1 **Letter**

2 Experimentally reduced insulin/IGF-1 signalling in adulthood extends 3 lifespan of parents and improves Darwinian fitness of their offspring

4

5 Running title: Lifespan extension improves offspring fitness

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Abstract

Classical theory maintains that ageing evolves via energy trade-offs between reproduction and survival leading to accumulation of unrepaired cellular damage with age. In contrast, the emerging new theory postulates that ageing evolves because of deleterious late-life hyper-function of reproduction-promoting genes leading to excessive biosynthesis in late-life. The hyper-function theory uniquely predicts that optimizing nutrient-sensing molecular signalling in adulthood can simultaneously postpone ageing and increase Darwinian fitness. Here we show that reducing evolutionarily conserved insulin/IGF-1 nutrient-sensing signalling via *daf-2* RNA interference (RNAi) fulfils this prediction in *Caenorhabditis elegans* nematodes. Long-lived *daf-2* RNAi parents showed normal fecundity as self-fertilizing hermaphrodites and improved late-life reproduction when mated to males. Remarkably, the offspring of *daf-2* RNAi parents had higher Darwinian fitness across three different genotypes. Thus, reduced nutrient-sensing signalling in adulthood improves both parental longevity and offspring fitness supporting the emerging view that sub-optimal gene expression in late-life lies at the heart of ageing.

Impact Statement

Understanding mechanisms underpinning ageing is fundamental to improving quality of life in an increasingly long-lived society. Recent breakthroughs have challenged the long-standing paradigm that the energy trade-off between reproduction and somatic maintenance causes organismal senescence via slow accumulation of unrepaired cellular damage with age. The emerging new theory of ageing provides a conceptually novel framework by proposing that ageing is a direct consequence of physiological processes optimized for early-life function, such as growth and early-life reproduction, that are running 'too high' (i.e. at hyperfunction) in late adulthood. Contrary to the classic view based on damage accumulation, the hyperfunction theory proposes that suboptimal gene expression in late-life causes ageing via excessive biosynthesis. Thus, the hyperfunction theory uniquely predicts that longevity and Darwinian fitness can be simultaneously increased by reducing unnecessarily high levels of nutrient-sensing signalling in adulthood. Here we show that reducing evolutionarily conserved nutrient-sensing signalling pathway fulfils this prediction in *Caenorhabditis elegans* nematodes. We found that downregulation of the insulin/IGF-1 signalling in adult *C. elegans* nematodes not only improves longevity but, most intriguingly, increases fitness of the resulting offspring in the next generation. We found support for increase in offspring fitness across different genetic backgrounds. Our findings contradict the theoretical conjecture that energy trade-offs between growth, reproduction and longevity is the universal cause of senescence and provide strong experimental support for the emerging hyperfunction theory of ageing.

Introduction

Understanding mechanisms underpinning healthy ageing is fundamental to improving quality of life in an increasingly long-lived society. The long-standing paradigm postulates that energy trade-offs between reproduction and somatic maintenance underlie organismal ageing (Kirkwood 1977, Kirkwood and Austad 2000, Kirkwood 2017). This theory is supported by a large number of studies in different taxa that reported a negative correlation between reproduction and survival (reviewed in Hughes and Reynolds 2005, Partridge et al. 2005, Edward and Chapman 2011, Flatt 2011, Nussey et al. 2013). However, the discoveries of environmental interventions that dramatically increase healthy lifespan in model organisms without the cost of reduced reproduction have challenged the current paradigm and suggested that our understanding of the evolution of ageing is incomplete (Dillin et al. 2002, Kenyon 2005, Antebi 2013, Gems and Partridge 2013, Maklakov and Immler 2016, Flatt and Partridge 2018). Specifically, experimental downregulation of nutrient-sensing insulin/IGF-like (IIS) signalling pathway that governs biosynthesis in response to nutrient availability can achieve increased longevity without a concomitant decrease in reproduction in model organisms (Dillin et al. 2002, Kenyon 2010, Kenyon 2011).

Since cost-free lifespan extension contradicts the traditional view of how ageing evolves, several studies investigated the fitness consequences of reduced IIS signalling (Gems et al. 1998, Walker et al. 2000, Jenkins et al. 2004, Savory et al. 2014, Maklakov et al. 2017). Indeed, mutations that reduce nutrient-sensing signalling during the whole life, as well as environmental interventions aimed at mimicking the mutational effect, often have detrimental pleiotropic effects on key life-history traits, such as development, growth rate, body size and early-life reproduction resulting in reduced Darwinian fitness even if total reproduction is

