Growing up and growing old
Briga, Michael

IMPORTANT NOTE: You are advised to consult the publisher's version (publisher's PDF) if you wish to cite from it. Please check the document version below.

Document Version
Publisher's PDF, also known as Version of record

Publication date:
2016

Link to publication in University of Groningen/UMCG research database

Citation for published version (APA):

Copyright
Other than for strictly personal use, it is not permitted to download or to forward/distribute the text or part of it without the consent of the author(s) and/or copyright holder(s), unless the work is under an open content license (like Creative Commons).

Take-down policy
If you believe that this document breaches copyright please contact us providing details, and we will remove access to the work immediately and investigate your claim.

Downloaded from the University of Groningen/UMCG research database (Pure): http://www.rug.nl/research/portal. For technical reasons the number of authors shown on this cover page is limited to 10 maximum.
Chapter 2

What can long-lived mutants tell us about mechanisms causing aging and lifespan variation in natural environments?

Michael Briga & Simon Verhulst

Experimental Gerontology 71, 21-26
Abstract

Long-lived mutants of model organisms have brought remarkable progress in our understanding of aging mechanisms. However, long-lived mutants are usually maintained in optimal standardized laboratory environments (SLEs), and it is not obvious to what extent insights from long-lived mutants in SLEs can be generalized to more natural environments. To address this question, we reviewed experiments that compared the fitness and lifespan advantage of long-lived mutants relative over wild type controls in SLEs and more challenging environments in various model organisms such as yeast *S. cerevisiae*, the nematode worm *C. elegans*, the fruitfly *D. melanogaster* and the mouse *Mus musculus*. In competition experiments over multiple generations, the long-lived mutants had a lower fitness relative to wild type controls, and this disadvantage was clearest when the environment included natural challenges such as limited food (N=6 studies). It is well known that most long-lived mutants have impaired reproduction, which provides one reason for the fitness disadvantage. However, based on 12 experiments, we found that the lifespan advantage of long-lived mutants is diminished in more challenging environments, often to the extent that the wild type controls outlive the long-lived mutants. Thus, it appears that information on aging mechanisms obtained from long-lived mutants in SLEs may be specific to such environments, because those same mechanisms do not extend lifespan in more natural environments. This suggests that different mechanisms cause variation in aging and lifespan in SLEs compared to natural populations.
Introduction

Aging is the decline in physiological function with age, associated with decreasing survival probability and reproduction. Remarkable progress in our understanding of aging mechanisms has been achieved through the study of model organisms such as yeast *Saccharomyces cerevisiae*, the nematode worm *Caenorhabditis elegans*, the fruitfly *Drosophila melanogaster* and the mouse *Mus musculus* (e.g. Sprott and Austad 1996). An important tool in the study of aging mechanisms is the use of genetic mutants with an extended lifespan (Kenyon 2005, 2010; Partridge 2010; Gems and Partridge 2013). The effect of these genetic mutations can be enormous, with for example some mutants living up to 10 times longer than their wild type controls (Ayyadevara et al. 2009). Aging pathways identified in this way include those involved in stress responses and nutrient sensing such as the ‘insulin/insulin-like growth factor 1 signaling’ (IIS) pathway and the ‘target of rapamycin’ (TOR) pathway (Kenyon 2005, 2010; Fontana et al. 2010; Gems and Partridge 2013). The study of long-lived mutants has thus provided insight into key mechanisms that affect aging and lifespan.

Long-lived mutants are usually studied in standardized laboratory environments (SLEs), characterized by a constant climate, minimal exposure to pathogens, no opportunity to reproduce (depending on the species) and *ad libitum* food that can be obtained with little or no physical effort. Standardizing the environment has the advantage that it may reduce environmentally caused variation in aging and lifespan. More importantly, when the SLE provides an optimal environment the animals may achieve a lifespan that is close to their maximum, determined only by intrinsic causes. On the other hand, an intrinsic aging phenotype can only be defined against the background of the environment, because intrinsic aging factors interact with the environment to determine intrinsic aging rate (Stearns 1992; Flatt et al. 2013). Thus the lifespan achieved by long-lived mutants in SLEs is only one of the many phenotypes that characterize the specific long-lived mutant genotype, and mechanisms causing an extended lifespan in SLEs may not have a similar effect in more natural environments.

How the aging phenotype of a long-lived mutant varies between environments will depend on the physiological mechanism through which the extended lifespan is achieved. Given that SLEs lack most challenges faced by organisms in natural environments, the optimality theory of aging (Partridge and Barton 1993), an umbrella covering the antagonistic pleiotropy (Williams 1957) and disposable soma (Kirkwood 1977) hypotheses, suggests that the extended lifespan of long-lived mutants may at least in part be due to a reallocation of resources saved on mechanisms that enhance fitness.
in natural environments (e.g. immune function, foraging, reproduction) to increased maintenance and repair (Fig. 1). If extended lifespans are achieved by saving resources that animals could not afford to save under more natural conditions, it is not clear how knowledge of the mechanisms giving these mutants an extended lifespan in SLEs will help understand variation in lifespan or the causes of aging in natural populations (including humans) where there would be strong natural selection against such savings. We thus question whether the mechanisms modulating lifespan in SLEs would be the same as those that explain variation in lifespan in the wild.

