The hidden costs of dietary restriction: implications for its evolutionary and mechanistic origins

Authors

Andrew W McCracken, Gracie Adams, Laura Hartshorne, Mirre J. P. Simons

Affiliation

Department of Animal and Plant Sciences & Bateson Centre, The University of Sheffield, Sheffield S10 2TN, UK.

Correspondence to be addressed to M.J.P.S.: m.simons@sheffield.ac.uk

Abstract

Dietary restriction (DR) consistently and universally extends health- and lifespan across taxa. Despite efforts to uncover the mechanisms underpinning DR - and ultimately translate its beneficial outcomes to humans - precise and universal mechanisms have not been identified. In biomedical science, the effects of DR are interpreted as regulating pro-longevity molecular pathways. This reasoning is guided by the conviction that DR evolved as an adaptive, pro-longevity physiological response to restricted food intake. Current evolutionary theory states that organisms should invest in their soma more heavily during periods of DR, and, when their resource availability improves, should outcompete age-matched rich-fed controls in survival and/or reproduction. Here we present a formal test of these key predictions utilising a large-scale demographic approach detailing mortality and fecundity in Drosophila melanogaster fed alternating dietary regimes (N > 66,000 flies across 11 genetic lines) . Our experiments reveal surprising and substantial mortality costs when returning to a rich diet after periods of DR. These results suggest the effects of DR are not necessarily intrinsically pro-longevity and could be considered an escape from costs incurred under nutrient-rich conditions, in addition to novel, discrete costs associated with restricting dietary protein. These insights question the relevance of current evolutionary explanations of DR in interpreting the mechanistic basis of dietary restriction.

Introduction

Ageing has attracted extensive scientific interest, both from a fundamental and biomedical perspective. Dietary restriction (DR) universally-extends health- and lifespan across taxa, from baker's yeast to mice (Fontana and Partridge 2015). The reduction of total calories - or more specifically macronutrients, like protein - extends lifespan reliably (Min *et al.* 2007, Lee *et al.* 2008, Solon-Biet *et al.* 2014, Jensen *et al.* 2015). Although the precise universal mechanisms that connect diet to ageing remain elusive, translating the health benefits of DR has been assumed possible (Dirks and Leeuwenburgh 2006, Balasubramanian *et al.* 2017). This widespread assumption follows the reasoning that DR's universal beneficial effects are facilitated by evolutionary conserved mechanisms - and as such, applicable to our own species. Although experiments on our close evolutionary relatives, rhesus monkeys (*Macaca mulatta*) have demonstrated that DR as a treatment of itself could be translational (Colman *et al.* 2009, Mattison *et al.* 2012, 2017), the mechanisms by which these benefits are accrued physiologically may be wholly disparate. No single genetic or pharmaceutical manipulation mimicking the benefits of DR across model organisms exists (Selman 2014). Mechanistic insight will be key, since DR as a lifestyle intervention has limited scope, given the degree of self-restraint required. It is therefore warranted to direct scrutiny towards the evolutionary theory of DR, since it underpins the assumed universality of physiological mechanisms by which DR confers health benefits.

The ubiquity of the DR longevity response and therefore shared universal mechanisms can only be accepted as an evolutionary argument when the selective pressures acting are understood. The DR response itself may have evolved once, and mechanisms might be conserved; alternatively DR could have undergone convergent evolution, either using similar mechanisms - or by adopting alternative ones - within individual species (Mair and Dillin 2008). These evolutionary scenarios provide distinct predictions for how informative mechanistic research on DR responses in other animals will be for human medicine. Current evolutionary theory on DR is limited, and its elemental phenotypic predictions have undergone minimal empirical examination (Zajitschek *et al.* 2018). DR is interpreted as an evolved, adaptive, pro-longevity physiological response to limiting food availability (Holliday 1989). Life-history theory - a central tenet of evolutionary biology - states all resources are limiting and thus predicts trade-offs between reproduction (Höglund et al. 1998) and survival (Stearns 1989) even in nutrient-rich environments. As such, DR presents an enigma: why do organisms live longer on a constrained energy budget?

The currently accepted evolutionary model for DR (Shanley and Kirkwood 2000) uses a life-history perspective on ageing - the Disposable Soma Theory (DS) (Kirkwood 1977, Kowald and Kirkwood 2016) to explain this enigma. It reconciles the apparent trade-off paradox by proposing that below a certain resource threshold, organisms will reallocate energy almost exclusively towards somatic maintenance. In certain ecological situations (e.g. severely reduced juvenile survival, or when the energy budget is lower than the cost of one unit of reproduction) investment into reproduction will cease to yield fitness. The optimal, fitness-maximising strategy under these harsh conditions would be to cease investment into reproduction and utilise this energy to gain fitness when conditions improve. Crucially, this life-history strategy could favour an increase in energy devoted to maintenance and repair - allowing organisms to survive periodic bouts of famine with an intact (or superior) soma. This 'somatic maintenance response' has been presumed to be the primary causative agent in the pro-longevity DR response (Kirkwood and Austad 2000, Masoro 2000, Ingram *et al.* 2006, Speakman and Mitchell 2011, Fontana and Partridge 2015).

This attractive evolutionary rationale has given credibility to the assumption that physiological changes in the DR animal are inherently pro-longevity. For example, transcriptomic responses to DR have been interpreted in this light. The upregulation of, what could be, maintenance processes under DR, have also lent credence to this hypothesis (Lee *et al.* 1999, Kirkwood and Austad 2000, Pletcher *et al.* 2005, Whitaker *et al.* 2014). Directionality is often ambiguous, however; downregulation of DNA repair under DR could be interpreted as either a reduction in DNA damage generation, or reduced investment into repair (Lee *et al.* 1999, Pletcher *et al.* 2005). As such, the surge of 'maintenance and repair' gene expression could be a mere stress response to metabolic disruption. The health benefits observed under DR might originate from a passive response - one not necessarily evolved as an adaptive regulatory response (or 'reaction norm') to diet. Under these circumstances, lifespan extension could be a simple correlated response to currently unknown, but strongly conserved, physiology. The limitation of metabolic rate or specific metabolites could reduce conserved associated physiological dysfunction, and extend lifespan. A similar example is if DR is a hormetic response - resulting in the stimulation of cellular stress reactions, and beneficial outcomes to health (Rattan 2008). Such correlated beneficial responses might also result in correlated negative effects as seen under DR. Dietary restricted organisms suffer compromised immune function (Kristan 2008) and cold tolerance (Adler and Bonduriansky 2014).

The distinction between more passive correlated responses versus adaptive programmed pro-longevity responses is clearly key to identifying the basic mechanisms of DR and

develop translation to humans. The widely-accepted evolutionary model of DR supports an adaptive phenotypic response and provides a key prediction: organisms should invest in their soma more heavily during periods of DR, and, when their resource availability improves, should outcompete age-matched rich-fed controls in survival and/or reproduction. Here we provide an experimental test of this prediction, utilising a large-scale demographic approach detailing mortality and fecundity in *Drosophila* melanogaster fed different dietary regimes.

Our results revealed substantial mortality and fecundity costs when returning to a rich diet after a period of DR, falsifying the key prediction provided by the evolutionary biology of DR . These effects were independent of genotype, duration of DR, number of dietary fluctuations, access to water, and microbiota abundance. Our results therefore suggest that the effects of DR are not intrinsically pro-longevity and could be considered an escape from costs incurred under nutrient-rich conditions in addition to novel, discrete costs associated with restricting dietary protein. These insights question the relevance of current evolutionary explanations of DR in guiding biomedical research into its mechanisms.

121 # Results

122 **Hidden costs of Dietary Restriction**

123 Our initial results were obtained from a large experiment using DGRP-195, comprising
124 11,084 individual deaths (Table S1). DR resulted in a significant reduction in mortality rate
125 across the lifespan (Fig. 1A, $P < 0.001$, 3 times lower hazard, Table S2). In addition,
126 switching flies to DR at older ages be instantly reduced mortality levels to the levels of flies
127 that had experienced continuous DR (Fig. 1D,E). Such mortality amnesia - a complete lack
128 of historic effect of diet - has been reported for flies previously (Good and Tatar 2001, Mair *et*
129 *al.* 2003).
130 Our expectation, based on the current evolutionary model of DR, was that if flies are
131 returned to rich food conditions after a period of DR they would have a superior soma
132 compared to flies that experienced rich food continuously. Energy reallocated from
133 reproduction towards maintenance should result in higher fitness and enhanced longevity. In
134 contrast, our long-switch treatment induced a substantial increase to mortality risk compared
135 to flies kept on a rich diet throughout life (Fig. 1A, $P < 0.001$, 3.7 times higher hazard, Table
136 S1). Mortality peaked immediately (48h, 5.1 times higher hazard) after the switch from a
137 restricted to a rich diet, but this difference slowly reduced to equal the continuous rich diet
138 mortality rates after 8 days (Fig. 1A, Table S3, $P < 0.001$).
139

140 **Repeated diet switching**

141 The effects of the long-switch treatment could be dependent on a prior sustained length of
142 DR. Indeed, it has been suggested that DR evolved to survive only relatively short
143 intermittent bouts of famine (Fontana and Partridge 2015). Secondly, it has been suggested
144 that the longevity effect of DR itself is selected for at relatively young ages (Shanley and
145 Kirkwood 2000). Thus, it was possible that only relatively old flies would be susceptible to
146 the heightened mortality we were observing. Lastly, it could be that a sudden change in diet
147 *per se* is harmful. To test these three potential confounds we used short recurring bouts of
148 DR, by alternating diets every four days between the rich and restricted- diet.
149 Simultaneously, this allowed us to test whether flies were able to repeatedly and rapidly
150 modulate their mortality risk in response to diet. Mortality costs on this switching diet
151 compared to the continuous rich-diet were likewise exacerbated (Fig 1B, $P < 0.001$, 2.4
152 times higher hazard, Table S1,2). Moreover, flies repeatedly and rapidly modulated their
153 mortality in direct response to the diet they were experiencing. Flies were able to reduce
154 their mortality close to levels that flies that were continuously exposed to DR experienced
155 (Fig 1B, Table S1): Mortality risk under DR, in the 4-day switch regime, was significantly
156 higher ($P < 0.001$, 1.6 times higher hazard, Table S1) which could be attributable to accrued

157 physiological costs or carry-over mortality induced by rich food conditions, but measured
158 within the DR condition.
159
160 **At which diet are costs incurred?**
161 An analysis of the timing of mortality (Table S4) within the 4-day interval showed that
162 mortality modulation by diet was strongest in the second 48 hrs under both DR and rich food
163 conditions ($P < 0.001$). This suggests a period of acclimation to the both the DR and rich diet
164 is necessary before their physiological effects are fully realised. Further insight in this timing
165 was achieved by imposing further dietary regimes. First, switching from DR to rich conditions
166 at increased frequency, repeatedly every 2 days (Table S1,2). Mortality within this dietary
167 regime confirmed that sustained exposure to diets was required for the phenotypes
168 observed. Although mortality in the 2-day switching regime compared to continuous diets, at
169 rich food conditions was slightly higher (1.13 times higher hazard, $P < 0.05$), however the
170 effect of DR was again reduced (1.3 times higher hazard, $P < 0.001$). Together this diet
171 modulation resulted in a lifespan extension (Table S2, Figure 1G). The reduction of mortality
172 under DR can therefore be considered to be relatively more rapid than the induction of
173 exacerbated mortality on rich food (after a period of DR). Such exacerbation of mortality on
174 rich food thus either requires an additional period on DR or on rich food. To test this directly,
175 asymmetrical diet regimes were imposed.
176
177 In an additional set of experiments we combined the 4-day with the 2-day switching regime,
178 either feeding on rich diet for 4 days followed by 2 days of DR, or switching from 2 days of
179 rich diet to 4 days of DR (each sequence repeated). These experiments showed no marked
180 increase of mortality on rich food on either regime (Table S5, Figure 1E,F). The effect of DR,
181 however, was markedly reduced, especially when flies were only restricted for 2 days, to be
182 refed directly after for 4 days (Table S5, Figure 1F). Compared to continuous DR, this
183 resulted in less than half the reduction in overall mortality risk, even though flies spend
184 spend two-thirds of their lives on DR, or only a quarter when flies spend a third of their life on
185 DR (Table S6, Figure 1E,F), again suggesting that a period of over 2 days on either diet is
186 required to observe marked effects. Note, however, that the exacerbation of mortality on rich
187 food in the long-switch paradigm was highest in the first 2-day interval (Fig 1A, Table S3),
188 and that mortality rapidly reverted back to (at least) that of continuously rich fed flies in each
189 dietary regime tested. In addition, in the reverse situation DR induced a full reduction to
190 continuous DR levels, i.e. mortality amnesia (Fig 1D,E), within 2 days. The additional
191 switching experiments (2-day and asymmetrical switches) also suggest that it is not the
192 frequency of switching in the diet *per se* that is causing premature mortality in the 4-day
193 switching regime. We therefore tentatively conclude from the combined results of the