unaffected (Gems et al. 1998, Briga and Verhulst 2015). The first longevity mutant discovered in *C. elegans*, *age-1*, is a good example because increased longevity, stress resistance and late-life reproduction come at a cost of reduced early-life reproduction and total individual fitness (Maklakov et al. 2017). Moreover, a recent literature survey suggests that all classic longevity-extending mutations across taxa from worms to flies to mice detrimentally affect life-history traits resulting in reduced fitness (Briga and Verhulst 2015). Similarly, experimental evolution studies showed that when longevity and fecundity are increased simultaneously through selection, the organisms pay the price in slow development and delayed sexual maturation, again resulting in reduced individual fitness (Lind et al. 2017). These results support the theoretical conjecture that genes with antagonistically pleiotropic effects between early-life and late-life fitness play an important role in the evolution of ageing (Williams 1957). However, the mechanisms of antagonistic pleiotropy (AP) remain elusive. The leading hypothesis, the "disposable soma" theory of ageing (DS) suggests that ageing results from competitive energy allocation between somatic maintenance and reproduction (Kirkwood 1977, Kirkwood and Holliday 1979, Kirkwood and Austad 2000). Indeed, increased reproductive performance in early-life correlates with reduced survival and/or reduced performance in late-life in natural populations (Gustafsson and Part 1990, Boonekamp et al. 2014, Lemaitre et al. 2015) and in laboratory experiments (Rose 1984, Charlesworth 1993, Partridge et al. 1999, but see Chen and Maklakov 2012, Kimber and Chippindale 2013, Chen and Maklakov 2014, Curtsinger 2019).

However, this hypothesis suffered several setbacks in recent years, with many empirical studies challenging the importance of energy trade-offs in organismal senescence (reviewed in Flatt 2011, Kenyon 2011, Antebi 2013, Gems and Partridge 2013, Maklakov and Immler 2016, Flatt and Partridge 2018). Instead, several authors proposed that ageing can result from

molecular signalling networks being optimized for development, growth and early-life reproduction rather than for late-life reproduction and longevity (Blagosklonny 2010, Kenyon 2010, Antebi 2013, Gems and Partridge 2013, Ezcurra et al. 2018). For example, the hyperfunction theory maintains that ageing is driven by excessive nutrient-sensing molecular signalling in adulthood, which results in cellular hypertrophy leading to age-related pathologies (Blagosklonny 2006, 2010, Ezcurra et al. 2018). These ideas can be traced back to the original AP theory by George Williams, who suggested that the same physiological processes that are beneficial for fitness early in life can become detrimental for organismal fitness with age because of the reduced strength of natural selection on late-life function (Williams 1957).

Williams's AP theory (Williams 1957) provides the population genetic framework for the evolution of ageing via two different physiological routes: energy trade-offs (the "disposable soma" theory of ageing) or functional trade-offs (e.g. "hyperfunction" theory of ageing) (Fig. 1). While both of these physiological theories rely on the same underlying principle, they make uniquely distinct predictions with respect to reproduction costs of longevity. The "disposable soma" theory maintains that organismal senescence is caused by slow accumulation of unrepaired cellular damage with age because insufficient energy resources are allocated to repair as organisms are maximising fitness rather than longevity (Kirkwood 1977, Kirkwood 2017). Therefore, the "disposable soma" theory predicts that increased allocation of resources to somatic maintenance will increase longevity at the cost of reduced resources available to current growth and reproduction. On the contrary, the functional trade-off theory suggests that longevity is compromised by suboptimal physiology in adulthood because, as discussed first by Williams (1957), selection is not strong enough to fully optimize the age-specific expression of an allele whose effects are strongly beneficial in

136 early-life (e.g. during development) and slightly detrimental in late-life (e.g. during
137 adulthood). Consequently, the functional trade-off theory predicts that experimental
138 optimization of physiology in adulthood can increase longevity without any cost to
139 reproduction, or even simultaneously increase longevity and reproduction.

140

141 Because the main cost of longevity appears to be associated with reduced early-life function,
142 it seems plausible that age-specific modification of gene expression can potentially
143 circumvent this problem. In their landmark study, Dillin et al. (2002) used age-specific RNA
144 interference (RNAi) approach to knock down *daf-2* gene expression in *C. elegans* nematodes
145 across the life cycle of the worms. While early-life feeding with bacteria expressing *daf-2*
146 double-stranded (ds) RNA resulted in reduced early-life reproduction, there was no
147 detrimental effect of *daf-2* RNAi in adult worms, which enjoyed two-fold lifespan extension
148 without any cost to reproduction (Dillin et al. 2002). This study provides the strongest
149 support to date for the hypothesis that ageing results from molecular nutrient-sensing
150 signalling that is optimized for early-life function but is suboptimal for late-life function.

151

152 Nevertheless, while this study provided a powerful example for the cost-free lifespan
153 extension, it is possible that certain fitness costs have been overlooked. One possibility is that
154 fecundity costs become apparent only in mated hermaphrodites. In nature, *C. elegans* live in
155 populations with small (~0.3%) yet appreciable number of males living among self-fertilising
156 hermaphrodites with sometimes high levels of outcrossing (Sivasundar and Hey 2005), and
157 mating, as well as mere presence of male-derived pheromones, has pronounced effects of the
158 life-history of hermaphrodites (Maures et al. 2013, Shi and Murphy 2014, Aprison and
159 Ruvinsky 2016). While it is certainly interesting to consider how male-derived effects affect

resource allocation in hermaphrodites, it is unlikely that putative trade-off that is only visible to selection in such rare circumstances can shape the evolution of ageing in this species. Perhaps more importantly, it is possible that the fitness of the offspring and, therefore, Darwinian fitness of the parents are compromised. The trade-off between offspring number and quality is well known from a number of study systems (Stearns 1992), and is a potential explanation for the apparent lack of fitness costs in the previous studies (Maklakov and Immler 2016). Alternatively, longevity and Darwinian fitness can be simultaneously increased by reducing unnecessarily high levels of nutrient-sensing signalling in adulthood. To distinguish between these possibilities, we need to understand how reduction in nutrient-sensing signalling in adulthood affects longevity, offspring number and offspring quality. Here we show that *daf-2* RNAi in adult *C. elegans* results in increased offspring fitness across three genetic backgrounds. We discuss these findings in the light of the emerging new theories of ageing and suggest that they support the hypothesis that functional trade-offs between early-life fitness and late-life fitness shape the evolution of ageing.

