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Review

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

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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 to wild type controls in SLEs and more challenging environments in various model organisms such as yeast *Saccharomyces cerevisiae*, the nematode worm *Caenorhabditis elegans*, the fruitfly *Drosophila 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 the 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.

Contents

1. 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 (Gems and Partridge, 2013; Kenyon, 2005, 2010; Partridge, 2010). The effect of these genetic mutations can be enormous, with for example some mutants living up to 10 times longer than wild type controls

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(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 (Fontana et al., 2010; Gems and Partridge, 2013; Kenyon, 2005, 2010). 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 (Flatt et al., 2013; Stearns, 1992). 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.

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 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 arise through differences in survival, in reproduction or a combination of the two, and while competition experiments quantify fitness, they rarely quantify survival 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 last only one generation and exclude 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.

2. Materials 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 wild type controls (Delaney et al., 2011; Jenkins et al., 2004; 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 (Flatt et al., 2013; Gems et al., 2002; Partridge and Gems, 2007; Tatar, 2007; Tatar et al., 2014; Van Voorhies et al., 2006). 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 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

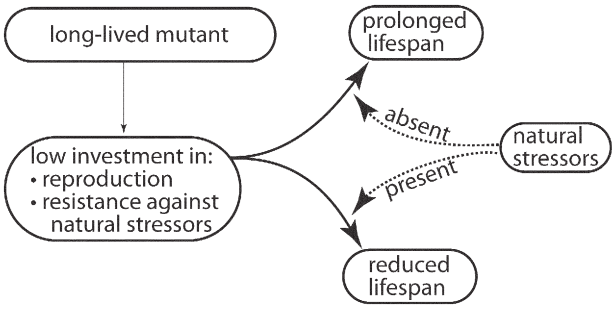

Fig. 1. Hypothesis, based on the optimality theory of aging (Partridge and Barton, 1993) stating that the lifespan advantage of long-lived mutants is diminished in the presence of natural stressors that are as a rule absent from standard laboratory environments.

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 a variety of diet concentrations can fulfill the challenging criteria if food dilution was applied to the extent that it shortened 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.

3. Results

3.1. 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 yeast (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; Savory et al., 2014; Wit et al., 2013), 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 wild type controls and this seems most pronounced in challenging environments.

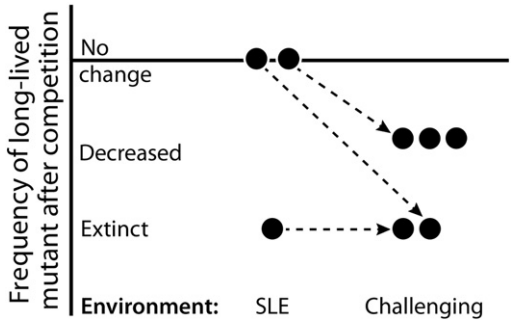

Fig. 2. Outcome of competition experiments between long-lived mutants and wild type controls. The outcome is from the perspective of the long-lived mutant. Arrows connect experiments that were done in the same study. One additional study in yeast is discussed separately in the main text because it consisted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

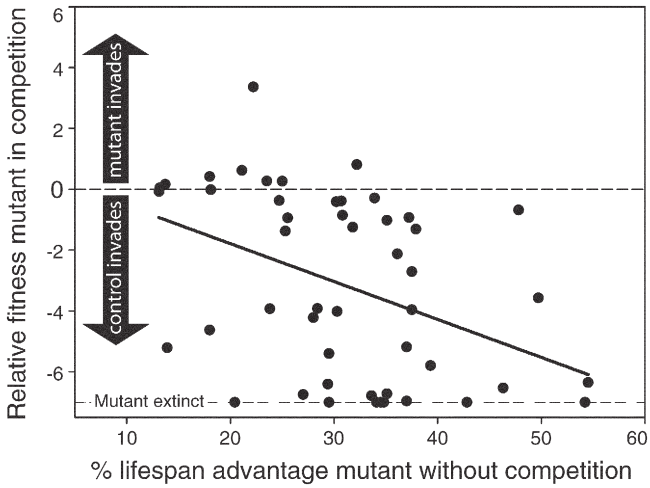

Fig. 3. Association between the lifespan advantage of 49 long-lived yeast mutants over controls in SLEs (standardized laboratory environments) and their fitness (dis)advantage in competition experiments. Relative fitness (RF) is defined as log base 2 ratio of mutant to wild type relative to the initial 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: $R^2 = 0.16$, $t = -3.13$, $p = 0.003$.