194 different dietary perturbations imposed, that the additional mortality costs observed on a rich
195 diet are contingent on the duration of DR.
196
197 **Genetic variance**
198 To eliminate the possibility that the dietary responses described above (in DGRP-195) were
199 the result of rare genetic effects, we performed the same dietary perturbations in a panel of
200 randomly selected genotypes (DGRP-105; 136; 195; 217; 239; 335; 362; 441; 705; 707;
201 853). Across our panel, we again observed that DR increased longevity (Fig. 2 A-F,H;
202 additive model, DR hazard = -0.21 ± 0.08, $P < 0.001$). We detected considerable genetic
203 effects in response to diet ($\chi^2 = 204.8$ (df=10), $P < 0.001$), however, with some genotypes
204 showing elevated mortality at DR compared to the rich diet (Fig. 2,3 A-F,H). Such variation
205 is expected, as reaction norms to diet will differ. As such, the same restricted diet will not
206 always cause a longevity extension across all genotypes (Lee *et al.* 2008, Tatar 2011,
207 Jensen *et al.* 2015).
208
209 Genetic variance in the response to the long-switch treatment was tested using age-
210 dependent interval based models. Across genotypes exposure to the rich diet after a period
211 of DR ('long switch') resulted in exacerbated mortality, exceeding that of flies fed a rich diet
212 for their whole lives (Figure 2; additive model, hazard = 0.997 ± 0.056, $P < 0.001$). There
213 was significant genetic variance for this trait ($\chi2 = 124$ (df=10), $P < 0.001$). Still, all
214 genotypes showed a sudden and marked mortality overshoot following a switch from DR to
215 high nutrient conditions (Figure 2, Table S7,8, 9 of 11 significantly), relative to a continuous
216 rich-diet (ranging from 1.12 to 5.21 times the hazard).
217
218 Alternating the diet from DR to rich every 4 days exacerbated mortality, compared to the
219 continuous rich diet, across the genetic panel (additive non-interval based model, hazard =
220 0.24 ± 0.047, $P < 0.001$). Again we found significant genetic variance for the response to this
221 dietary regime ($\chi2 = 117$ (df=10), $P < 0.001$). Lines were split in their responses with 5 of the
222 11 showing marked decreases in survival, 1 showing an increase in survival, and the
223 remaining 5 showed statistically non-significant effects (Table S8). Interval-based models
224 showed that mortality rate was increased at the rich diets following a period of DR, as in the
225 long-switch, in all lines (significant in 7 of 11, table S10,11). There was a modest positive
226 genetic correlation in the increase in mortality induced by the long-switch and 4-day-switch
227 dietary regime when switched from DR to rich conditions (correlation of coefficients from
228 Table S9, S11, $r_s = 0.45$, $P = 0.17$), suggesting these dietary phenotypes originate from
229 similar physiology.
230

231 **Starvation and hidden costs of DR**

232 Our restricted diet unexpectedly induced a putative starvation response - observable as an
233 increased mortality rate - in four lines (136, 239, 335, 853; Figure 2,3; Table S7,9,10,11).
234 These contrasting responses to DR serendipitously allowed us to see whether the dietary
235 switching phenotypes were contingent on the direction of the DR response. Surprisingly
236 when flies were refed on rich a rich diet, after a period of starvation on DR, mortality did not
237 decrease, but increased (Table S7,8; 3 out of 4 showed a significant increase), even beyond
238 the starvation diet (Figure 2, Table S12). Similarly, in the 4 day switching regime, mortality
239 was higher at the rich diet, and the pattern of mortality even reversed compared to flies fed
240 DR and rich diets continuously, with flies now showing a putative DR-longevity response
241 (Figure S1; Table S13; 3 out of 4 showed a significant increase). These outcomes were
242 particularly remarkable since exposure to a richer diet was expected to rescue the starvation
243 response. In essence, upon a return to the diet where convalescence would be expected to
244 occur, individual mortality risk surged even higher.

245

246 **Cost of mortality not compensated for by fecundity increase**

247 We recognised our results would not necessarily discredit the consensus DR model should
248 the observed costs in mortality be compensated fully, or partially, by an increase in
249 fecundity. Egg produced in across the DGRP panel of both dietary regimes were counted
250 from vials and expressed both as a total count (age-specific fitness of the population, Tables
251 S14,16; Figure S2,3) or eggs per fly (age-specific reproductive output, corrected for mortality
252 differences, Tables S15,17, Figure S2,3). All lines responded strongly to DR, by lowering
253 fecundity. Within the 4-day switching paradigm, the restricted diet also induced a rapid
254 reduction in fecundity (Figure S3). As with the mortality response, genetic lines responded
255 somewhat differently in fecundity in response to the dietary treatments (long switch: $F = 57$
256 ($df=2$), $P < 0.001$; 4-day switch: $\chi2 = 187$ ($df=9$), $P < 0.001$). However, in both metrics, our
257 switching diets underperformed in comparison to the continuous rich-diet (Table S16-18,
258 Figures S2-3). Clearly, the costs of heightened mortality after a period of DR were not
259 compensated by higher fecundity upon a return to nutrient rich conditions.

260

261 **Mortality phenotypes were not contingent on condition of the microbiome, social**
262 **housing, water or sex.**

263 A switch to rich diets after a sustained period of DR ('long switch') still resulted in an
264 increase of mortality when flies were treated with antibiotics (Table S18, $P < 0.001$) or water
265 (Table S19, $P = 0.002$), or when mortality was assessed in isolation (Table S20, $P = 0.014$).
266 Males responded, similar to females, by increasing mortality on rich diets if this was
267 preceded by 4 days of DR (4 day switch, Table S21, $P = 0.001$, 'long switch' not tested).

Discussion

Across the many studies of DR across multiple species, the resultant longevity extension has consistently - with very few exceptions (Adler and Bonduriansky 2014) - been interpreted as provoking anti-ageing, pro-longevity physiology. Such reasoning is founded upon the widely-accepted evolutionary theory of DR (Shanley and Kirkwood 2000) that suggests that during periods of famine resources are actively invested into the soma, to await better times when Darwinian fitness can be gained. In contrast, we find that periods of DR did not result in a superior soma as predicted, but resulted in a large increase in mortality and reduction in fecundity when nutrient availability returned to plentiful. These results therefore question the relevance of using the current explanations of DR's evolutionary origins in interpreting experiments on DR's mechanistic origins.

Other studies have raised similar concerns, but have only very rarely measured the consequences of the relevant life-history event -- a period of DR followed by a period of rich food conditions. Direct measurement of investment into the soma using stable isotopes showed no increased investment under DR (O'Brien *et al.* 2008). Experimental evolution across fifty generations at DR to test this hypothesis also failed to find corroborative evidence (Zajitschek *et al.* 2018). A limited amount of studies have shown that DR in flies results in the remarkable immediate reduction in mortality -- a reduction in frailty. Importantly, lowering mortality to the levels seen in flies that were continuously fed DR diets their whole lives (Good and Tatar 2001, Mair *et al.* 2003), with effects on how fast mortality increases with age (actuarial ageing rate, (Mair *et al.* 2003, Simons *et al.* 2013). We confirmed these results (Fig.1D), but also show for the first time that flies are capable of doing this repeatedly in response to repeated switching in diet. We note that when DR does not slow ageing demographically, but results in an instant lowering of mortality, without any accrued beneficial effects, this is in itself evidence against investment into the soma under DR (Simons *et al.* 2013, Garratt *et al.* 2016).

In the reverse scenario, when we returned flies after long-term (and short-term) DR to rich diets, their performance was markedly lower than that of flies that were fed rich diets for their entire lives. Surprisingly, these effects even held when DR caused higher mortality - interpreted as starvation (Tatar 2011) - resulting in higher mortality on the diet that should provide an opportunity to refeed. Other experiments conducting this 'reverse switch paradigm' did not detect the same costs (Mair *et al.* 2003, 2005), although in the raw non-smoothed data, some mortality excessarbation can be seen in some conditions. Note that

304 even a return to similar mortality rates as flies kept on rich diet for their whole lives, suggests
305 that DR did not result in a superior soma compared to flies kept on rich diets. There are a
306 number of potential variables which could explain these differences. First, the duration of DR
307 prior to a rich diet appears to be integral to induce exacerbated mortality on rich diets (Fig.
308 1). Secondly, the existence and intensity of both the longswitch and 4-day switch phenotype,
309 are genotype-dependent (Fig. 2,S1). This matter is further complicated by the lack of
310 complete synchronicity between both phenotypes, across genotypes (Fig. 2,S1). Lastly, the
311 longevity response to both a restricted diet, and the re-introduction of a rich one, may be
312 contingent on the macronutrient composition of both diets (Lee *et al.* 2008, Jensen *et al.*
313 2015). Previous work on the 'reverse switch' has used dilution of diets to induce DR (Mair *et*
314 *al.* 2003, 2005), whereas we reduce yeast concentration alone.
315
316 Genotypes will differ in their longevity reaction norm to diet, rendering it is impossible to
317 know *a priori* whether a certain dietary composition constitutes the exact optimal longevity-
318 directed diet (Tatar 2011). We propose that our phenotypes may also be contingent upon the
319 direction and degree in which these diets deviate from the optimum, which may be one
320 explanation for the dissimilarity of results observed in similar experiments. These
321 considerations can also explain why the precise duration of dietary switches is important
322 (Fig.1B,C/E,F), which is in line with the recent observation that the precise duration of
323 starvation is critical in achieving life-extension from intermittent fasting (Catterson et al.
324 2018). In addition, larval diet, timing and the order of how diets were fluctuated contributed to
325 differential mortality that has been observed in response to fluctuating diets (van den Heuvel
326 *et al.* 2014). Interestingly, when a poor and a rich diet are offered to flies at the same time,
327 this results in a reduced lifespan (similar to Fig.1B,S1), compared to flies fed either diet
328 separately. Furthermore, these effects are dependent on serotonin signalling (Ro *et al.* 2016)
329 perhaps suggesting that how animals appreciate food (Libert *et al.* 2007) affects how they
330 process or consume their diets, thereby modulating ageing.
331
332 At present, no mechanistic explanation is apparent that explains the premature mortality
333 seen when flies are switched from DR to rich diets. We have excluded water balance,
334 microbiome, sex-specific and social effects being wholly responsible for our observations.
335 We therefore conclude that in conjunction with physiological costs associated with a rich diet
336 there are, until now hidden, costs associated with DR. These costs appear only when a rich
337 diet is resumed after DR. A period on DR is necessary to induce this additional risk of DR,
338 suggesting a physiological change at DR that makes animals more sensitive to rich diets,
339 directly contrary to expectations that follow from evolutionary theory. The question remains
340 what these physiological costs could be.

Reasoning from our observation of exacerbated mortality upon resumption of a rich diet even if DR caused starvation - we suggest the exacerbation of mortality on a rich diet results from physiological adaptations that compensate for the lack of specific components of the DR diet. Such compensation predisposes animals to the physiological costs associated with the intake or metabolism of such a dietary component. Given the generality of this response across the majority of genotypes and dietary manipulations tested, we suggest these same physiological costs might underlie why animals fed rich diet continuously are shorter lived than those on DR. Interestingly, this novel paradigm also explains why flies respond rapidly and consistently to DR, as this could be the result of an escape from costs associated with the intake or metabolism of this (or several) dietary component(s).