Materials and Methods

Strains

We used the *Caenorhabditis elegans* strains Bristol N2 wild-type (Brenner, Genetics 1974), as well as the mutants *ppw-1(pk2505)* and *rrf-1(pk1417)*, obtained from Caenorhabditis Genetics Center (CGC, Missouri, USA).

Maintenance

Before each assay, worms were recovered from freezing and synchronised by bleaching for

184 two generations to remove any freezing effects. The nematode populations were maintained
185 at 20°C and 60% relative humidity in an environmental test chamber. For regular
186 maintenance, the worms were kept on NGM agar supplemented with the antibiotics
187 streptomycin, kanamycin and nystatin (following Lionaki and Tavernarakis 2013), seeded
188 with the antibiotic-resistant *E. coli* strain OP50-1 (pUC4K).

189

190 ***Outline of the study***

191 The study was run in three separate experiments. In the first experiment, we investigated
192 lifespan and reproduction of mated and unmated N2 hermaphrodites reared from sexual
193 maturity onwards on *daf-2* RNAi or empty vector (EV, control) plates. For logistic reasons,
194 this experiment was conducted in two blocks for mated worms and one block for unmated
195 worms. In the second experiment, we investigated the lifespan and egg size of unmated N2,
196 *rrf-1(pk1417)* and *ppw-1(pk2505)* hermaphrodites on raised from sexual maturity onwards on
197 *daf-2* RNAi or EV plates. In the third experiment, we collected one egg from each parent at
198 their second day of adulthood (from *daf-2* RNAi and EV treatments) and investigated the
199 lifespan and reproduction of these offspring on control plates. Because different experiments
200 differed in setup time, daily reproduction values (and calculations based upon these, such as
201 λ_{ind}) are only meaningful for comparison between treatments within each experiment.

202

203 ***RNAi***

204 RNase-III deficient, IPTG-inducible HT115 *Escherichia coli* bacteria with empty plasmid
205 vector (L4440) was used as control (Timmons et al. 2001) and the same HT115 bacteria with
206 *daf-2* RNAi construct from the Vidal library was used as RNAi treatment. RNAi treatment
207 started from sexual maturity, and continued until the death of the individual. During the
208 experiments, worms were maintained on 35 mm NGM agar plates (supplemented with 1 mM

209 IPTG and 50 µg/ml ampicillin) seeded with 0.1 ml L4440 empty vector control or *daf-2*
210 bacteria grown in LB supplemented with 50 µg/ml ampicillin for 16-20 hours and seeded
211 (incubated) on the NGM agar plates again for 24 hours (following. Hinas et al. 2012).

212

213 ***Lifespan Assays***

214 Lifespan assays were set up for all treatment combinations described above. In the lifespan
215 assays, the individual age-synchronised L4 worms were placed on separate 35 mm plates and
216 the plates were checked daily to record any instances of death. The surviving worms were
217 moved to new plates daily until their death. Fertile worms, which showed odd developmental
218 characteristics and low offspring numbers (<36 offspring), were excluded from the final
219 analysis (3 mated control worms and 7 mated *daf-2* worms).

220

221 ***Reproduction assays***

222 Offspring production was scored in the reproduction assays using the same worms as those
223 scored for lifespan, except for the parental N2, *ppw-1* and *rrf-1* worms in the second
224 experiment, where only lifespan was recorded. Unmated individual hermaphrodites were
225 moved to new plates daily and scored for offspring produced 2.5 days later. In the "mated"
226 treatment, two male *C. elegans* (from the initial sample population of N2 strain) were placed
227 on a plate with a single hermaphrodite for two hours every day to allow time for mating.
228 Offspring production was scored 2.5 days later, as in the "unmated" treatment.

229

230 ***Egg size assays***

231 Egg size was measured in N2, *ppw-1* and *rrf-1* strains (unmated hermpahrodites) growing on
232 either *daf-2* RNAi or empty vector (EV) plates. Two days after maturation, worms were
233 placed individually on new plates and observed continually during five hours for the presence
234 of newly laid eggs, of which the first two eggs were collected. Eggs were picked immediately
235 after laying and placed under a Leica M165C microscope set on 12x magnification; photos
236 were taken using a Lumenera Infinity 2-6C digital microscope camera. Egg size was analysed
237 from photos using *ImageJ* (https://imagej.nih.gov/ij/). Only eggs laid during gastrulation
238 stage (the normal developmental stage at egg laying) were included in the analyses.