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 matched wild type controls. 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 life 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.

3.2. Lifespan of long-lived mutants in environments other than SLEs

The competitive disadvantage of long-lived mutants relative to 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 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 (Hwangbo et al., 2004; Marden et al., 2003). 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

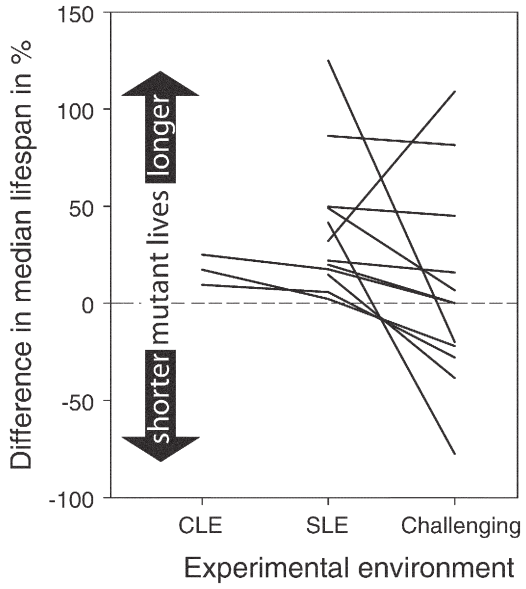

Fig. 4. Lifespan advantage of the long-lived mutants over the wild type controls is environment dependent. Lines connect environmental manipulations carried out within one study. CLE: cafeteria style laboratory environment, SLE: standardized laboratory environment, and challenging: environment was made 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.

this question we reviewed the studies that compared the lifespan advantage of long-lived mutants over 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 Section 2, 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) 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.

Of the studies listed above only two were on vertebrates (mice). Snell dwarf mice 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 (Flurkey et al., 2001; Schneider, 1976; Shire, 1973; Silderberg, 1972) 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 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 $p66^{Shc}$ knockout mouse. $P66^{Shc}$ 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 (Berniakovich et al., 2008; Fadini et al., 2010; Menini et al., 2006; Migliaccio et al., 1999; Ranieri et al., 2010). However, in an outdoor enclosure where mice were exposed to natural variation in temperature, food competition and to predators, their survival advantage became a disadvantage: after 8 months, 18% of controls were alive while only 5% of $p66^{Shc}$ 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.

3.3. 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% (Broughton et al., 2010; Clancy et al., 2002). 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 in CLEs (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.

4. Discussion

We investigated to what extent the performance of long-lived mutants over wild type controls depends on the study environment, because this sheds light on the question whether mechanisms causing the extended lifespan in SLEs 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; Linnen et al., 2001; Sgrò and Partridge, 2000; Sgrò et al., 2013). This argument also applies when the underlying mechanism is not related to reallocation 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 (Boonekamp et al., 2014; Lee et al., 2015, 2013) 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 *D. melanogaster* and the mouse *M. 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 $p66^{Shc}$) are one way or another involved in metabolism and energy balance. When these long-lived mutants are faced with food related challenges, genotype × environment interactions can be expected, but this does not make them less relevant given that food related challenges are common in nature. It needs to be investigated whether metabolism related 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.

Acknowledgments

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Appendix A. Supplementary data

Supplementary data to this article can be found online at http://dx.doi.org/10.1016/j.exger.2015.09.002.

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Supplementary information to: What can long-lived mutants tell us about mechanisms causing ageing and lifespan variation in natural environments?

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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.