**Fig. 1** Hypothesis, based on the optimality theory of aging (Partridge and Barton 1993) stating that the lifespan advantage of long-lived mutants is diminished in the presence of natural stressors that are as a rule absent from standard laboratory environments.

Given that much of our understanding of the mechanisms of aging comes from studies of long-lived mutants in SLEs, and that the environment can have profound effects on lifespan, we here ask to what extent insights from long-lived mutants in SLEs can be generalized to more natural environments. Is it possible that the longer lifespans of long-lived mutants are achieved at the expense of defenses against natural environmental challenges? And if so, what are the consequences for mechanisms involved in lifespan determination and variation in the wild? These questions are of importance when the aim is to apply insights from long-lived mutants in SLEs to other organisms such as humans, which are invariably exposed to a variety of environmental challenges. To address these questions we reviewed two kinds of studies. Firstly, we reviewed experiments that quantified the performance of long-lived mutants and their wild type controls on evolutionary timescales by measuring the fitness of both genotypes in either SLEs or more challenging environments. These studies carried out competition experiments, which consist of mixing two genotypes (the long-lived mutant and the wild type control) in a common environment (SLE or challenging) usually for several generations, after which the relative frequency of each genotype was quantified.
However, fitness (dis)advantages in competition experiments may have arisen through differences in lifespan, in reproduction or a combination of the two, and while competition experiments quantified fitness, they rarely quantified lifespan per se. In the second part, we therefore reviewed studies that quantified the lifespan advantage of long-lived mutants over the wild type controls in SLEs and environments containing more natural challenges. These experiments often lasted only one generation and excluded competition, i.e. long-lived mutant and wild type populations are not mixed. When the life-extending effect of mutations is largely independent of the environment, this indicates that the underlying mechanisms may be of general importance in causing variation in lifespan. Conversely, a strong dependence of the life extending effect on environmental conditions would give reason to question the generality of the mechanism causing the life extending effect in SLEs.

**Material and Methods**

To find papers that reported competition experiments including long-lived mutants, we searched the Web of Science database using the keywords ‘long-lived mutant’ and ‘evolution’ (last search on May 31st 2015). This search resulted in 42 articles, of which we selected all articles that had long-lived mutants compete with their wild type counterparts (Jenkins et al. 2004; Delaney et al. 2011; Savory et al. 2014). We then cross-searched all the references and citations of these articles.

For the lifespan studies, articles were only selected if the following criteria were met (i) a long-lived mutant had an extended lifespan in a SLE, (ii) an experimental manipulation of the environment affected the lifespan of either the long-lived mutant or the wild type control and (iii) an estimation of lifespan of the long-lived mutant and the wild type control in both environments. We searched the literature using (i) the above search and (ii) the Web of Science database using the keywords ‘long-lived mutant’ and ‘environment’ or ‘long-lived mutant’ and ‘natural’ (last search on May 31st 2015). In addition, we used influential reviews and perspective papers on long-lived mutants and genotype x environment interactions (Gems et al. 2002; Van Voorhies et al. 2006; Partridge and Gems 2007; Tatar 2007; Flatt et al. 2013; Tatar et al. 2014). For each of the three searches we searched all the references and citations of these articles before May 31st 2015 in the Web of Science database.

We define a stressor as a factor that shortens the lifespan of wild type controls and/or long-lived mutants relative to the lifespan in a SLE. When examining effects of stressors
on lifespan we distinguished between the application of short-term acute stressors (heat stress, UV-radiation, toxic chemicals) that cause more or less immediate death of part of the population (e.g. Barsyte et al. 2001; Clancy et al. 2001), and more moderate long-term stressors that were applied permanently. Long-lived mutants appear more resistant to short-term acute stressors than their wild type controls (see e.g. Zhou et al. 2011 for a review). Hence, when an environment is made more challenging by applying short-term acute stressors the lifespan advantage of the long-lived mutants may increase (Zhou et al. 2011). However, we considered such acute stressors to be generally outside of the range that animals under more natural conditions would encounter. Thus, we reviewed only studies that permanently applied more natural and/or moderate stressors, such as a more natural medium, food competition or exposure to pathogens. Note that in dietary restriction experiments, lifespan differences between long-lived mutants and wild type controls can also be environment dependent (Clancy et al. 2002; Gems et al. 2002; Tatar et al. 2014). Yet we did not consider dietary restriction to be a stressor or a natural challenge because it extends the lifespan of wild type controls. However dietary restriction experiments that used variety of diet concentrations can fulfill the challenging criteria if food dilution is applied to the extent that it shortens lifespan of the wild type controls (e.g. Broughton et al. 2010; Clancy et al. 2002; Tatar et al. 2014).