239

240 ***Statistical analyses***

241 Survival was analysed for each experiment in Cox proportional hazard models in *R 3.3.3*.
242 Mated (EV: n=72, *daf-2* n=68) and unmated (n=25 per treatment) individuals were analysed
243 separately, as they were run in different blocks. Unmated individuals were analysed using the
244 *coxph* function in the package *survival*, with *daf-2* RNAi treatment as a fixed factor. For
245 mated individuals, we used the *coxme* package in order to fit block as a random effect, in
246 addition to the fixed effect of RNAi treatment. In the second experiment (n=25 per
247 treatment), in addition to RNAi treatment, we also fitted the fixed factor strain (N2, *ppw-1*,
248 *rrf-1*) and its interaction with treatment using the *coxph* function in the *survival* package.

249 Reproduction was analysed as total reproduction as well as rate-sensitive individual fitness
250 λ_{ind}, which encompasses the timing and number of offspring (Brommer et al. 2002, Lind et al.
251 2016). λ_{ind} is estimated by solving the Euler-Lotka equation for each individual using the
252 *lambda* function in the *popbio* package and is analogous to the intrinsic rate of population
253 growth (Stearns 1992). For all unmated worms (n=25 per treatment), we estimated the fixed
254 effect of treatment (*daf-2* RNAi or empty vector). For offspring of the three mutants (n=25

255 per treatment), we also estimated the fixed effect or strain, using linear models. For the mated
256 worms (EV: n=72, *daf-2* n=68), we also estimated the random effect of block, in addition to
257 RNAi treatment. These models were implemented as mixed effect models using the *lme4*
258 package in *R 3.3.3*, and chi-square tests of fixed effects were performed using the *car*
259 package. Egg size was analysed in a mixed effect model in *lme4*, treating strain and RNAi
260 treatment as crossed fixed effects, and parent ID as well as block as random effects. We
261 obtained the following n: N2 on EV: 56, N2 on *daf-2*: 54, *ppw-1* on EV: 44, *ppw-1* on *daf-2*:
262 42, *rrf-1* on EV: 59, *rrf-1* on *daf-2*: 42.

263

264 **Results**

265 First off, we confirmed that *daf-2* RNAi significantly extended the lifespan of unmated N2
266 wild-type hermaphrodite worms (censoring matricide: $z = -4.94$, $df = 1$, $p < 0.001$, Fig. 2A;
267 including matricide as dead: $z = -4.97$, $df = 1$, $p < 0.001$), as expected from previous studies
268 (Dillin et al. 2002). In addition, for mated N2, *daf-2* RNAi extended lifespan when matricide
269 was censored ($z = -2.42$, $df = 1$, $p = 0.016$, Fig. 2B) but not if matricidal worms were
270 included as dead ($z = 0.16$, $df = 1$, $p = 0.87$) because of an increase in matricide in the late
271 reproducing mated *daf-2* RNAi N2.

272

273 We did not find any effect of *daf-2* RNAi on total reproduction (unmated: $F = 0.32$, $df = 1$, p
274 $= 0.58$; mated: $\chi^2 = 1.11$, $df = 1$, $p = 0.29$) or individual fitness λ_{ind} (unmated: $F = 0.30$, $df = 1$,
275 $p = 0.59$; mated: $\chi^2 = 0.43$, $df = 1$, $p = 0.51$) for neither unmated nor mated N2 (Table 1, Fig.
276 3). However, *daf-2* RNAi had a positive effect on late (day 5+) reproduction for mated
277 hermaphrodites ($\chi^2 = 24.76$, $df = 1$, $p < 0.001$, Fig. 3B).

278

279 In a second experiment, using unmated hermaphrodites only, we investigated the effect of
280 *daf-2* RNAi on parent lifespan and offspring lifespan and reproduction across three genetic
281 backgrounds (N2 wild-type and the mutants *ppw-1* and *rrf-1*, that are deficient for germline
282 and somatic RNAi, respectively). Parental treatment with *daf-2* RNAi increased lifespan
283 across all genetic backgrounds, both when matricide was censored (treatment: $\chi^2 = 90.39$, df
284 = 1, p <0.001; strain: $\chi^2 = 21.8$, df = 2, p <0.001; treatment × strain: $\chi^2 = 10.46$, df = 2, p =
285 0.005, Fig. 2A) and included as dead (treatment: $\chi^2 = 85.25$, df = 1, p <0.001; strain: $\chi^2 =$
286 20.45, df = 2, p <0.001; treatment × strain: $\chi^2 = 9.43$, df = 2, p = 0.009). In accordance with
287 previously published research (Hibshman et al. 2016), parental *daf-2* RNAi increased egg
288 size (treatment: $\chi^2 = 5.11$, df = 1, p =0.024; strain: $\chi^2 = 13.89$, df = 2, p <0.001; treatment ×
289 strain: $\chi^2 = 2.68$, df = 2, p = 0.262, Fig. 4). However, we found that the effect was most
290 pronounced in N2 wildtype worms, and relatively weak in both somatic and germline *daf-2*
291 knockdown (see Fig. 4), suggesting that *daf-2* knockdown in both somatic and reproductive
292 tissues is required to maximize the effect on egg size.

293

294 Parental *daf-2* RNAi treatment did not, however, influence the lifespan of their offspring,
295 neither when matricidal worms were censored (treatment: $\chi^2 = 0.04$, df = 1, p = 0.85; strain:
296 $\chi^2 = 24.2$, df = 2, p <0.001; treatment × strain: $\chi^2 = 0.61$, df = 2, p = 0.74, Fig. 5A) nor when
297 included as dead (treatment: $\chi^2 = 0.01$, df = 1, p = 0.92; strain: $\chi^2 = 21.8$, df = 2, p <0.001;
298 treatment × strain: $\chi^2 = 0.48$, df = 2, p = 0.79).