All current evidence so far suggests that uptake of the macronutrient protein is responsible for the effects of diet on longevity (Min *et al.* 2007, Lee *et al.* 2008, Maklakov *et al.* 2008, Solon-Biet *et al.* 2014, Jensen *et al.* 2015, Fontana *et al.* 2016). We suggest that DR's effect on longevity is not via increased investment in somatic maintenance but the result from a forced escape from the intrinsically harmful effects of dietary protein. The reason why animals would still choose to eat or absorb intrinsically harmful components, such as protein from their diets, is most probable for their need in reproduction (Speakman and Mitchell 2011, Jensen *et al.* 2015). The specific physiological mechanisms that underlie these costs are at the heart of why DR extends lifespan. We suggest that the quest to identify these costs is helped by the acceptance that investment in somatic maintenance is not necessarily responsible for DR's life-extending effects. In addition, our identification of novel dietary phenotypes in the fly could prove a powerful experimental phenotype for mechanistic study of DR.

368 ## Materials and Methods

369

370 **Fly husbandry**

371 Wild-type inbred isofemale flies from the Drosophila melanogaster Genetic Reference Panel
372 (Mackay *et al.* 2012) were acquired from the Bloomington Stock Centre and the lab of Bart
373 Deplancke (EPFL). Flies were cultured on rich media (8% autolysed yeast, 13% table sugar,
374 6% cornmeal, 1% agar and nipagin 0.225% [w/v]) with bottles for growing and mating,
375 containing an additional 1% [w/v] propanoic acid.. For lifespan experiments, adult flies were
376 subsequently provided with either the same rich media, or a restricted media (2% autolysed
377 yeast) in vials. Restricted media retained the composition of all other media components,
378 given the dietary protein axis is the main lifespan determinant in flies (Lee *et al.* 2008,
379 Jensen *et al.* 2015). Cooked fly media was kept for a maximum of 2 weeks at 4-6 °C and
380 was warmed to 25°C before use.

381

382 **Experimental mortality protocol and demography cages**

383 Flies were expanded in bottles (Drosophila PP Flask Square Bottom; Flystuff) on a rich diet.
384 Experimental flies were grown in bottles (incubated at 25°C) sprinkled with granulated live
385 yeast, in which 12 females and 2 males had been egg-laying for a period of ~60 hours.
386 Bottles were sprinkled with water, daily, if media appeared dry until pupation began. Upon
387 eclosion, the adult F1 generation was transferred, daily to generate age-matched cohorts, to
388 mating bottles for 48 hours before being sorted under light CO_2 anaesthesia (Flystuff
389 Flowbuddy; < 5L / min) (Bartholomew *et al.* 2015) and transferred to purpose-built
390 demography cages (Good and Tatar 2001). Lifespan experiments were carried out in a
391 climate-controlled room (12:12 LD, 25°C and 50-60% relative humidity). Cages contained
392 between 100-125 females each; the number of cages was treatment-dependent. All flies
393 were kept on rich media until age 3-6 days whereupon they were divided between the
394 dietary treatments. Individual lifespan was determined from the time when the individual
395 entered the experimental cage (at two days of age) until death or censoring. A census of
396 flies was taken every other day: dead flies were counted and removed, and fresh media was
397 provided at this time. Flies that were alive, but stuck to the side of the vial; escaped flies and
398 individuals affixed to the food (~10.5% of deaths) were right-censored.

399

400 **Fecundity**

401 A subsection of fly feeding vials were imaged and analysed using QuantiFly (Waithe et al.
402 2015) to determine relative amounts of egg laying.

403

404 **Dietary regimes**
405 Two main temporal dietary regimes were imposed on several genotypes of mainly female
406 flies using two diets, restricted (2% yeast) and rich (8% yeast) with controls of continuous
407 exposure to these diets.
408 1) To test whether a prolonged period of DR resulted in superior survival and reproduction
409 when conditions improved, flies were exposed to continuous restricted diet that was
410 switched to a rich diet at ~45-60% survival of the continuous rich diet group ('long-switch').
411 All flies of the same genotype were switched on the same day, irrespective of eclosion date.
412 2) We further tested whether short bouts of DR had similar effects, which also allowed us to
413 test whether effects observed in the long-switch regime were exclusive to older flies. In these
414 diets flies were repeatedly switched between restricted and rich diets at four-day intervals
415 ('four-day switch'). By starting half of the experimental cohort on restricted or rich diets,
416 current dietary treatments were mirrored and balanced across the cohort.
417
418 These experiments were performed on DGRP-195 at high sample size (N = 14,102).
419 Subsequently, to test whether these effects were general, these experiments were expanded
420 to a panel of DGRP lines (DGRP-105; 136; 195; 217; 239; 335; 362; 441; 705; 707; 853) in
421 one large experiment of N = 37,897. Several other parts of the experiments (see below)
422 were run separately (for specific grouping see supplementary data). Dietary treatments were
423 balanced for age. From this experiment, fecundity estimates were also taken from feeding
424 vials, on four consecutive scoring days (for 4-day switch, and continuous treatments) and
425 one scoring day before, and after, the dietary switch (for longswitch, and continuous rich
426 treatment).
427
428 **Supplementary dietary regimes**
429 We tested a range of other dietary regimes to test specific hypotheses, alongside the
430 treatments listed above, using line DGRP-195. 1) We tested whether DR could instantly
431 reduce mortality by imposing a short duration (four days) of DR, in late-life, *sensu* (Mair *et al.*
432 2003), before returning to a rich-diet ('short-switch'). 2) We increased the frequency of the
433 dietary switch to two days ('two-day switch') to investigate the length of DR necessary for the
434 observed phenotypes, and 3) further changed the ratio of the time spend on either diet; two
435 days of either rich or restricted-diet, to four days of the reverse ('4-to-2-day 2-to-4-day').
436
437 **Tests of specific hypotheses: microbiome, water balance, sex and social effects**
438 We tested whether the dietary phenotypes observed were due to four potential previously
439 suggested confounding factors: 1) the microbiome (Wong *et al.* 2014), 2) water balance
440 (Fanson *et al.* 2012) and 3) social effects (Leech *et al.* 2017). 4) Sex-differences in the DR

441 response (Magwere *et al.* 2004, Regan *et al.* 2016). These were confirmed not to interfere
442 with the observed phenotype (see results). DGRP-195 were used exclusively for these
443 experiments, under the continuous restricted, continuous rich, and long-switch diets. 1) We
444 assessed whether disruption of the gut microbiome was responsible for the mortality
445 phenotype observed by wholesale abating the microbiome. Flies were provided media upon
446 which an array of broad-spectrum antibiotics (50 µl of a stock solution, comprised of 100
447 µg/ml ampicillin, 50 µg/ml vancomycin, 100 µg/ml neomycin, and 100 µg/ml metronidazole)
448 were pipetted and left for 24hrs. We assumed dissolution incorporation in the top 1 ml of
449 food (Ren *et al.* 2009). Antibiotic treatment began four days prior to dietary switch
450 treatments, and concluded eight days thereafter. Ablation of the microbiome was confirmed
451 by whole-fly homogenisation (age 20 days; 8 days post-antibiotic treatment) and growth of
452 solution on MRS agar plates (Oxoid; see supplementary figure S7). Individuals (6 control
453 and 6 antibiotic treated) were removed from cages containing a continuous restricted diet,
454 washed in ethanol, and rinsed in PBS (Gibco). Homogenisation took place in 500 µl of PBS,
455 and solute was transferred to a 96-well plate for 1:10 serial dilutions. Dilutions were spotted
456 on plates with, and without antibiotic (500 µl of stock solution) and incubated at 25°C for 72
457 hours. Plates were coated with parafilm to mimic anoxic conditions. 2) Flies were provided
458 with ~1cm^3 portion of water-agar (2%[w/v]) accompanying media in vials, to eliminate
459 desiccation as a proximal cause. Water-agar supplementation began at age four, and
460 continued throughout the flies' full lifecourse. 3) Social effects were excluded by housing flies
461 individually in vials. These flies were taken from experimental cages and put on the
462 experimental diets at the dietary switch 4) Males were assessed for mortality in the 4 day
463 switch dietary regime.
464
465 **Experimental batches**
466 All demography experiments contained the relevant controls, grown and assayed for
467 mortality at the same time. Where data are plotted in a single figure, this constitutes results
468 gathered from a batch of flies at the same chronological time.
469
470 **Data Analysis**
471 Mixed cox-proportional hazard models were used that included 'cage' as random term to
472 correct for uncertainty of pseudo-replicated effects within demography cages (Ripatti and
473 Palmgren 2000, Therneau *et al.* 2003). We used interval-based models that used time-
474 dependent covariates to estimate the differential mortality risks associated with diet (and with
475 time spend on a diet - after diets changed), as imposed in the different dietary regimes
476 (Therneau *et al.* 2018). These models allow a statistical association, within the cox-
477 proportional hazard risk, with the current state (e.g. diet) and mortality. Our models included

478 dietary regime (i.e. treatment) and as time-dependent covariate, diet. Flies in the long-switch
479 dietary regime were also analysed in a state-dependent manner, coding for long switch only
480 when this state change occured. Repeated switching regimes were considered life-long
481 treatment and tested in interaction with the state variable diet. Each model used continuous
482 rich food and DGRP-195 as reference category, except if otherwise stated.
483
484 Interactions between dietary regime, diet and genotype were fitted to test for differential
485 effects of diet on mortality depending on the regime it was provided. Additional specific test
486 of coefficients are provided that combine the single and interaction term (in a z-test, using
487 the maximum s.e. of the factor compared) to test how mortality risk was changing compared
488 to specific reference categories of interest (e.g. compared to continuous DR). For
489 comparisons between genotypes we report full models including all data and models fitted
490 within each genotype separately. The latter corrects for deviations in proportionality of
491 hazards between the genotypes. Qualitative conclusions remain similar and note formal
492 tests for proportionality of hazards are not available for mixed effects cox regressions.
493 Models without a time-dependent covariate for diet were also run to compare overall
494 longevity differences as a result of alternating exposure to DR (2-day switch, 4-day switch
495 and their combination). These models therefore test the integrated effect on mortality
496 disregarding any within dietary treatment diet effects. Coefficients are reported as logged
497 hazards with significance based on z-tests. Right-censoring was included, as indicated
498 above.
499
500 Egg laying was analysed as a mixed generalized Poisson model using cage as random
501 term, and fitting age as a non-continuous factor in the analysis. Estimates from models are
502 presented (effects of dietary regime) as well as model comparisons using log-likelihood
503 comparison with chi-square to test overall effects of genotype. Effects of different dietary
504 regimes were estimated within the same model. Comparisons of genotypic effects were
505 performed for each different dietary regime separately compared to continuous treatment, as
506 not to conflate genetic variance across different categories with each other.
507
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512

513 **Acknowledgements**
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520