Several studies applied combinations of stressors, for example a variety of pathogens (Garsin et al. 2003), or different degrees of a stressor. To avoid pseudo-replication due to repeated testing, we restricted our analysis to those environmental manipulations that had the strongest effect on the lifespan of wild type controls, because these manipulations best represent a challenging environment.

Unfortunately, most studies did not statistically test genotype x environment interactions (Table S2), prohibiting a formal meta-analysis. However, given the results (e.g. Fig. 4), we see no reason to expect that a formal meta-analysis would change our findings.

Results

Competition performance of long-lived mutants

Very few competition experiments have been conducted in SLEs (n=3) and all have used C. elegans (Table S1). In two experiments, the relative fitness between the long-lived mutant and the wild type control did not differ and in one experiment the long-lived mutant went extinct while the wild type control persisted (Fig. 2). While the sample size is low, there is no evidence that long-lived mutants have a consistent competitive advantage or disadvantage over the wild type controls in SLEs.
We found five competition experiments carried out in more challenging environments, covering most model species (Table S1). In addition, we also found one study that carried out 49 competition experiments with long-lived yeast mutants \textit{S. cerevisiae} (Delaney et al. 2011), which we discuss separately below. In all experiments, the challenge consisted of competition for food. The outcome of these experiments was consistent (Fig. 2): the frequency of the long-lived mutant decreased (Giorgio et al. 2012; Wit et al. 2013; Savory et al. 2014), and even went extinct in two out of five experiments (Jenkins et al. 2004; Walker et al. 2000). This outcome stands in contrast with what we found in SLEs, especially given that three out of these five experiments came from the same study as those from SLEs (Table S1). Thus, in competition experiments long-lived mutants have lower fitness relative to their wild type controls and this seems most pronounced in challenging environments.

![Image](image.png)

**Fig. 2** Outcome of competition experiments between long-lived mutants and their wild type controls. The outcome is from the perspective of the long-lived mutant. Arrows connect experiments that were done in the same study. One additional study in yeast is discussed separately in the main text because it consisted of 49 experiments (Delaney et al. 2011). Studies are summarized in table S1. SLE: standardized laboratory environments.

In addition to the competition experiments discussed above, there is one study that comprised 49 experiments with 49 different long-lived yeast mutants (Delaney et al. 2011). In this study, 84% (41/49) of the long-lived mutants decreased in relative frequency (statistically significant for 32 mutants). In contrast, 16% (8/49) of the mutants increased in relative frequency (statistically significant for two mutants). Thus, the mutants were clearly outcompeted by the wild type yeast strain. In this study, the mutants differed strongly in the extent to which their lifespan was increased relative to wild type controls in the SLE (range 13-55% without competition). This allowed us to investigate whether the mutants with the largest lifespan advantage in a non-competitive environment also have the lowest fitness in a competitive environment. If
Chapter 2

Lifespan extension generally is achieved at the expense of competitive performance, we expect a negative correlation between the two variables. Indeed, yeast mutants with the largest lifespan advantage were, in evolutionary terms, least fit relative to the wild type controls in the competitive environment (Fig. 3). This finding confirms that extended lifespan is achieved at the expense of fitness in competitive environments. In conclusion, the competition experiments indicate that when having to reproduce and compete with wild type controls in the face of natural challenges such as food limitation, long-lived mutants have decreased fitness relative to wild type controls.

![Fig. 3](image)

**Fig. 3** Association between the lifespan advantage of 49 long-lived yeast mutants over controls in SLEs (standardized laboratory environments) and their fitness (dis)advantage in competition experiments. Relative fitness (RF) is defined as log base 2 ratio of mutant to wild type relative to the initial ratio, such that RF = 0 indicates no change in the ratio of mutant to wild type, an RF = 1 corresponds to twice as many mutant cells as wild type cells relative to the initial ratio, while an RF = -1 corresponds to twice as many wild type cells as mutant cells. A RF of -7 refers to extinction of the long-lived mutant. Competition experiments were carried out for all 49 mutants separately. Data from Delaney et al. (2011). Best fit: R²=0.16, t=-3.13, p=0.003.

**Lifespan of long-lived mutants in environments other than SLEs**

The competitive disadvantage of long-lived mutants relative to their wild type controls can arise via diminished survival and/or diminished fecundity. It is a general finding, reviewed elsewhere, that long-lived mutants have diminished fecundity relative to their wild type controls (Flatt 2011; Kenyon 2005; Leroi et al. 2005; Partridge et al. 2005; Tatar 2010) although there are exceptions where the fecundity of both genotypes is similar (Rogina et al. 2003; Hwangbo et al. 2004). It is likely therefore that the reduced competitive ability of long-lived mutants is at least in part due to lower fecundity. However, lifespan was not monitored in the competition experiments, and the possibility
remains that a shortened lifespan of the long-lived mutants also contributed to the low competition success in more natural environments. To address this question we reviewed the studies that compared the lifespan advantage of long-lived mutants over their wild type controls in SLEs and in more challenging environments.