299

300 In contrast, parental *daf-2* RNAi treatment significantly increased offspring total reproduction
301 (treatment: F = 15.9, df = 1, p <0.001; strain: F = 33.7, df = 2, p <0.001; treatment × strain: F
302 = 0.09, df = 2, p = 0.91, Fig. 5B-C) and individual fitness λ_{ind} (treatment: F = 11.8, df = 1, p

303 <0.001; strain: F = 13.1, df = 2, p <0.001; treatment × strain: F = 0.18, df = 2, p = 0.84, Fig.
304 5D) across all genetic backgrounds. Importantly, there was no correspondence between the
305 effect of parental *daf-2* RNAi on egg size (see above) and offspring total reproduction /
306 individual fitness, suggesting that factors beyond the amount of resources in the egg
307 contribute to increased fitness of offspring of *daf-2* RNAi parents.

308

309 **Discussion**

310 The "disposable soma" theory of ageing postulates that senescence results from competitive
311 energy allocation between key life-history traits, such as growth, reproduction and somatic
312 maintenance (Kirkwood 1977, Kirkwood 2017). This theory predicts that genetic and
313 environmental manipulations that increase energy allocation to growth and somatic
314 maintenance will result in detrimental effects to reproduction. This is why the findings by
315 Dillin et al. (2002), which suggested that adult-only downregulation of insulin/IGF-1 by *daf-2*
316 RNAi can substantially increase lifespan without any detrimental effect to reproduction, were
317 subsequently scrutinized in an attempt to find the hidden costs of longevity (Jenkins et al.
318 2004, Partridge et al. 2005). Nonetheless, both the original findings (Dillin et al. 2002) and
319 our results here, suggest that adult-only *daf-2* RNAi can more than double longevity without
320 any negative effect on reproduction. Moreover, when supplied with sperm from males, *daf-2*
321 RNAi-treated parents have improved fecundity in late-life. However, the key question that we
322 asked in this study was whether treatments that improve parental performance have positive
323 or negative effects on their offspring. The trade-off between offspring number and offspring
324 quality is a well known concept in life-history evolution (Stearns 1992) but is rarely
325 considered in biogerontological research (reviewed in Maklakov and Immler 2016). Germline
326 maintenance is costly (Sniegowski et al. 2000, Agrawal and Wang 2008, Maklakov and

Immler 2016, Berger et al. 2017), and increased investment into somatic maintenance can, in theory, result in increased mutation rate and reduced fitness of progeny.

Alternatively, it is possible that instead of energy trade-offs, the evolution of senescence is governed by functional trade-offs. Functional trade-offs can occur because the physiological requirements of a young organism can differ substantially from those of a mature one (Williams 1957). In his classic 1957 paper, George Williams (Williams 1957) described a hypothetical example of a mutation that positively affects bone calcification in a developing young organism but increases calcification of the connective tissues of arteries in a mature one with detrimental consequences. More recently, it has been suggested that nutrient sensing IIS/TOR molecular signalling pathways that govern growth and development result in excessive biosynthesis in late-life leading to different pathologies and increased mortality (Blagosklonny 2006, 2010, Gems and Partridge 2013, Ezcurra et al. 2018). These proximate explanations rest on the fundamental assumption that the strength of natural selection declines with age because of environmental mortality from a range of biotic and abiotic hazards (e.g. predation, pathogens, competition, starvation) (Williams 1957). Because of such environmental mortality, optimizing development, growth and instantaneous reproduction may be more important for organismal fitness that optimizing future survival and reproduction (Williams 1957, Hamilton 1966). Thus, progressively weakening selection in adulthood may result in suboptimal levels of IIS/TOR signalling leading to pathology and senescence (Ezcurra et al. 2018). However, unlike the classic energy trade-off theory, the functional trade-off hypothesis predicts that it should be possible to modify adult physiology to improve both longevity and fitness.

351 Here we found that reduced insulin/IGF-1 signalling in adult worms not only improved
352 longevity, but also increased reproduction and Darwinian fitness of the resulting offspring in
353 three different genetic backgrounds. This result contradicts the hypothesis that improved
354 longevity and postponed ageing of *daf-2* RNAi parents comes at the cost of offspring fitness.
355 Instead, our findings are in line with the hypothesis that suboptimal levels of nutrient-sensing
356 signalling in adult life accelerate ageing, curtail lifespan and reduce individual fitness. This
357 result was not caused by direct inheritance of *daf-2* RNAi, since we did not recover the
358 lifespan extension effect of *daf-2* knockdown in these offspring. Because *daf-2* is essential for
359 successful development and growth of a young worm (Dillin et al. 2002), these results
360 suggest that wildtype *C. elegans* nematodes trade-off improved pre-adult performance for
361 reduced offspring quality. Such trade-offs are at heart of AP theory, but are usually
362 interpreted as evidence for energy-based trade-offs. Our results clearly demonstrate that this
363 is not the case, and that adulthood-only *daf-2* RNAi increases offspring fitness. In summary,
364 our findings suggest that selection on expression of *daf-2* in adulthood is not sufficiently
365 strong in nature. We predict that such effects may be very common, and suggest that future
366 studies should aim to quantify the fitness consequences of experimental manipulation of age-
367 specific gene expression across a broad range of taxa. Such approach will allow us to
368 estimate the relative importance of energy trade-offs versus functional trade-offs in the
369 evolution of ageing across the tree of life.