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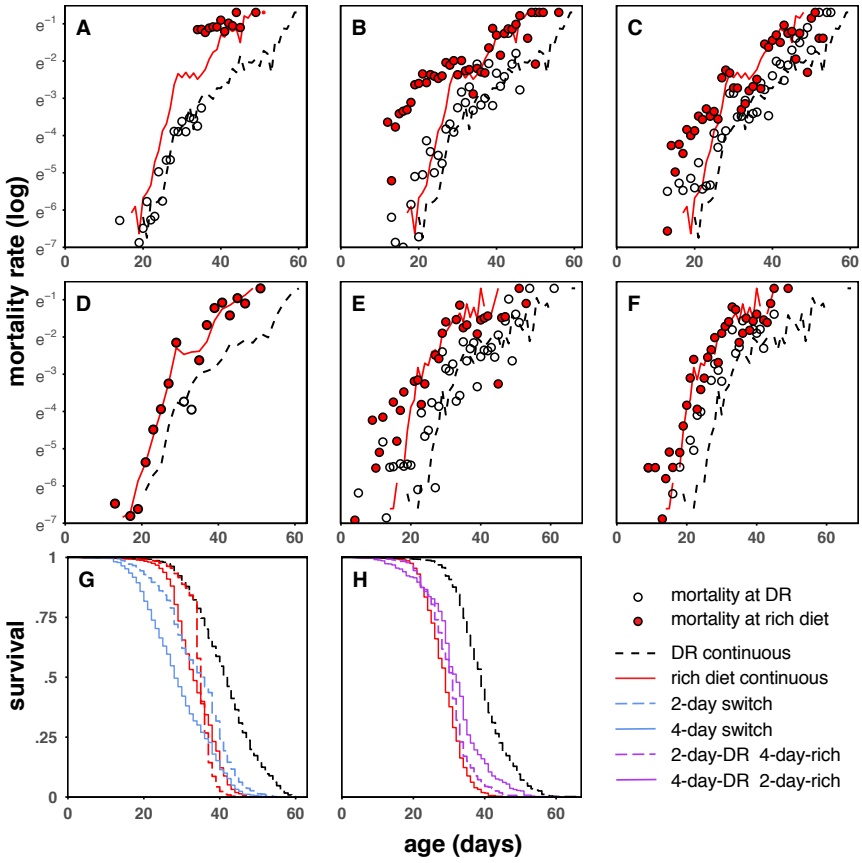


Figure 1. The effect of different dietary regimes in DGRP-195. Continuous rich, and restricted treatments plotted as lines (solid red and dashed black, respectively). Switch treatments plotted as points (white and red). The exacerbation of mortality due to switch phenotypes is observable as the difference between mortality at continuous rich diet (red line), and mortality of switch treatment when on a rich diet (red points). A – long switch; After a long period of DR, mortality is exacerbated compared to flies fed a rich diet continuously. B – 4-day switch; Switching from a DR to a rich diet, repeatedly, for every four days, increases mortality on rich diets, compared to continuously rich fed flies. Flies are still able to modulate their mortality in response to DR even when diet fluctuates rapidly. C – 2-day switch; Mortality on rich diets is only mildly increased and flies still respond to DR even if it is only imposed for two days. D – age-adjusted short reverse switch; after a long period of rich diets, DR for 4 days E – 4-day DR, 2-day rich switch; Flies respond to DR, but slightly blunted compared to continuous DR. F – 4-day rich, 2-day DR switch; The effect of DR is reduced when imposed for 2 days following 4 days of DR. G – survival plot of panels A-C with associated continuous diet controls; Total survival of both the 4-day switching dietary regime and the long-switch is lowered compared to continuously rich diets, even though flies spend a considerable amount of their lives on restricted diets. Flies on DR outlive all other categories. H – survival plot of panels E/F with associated continuous diet controls. Even though in these asymmetrical dietary regimes flies spend up to two-thirds of their lives on DR survival benefits are only small compared to continuous DR. N = 19,086 total. N = 995-3,769 per treatment. Panel D has been aligned at the time of the dietary switch, with ages being adjusted by ≤ 1 day.

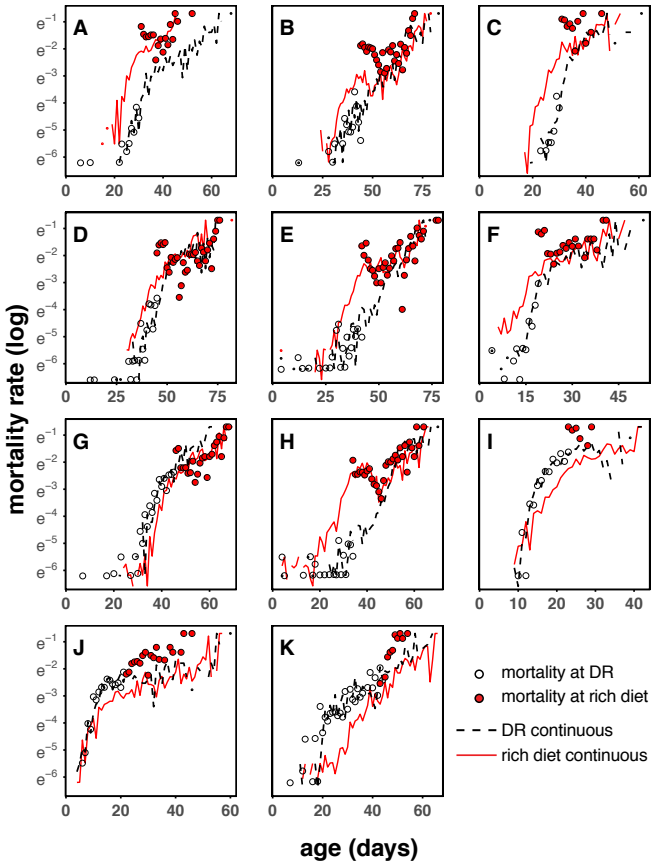


Figure 2. Long switch treatment in a panel of 11 DGRP genotypes. A – 195; B – 105; C – 217; D – 441; E – 705; F – 707; G – 136; H – 362; I – 239; J – 335; K – 853. Exposure to a high nutrient diet after a period of DR resulted in marked increase in mortality compared to a continuous rich diet in all lines (9 out of 11 significant). There was genetic variation in this response with DGRP-136 (G) and DGRP-362 (H) showed the smallest effects. Interestingly this marked overshoot was not dependent on DR extending longevity. Even in lines that showed 'starvation' on our DR diet showed marked overshoots, even compared to continuous DR (I, J, K), where recuperation of starvation was expected. Continuous rich, and restricted treatments plotted as lines (solid red and dashed black, respectively). Switch treatments plotted as points (white and red). The exacerbation of mortality due to switch phenotypes is observable as the difference between mortality at continuous rich diet (red line), and mortality of switch treatment when on a rich diet (red points). N = 29,702 total; ~2,725 per genotype; 13,375 for continuous rich treatments, and ~8,170 for all other treatments. Dietary switch for long switch treatment group occurred at 45-65% of continuous rich treatment flies.

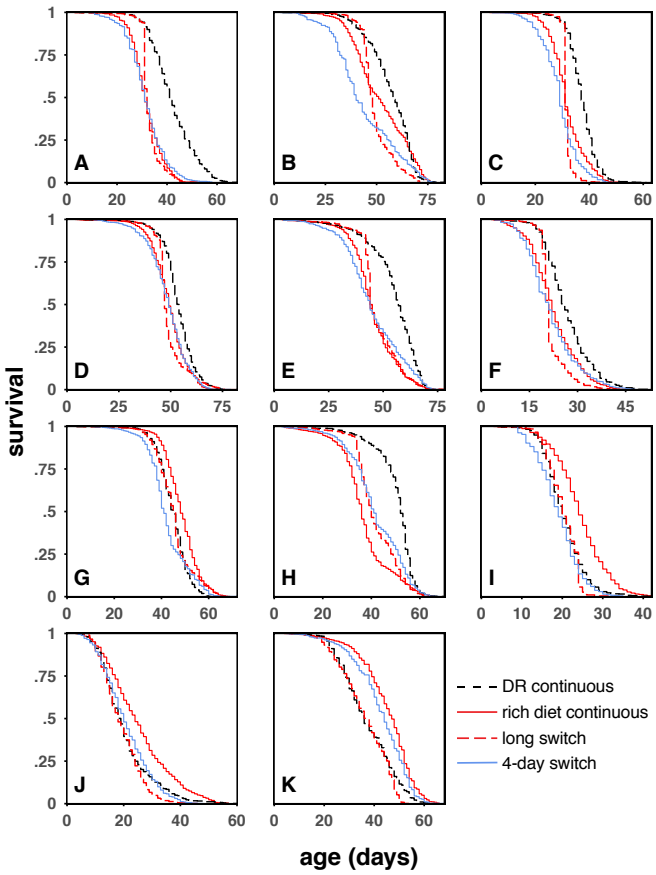


Figure 3. Survival curves of DGRP panel. A – 195; B – 105; C – 217; D – 441; E – 705; F – 707; G – 136; H – 362; I – 239; J – 335; K – 853. Total survival on the different dietary regimes across the genetic panel tested. Rich diets after a period of DR, in the case where this increased longevity and investment into the soma is assumed, resulted in such an increase in mortality that total survival of the cohort was lower or equal to those fed a continuous rich diets for their whole lives (A-F). Continuous rich, and restricted treatments plotted as lines (solid red and dashed black, respectively). Switch treatments plotted as dashed red, and solid blue lines (see Fig.S1 for 4-day switch mortality). N = 37,897 total; ~3,450 per genotype; 13,375 for continuous rich treatments, and ~8,170 for all other treatments. Dietary switch for long switch treatment group occurred at 45-65% of continuous rich treatment flies (see Fig.2).

Supplementary figures

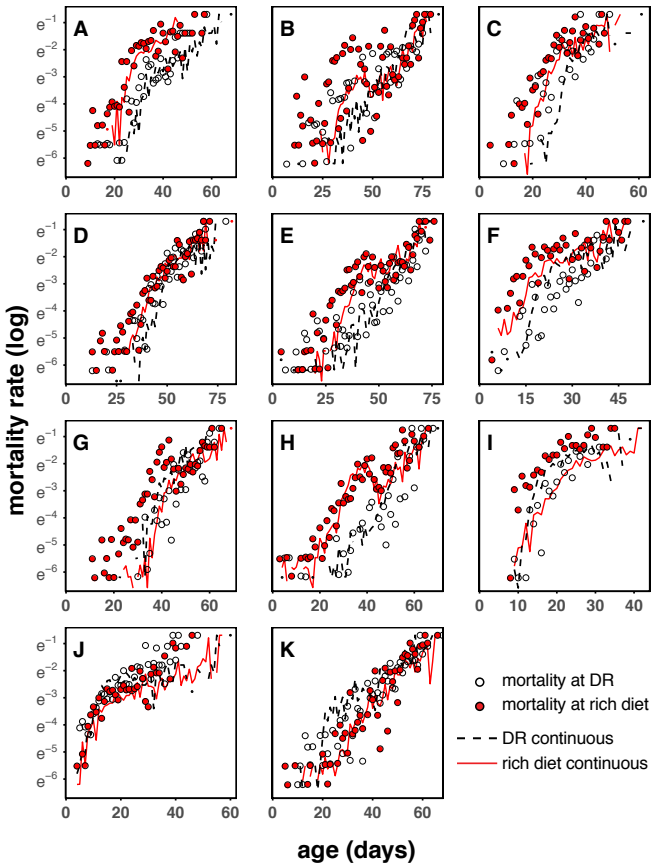


Figure S1. 4-day switch treatment in a panel of 11 DGRP genotypes. A – 195; B – 105; C – 217; D – 441; E – 705; F – 707; G – 136; H – 362; I – 239; J – 335; K – 853. Continuous rich, and restricted treatments plotted as lines (solid red and dashed black, respectively). Switch treatments plotted as points (white and red). The exacerbation of mortality due to switch phenotypes is observable as the difference between mortality at continuous rich diet (red line), and mortality of switch treatment when on a rich diet (red points). N = 29,740 total; ~2,725 per genotype; 13,375 for continuous rich treatments, and ~8,170 for continuous rich and 4-day switch treatments. Dietary switch for 4-day switch treatment group occurred every 4 days, and was mirrored at each time point. Continuous rich and restricted treatments are twinned with long switch treatment experiment (Fig. 2).