We found a total of 19 experiments in 10 studies where the lifespan of long-lived mutants relative to wild type controls was compared between SLEs and challenging environments, in three different species: *C. elegans, D. melanogaster* and *M. musculus*. Several studies exposed different populations to different stressors or different levels of a stressor. Following the pseudo-replication standards as explained in the 'Material and Methods' section, we used 12 experiments in three species (Table S2). In 5 out of 12 experiments, the long-lived mutants lived significantly shorter than the wild type controls in the challenging environment (e.g. Mockett and Sohal 2006; Van Voorhies et al. 2005; Fig. 4). In another six experiments, the lifespan advantage of the long-lived mutants decreased, but long-lived mutants still lived as long as or longer than the wild type controls (e.g. Baldal et al. 2006; Broughton et al. 2010; Toivonen et al. 2007; Fig. 4). In only one case, the lifespan advantage of long-lived mutants over the wild type controls was larger in the challenging environment than in the SLE (Merino et al. 2015). Thus overall, the lifespan advantage of long-lived mutants decreased in the challenging environment in 92% (11/12) percent of studies and a two-tailed sign-test shows this deviation from 50:50 to be larger than expected by chance (p=0.006). Furthermore, we note that in studies with multiple levels of a stressor, the intensity of the stressor correlated negatively with the lifespan advantage of the long-lived mutants over the wild type controls. In other words, in response to high intensity stressors, the advantage of long-lived mutants over wild type controls was smaller than in response to low intensity stressors (e.g. Clancy et al. 2002). We anticipate therefore that in the studies where the long-lived mutants retained a lifespan advantage over the wild type controls in the challenging environment, long-lived mutants would end up living shorter than the wild type controls if the intensity of the challenge had been further increased. Thus, there is strong evidence that long-lived mutants cope less well with environmental challenges than the wild type controls.
Fig. 4 Lifespan advantage of long-lived mutants over the controls is environment dependent. Lines connect environmental manipulations carried out within one study. CLE: Cafeteria style laboratory environment, SLE: Standardized laboratory environment, Challenging: environment was made more challenging in various ways as evidenced by a reduced lifespan of the control lines (see main text for details). Studies are summarized in table S2.

Of the studies listed above only two were on vertebrates (mice). One study was on Snell dwarf mice. This strain originated as a spontaneous mutation and animals homozygous for this mutation grow to approximately one third of the mass of their wild type siblings (Snell 1929). The impaired growth is due to defects in production of growth hormone, insulin-like growth factor-1 (IGF-1), thyroid hormones, and prolactin (reviewed e.g. in Bartke 2006). Snell dwarf mice were initially found to be a short-lived mutant due to increased susceptibility to infectious disease (Fabris et al. 1972). However, other laboratories later found that Snell dwarf mice had lifespans up to 40% longer than standard laboratory mice (Silderberg 1972; Shire 1973; Schneider 1976; Flurkey et al. 2001) when housing conditions were made more hygienic (Bartke 2006) and mutants were provided a companion mouse to keep them warm. This suggests that the increased lifespan of Snell dwarf mice might trade-off against the immune response and/or body temperature homeostasis. To our best knowledge, this dependence of the lifespan of Snell’s dwarf mice on environmental conditions was not explicitly tested, but the contrasts are clear enough in our view to include this strain in Table S2. The second long-lived vertebrate mutant that was studied in a challenging environment was the p66Shc knockout mouse. P66Shc is a vertebrate protein that is involved in metabolism.
and intracellular redox balance and its knockout results in mice that are leaner, more resistant to obesity and diabetes, with reduced oxidative stress and a 30% increased lifespan in SLEs (Migliaccio et al. 1999; Menini et al. 2006; Berniakovich et al. 2008; Fadini et al. 2010; Ranieri et al. 2010). However, in an outdoor enclosure where mice were exposed to natural variation in temperature, food competition and exposure to predators their survival advantage was overturned: after 8 months, 18% of controls were alive while only 5% of p66shc knock outs were alive (Giorgio et al. 2012). Thus, the limited information available for rodents confirms the finding in invertebrates that the lifespan advantage of long-lived mutants is restricted to specific laboratory environments.