370

371 Because previous research found that both dietary restriction and reduction in insulin-like
372 signalling by *daf-2* RNAi knockdown increased embryo size in *C. elegans* nematodes
373 (Hibshman et al. 2016), we replicated these results to test whether increased fitness of adult
374 progeny results from increased resource allocation to eggs by *daf-2* RNAi mothers. While
375 *daf-2* knockdown increased egg size to a different degree in N2, *ppw-1* and *rrf-1* strains, there

was no correlation between the effect of parental *daf-2* RNAi on egg size and offspring reproductive performance. We provisionally conclude that increased egg size under reduced maternal insulin-like signalling can contribute to increased offspring fitness, but it is likely not the sole source of variation in this trait. Recent work has identified oocyte-specific IIS targets that are different from soma-specific IIS targets suggesting that IIS signalling regulates reproduction and longevity through different mechanisms (Templeman et al. 2018). In the future, it will be interesting to test for individual fitness of offspring produced via genetic manipulation of oocyte-specific targets of IIS signalling pathway. In recent years, there has been a vigorous debate regarding whether mechanistic understanding of life-history trade-offs is necessary to advance life-history theory (Flatt and Heyland 2011, Stearns 2011a, b). Here we used the mechanistic approach to separate between two conceptually different evolutionary theories of ageing – energy trade-offs and functional trade-offs – in an empirical study. We argue that unification between the conceptual approach and the mechanistic understanding may often prove fruitful in this regard.

Author contributions

MIL and AAM designed the study, with the aid of AH. SR, ZS, MIL and HC collected the data, MIL analysed the data, MIL and AAM drafted the manuscript. All authors contributed to the revision of the manuscript.

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Data accessibility

Upon acceptance, the data will be archived at Dryad.

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549 **Table 1. The effect of daf-2 RNAi on reproduction.** Total reproduction and individual

550 fitness (λ_{ind}) for unmated and mated *C. elegans* N2 wild-type treated with either empty vector

551 (Control) or *daf-2* RNAi from adulthood onwards. All values expressed as mean ± SE.

	Total reproduction		Fitness (λ_{ind})	
RNAi treatment	unmated	mated	unmated	mated
Control	311.0 ± 7.0	595.7 ± 24.3	4.66 ± 0.03	4.47 ± 0.05
daf-2 RNAi	317.4 ± 8.9	630.5 ± 22.2	4.63 ± 0.05	4.50 ± 0.05

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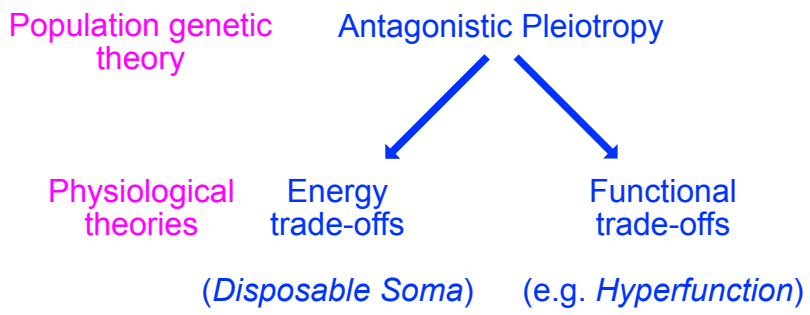


Fig. 1. Theoretical framework. The relationship between population genetic theory of ageing ("antagonistic pleiotropy", Williams 1957) and physiological theories of ageing based on either energy trade-offs ("disposable soma" (Kirkwood 1977) or functional trade-offs (e.g. "hyperfunction" (Blagosklonny 2006)). Note that Williams (1957) was the first to provide an abstract example of a functional trade-off between early-life and late-life allelic effects on organismal physiology. Recently, Blagosklonny (2006, 2010) put forward a "hyperfunction" hypothesis that specifically links suboptimal levels of nutrient-sensing signalling to excessive biosynthesis (hence, hyperfunction) leading to cellular and organismal senescence.