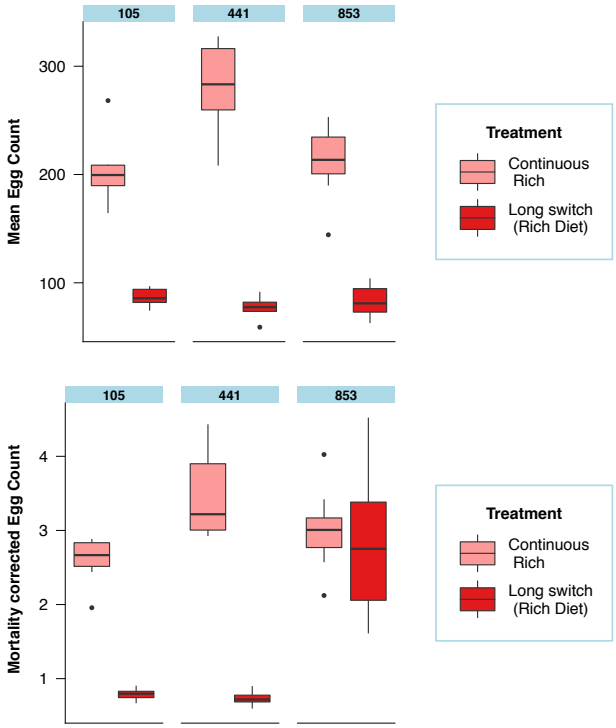


Figure S2. Fecundity analysis of long switch treatment from 3 DGRP genotypes. No compensation via fecundity for reduced lifespans in switch treatment. Raw (above) and mortality corrected (below) egg counts of DGRP-105; 441; 853 from long switch treatment experiment (Fig. 2). Counts generated using QuantiFly software. Counts are relative, but directly comparable. Flies assayed between age 44-47 days, with boxplots (median with the box depicting a quartile each was and whiskers showing the range, outliers plotted as dots) aggregating totals. Each cage was assayed once, on the first scoring day post dietary switch. Mortality corrected counts (below) generated by dividing raw counts, by N flies remaining in cage at the time of assaying. N = on average, 7 cages assayed, per treatment, per genotype.

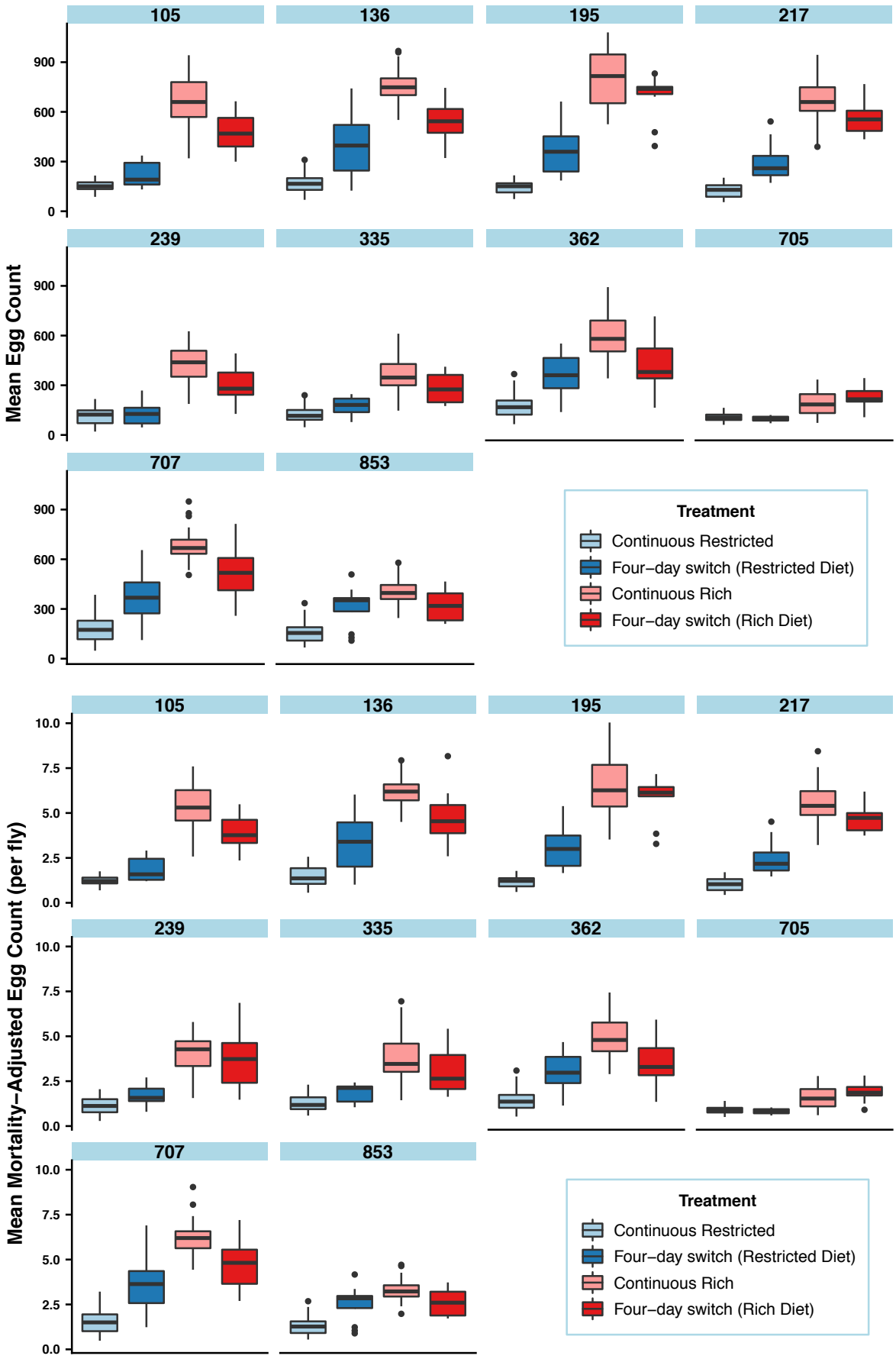

105
136
195
217
239
335
362
705
707
853
Mean Egg Count
0
300
600
900
Mean Mortality−Adjusted Egg Count (per fly)
0.0
2.5
5.0
7.5
10.0
Treatment
Continuous Restricted
Four−day switch (Restricted Diet)
Continuous Rich
Four−day switch (Rich Diet)

Figure S3. Fecundity analysis of 4-day switch treatment from 10 DGRP genotypes. No compensation via fecundity for reduced lifespans in switch treatment. Raw (above) and mortality corrected (below) egg counts of DGRP-105; 136; 195; 217; 239; 335; 362; 705; 707; 853 from long switch treatment experiment (Fig. 2). Counts generated using QuantiFly software. Counts are relative, but directly comparable. Flies assayed between age 8-21 days, with boxplots aggregating totals (median with the box depicting a quartile each was and whiskers showing the range, outliers plotted as dots). Each cage was assayed on 4 consecutive scoring days. Mortality corrected counts (below) generated by dividing raw counts, by N flies remaining in cage at the time of assaying. N = on average, 7 cages assayed, per treatment, per genotype.

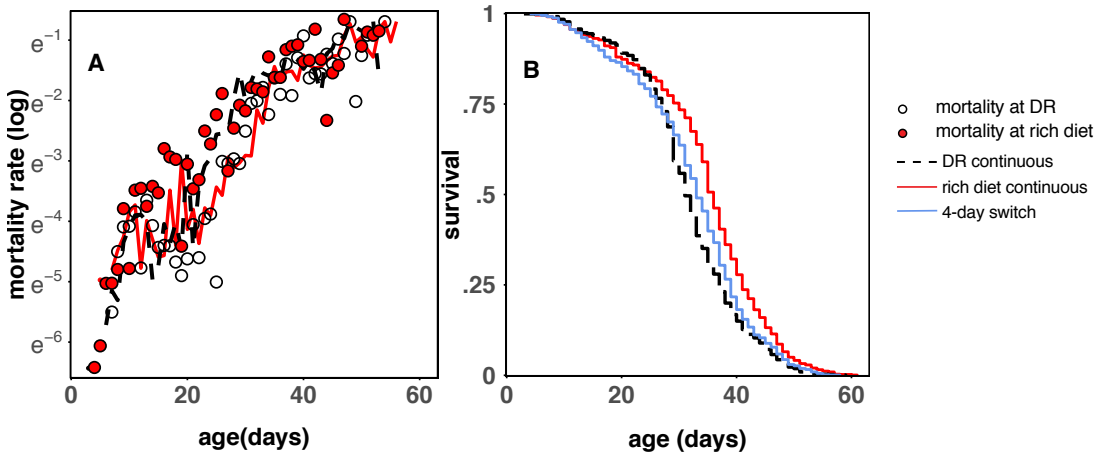


Figure S4. 4-day switch treatment of DGRP-195 males. A – 4-day switch mortality; B – 4-day switch survival. Muted response to 4-day switch treatment in males. Rich diet in the 4-day switch increased mortality compared to continuously rich fed flies. Continuous rich, and restricted treatments plotted as lines (solid red and dashed black, respectively). Switch treatment plotted as points (white and red). The exacerbation of mortality due to switch phenotypes is observable as the difference between mortality at continuous rich diet (red line), and mortality of switch treatment when on a rich diet (red points). N = 4,429 total; ~1,475 per treatment. Dietary switch for 4-day switch treatment group occurred every 4 days, and was mirrored at each time point.

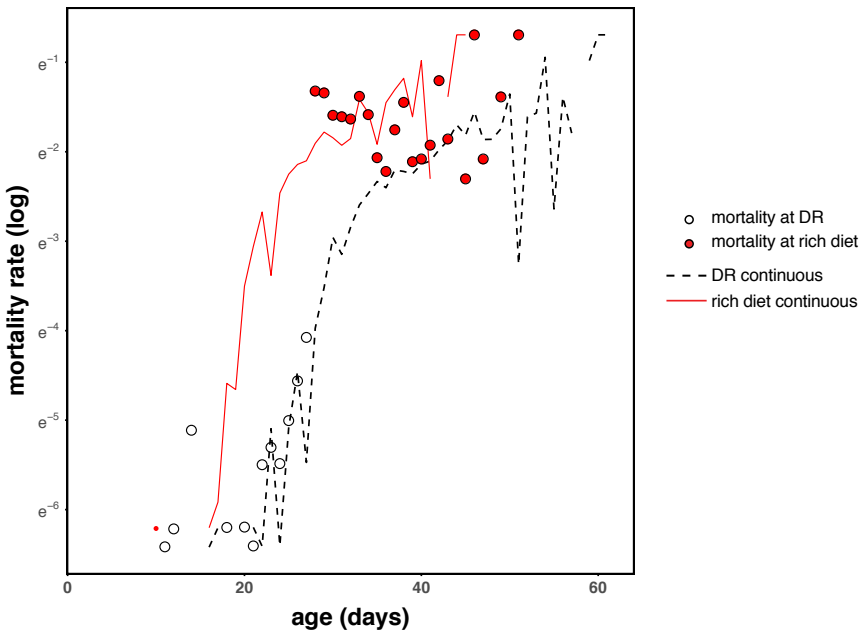


Figure S5. Antibiotic long switch treatment of DGRP-195. Long switch phenotype independent of antibiotic treatment. Antibiotic treatment took place in all treatments four days prior to dietary switch, and concluded eight days thereafter. Continuous rich, and restricted treatments plotted as lines (solid red and dashed black, respectively). Switch treatment plotted as points (white and red). The exacerbation of mortality due to switch phenotypes is observable as the difference between mortality at continuous rich diet (red line), and mortality of switch treatment when on a rich diet (red points). N = 2,605 total; ~870 per treatment. (See Fig. S7 for confirmation of ablation of microbiome).