Lifespan in cafeteria environments

In the studies discussed above, the environment was made more challenging in different ways, for example by increasing the effort required to obtain a unit of food relative to SLEs. In contrast, a few studies decreased the effort required to obtain a unit of food, i.e. animals were offered a so-called ‘cafeteria-style’ laboratory environment (CLE). Such manipulations decrease lifespan (Ozanne and Hales 2004) and show strong similarities to the sedentary lifestyles that decrease lifespan in humans (Flegal et al. 2013). In Drosophila, CLEs induced an increase in calorie intake of up to 1.5 times that in SLEs and reduced the lifespan of controls and long-lived Indy, chico and IPC KO (insulin-producing cells knock out) mutants (Clancy et al. 2002; Wang et al. 2009; Broughton et al. 2010). In CLEs long-lived Indy mutants increased their lifespan advantage over that of controls (Wang et al. 2009). For chico and IPC KO mutants there was also an increase in lifespan advantage in CLEs relative to SLEs, but that increase was small, i.e. between 3 and 7% (Clancy et al. 2002; Broughton et al. 2010). CLEs consist of manipulations that make SLEs even more ‘sedentary’ (and thus are in the opposite direction to the experiments in which SLEs were made more challenging, Fig. 4). Thus, the few studies available suggest that long-lived mutants appear to increase their lifespan advantage relative to wild type controls (Fig. 4). This is consistent with our conclusion that the lifespan advantage of long-lived mutants over the wild type controls is most pronounced in environments with few environmental challenges.

Discussion

We investigated to what extent the performance of long-lived mutants depends on the environment in which they were studied, because this sheds light on the question whether mechanisms causing the extended lifespan may have similar effects in more natural environments. In competition experiments, the long-lived mutants almost always had
lower fitness relative to the wild type controls, especially in challenging environments (Fig. 2). It is well known that the fecundity of long-lived mutants is generally reduced (Flatt 2011; Kenyon 2005; Leroi et al. 2005; Partridge et al. 2005; Tatar 2010), but we find that the lifespan advantage of long-lived mutants is also diminished in more challenging environments (Fig. 4). This effect was such that the lifespan difference was reversed in 5/12 studies and we speculate that this proportion would increase further when environments are made more challenging, as graded dietary restriction studies in *Drosophila* suggest (Clancy et al. 2002; Tatar et al. 2014).

The observation that long-lived mutants are more susceptible to environmental challenges than the wild type controls suggests that they lack the required mechanisms to cope with such challenges. Indeed, in agreement with the optimality theory of aging (Partridge and Barton 1993), the extended lifespan of long-lived mutants may be due to a reallocation of resources saved on coping mechanisms (e.g. immune function) to increased maintenance and repair (Fig. 1). Unraveling the mechanisms that extend the lifespan of long-lived mutants is very interesting in itself. Yet the extended lifespans of long-lived mutants in SLEs are at least partially achieved by saving resources that animals could not afford to save under more natural conditions. Thus, in natural environments there would be strong natural selection against such savings and we therefore believe that variation in lifespan in natural populations (including humans) is unlikely to have the same mechanistic basis as that indicated by work on long-lived mutants in SLEs. The artificial conditions and selection pressures imposed by SLEs can do much to skew the physiological traits among model organisms that are relevant to the aging process in SLEs but not under natural conditions (Harshman and Hoffmann 2000; Sgrò and Partridge 2000; Linnen et al. 2001; Sgrò et al. 2013). This argument also applies when the underlying mechanism is not related to re-allocation of resources, because it is the finding that mechanisms can have the opposite effect on lifespan in more challenging environments that gives reason to question the relevance of these mechanisms in natural populations. Instead, with respect to aging mechanisms in natural environments, we believe there is a need for ecologically relevant manipulations that modulate lifespan and aging in a way that invokes mechanisms that have evolved naturally. Manipulation of reproductive effort or developmental conditions, which can both affect lifespan and aging (Lee et al. 2013, 2016; Boonekamp et al. 2014) come to mind as promising avenues to explore.

Our findings hold in all taxonomic groups where they were studied, including the nematode *C. elegans*, the fly *Drosophila*, and the mouse *Mus musculus*. Our review includes a variety of environmental challenges including exposure to pathogens, cold exposure
and competition for food or starvation (Table S2). Our review also included a variety of long-lived mutations involving multiple pathways. Several of these mutations (Indy, chico, IPC KO and p66<sup>shc</sup>) are one way or another involved in metabolism and energy balance. When these long-lived mutants are faced with food related challenges, genotype x environment interactions can be expected, but this does not make them less relevant given that food related challenges are common in nature. Further research is required to address whether metabolism-related mechanisms pathways extend lifespan in the wild.

More generally, we need to understand better which life-extending pathways are susceptible to which environmental challenges. This is important because insights gained from studying long-lived mutants in SLEs can provide an important source of inspiration for the development of interventions that postpone or slow down aging (Longo et al. 2015). Yet the trade-offs involved in extending the lifespan of long-lived mutants, and the environment dependent outcome of mutations that affect aging and lifespan, need to be taken into account for interventions to be effective (see also Kuningas et al. 2008; Vijg and Campisi 2008). We believe that ecologically relevant manipulations such as those mentioned above can uncover mechanisms and trade-offs involved in aging and lifespan variation and may provide essential insights for possible ‘anti-aging’ interventions.

