the eggs that produced the F2
369 generation.
370
371 We then collected virgin F2 flies – the grandoffspring of the F0 flies – from each of the four
372 combinations of F0 diet treatments (per sex), and placed them in their respective sexes in
373 vials of 10 flies, across vial replicates per treatment per sex (4080 flies, 2040 male, 2040
374 female). We then assigned these F2 flies, produced by each dietary treatment combination of
375 F0 flies, to either the lower sucrose or higher sucrose diet. At day 6 of adulthood, we snap
376 froze F2 flies of six randomly chosen vials per grand dam × grand sire × grandoffspring
377 combination. On the same day, 10 virgin focal F2 flies of each grand dam × grand sire ×
378 grandoffspring combination and each sex were placed together with 10 age-matched tester
379 flies of the opposite sex from the Dahomey population, entering into a cohabitation phase of
380 96 h (during which time the number of eggs laid by females of each vial was assessed). After
381 96 hours flies were separated again into their respective sexes (in vials of 20 flies), and
382 assigned back onto either the lower sucrose or higher sucrose diets that they had been on
383 prior to cohabitation, and a lifespan assay carried out.
384

385 **Lifespan**

386 We scored the lifespan of experimental flies of the F2 generation. Each vial in the assay
387 commenced with 20 flies of single sex in each, and we included 10 vial replicates per
388 treatment (grand dam × grand sire × grandoffspring) (3400 flies total, the original amount
389 collected, minus the snap frozen samples). The number of dead flies per vial was scored three
390 times per week (Monday, Wednesday, Friday), and surviving flies at each check transferred
391 to vials with fresh food of the assigned diet treatment—until all flies were deceased. During

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392 the lifespan assay, vials were stored in boxes (of 85 vials per box) that were moved to
393 randomised locations in a (25°C) control temperature cabinet every few days to decrease the
394 potential for confounding effects of extraneous sources of environmental variation within the
395 cabinet from affecting the results.
396
397 **Fecundity**
398 We measured the egg output of female flies from generations F0 and F2 at eight days
399 following eclosion, as a proxy of female fecundity. On day eight, female flies oviposited for a
400 23 hour period, and were then transferred to fresh vials. Day eight was selected because
401 fecundity over 24 hours at this age has been shown to correlate with total lifetime fecundity
402 of females in this Dahomey population[39] and early, short term measures of reproduction of
403 between one and seven days can be used to accurately predict total lifelong fecundity in *D.*
404 *melanogaster* [40]. Moreover, data shows that varying the range of sucrose concentrations did
405 not alter the timing reproductive peaks between treatments [39].
406
407 For the F0 generation, we counted eggs from vials, each containing 10 female flies, that had
408 been mated with 10 male flies, across 2 different sucrose levels (2.5% and 20% sucrose), and
409 different mate combinations, as above. For the F2 generation, we counted eggs from each
410 grand dam × grand sire × grandoffspring dietary treatment combination; each combination
411 was represented by 10 vial replicates, each containing 10 focal females (females from the
412 experiment) combined with 10 tester male flies. Additionally, we counted the number of
413 adult flies that eclosed within 10.5 days from the eggs laid by F2 females (a composite of
414 clutch viability and juvenile developmental speed). F2 females cohabited and mated with
415 age-matched tester males of the Dahomey population (in the experimental process described
416 above, rather the standard medium of 5% sucrose), for 24 hours at 6 days of life, and the vials

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417 containing these eggs were left to develop into adult offspring, for 10 days at 25°C; 12:12
418 light/dark cycle in a temperature-controlled cabinet (Panasonic MLR-352H-PE incubator).
419

420 **Lipids and protein**

421 Whole-body triglyceride levels were measured in adult flies from the F2 generation (six days
422 of adult age, corresponding with six days of exposure to the relevant F2 dietary treatment,
423 prior to mating) and normalized to protein content (full protocols reported in the
424 Supplementary Material). Three biological replicates per treatment level, with three technical
425 replicates per biological replicate were used. Five female flies and eight male flies
426 respectively, were used for each biological replicate in the assay.
427

428

429 **Statistical Analyses**

430

431 We used R (Version 3.6.1) and RStudio (Version 1.2.1335) (R Core Team, 2019) for
432 statistical analyses. To test the effects of F0 female diet, F0 male diet, F2 diet, and sex on
433 lifespan, TAG, and F2 offspring production, we fitted linear mixed effects models, using the
434 R package lme4[41], to the lifespan data for the F2 generation. We use the term lifespan to
435 denote the age of recorded death for each individual fly within a margin of 72 hours (for
436 example, a lifespan of 30 days indicates that a fly died between 27-30 days post eclosion). To
437 test the effects of grand maternal diet, grand paternal diet, grand-offspring diet, and sex on
438 female fecundity, we fit a linear model to the egg output data for both generations.
439 We included F0 male, F0 female, F2 diets, and F2 sex as fixed effects in each model,
440 exploring interactions between these factors. We included the vial identification number as a

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441 random effect in the lifespan models. The fecundity models only included one observation
442 per vial because we counted eggs per vial, and divided by the number of females in the vial
443 (approx. 10 females); therefore no random effects were included in this model. We used log-
444 likelihood ratio tests that reduce the full model, via the sequential removal of highest order
445 terms that did not (significantly) change the deviance of the model, using a *p* value
446 significance level of <0.05. The final reduced models (except fecundity measures) were fit by
447 restricted maximum likelihood, applying type III ANOVA with Kenwood-Roger's F test and
448 approximation of denominator degrees of freedom. We used sum to zero constraints in all
449 models, and we visually inspected diagnostic plots for the linear mixed effect models, to
450 ensure that the assumptions of normality and equal variances were met.
451

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454

455 **Conflicts of Interest**

456 The authors have no conflicts of interest to declare.

457

458 ## Author contributions

459 TLC, DKD, MDWP & RLR designed the experiment, TLC planned and carried out the
460 experiment, and wrote the initial draft of the manuscript, and TLC, DKD, MDWP & RLR

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461 all contributed to the writing and editing of the manuscript. TLC performed statistical
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468

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