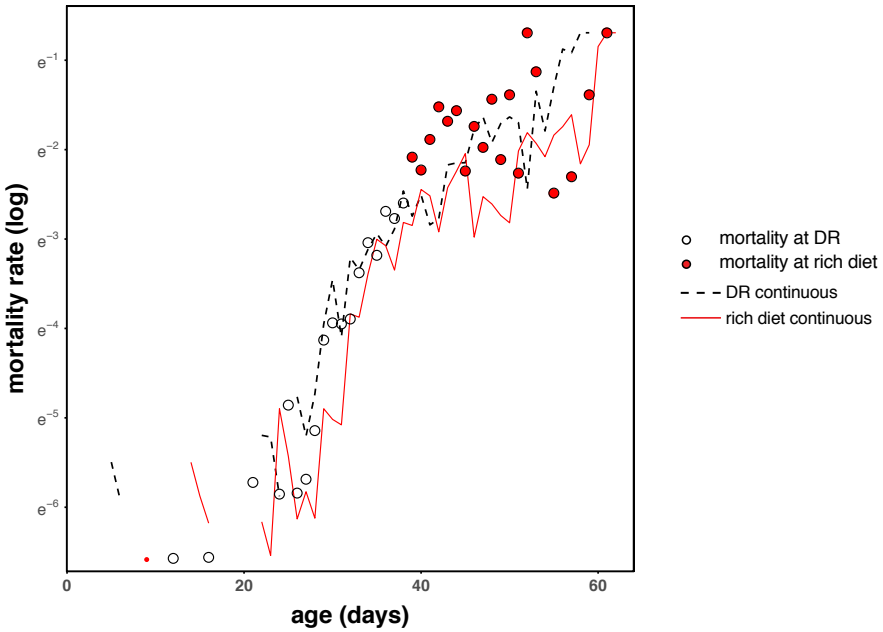
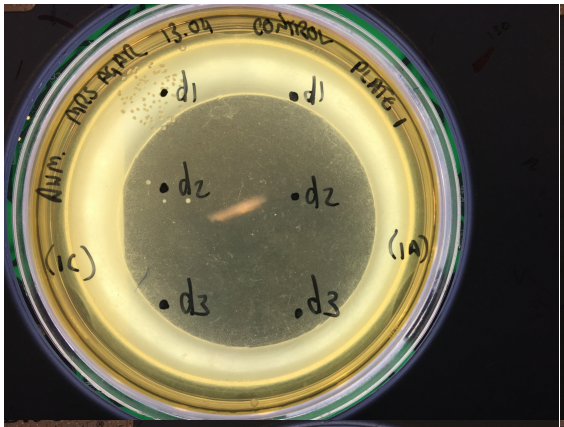
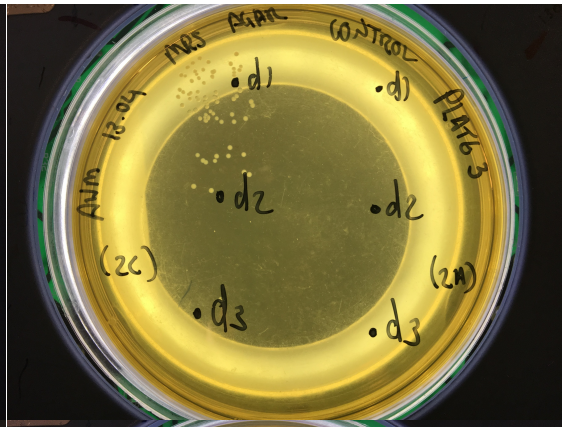
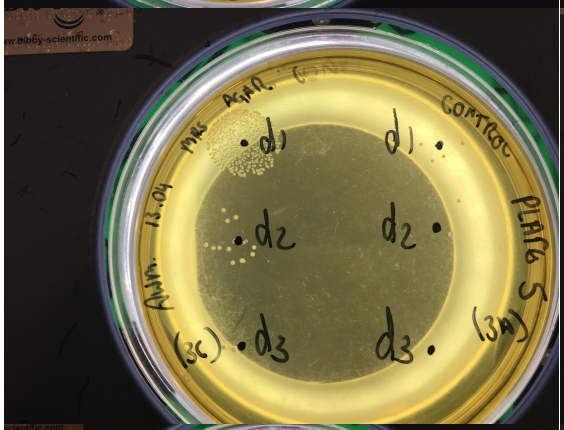
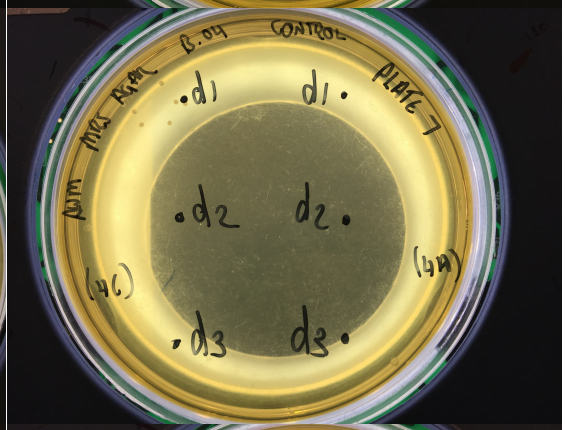


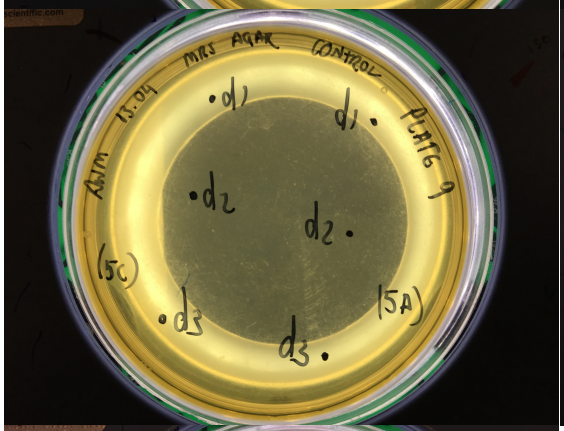
Figure S6. Water supplemented long switch treatment of DGRP-195. Long switch phenotype independent of water supplementation. Water supplementation took place in all treatments throughout life of the cage. Continuous rich, and restricted treatments plotted as lines (solid red and dashed black, respectively). Switch treatment plotted as points (white and red). The exacerbation of mortality due to switch phenotypes is observable as the difference between mortality at continuous rich diet (red line), and mortality of switch treatment when on a rich diet (red points). N = 2,562 total; ~850 per treatment. **NB** water supplementation did change the response to DR. This effect was followed up with five different genotypes across a reaction norm of diets, with only a shift in reaction norm detected (manuscript in preparation). DR is not explained by dehydration as sometimes suggested, nor is the long switch phenotype.

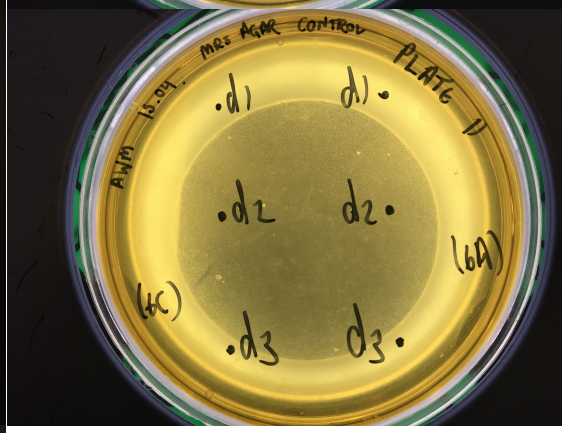

AWM. MRS AGAR 13.04 CONTROL PLATE 1
d1
d1
d2
d2
(1C)
(1A)
d3
d3

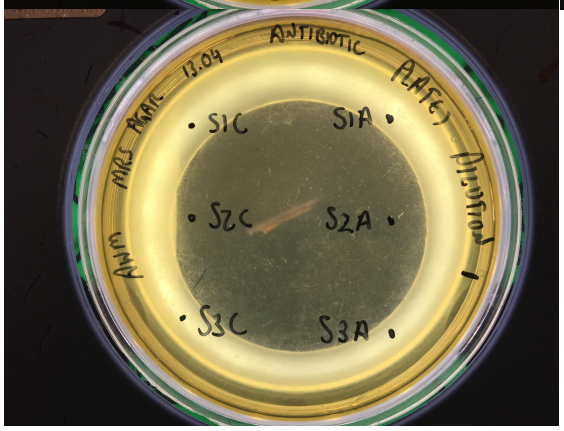

AWM 13.04 MRS AGAR CONTROL PLATE 3
d1
d1
d2
d2
(2C)
(2A)
d3
d3


AWM. 13.04 MRS AGAR CONTROL PLATE 5
d1
d1
d2
d2
(3C)
(3A)
d3
d3


AWM MRS AGAR 13.04 CONTROL PLATE 7
d1
d1
d2
d2
(4C)
(4A)
d3
d3


AWM 13.04 MRS AGAR CONTROL PLATE 9
d1
d1
d2
d2
(5C)
(5A)
d3
d3


AWM 13.04 MRS AGAR CONTROL PLATE 11
d1
d1
d2
d2
(6C)
(6A)
d3
d3


AWM MRS AGAR 13.04 ANTIBIOTIC PLATE, DILUTION 1
S1C
S1A
S2C
S2A
S3C
S3A

Sample	Colonies at dilution 1	Colonies at dilution 2	Colonies at dilution 3	Colonies at antibiotic plate dilutions
1 (control)	75	3	0	0
2 (control)	50	12	1	0
3 (control)	250	12	2	0
4 (control)	8	0	0	NA
5 (control)	0	0	0	NA
6 (control)	1	0	0	NA
7 (antibiotic treated)	0	0	0	0
8 (antibiotic treated)	0	0	0	0
9 (antibiotic treated)	6	2	0	0
10 (antibiotic treated)	0	0	0	NA
11 (antibiotic treated)	0	0	0	NA
12 (antibiotic treated)	0	0	0	NA

Figure S7. Confirmation of ablation of microbiome. Images of bacterial colonies visible on MRS agar plates (above) and estimated colony count (below). Twelve samples ceded from control, or antibiotic-treated cages. Lysate was diluted post-homogenisation and grown on control, or antibiotic-treated plates. No growth visible under antibiotic treated plate conditions. 98.4% reduction of total microbiota observed at dilution 1. 92.5% reduction of total microbiota observed at dilution 2.

Table S1. Effect of dietary regimes on interval-based log hazard ratios of mortality in DGRP-195.

	Full Model				Versus continuous DR		
coefficient	estimate	exp (estimate)	s.e.	p	estimate	exp (estimate)	p
DR	-1.179	0.308	0.047	**<0.001**			
long switch	1.310	3.705	0.055	**<0.001**			
4 day switch	0.858	2.358	0.062	**<0.001**			
2 day switch	0.125	1.133	0.063	**0.047**			
short switch	0.272	1.312	0.051	**<0.001**			
4 day switch * DR	-0.409	0.665	0.085	**<0.001**	0.449	1.567	**<0.001**
2 day switch * DR	0.255	1.291	0.074	**0.001**	0.380	1.462	**<0.001**
short switch * DR	-0.466	0.627	0.116	**<0.001**	-0.195	0.823	0.092

Table S2. Effect of dietary regimes on longevity in DGRP-195.

	Full Model			
coefficient	estimate	exp (estimate)	s.e.	p
DR	-1.136	0.321	0.058	**<0.001**
2 day switch	-0.224	0.799	0.064	**<0.001**
4 day switch	0.386	1.471	0.064	**<0.001**

Table S3. Time-dependent effect of mortality increase induced by a ('long') switch from reduced to rich diets in DGRP-195

	Full Model			
coefficient	estimate	exp (estimate)	s.e.	p
day 2	1.629	5.101	0.085	**<0.001**
day 4	0.847	2.334	0.093	**<0.001**
day 6	0.452	1.572	0.121	**<0.001**
day 8	-0.045	0.956	0.216	0.84
day 10	-0.226	0.798	0.369	0.54
day 12	-0.077	0.926	0.601	0.9
> day 14	-1.038	0.354	1.066	0.33

Table S4. Time-dependent effect of mortality increase induced by a 4-day swith in DGRP-195

	Full Model			
coefficient	estimate	exp (estimate)	s.e.	p
DR	-1.303	0.272	0.094	**<0.001**
2nd interval	0.227	1.255	0.055	**<0.001**
2nd interval * DR	-0.715	0.489	0.143	**<0.001**

Table S5. Effect of asymmetrical dietary regimes on mortalty in DGRP-195.

	Full Model				Versus continuous DR		
coefficient	estimate	exp (estimate)	s.e.	p	estimate	exp (estimate)	p
DR	-1.684	0.186	0.087	**<0.001**			
4d-DR|2d-Rich	-0.209	0.811	0.097	**0.032**			
2d-DR|4d-Rich	-0.157	0.854	0.095	0.097			
4d-DR|2d-Rich * DR	0.458	1.581	0.113	**<0.001**	0.249	1.283	**0.027**
2d-DR|4d-Rich * DR	0.929	2.531	0.124	**<0.001**	0.771	2.163	**<0.001**

Table S6. Effect of asymmetrical dietary regimes on longevity in DGRP-195.