Acknowledgements

We like to thank the Quinn Fletcher and two anonymous reviewers for valuable comments that improved the manuscript.
Table S1: Overview of competition experiments with long-lived mutants carried out in various environments. The outcome of the competition experiment is from the perspective of the long-lived mutant. Abbreviations: NA: not applicable, NS: not significant.

<table>
<thead>
<tr>
<th>Species</th>
<th>Mutant</th>
<th>Function</th>
<th>Outcome in SLE</th>
<th>Challenge</th>
<th>Outcome in challenging environment</th>
<th>Reference (Location)</th>
</tr>
</thead>
<tbody>
<tr>
<td>S. cerevisae</td>
<td>49</td>
<td>Various</td>
<td>NA</td>
<td>Cyclic starvation</td>
<td>84% decrease or extinct (65% significant); 16% increase (4% significant), no invading genotypes</td>
<td>Delaney et al. 2011 (Table 1)</td>
</tr>
<tr>
<td>C. elegans</td>
<td>daf-2</td>
<td>Insulin signaling</td>
<td>Extinct</td>
<td>Cyclic starvation</td>
<td>Extinct</td>
<td>Jenkins et al. 2004 (Fig. 1)</td>
</tr>
<tr>
<td>C. elegans</td>
<td>age-1</td>
<td>Insulin signaling</td>
<td>NS</td>
<td>Cyclic starvation</td>
<td>Extinct</td>
<td>Walker et al. 2000 (Fig. 1)</td>
</tr>
<tr>
<td>C. elegans</td>
<td>age-1</td>
<td>Insulin signaling</td>
<td>NS</td>
<td>Limited food</td>
<td>Decrease</td>
<td>Savory et al. 2014 (Fig. 1)</td>
</tr>
<tr>
<td>D. melanogaster</td>
<td>3 longevity lines</td>
<td>Unclear</td>
<td>NA</td>
<td>Field release with food searching</td>
<td>Decreased recapture probability</td>
<td>Wit et al. 2013 (Fig. 2, Table 5)</td>
</tr>
<tr>
<td>M. musculus</td>
<td>p66Shc</td>
<td>Various</td>
<td>NA</td>
<td>Outdoor enclosure with food competition</td>
<td>Decrease significantly within 1 or few generations. Wild type invaded to 75%</td>
<td>Giorgio et al. 2012 (Fig. 1)</td>
</tr>
</tbody>
</table>
Table S2 Overview of experiments in which the lifespan of long-lived mutants was compared with that of controls in SLEs and more challenging environments. Data was split in two types of environmental manipulations natural like challenges (top) and cafeteria style laboratory environments (CLE, bottom). To avoid pseudo replication of studies, per study we included only the experimental challenge that had the strongest negative effect on the lifespan of controls. Abbreviations: manip: manipulated, neg: negative, pos: positive, NST: not statistically tested, NS: not significant. For NST cases, where possible we derived statistical significance ourselves from the SE or SD given in manuscript.