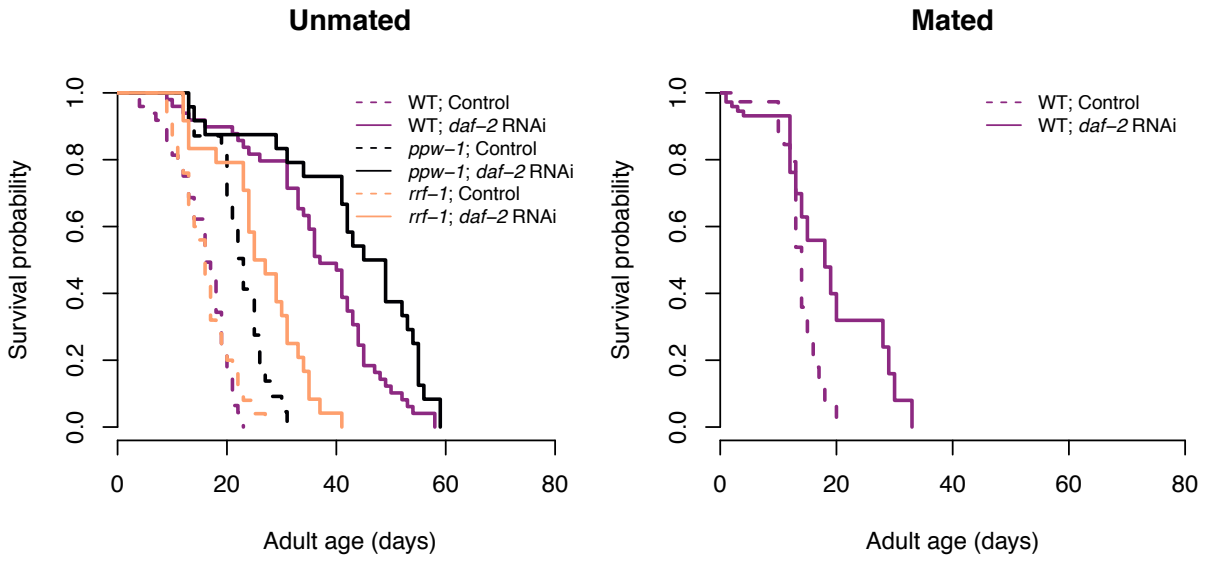


Fig. 2. The effect of *daf-2* RNAi on lifespan. Survival probability for (**A**) unmated or (**B**) mated N2 wild-type (purple), *ppw-1* (black) and *rrf-1* (orange) mutants, treated with either *daf-2* RNAi (solid lines, filled symbols) or control empty vector (broken lines, open symbols) from adulthood onwards.

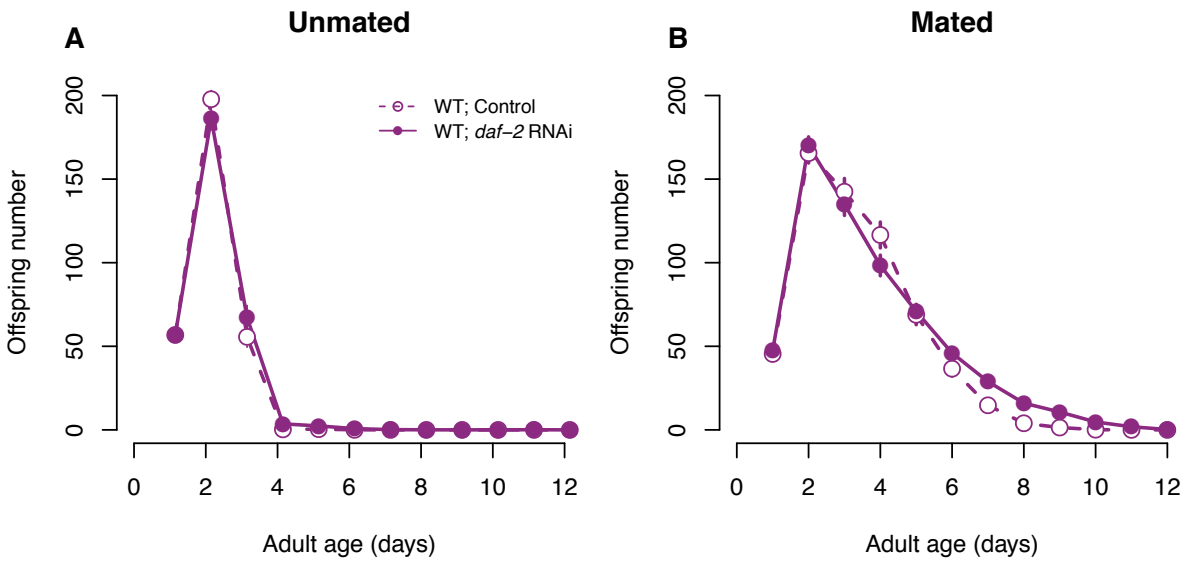


Fig. 3. The effect of *daf-2* RNAi on reproduction. Daily offspring number for (**A**) unmated or (**B**) mated N2 wild-type worms, treated with either *daf-2* RNAi (solid lines, filled symbols) or control empty vector (broken lines, open symbols) from adulthood onwards. Symbols represent mean ± SE.

