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Fitness cost of extended lifespan in *Caenorhabditis elegans*

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An insulin/IGF-I-like signalling pathway determines the rate of aging of the adult nematode, *Caenorhabditis elegans*. Mutations in genes encoding this pathway can result in a doubling of lifespan. While such mutations may appear to have little effect on development or fertility, evolutionary theory predicts that large increases in lifespan will not be optimal for fitness. We demonstrate by laboratory natural selection that partial loss of function of the insulin receptor-like protein DAF-2 results in dramatically reduced fitness even under laboratory conditions. Despite long-lived mutants appearing healthy, they exhibit a heavy fitness cost consistent with an evolutionary theory of aging.

Keywords: longevity; trade-off; *daf-2*; evolution

1. INTRODUCTION

Increasing evidence suggests conservation of the role of the insulin/insulin-like growth factor type-I (IGF-I) signalling pathway in lifespan regulation across taxa, including mice, *Drosophila* and *Caenorhabditis elegans* (Tatar *et al.* 2003). An IGF-I receptor mutation ($Igf1r^{+/-}$) in mice has been reported that increases lifespan and stress resistance while maintaining normal metabolism, fertility and reproduction (Holzenberger *et al.* 2002). Together with the apparent healthiness of nematodes with extreme lifespan extension (Arantes-Oliveira *et al.* 2003), this suggests that lifespan can be increased through manipulation of the insulin receptor (Bluher *et al.* 2003) or IGF receptor without detrimental effects.

The antagonistic pleiotropy (AP) theory of aging suggests that the wild-type variant of longevity genes has been favoured over evolutionary time owing to benefits on early life fitness traits (Williams 1957). The mutations that confer long life would be predicted to have a detrimental effect on some aspect of early fitness, with longevity and fitness coupled at the genetic level. A correlate is the disposable-soma theory in which a trade-off between reproductive and maintenance functions ensures that optimal fitness does not coincide with optimal longevity (Kirkwood & Holliday 1979). In terms of Darwinian fitness, this suggests that laboratory-derived long-lived variants would be disadvantaged. Contrary to these theories, we previously demonstrated by laboratory natural selection experiments that lifespan extension by mutation of the *age-1* gene in *C. elegans* was not associated with a fitness cost under normal laboratory growth conditions (Walker *et al.* 2000). Similarly, the longevity mutation in the *Drosophila Indy* gene is not associated with any cost when flies are well fed (Marden *et al.* 2003). However, mutant forms of both *age-1* and *Indy* do result in lowered fitness when the animals are subject to nutritional stress (Walker *et al.* 2000) and thus AP is apparent when animals are observed in laboratory conditions that more effectively mimic the natural environment. We investigate the fitness costs of insulin/IGF-1 signalling at the level of the insulin/IGF-1 receptor. We find that a mutation that confers longevity causes a severe fitness deficit even in a high-nutrition environment. We apply our results to another lifespan extension intervention, RNA interference (RNAi), and show that this longevity is also associated with a fitness cost.

2. MATERIAL AND METHODS

(a) *Nematode strains*

Worms were grown on nutrient growth media (NGM) agar plates, at 20 °C or 25 °C. The wild-type N2 strain and DR1572 [*daf-2(e1368)III*] were obtained from the *Caenorhabditis* Genetics Center in 2001.

(b) *Laboratory natural selection without starvation cycling*

Six replicate populations were established with 50 N2 and 50 *daf-2(e1368)* eggs laid within 30 min from age-synchronous parental populations transferred to 50 mm diameter NGM agar plates. Each plate contained 8 ml NGM and was spotted with 300 µl of *E. coli* (strain OP50, 1.5×10^9 cells ml^{-1}). All populations were maintained at 20 °C for 3 days. Transferring 100 eggs maintained subsequent generations. Under these conditions, populations were never starved.

(c) *Laboratory natural selection with starvation cycling*

Five replicate populations were initiated as above. Plates were incubated at 20 °C and were monitored for exhaustion of food, which occurred at day five for all populations and all cycles. Populations were maintained at 20 °C for a further 4 days, and then plugs (9 mm in diameter) were taken and transferred to a spotted plate. Two plugs were taken from opposite sides of each plate (together representing *ca.* 6.5% of the total agar volume of the starved plate). Plugs were transferred to 90 mm diameter plates containing 25 ml NGM and spotted with 700 µl of OP50 (as above). Approximately 24 h later 100 eggs were sampled from