<table>
<thead>
<tr>
<th>Study organism</th>
<th>Mutation</th>
<th>Function</th>
<th>Description environmental challenge</th>
<th>Trait</th>
<th>Lifespan [Days]</th>
<th>Statistics</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>SLE</td>
<td>Challenging</td>
<td>G x E</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Controls</td>
<td>Mutants</td>
<td>Controls</td>
<td>Mutants</td>
</tr>
<tr>
<td>C. elegans</td>
<td>daf-2</td>
<td>Insulin signaling</td>
<td>Heat treated soil</td>
<td>Median</td>
<td>12</td>
<td>27</td>
<td>1</td>
</tr>
<tr>
<td>C. elegans</td>
<td>daf-2 (mean)</td>
<td>Insulin signaling</td>
<td>Pathogen</td>
<td>Median</td>
<td>13.1</td>
<td>24.4</td>
<td>2</td>
</tr>
<tr>
<td>C. elegans</td>
<td>age-1</td>
<td>Insulin signaling</td>
<td>Pathogen</td>
<td>Median</td>
<td>13.1</td>
<td>19.6</td>
<td>2</td>
</tr>
<tr>
<td>D. melanogaster</td>
<td>mth (heterozygote)</td>
<td>Stress response</td>
<td>Reproduction</td>
<td>Mean</td>
<td>26</td>
<td>31</td>
<td>23</td>
</tr>
<tr>
<td>D. melanogaster</td>
<td>mth (heterozygote)</td>
<td>Stress response</td>
<td>Constant moderate heat stress</td>
<td>Mean</td>
<td>41</td>
<td>50</td>
<td>25</td>
</tr>
<tr>
<td>D. melanogaster</td>
<td>chico (homozygote)</td>
<td>Insulin signaling</td>
<td>Starvation</td>
<td>Mean</td>
<td>52</td>
<td>55</td>
<td>43</td>
</tr>
<tr>
<td>D. melanogaster</td>
<td>IPC KO (dilp2)</td>
<td>Insulin signaling</td>
<td>Starvation</td>
<td>Median</td>
<td>66</td>
<td>78</td>
<td>22</td>
</tr>
<tr>
<td>D. melanogaster</td>
<td>Azot (homozygote)</td>
<td>Elimination of malfunctioning cells</td>
<td>Constant moderate heat stress</td>
<td>Median</td>
<td>25.9</td>
<td>34.2</td>
<td>7.8</td>
</tr>
<tr>
<td>D. melanogaster</td>
<td>indy206 (heterozygote)</td>
<td>Krebs cycle</td>
<td>Eliminating Wolbachia infection</td>
<td>Median</td>
<td>45</td>
<td>67</td>
<td>45</td>
</tr>
<tr>
<td>D. melanogaster</td>
<td>mth (homozygote)</td>
<td>Stress response</td>
<td>Cold stress</td>
<td>Mean</td>
<td>138</td>
<td>141</td>
<td>5</td>
</tr>
<tr>
<td>M. musculus</td>
<td>Snell dwarf mice</td>
<td>Insulin-like growth factor-1</td>
<td>Pathogen?</td>
<td>Mean</td>
<td>831</td>
<td>1178</td>
<td>600</td>
</tr>
<tr>
<td>M. musculus</td>
<td>p66shc (heterozygote)</td>
<td>Metabolism</td>
<td>Outdoors with food competition and predators</td>
<td>15% Survival</td>
<td>820</td>
<td>940</td>
<td>390</td>
</tr>
<tr>
<td>Study organism</td>
<td>Mutation</td>
<td>Function</td>
<td>Description of environmental challenge</td>
<td>Trait</td>
<td>Lifespan [Days]</td>
<td>Statistics</td>
<td>G x E Interaction</td>
</tr>
<tr>
<td>----------------</td>
<td>----------</td>
<td>----------</td>
<td>----------------------------------------</td>
<td>-------</td>
<td>----------------</td>
<td>------------</td>
<td>------------------</td>
</tr>
<tr>
<td><em>D. melanogaster</em></td>
<td>indy (homozygote)</td>
<td>Krebs cycle</td>
<td>CLE</td>
<td>Median</td>
<td>43</td>
<td>44</td>
<td>35</td>
</tr>
<tr>
<td><em>D. melanogaster</em></td>
<td>chico</td>
<td>Insulin signaling</td>
<td>CLE</td>
<td>Mean</td>
<td>52</td>
<td>55</td>
<td>42</td>
</tr>
<tr>
<td><em>D. melanogaster</em></td>
<td>IPC KO (dilp2)</td>
<td>Insulin signaling</td>
<td>CLE</td>
<td>Median</td>
<td>66</td>
<td>78</td>
<td>60</td>
</tr>
</tbody>
</table>
References


Chapter 2


Box A

Growing up in large broods impairs development in zebra finches

Michael Briga, Egbert Koetsier & Simon Verhulst
Study 1: Growing up in large broods increases the effort made per item food reward

Experimental manipulation of developmental conditions are commonly done through changes in food abundance or brood size (Griffith and Buchanan 2010). In our study, we manipulated the brood size and here investigate the consequences on chick behaviour and growth. We paid particular attention to begging behaviour, because various studies have indicated that begging incurs costs, in terms of energy (Bachman and Chappell 1996; McCarty 1996; Moreno-Rueda 2007) or physiology. For example, in various bird species, experimental increases of begging behaviour were found to impair growth (Kilner 2001; Rodríguez-Gironés et al. 2001; Moreno-Rueda and Redondo 2011; Moreno-Rueda et al. 2012), immunocompetence (Moreno-Rueda 2010; Moreno-Rueda and Redondo 2011; Moreno-Rueda et al. 2012; Redondo et al. in press) and to increase oxidative stress (Moreno-Rueda et al. 2012). To investigate whether the brood size manipulation affected begging behaviour we recorded 7 small and 8 large brood nests. Recordings were done at two growth points, halfway through the chick stage and just before fledging, i.e. at the age of 7 and 15 days. We recorded on average 1.5 hour (95% CI 1-2 hours) per hour per age class, giving a total of 50 hours of recording. For each nest we quantified the time budget of two chicks. We found that chicks in large broods begged more (Fig. 1; $\chi^2=7.56; p=0.006$) and received less regurgitations per hour compared to chicks reared in small broods (Fig. 1; $F=8.14; p=0.01$). These results are consistent with other studies showing increased begging in chicks from large broods (Leonard et al. 2000; Neuenschwander et al. 2003; Kim et al. 2011). Thus, growing up in large broods increased the effort made per item food reward.