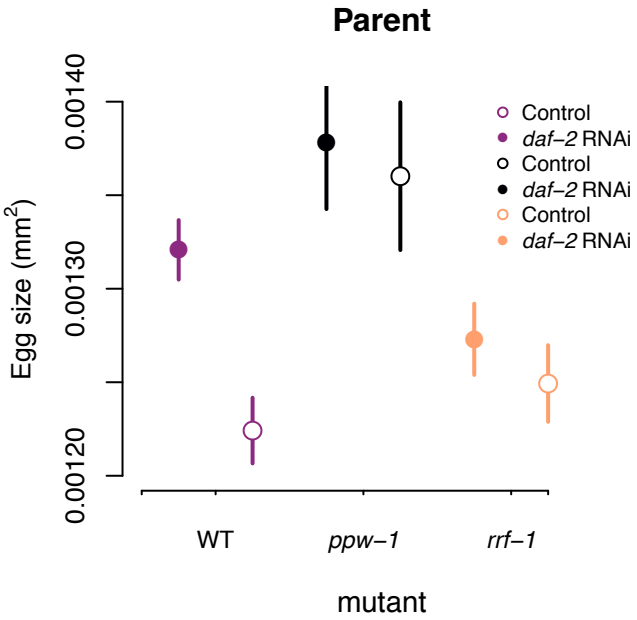
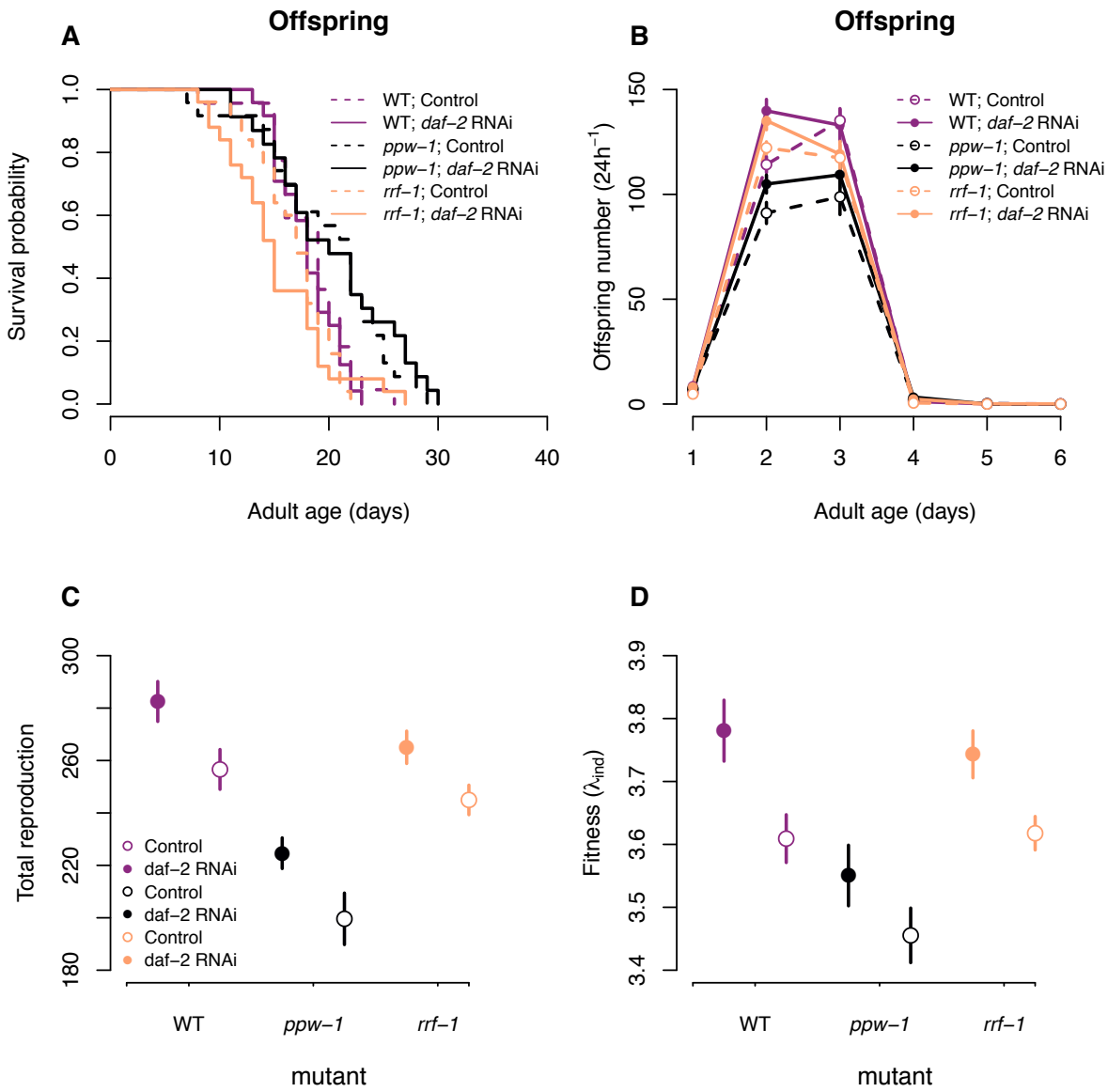


Fig. 4. The effect of *daf-2* RNAi on egg size. Egg size of unmated, parental worms. N2 wild-type (purple), *ppw-1* (black) and *rrf-1* (orange) mutants, treated with either *daf-2* RNAi (solid

577 lines, filled symbols) or control empty vector (broken lines, open symbols) from adulthood

578 onwards. Symbols represent mean ± SE.



579

580 **Fig. 5. The effect of parental *daf-2* RNAi on offspring survival and reproduction.**

581 Offspring worms, unmated, on control (empty vector) plates from parents exposed to *daf-2*

582 RNAi or control treatment. (**A**) Survival probability, (**B**) daily offspring number, (**C**) Total

583 reproduction and (**D**) individual fitness (λ_{ind}) of offspring (on control plates) from parents

584 either exposed to *daf-2* RNAi (solid lines, filled symbols) or control empty vector (broken

585 lines, open symbols). The colors reflect N2 wild-type (purple), *ppw-1* (black) and *rrf-1*

586 (orange) mutants. Symbols represent mean ± SE.