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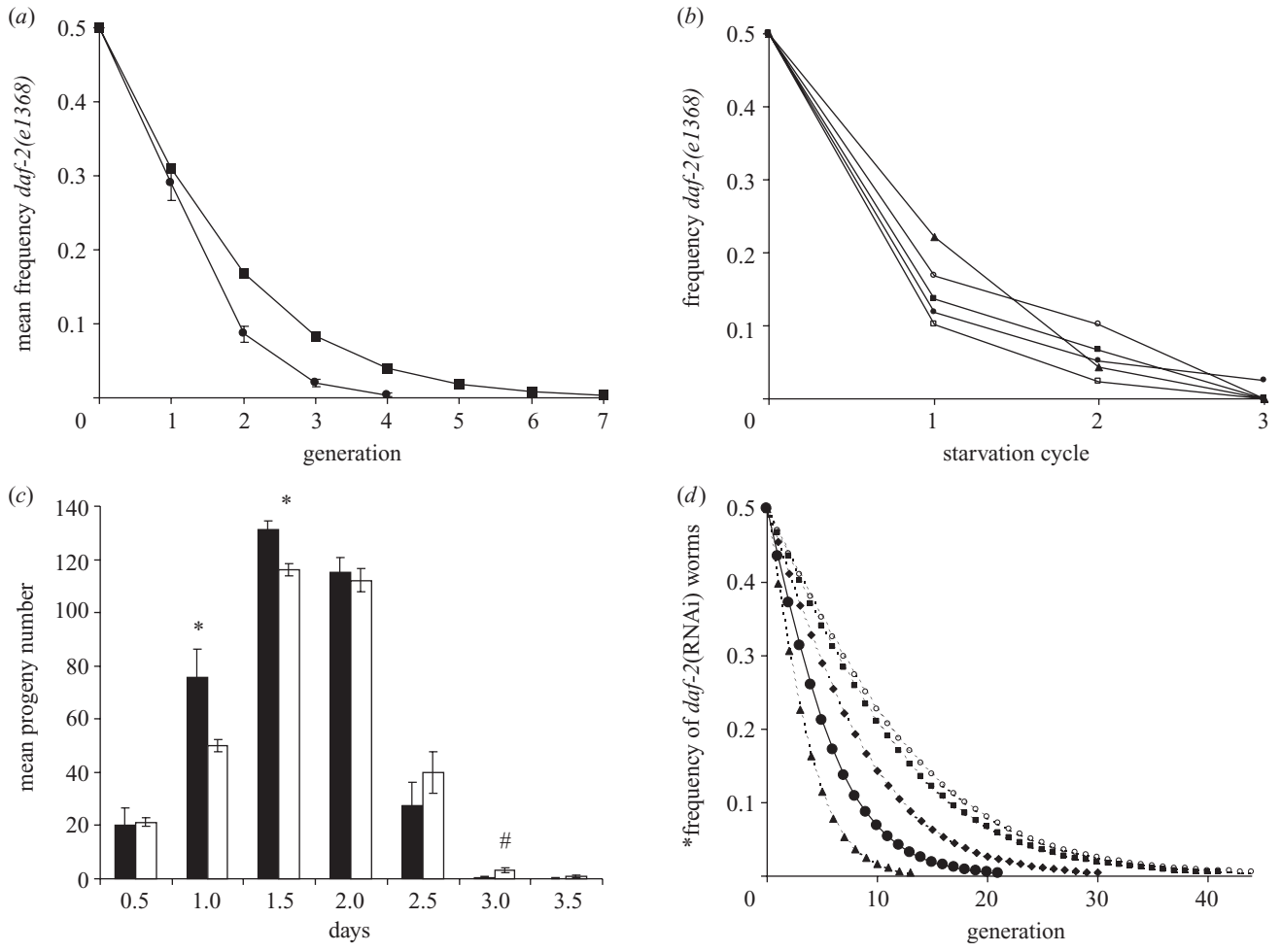

Figure 1. (*a*) Laboratory natural selection for the wild-type *daf-2* allele. Mean frequency of *daf-2(e1368)* from six replicate populations under non-starvation conditions at 20 °C in mixed cultures of N2 (wild-type) and *daf-2(e1368)* worms. Comparison of observed mean (circles) and expected (squares) allele frequency of *daf-2(e1368)*. Error bars are ± s.e.m. A homogeneous response ($p > 0.8$, contingency χ^2 tests) to selection was observed across the six populations in all generations. Expected frequency calculated from fertility measurements (see § 3a). (*b*) Frequency of *daf-2(e1368)* in five replicate populations undergoing cyclical starvation at 20 °C, where all food was depleted by 5 days and populations remained starved for a further 4 days. (*c*) Mean progeny number laid over half-day intervals in N2 (wild-type) worms treated with vector control (black bars) and *daf-2*(RNAi) (open bars). Error bars are ± s.e.m.. Data from Dillin *et al.* (2002). ∗Vector control > *daf-2*(RNAi), #*daf-2*(RNAi) > vector control, $p < 0.05$, 2-tailed *t*-tests. (*d*) Predicted loss of worms with reduced *daf-2* activity, based on time periods where there is a significant difference in accumulated progeny number between vector control and *daf-2*(RNAi)-treated worms. Time periods represented are 0.5–1.0 (filled circles); 0.5–1.5 (diamonds); 0.5–2.0 (squares); 1 (triangles) and 1.5 (open circles) days. (Data from Dillin *et al.* (2002).)