Species	Mutant	Function	Outcome in SLE	Challenge	Outcome in challenging environment	Reference (Location)
S. cerevisae	49 genotypes	Various	NA	Cyclic starvation	84% decrease or extinct (65% siginificant); 16% increase (4% significant), no invading genotypes	Delaney et al., 2011 (Table 1)
C. elegans	*daf-2*	Insulin signaling	Extinct	Cyclic starvation	Extinct	Jenkins et al., 2004 (Fig. 1)
C. elegans	*age-1*	Insulin signaling	NS	Cyclic starvation	Extinct	Walker et al., 2000 (Fig. 1)
C. elegans	*age-1*	Insulin signaling	NS	Limited food	Decrease	Savory et al., 2014 (Fig. 1)
D. melanogaster	3 longevity lines	Unclear	NA	Field release with food searching	Decreased recapture probability	Wit et al., 2013 (Fig. 2, Table 5)
M. musculus	*p66Shc*	Various	NA	Outdoor enclosure with food competition	Decrease significantly within 1 or few generations. Wild type invaded to 75%	Giorgio et al., 2012 (Fig. 1)

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.

				Lifespan [Days]						
					SLE		Challenging		Statistics G x E	
Study organism	Mutation	Function	Description environmental challenge	Trait	Controls	Mutants	Controls	Mutants	Interaction	Reference
Environmental manipulation: natural challenge										
C. elegans	daf-2	Insulin signaling	Heat treated soil	Median	12	27	1	0.8	Yes	Van Voorhies et al. 2005 (Fig.2)
C. elegans	daf-2 (mean)	Insulin signaling	Pathogen	Median	13.1	24.4	2	3.63	NST	Garsin et al. 2003 (Table S1)
C. elegans	age-1	Insulin signaling	Pathogen	Median	13.1	19.6	2	2.9	NST	Garsin et al. 2003 (Table S1)
D. melanogaster	mth (heterozygote)	Stress response	Reproduction	Mean	26	31	23	23	Yes	Baldal et al. 2006 (Fig.3)
D. melanogaster	mth (heterozygote)	Stress response	Constant moderate heat stress	Mean	41	50	25	29	NST: Yes	Baldal et al. 2006 (Fig.3)
D. melanogaster	chico (homozygote)	Insulin signaling	Starvation	Mean	52	55	43	31	NST: Yes	Clancy et al. 2002 (Fig.1)
D. melanogaster	IPC KO (dilp2)	Insulin signaling	Starvation	Median	66	78	22	22	NST: Yes	Broughton et al., 2010 (Table 1)
D. melanogaster	Azot (homozygote)	Elimination of malfunctioning cells	Constant moderate heat stress	Median	25.9	34.2	7.8	16.3	NST	Merino et al. 2015 (Fig.7N vs. Fig.6Y)
D. melanogaster	indy206 (heterozygote)	Krebs cycle	Eliminating Wolbachia infection	Median	45	67	45	48	Yes	Toivonen et al. 2007 (Fig.5B)
D. melanogaster	mth (homozygote)	Stress response	Cold stress	Mean	138	141	5	3.9	NST: Yes	Mockett and Sohal 2006 (Table 1)
M. musculus	Snell dwarf mice	Insulin-like growth factor-1	Pathogen? Body Temperature homeostasis?	Mean	831	1178	600	135	NST: Yes	Bartke 2006 (p. 404); Fabris et al. 1972 (Fig.1); Flurkey et al., 2001 (Fig.1)
M. musculus	Hspc66 (heterozygote)	Metabolism	Outdoors with food competition and predators	15% Survival	820	940	390	240	NST: Yes	Giorgio et al., 2012 (p. 163 paragraph 2); Migliaccio et al., 1999 (Fig.6)

Study organism	Mutation	Function	Description environmental challenge	Lifespan [Days]					Statistics G x E Interaction	Reference
				Trait	SLE		Challenging			
					Controls	Mutants	Controls	Mutants		
Environmental manipulation: CLE										
D. melanogaster	indy (homozygote)	Krebs cycle	CLE	Median	43	44	35	41	Yes	Wang et al. 2009 (Fig.1A; Table S1)
D. melanogaster	chico	Insulin signaling	CLE	Mean	52	55	42	46	NST: No	Clancy et al. 2002 (Fig.1)
D. melanogaster	IPC KO (dilp2)	Insulin signaling	CLE	Median	66	78	60	75	NST	Broughton et al., 2010 (Table 1)

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