	Full Model			
coefficient	estimate	exp (estimate)	s.e.	p
DR	-1.692	0.184	0.087	**<0.001**
4d-DR|2d-Rich	-0.805	0.447	0.095	**<0.001**
2d-DR|4d-Rich	-0.343	0.710	0.094	**<0.001**

Table S7. Mortality increases in response to a rich diet after a period of DR ('long switch') across a panel of 11 DGRP lines (195 is reference)

	Full Model				Effect of DR			Long switch versus rich diet		
coefficient	estimate	exp	s.e.	p	estimate	exp	p	estimate	exp	p
DR	-1.723	0.179	0.157	**<0.001**						
long switch	0.973	2.645	0.160	**<0.001**						
105	-2.681	0.068	0.146	**<0.001**						
136	-1.969	0.140	0.152	**<0.001**						
217	-0.080	0.923	0.159	0.61						
239	0.899	2.457	0.156	**<0.001**						
335	0.190	1.209	0.155	0.22						
362	-1.022	0.360	0.155	**<0.001**						
441	-2.107	0.122	0.152	**<0.001**						
705	-1.861	0.156	0.154	**<0.001**						
707	1.073	2.925	0.156	**<0.001**						
853	-1.803	0.165	0.152	**<0.001**						
105 * DR	1.296	3.655	0.217	**<0.001**	-0.427	0.653	**0.049**			
136 * DR	1.995	7.355	0.213	**<0.001**	0.273	1.314	0.199			
217 * DR	0.622	1.863	0.219	**0.004**	-1.100	0.333	**<0.001**			
239 * DR	2.427	11.319	0.211	**<0.001**	0.704	2.022	**0.001**			
335 * DR	2.947	19.052	0.208	**<0.001**	1.225	3.403	**<0.001**			
362 * DR	0.343	1.409	0.219	0.12	-1.379	0.252	**<0.001**			
441 * DR	1.002	2.724	0.215	**<0.001**	-0.720	0.487	**0.001**			
705 * DR	0.602	1.826	0.217	**0.006**	-1.120	0.326	**<0.001**			
707 * DR	0.886	2.424	0.215	**<0.001**	-0.837	0.433	**<0.001**			
853 * DR	2.549	12.796	0.210	**<0.001**	0.827	2.286	**<0.001**			
105 * long switch	0.168	1.183	0.220	0.44				1.141	3.130	**<0.001**
136 * long switch	-0.381	0.683	0.222	0.086				0.592	1.807	**0.008**
217 * long switch	1.152	3.165	0.226	**<0.001**				2.125	8.370	**<0.001**
239 * long switch	0.870	2.387	0.224	**<0.001**				1.843	6.313	**<0.001**
335 * long switch	-0.305	0.737	0.221	0.17				0.668	1.950	**0.002**
362 * long switch	-0.982	0.375	0.223	**<0.001**				-0.009	0.991	0.968
441 * long switch	-0.172	0.842	0.220	0.43				0.800	2.226	**<0.001**
705 * long switch	-0.605	0.546	0.222	**0.006**				0.368	1.445	0.097
707 * long switch	0.188	1.206	0.218	0.39				1.160	3.191	**<0.001**
853 * long switch	0.117	1.124	0.225	0.6				1.090	2.973	**<0.001**

Table S8. Models run within each genotype testing for increases in response to a rich diet after a period of DR ('long switch')

	Estimates from individual models			
coefficient	estimate	exp	s.e.	p
105 DR	-0.501	0.149	0.606	**0.001**
136 DR	0.647	0.120	1.910	**<0.001**
195 DR	-1.737	0.197	0.176	**<0.001**
217 DR	-1.102	0.178	0.332	**<0.001**
239 DR	0.775	0.071	2.170	**<0.001**
335 DR	0.570	0.115	1.768	**<0.001**
362 DR	-1.201	0.130	0.301	**<0.001**
441 DR	-0.503	0.101	0.605	**<0.001**
705 DR	-0.935	0.095	0.393	**<0.001**
707 DR	-0.646	0.076	0.524	**<0.001**
853 DR	0.828	0.093	2.288	**<0.001**
105 long switch	1.258	0.156	3.520	**<0.001**
136 long switch	0.113	0.132	1.120	0.39
195 long switch	0.453	0.203	1.573	**0.025**
217 long switch	1.650	0.187	5.205	**<0.001**
239 long switch	1.483	0.103	4.406	**<0.001**
335 long switch	0.881	0.136	2.413	**<0.001**
362 long switch	0.145	0.137	1.157	0.29
441 long switch	0.313	0.109	1.368	**0.004**
705 long switch	0.329	0.102	1.389	**0.001**
707 long switch	0.854	0.083	2.348	**<0.001**
853 long switch	1.373	0.122	3.949	**<0.001**

Table S9. Effect of alterating DR and rich diets every 4 days (4 day switch) on longevity across 11 DGRP lines (195 is reference)

	Full Model				Effect compared to rich diet		
coefficient	estimate	exp	s.e.	p	estimate	exp	p
4 day switch	-0.140	0.869	0.114	0.22			
105	-2.476	0.084	0.096	**<0.001**			
136	-1.858	0.156	0.098	**<0.001**			
217	-0.065	0.937	0.100	0.52			
239	0.918	2.505	0.099	**<0.001**			
335	0.226	1.254	0.100	**0.024**			
362	-0.988	0.373	0.099	**<0.001**			
441	-1.969	0.140	0.097	**<0.001**			
705	-1.769	0.171	0.098	**<0.001**			
707	1.054	2.869	0.100	**<0.001**			
853	-1.697	0.183	0.098	**<0.001**			
105 * 4 day switch	0.644	1.904	0.159	**<0.001**	0.504	1.656	**0.002**
136 * 4 day switch	0.632	1.880	0.157	**<0.001**	0.492	1.635	**0.002**
217 * 4 day switch	0.490	1.632	0.158	**0.002**	0.350	1.419	**0.027**
239 * 4 day switch	1.053	2.866	0.157	**<0.001**	0.913	2.491	**<0.001**
335 * 4 day switch	1.026	2.789	0.158	**<0.001**	0.886	2.425	**<0.001**
362 * 4 day switch	-0.343	0.710	0.157	**0.029**	-0.483	0.617	**0.002**
441 * 4 day switch	0.163	1.177	0.158	0.3	0.023	1.023	0.885
705 * 4 day switch	-0.032	0.968	0.157	0.84	-0.172	0.842	0.273
707 * 4 day switch	0.153	1.165	0.158	0.33	0.013	1.013	0.935
853 * 4 day switch	0.365	1.441	0.157	**0.02**	0.225	1.253	0.152

Table S10. Effect of alterating DR and rich diets every 4 days (4 day switch) on mortality at each diet, across 11 DGRP lines (195 is reference)

								Compared to continious diets					
	Full Model				Effect of DR			4 day switch at rich diet			4 day switch at DR		
coefficient	estimate	exp	s.e.	p	estimate	exp	p	estimate	exp	p	estimate	exp	p
DR	-1.378	0.252	0.129	**<0.001**									
4 day switch	0.305	1.357	0.132	**0.02**									
4 day switch * DR	-0.078	0.925	0.164	0.63									
105	-2.649	0.071	0.109	**<0.001**									
136	-1.907	0.148	0.111	**<0.001**									
217	-0.064	0.938	0.115	0.58									
239	0.907	2.476	0.113	**<0.001**									
335	0.227	1.255	0.114	**0.046**									
362	-1.024	0.359	0.113	**<0.001**									
441	-2.046	0.129	0.111	**<0.001**									
705	-1.846	0.158	0.112	**<0.001**									
707	1.051	2.859	0.113	**<0.001**									
853	-1.733	0.177	0.111	**<0.001**									
105 * DR	1.171	3.224	0.180	**<0.001**	-0.207	0.813	0.248						
136 * DR	1.804	6.071	0.178	**<0.001**	0.426	1.531	**0.017**						
217 * DR	0.822	2.275	0.178	**<0.001**	-0.556	0.574	**0.002**						
239 * DR	2.046	7.740	0.179	**<0.001**	0.668	1.951	**<0.001**						
335 * DR	2.104	8.197	0.178	**<0.001**	0.726	2.066	**<0.001**						
362 * DR	0.307	1.359	0.179	0.087	-1.071	0.343	**<0.001**						
441 * DR	0.994	2.701	0.179	**<0.001**	-0.384	0.681	**0.031**						
705 * DR	0.527	1.695	0.178	**0.003**	-0.851	0.427	**<0.001**						
707 * DR	0.797	2.218	0.179	**<0.001**	-0.581	0.559	**0.001**						
853 * DR	2.090	8.086	0.178	**<0.001**	0.712	2.038	**<0.001**						
105 * 4 day switch	0.530	1.699	0.184	**0.004**				0.835	2.305	**<0.001**			
136 * 4 day switch	0.648	1.912	0.182	**<0.001**				0.953	2.595	**<0.001**			
217 * 4 day switch	0.372	1.451	0.183	**0.042**				0.677	1.969	**<0.001**			
239 * 4 day switch	1.019	2.771	0.182	**<0.001**				1.324	3.759	**<0.001**			
335 * 4 day switch	0.451	1.569	0.186	**0.015**				0.756	2.129	**<0.001**			
362 * 4 day switch	-0.285	0.752	0.182	0.12				0.020	1.020	0.914			
441 * 4 day switch	-0.158	0.854	0.184	0.39				0.148	1.159	0.421			
705 * 4 day switch	-0.185	0.831	0.182	0.31				0.120	1.127	0.511			
707 * 4 day switch	0.167	1.182	0.183	0.36				0.472	1.603	**0.01**			
853 * 4 day switch	-0.140	0.869	0.185	0.45				0.165	1.179	0.372			
105 * 4 day switch * DR	-0.580	0.560	0.223	**0.009**							0.177	1.194	0.427
136 * 4 day switch * DR	-1.735	0.176	0.226	**<0.001**							-0.859	0.423	**<0.001**
217 * 4 day switch * DR	-0.272	0.762	0.223	0.22							0.327	1.387	0.142
239 * 4 day switch * DR	-1.846	0.158	0.225	**<0.001**							-0.600	0.549	**0.008**
335 * 4 day switch * DR	-0.400	0.670	0.219	0.068							0.277	1.319	0.207
362 * 4 day switch * DR	-0.838	0.433	0.238	**<0.001**							-0.896	0.408	**<0.001**
441 * 4 day switch * DR	0.179	1.196	0.219	0.41							0.249	1.282	0.256
705 * 4 day switch * DR	-0.050	0.951	0.222	0.82							-0.009	0.992	0.969
707 * 4 day switch * DR	-0.910	0.403	0.232	**<0.001**							-0.516	0.597	**0.026**
853 * 4 day switch * DR	-0.477	0.621	0.218	**0.028**							-0.390	0.677	0.073

Table S11. Interval models run within each genotype testing for differential effects of diet in the four day switch dietary regime

	Estimates from individual models			
coefficient	estimate	exp	s.e.	p
105 DR	-0.162	0.129	0.850	0.21
136 DR	0.576	0.134	1.780	**<0.001**
195 DR	-1.464	0.197	0.231	**<0.001**
217 DR	-0.695	0.132	0.499	**<0.001**
239 DR	0.697	0.075	2.008	**<0.001**
335 DR	0.516	0.128	1.675	**<0.001**
362 DR	-0.954	0.105	0.385	**<0.001**
441 DR	-0.466	0.140	0.628	**0.001**
705 DR	-0.741	0.078	0.476	**<0.001**
707 DR	-0.483	0.053	0.617	**<0.001**
853 DR	0.738	0.106	2.093	**<0.001**
105 four day switch	0.789	0.132	2.201	**<0.001**
136 four day switch	1.090	0.135	2.974	**<0.001**
195 four day switch	0.265	0.195	1.303	0.18
217 four day switch	0.756	0.134	2.131	**<0.001**
239 four day switch	1.367	0.078	3.925	**<0.001**
335 four day switch	0.351	0.137	1.421	**0.01**
362 four day switch	0.065	0.106	1.068	0.54
441 four day switch	0.139	0.143	1.149	0.33
705 four day switch	0.171	0.081	1.186	**0.036**
707 four day switch	0.543	0.054	1.720	**<0.001**
853 four day switch	0.176	0.113	1.192	0.12
105 four day switch * DR	-0.686	0.155	0.503	**<0.001**
136 four day switch * DR	-1.965	0.165	0.140	**<0.001**
195 four day switch * DR	0.018	0.221	1.018	0.94
217 four day switch * DR	-0.166	0.158	0.847	0.29
239 four day switch * DR	-1.956	0.119	0.141	**<0.001**
335 four day switch * DR	-0.292	0.150	0.746	0.052
362 four day switch * DR	-1.023	0.160	0.360	**<0.001**
441 four day switch * DR	0.199	0.159	1.220	0.21
705 four day switch * DR	-0.242	0.116	0.785	**0.037**
707 four day switch * DR	-1.113	0.120	0.329	**<0.001**
853 four day switch * DR	-0.579	0.129	0.560	**<0.001**