each population to assay allele frequency and a further 100 to initiate the next starvation cycle.

(d) *Determination of allele frequency*

The formation of dauers by *daf-2 (e1368)* at 25 °C (Riddle & Albert 1997; Walker *et al.* 2000) was used to assess allele frequency. Control plates of the individual strains, N2 and *daf-2(e1368),* were included to ensure the accuracy of this assay. Two replicate plates of 50 eggs were assayed for each population. Not all eggs hatched, and some worms were not scored, having desiccated by moving off the media, with the loss varying from 0% to 50%. Analysis of N2 and *daf-2(e1368)* controls indicated that neither genotype was preferentially lost.

3. RESULTS AND DISCUSSION

Since mutation of the mouse IGF-I receptor gene, and the *C. elegans* orthologue *daf-2,* results in healthy long-lived animals, we tested whether reducing DAF-2 function would have a fitness cost. We chose the *daf-2(e1368)* mutation in *C. elegans* that, akin to the *Igf1r*$^{+/-}$ mouse, is reported to have normal growth and fertility at 20 °C (Gems *et al.* 1998). To test the fitness of this long-lived variant, we undertook laboratory natural selection experiments as previously described (Walker *et al.* 2000) in which we mixed wild-type worms with *daf-2* mutants and cultured these populations over multiple generations. Consequently the wild-type and *daf-2* mutant worms were subject to identical environmental conditions and were directly competing for resources. We found that there was a significant cost associated with the *daf-2(e1368)* mutation even under a regime of constant food (figure 1*a*), with the *daf-2* mutation becoming extinct in all populations in only four generations. This rapid extinction suggested a major fitness cost. As *C. elegans* is a self-fertilizing hermaphrodite, a haploid model can be used to determine relative fitness (Spiess 1978; Walker *et al.* 2000). Because we are assaying

100 individuals, the resolution of our data is 0.01. It took only three generations for *daf-2(e1368)* to be reduced to this level, consistent with a relative fitness of 0.35.

There are a number of factors that may contribute to the fitness deficit observed in the *daf-2(e1368)* mutant including reduced fertility, reduced egg to adult viability, and increased development time. Previous studies have found that generation time can be a critical factor in the competitive ability of *C. elegans* (Hodgkin & Barnes 1991). The development time of the *daf-2(e1368)* mutants was found to be similar to the wild-type worms. However, when we measured the fertility of the mutant and wild-type strains under the culture conditions described, *daf-2(e1368)* had a significantly reduced early fertility compared with wild type. We incorporated this data into a simple model of expected allele change over time to determine if this was a principal reason for the observed results.

(a) *Fitness model*

If we assign p to the frequency of the wild-type allele and q to the frequency of the *daf-2(e1368)* allele, and w is the relative fitness of *daf-2(e1368)* compared with wild-type, the predicted frequency of *daf-2(e1368)* allele over time (t) is given by the following equation

$$q_t = q_{(t-1)}w/(q_{(t-1)}w + p_{(t-1)}).$$

In the time period selected for the non-starved competition experiments, wild-type worms laid an average of 50 eggs, compared with 22.5 laid by *daf-2(e1368)*. This means that the relative fitness of worms carrying the *daf-2(e1368)* mutation, $w = 22.5/50 \approx 0.45$. From a starting frequency of 0.5, the expected frequency of *daf-2(e1368)* in the next generation is

$$q_1 = 0.5 \times 0.45/(0.5 \times 0.45 + 0.5) = 0.31.$$

In a model of discrete generations, this decline in allele frequency would lead to total loss of the *daf-2* mutation in only seven generations. We found that the predicted response to selection was very similar to that observed (figure 1*a*) suggesting that this early fertility difference is a major contributor to the rapid extinction of the mutant allele. The culture conditions used here may emphasize differences in early fertility. However, the use of direct competition assays incorporates total fitness rather than simply measuring predictions based on easy-to-measure fitness components such as fertility. We observed complete loss of *daf-2(e1368)* in only four generations, rather than the seven generations predicted if the fitness deficit was due to fertility differences alone. This indicates that overall fitness rather than simple fertility is reduced in the *daf-2* mutant strain, as reflected by the difference in observed (0.35) and expected (0.45) relative fitness measures.