![Fig. 1 Chicks in large broods spent more time begging (left), but nevertheless received fewer regurgitations from the parents (right). Shown are means per chick ± SE. Results are based on 50 hours of recording in 7 small and 8 large brood nests.](image-url)
Study 2: Growing up in large broods impairs growth

Given that large broods increased the effort made per item food reward, we expected impaired growth in chicks from large broods. We quantified the growth curve by measuring chicks at three age stages: just before fledging (15 days) and at the age of 35 and 100 days, when birds are approximately fully grown. Data included here are 3263 measurements on 295 individuals from three breeding rounds in 2006, 2007 and 2008 and all these were allocated to the foraging cost treatment (Chapters 3-11). At each age, we measured weight and the length of tarsus, headbill and wing. We also devised a more general of structural body size, using the average of the tarsus and the headbill after transforming both to a standard normal distribution. As a control we weighed chicks before manipulation (day 5) and there was no difference in mass between chicks going to large broods or small broods (Fig. 2; F=0.40; p=0.52). All analyzes were performed in SAS JMP 7 using general linear models including as fixed effects brood size and age and as random effects individual, genetic father and genetic mother. Residuals of all models had a normal distribution and without outliers. To allow comparison of the effect of the brood size manipulation across ages and traits, we report the effect size as Cohen’s d (Cohen 1988), which in brief, is the ratio of the difference between two groups over their standard deviation (Fig. 2). Confidence intervals were estimated following equations 15 and 16 in Nakagawa and Cuthill (2007). As a simple rule of thumb, an effect size between 0.1 and 0.5 is usually considered moderate (Cohen 1988), with 0.5 being the average effect size of published results in the fields of ecology and evolution (Moller and Jennions 2002). Note however that many studies with smaller effects do not make it till publication, i.e. there is a publication bias of positive, significant or ‘stronger’ results (Rosenthal 1979; Csada et al. 1996; Cassey et al. 2004; Fanelli 2010).

At the age of 5 days, i.e. before the brood size manipulation, there was no difference in mass between chicks that went to small or large broods (F_{272}=0.04; p=0.92; Fig. 2). The brood size manipulation had a major effect on mass at the age of 15 days: birds from large broods were 1.2 g lighter (11% at 10.0 g) than those from small broods (F_{220.6}=51.2; p=0.0002; Fig. 2). This effect decreased with age (F_{780}=18.8; p<0.0001; Fig. 2) and at the age of 100 days they were still 0.65 g (4% at 14.2 g) lighter which remained significant (F_{246.5}=14.5; p=0.0002; Fig. 2). Thus growing up in large broods impaired growth, which effect was followed by a partial compensation response. Note that the effect of the brood size manipulation on mass remained throughout adulthood at 0.56 g (Chapter 11).
Fig. 2 Birds from large broods have impaired growth which effect decreases with age. Shown are effect sizes ± 95%CI, i.e. a standardized difference quantified as Cohen’s d: (m_{large} - m_{small}) / SD, with m_{large} and m_{small} being the mean value for birds from large and small broods respectively. Vertical dotted line shows d=0 no difference between birds from large and small broods and left of the vertical line shows better growth in small broods. Open dot shows an effect size on mass before manipulation, i.e. where there should be no brood size effect. Size refers to structural size, a standard normally distributed pooled measure of tarsus and headbill (see above).

The effects on mass can partially be mediated by size. Indeed, birds from large broods tended to have smaller tarsi ($F_{250}=2.83; p=0.09$) than birds from small broods and this was independent of age ($F_{502}=0.01; p=0.99$; Fig. 2). They also had smaller headbills, which effect was most pronounced at young age ($F_{248}=6.88; p=0.0092$) and decreased with age ($F_{466}=8.31; p=0.0003$; Fig. 2) until the age of 100 days at which headbills were slightly but not significantly smaller ($F_{248}=1.67; p=0.19$; Fig. 2). Overall young from large broods were smaller in size ($F_{250}=9.82; p=0.0019$), which effect weakly decreased with age ($F_{495}=2.12; p=0.12$; Fig. 2). Birds from large broods also had smaller wings ($F_{264}=4.58; p=0.03$), which effect did not change with age ($F_{471}=0.01; p=0.98$; Fig.
2). Taking the effects of size into account, the effect of the brood size manipulation on size corrected mass followed a very similar growth trajectory as that of mass, with the strongest effect at early growth ($F_{225}=56.6; p<0.0001$), followed by a partial compensation response ($F_{492}=4.8; p=0.0088$; Fig. 2). Note that the brood size effect on size corrected mass also remained throughout adulthood (Chapter 11). Thus, birds from large broods were lighter and smaller in size than birds from small broods and partially compensated in both mass and size.
References