Table S12. Models run within each genotype testing for increases in response to a rich diet after a period of DR ('long switch') but within lines that showed starvation only, and with DR as reference category

	Estimates from individual models			
coefficient	estimate	exp	s.e.	p
136 rich diet	-0.647	0.524	0.120	**<0.001**
239 rich diet	-0.775	0.461	0.071	**<0.001**
335 rich diet	-0.570	0.566	0.115	**<0.001**
853 rich diet	-0.828	0.437	0.093	**<0.001**
136 long switch	0.113	1.120	0.132	0.39
239 long switch	1.483	4.406	0.103	**<0.001**
335 long switch	0.881	2.413	0.136	**<0.001**
853 long switch	1.373	3.949	0.122	**<0.001**

Table S13. Interval models run within each genotype testing for differential effects of diet in the four day switch dietary regime, but within lines that showed starvation only, and with DR as reference category.

	Estimates from individual models			
coefficient	estimate	exp	s.e.	p
136 rich diet	-0.576	0.562	0.134	**<0.001**
239 rich diet	-0.697	0.498	0.075	**<0.001**
335 rich diet	-0.516	0.597	0.128	**<0.001**
853 rich diet	-0.738	0.478	0.106	**<0.001**
136 four day switch	-0.875	0.417	0.169	**<0.001**
239 four day switch	-0.589	0.555	0.111	**<0.001**
335 four day switch	0.059	1.061	0.148	0.69
853 four day switch	-0.403	0.668	0.124	**0.001**
136 four day switch * rich diet	1.965	7.135	0.165	**<0.001**
239 four day switch * rich diet	1.956	7.071	0.119	**<0.001**
335 four day switch * rich diet	0.292	1.340	0.150	0.052
853 four day switch * rich diet	0.579	1.785	0.129	**<0.001**

Table S14. Linear model of estimates of (log-transformed) fecundity (from Quantifly) in the long-switch dietary treatment. A return to rich conditions from DR, resulted in reduced fecundity, rather than the predicted increase.

	Full model		
coefficient	estimate	s.e.	p
rich diet	2.281	0.038	**<0.001**
long switch	-0.367	0.037	**<0.001**
441	0.149	0.036	**<0.001**
853	0.010	0.035	0.768
age 45	0.040	0.041	0.339
age 46	0.028	0.042	0.512
age 47	-0.018	0.054	0.736
441 * long switch	-0.195	0.050	**<0.001**
853 * long switch	-0.043	0.050	0.4

Table S15. Linear model of estimates of (log-transformed) fecundity (from Quantifly), corrected for number of flies in the cage, in the long-switch dietary treatment. A return to rich conditions from DR, resulted in reduced fecundity, rather than the predicted increase. Note, this correction uses the census after egg laying and thus overcorrects for mortality, and hence estimates compared are biased upwards, and provide the most senstive test for an upregulation in in response to this dietary treatment.

	Full model		
coefficient	estimate	s.e.	p
Intercept	0.374	0.048	**<0.001**
long switch	-0.515	0.046	**<0.001**
441	0.079	0.046	0.092
853	0.041	0.044	0.355
age 45	0.049	0.052	0.349
age 46	0.060	0.053	0.262
age 47	0.132	0.067	0.058
441 * long switch	-0.158	0.063	**0.017**
853 * long switch	0.471	0.063	**<0.001**

Table S16. Mixed model (correcting for Cage) of estimates of (log-transformed) fecundity (from Quantifly) in the four day switching paradigm. Repeated short-term exposure to DR did not increase, but rather decreased fecundity.

	Full model		
coefficient	estimate	s.e.	p
Intercept	2.924	0.057	**<0.001**
DR	-0.642	0.030	**<0.001**
four day	-0.131	0.047	**0.005**
136	0.028	0.032	0.39
195	0.092	0.033	**0.006**
217	0.018	0.032	0.569
239	-0.162	0.032	**<0.001**
335	-0.302	0.032	**<0.001**
362	-0.092	0.033	**0.005**
705	-0.570	0.032	**<0.001**
707	-0.021	0.032	0.513
853	-0.248	0.032	**<0.001**
Age 9	-0.046	0.059	0.444
Age 10	-0.073	0.054	0.171
Age 11	-0.040	0.055	0.474
Age 12	0.011	0.053	0.841
Age 13	-0.086	0.054	0.11
Age 14	-0.104	0.053	**0.049**
Age 15	-0.115	0.054	**0.032**
Age 16	-0.139	0.053	**0.009**
Age 17	-0.070	0.054	0.196
Age 18	-0.049	0.055	0.375
Age 19	-0.202	0.056	**<0.001**
Age 20	-0.212	0.056	**<0.001**
Age 21	-0.367	0.072	**<0.001**
four day * DR	0.287	0.065	**<0.001**
136 * DR	-0.036	0.043	0.401
195 * DR	-0.114	0.044	**0.01**
217 * DR	-0.109	0.043	**0.011**
239 * DR	0.017	0.043	0.691
335 * DR	0.164	0.043	**<0.001**
362 * DR	0.072	0.043	0.09
705 * DR	0.422	0.043	**<0.001**
707 * DR	0.032	0.043	0.447
853 * DR	0.211	0.043	**<0.001**
136 * four day	-0.014	0.066	0.829
195 * four day	0.071	0.067	0.285
217 * four day	0.053	0.066	0.424
239 * four day	-0.029	0.066	0.656
335 * four day	0.015	0.066	0.818
362 * four day	-0.060	0.066	0.362
705 * four day	0.219	0.066	**0.001**
707 * four day	0.002	0.066	0.976
853 * four day	0.017	0.066	0.795
136 * four day * DR	0.215	0.092	**0.02**
195 * four day * DR	0.162	0.093	0.081
217 * four day * DR	0.166	0.092	0.071
239 * four day * DR	-0.048	0.092	0.601
335 * four day * DR	-0.023	0.092	0.805
362 * four day * DR	0.218	0.092	**0.018**
705 * four day * DR	-0.422	0.092	**<0.001**
707 * four day * DR	0.149	0.092	0.106
853 * four day * DR	0.112	0.092	0.225

Table S17. Mixed model (correcting for Cage) of estimates of (log-transformed) fecundity (from Quantifly), corrected for number of flies in the cage, in the four day switching paradigm. Repeated short-term exposure to DR did not increase, but rather decreased fecundity. Note, this correction uses the census after egg laying and thus overcorrects for mortality, and hence estimates compared are biased upwards, and provide the most senstive test for an upregulation in in response to this dietary treatment.

	Full model		
coefficient	estimate	s.e.	p
Intercept	0.812	0.056	**<0.001**
DR	-0.640	0.030	**<0.001**
four day	-0.126	0.046	**0.006**
136	0.051	0.032	0.111
195	0.094	0.033	**0.004**
217	0.029	0.031	0.363
239	-0.124	0.031	**<0.001**
335	-0.180	0.032	**<0.001**
362	-0.057	0.032	0.08
705	-0.556	0.031	**<0.001**
707	0.048	0.032	0.133
853	-0.225	0.032	**<0.001**
Age 9	-0.053	0.059	0.37
Age 10	-0.089	0.053	0.094
Age 11	-0.059	0.055	0.283
Age 12	0.008	0.053	0.883
Age 13	-0.083	0.053	0.12
Age 14	-0.099	0.052	0.06
Age 15	-0.107	0.053	**0.045**
Age 16	-0.120	0.053	**0.022**
Age 17	-0.043	0.054	0.419
Age 18	-0.027	0.055	0.626
Age 19	-0.161	0.055	**0.003**
Age 20	-0.167	0.055	**0.003**
Age 21	-0.151	0.071	**0.034**
four day * DR	0.297	0.064	**<0.001**
136 * DR	-0.027	0.042	0.517
195 * DR	-0.119	0.044	**0.007**
217 * DR	-0.114	0.042	**0.007**
239 * DR	0.074	0.042	0.081
335 * DR	0.170	0.042	**<0.001**
362 * DR	0.064	0.042	0.129
705 * DR	0.418	0.042	**<0.001**
707 * DR	0.000	0.042	0.992
853 * DR	0.213	0.042	**<0.001**
136 * four day	0.003	0.065	0.969
195 * four day	0.078	0.066	0.236
217 * four day	0.050	0.065	0.446
239 * four day	0.076	0.065	0.243
335 * four day	0.019	0.065	0.767
362 * four day	-0.062	0.065	0.342
705 * four day	0.217	0.065	**0.001**
707 * four day	-0.002	0.065	0.97
853 * four day	0.013	0.065	0.841
136 * four day * DR	0.178	0.091	0.051
195 * four day * DR	0.156	0.092	0.089
217 * four day * DR	0.164	0.091	0.072
239 * four day * DR	-0.060	0.091	0.511
335 * four day * DR	-0.030	0.091	0.738
362 * four day * DR	0.215	0.091	**0.018**
705 * four day * DR	-0.431	0.091	**<0.001**
707 * four day * DR	0.207	0.091	**0.023**
853 * four day * DR	0.098	0.091	0.283

Table S18. Effect of returning to a rich diet after a period of DR ('long switch') after ablation of the microbiome (Antibiotics on rich diet is reference)

	Full Model			
coefficient	estimate	exp (estimate)	s.e.	p
DR on Antibiotics	-0.894	0.409	0.181	**<0.001**
long switch on Antibiotics	1.313	3.717	0.142	**<0.001**
DR	-1.201	0.301	0.223	**<0.001**
Rich diet	-0.087	0.917	0.159	0.59

Table S19. Effect of returning to a rich diet after a period of DR ('long switch') with supplementation of water

	Full Model			
coefficient	estimate	exp (estimate)	s.e.	p
DR with water supplementation	0.648	1.911	0.138	**<0.001**
long switch with water supplementation	0.385	1.470	0.126	**0.002**

Table S20. Effect of returning to a rich diet after a period of DR ('long switch') with flies in isolation in vials. Test from chi-square tests on proportions by age.

	Dead				Mortality rate				P value from proportion test	
Age	DR	Rich to DR	Rich	DR to Rich (long switch)	DR	Rich to DR	Rich	DR to Rich (long switch)	Rich versus DR	Rich versus long switch
28	0	0	5	16	0.000	0	0.100	0.320	0.063	**0.014**
30	0	0	8	5	0.000	0	0.178	0.152	**0.006**	1
32	1	0	16	13	0.020	0	0.432	0.464	**<0.001**	0.997
34	1	0	8	4	0.020	0	0.381	0.267	**<0.001**	0.72
36	1	0	8	2	0.020	0	0.667	0.200	**<0.001**	0.079
38	4	0	2	2	0.087	0	0.500	0.250	0.102	0.829
40	3	0	0	3	0.071	0	0.000	0.500	1	1
42	2	0	0	3	0.051	0	0.000	1.000	1	0.505

Table S21. Effect of switching from DR to rich food every four days ('4 day switch') in males

	Full Model			
coefficient	estimate	exp (estimate)	s.e.	p
DR	0.362	1.437	0.117	**0.002**
4 day switch	0.410	1.507	0.120	**0.001**
4 day switch * DR	-0.768	0.464	0.129	**<0.001**