We also tested the effect of the longevity mutation under nutritional stress. Here we subjected the mixed populations of competing worms to periods of starvation. Under these conditions we also found that there was rapid extinction of the *daf-2(e1368)* allele (figure 1*b*). Indeed it appears that the extinction is more rapid under these conditions, consistent with that observed for mutant *age-1* and *Indy*. Preliminary analysis suggests that this may be due to maternal effects, with reduced fertility observed in progeny of starved worms (data not shown).

Although our observations are consistent with the predictions of AP theory, we considered the possibility that the fitness deficits were the result of unknown allelic variation tightly linked to the *daf-2* locus. Longevity and gross effects on fertility can reportedly be separated using RNAi to reduce the levels of DAF-2 in wild-type worms (Dillin *et al.* 2002) Considerable effects on both lifespan and fertility are seen when *daf-2* is absent throughout development, but administering RNAi in adult worms increases lifespan without apparently affecting fertility. Since RNAi methodology circumvents possible confounding genetic background effects, we analysed the data of Dillin *et al.* (2002; for RNAi methods refer to this original reference) to examine the effect of loss of *daf-2* activity on early fertility. Loss of *daf-2* function in the young adult pre-fertile period results in a significant reduction in early fertility (figure 1*c*).

The data from the RNAi experiment were applied to the simple model of competitive fitness. For the case of *daf-2*(RNAi) administered from the pre-fertile period of young adults, the mean progeny production in the first 24 h is 72 compared with 93 for the vector-treated control worms. From the model described above, this difference results in the relative fitness of worms with reduced *daf-2* activity, $w = 72/93 \approx 0.77$. If we consider a situation where there is a starting population with an equal proportion of wild-type and individuals with reduced *daf-2* activity, in the next generation the proportion of those with reduced activity would be:

$$q_1 = 0.5 \times 0.77/(0.5 \times 0.77 + 0.5) = 0.43.$$

When the progeny produced in the first 24 h are compared (as in our competition experiment represented in figure 1*a*), worms with reduced *daf-2* function would become extinct in 21 generations (figure 1*d*). Furthermore, if other periods of reduced fertility in *daf-2*(RNAi)-treated worms are considered, the time to extinction simply varies (figure 1*d*). RNAi treatment of older adult worms does not reduce early fertility, as expected by the delay in the RNAi effect (Dillin *et al.* 2002). However, early fertility is affected when *daf-2*(RNAi) is administered during all larval stages, with the time to extinction of worms with reduced *daf-2* activity closer to that observed in our selection experiments (data not shown). Considering that the model incorporates only one measure of fitness and underestimated the fitness cost of the *daf-2(e1368)* mutation, this is also potentially a conservative estimate of the fitness cost associated with reduced *daf-2* activity. We conclude that the RNAi approach to reduce *daf-2* activity also reveals a fitness cost of longevity.

It is interesting that loss of *daf-2* during the young adult period only reduced early fertility during the 1.5 days after first egg lay and did not reduce later fertility. Early progeny are paramount in *C. elegans* populations because an egg progresses to become a fertile adult in less than 3 days at 20 °C. Therefore, late progeny will compete for resources with the far more numerous progeny of their older sibs. An effect on progeny number is apparent only if insulin/IGF-I signalling is disrupted at an early stage when the reproductive pattern is being determined. This is analogous to the situation observed in *Drosophila* where gene action

during the early reproductive period had a delayed effect on lifespan (Sgro & Partridge 1999). Hodgkin & Barnes (1991) also demonstrated that a mutant with increased fertility was at a competitive disadvantage because the fertility increase caused an increase in generation time. Thus, early fitness traits may be of the utmost importance. Our results demonstrate that early fitness traits rather than lifetime fertility are important when assessing the possibility of trade-offs between longevity and Darwinian fitness.

In many longevity mutants, fitness costs may be readily apparent, such as those with altered development (Lakowski & Hekimi 1996) and greatly reduced fertility (Gems *et al.* 1998). Our results demonstrate that even in instances where the mutants are reportedly healthy, their increased longevity is still associated with reduced fitness, as predicted by the AP theory of aging. We conclude that even though the longevity mutation confers apparent benefits on the individual in terms of lifespan and stress resistance (Gems *et al.* 1998), competitive fitness is lowered, resulting in rapid extinction.

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