Targeting environmental and genetic aspects affecting life history traits

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Chapter 6

Methuselah life history in a variety of conditions, implications for the use of mutants in longevity research.

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Abstract

The laboratory has yielded many long-lived mutants of several model-organisms in the past few years. Many of the resulting claims for extended longevity have been nuanced or shown to be restricted to specific conditions, including environments and genetic backgrounds. Here, we test whether the long-lived mutant fruit fly *methuselah* (*mth*^{1}) displays its apparent superiority in longevity and stress resistance in different environments, at different ages and in correlated traits. The results demonstrate that stress resistance at different times in life is not consistently higher in the mutant relative to its progenitor strain (*w*^{1118}). Furthermore, the *mth*^{1} genotype only leads to an increase in longevity in an environment where reproduction is not stimulated. Also, virgin and mated life span were compared and showed that mating negatively affects life span, especially in the *mth*^{1} individuals. This reduced the life span enhancing effect of the mutation to zero. This apparent environment and mating dependent trade-off between longevity and reproduction supports the disposable soma theory of ageing. We conclude that these data can only provide limited information on natural variation. The data show the need to uncover the full complexity of variation in such traits in natural environments.

Keywords
Methuselah, longevity, mutant, *Drosophila*

Introduction

Several different long-lived *Drosophila melanogaster* mutants have been identified in the past years (e.g. Lin et al. 1998; Rogina et al. 2000; Clancy et al. 2001; Tatar et al. 2001). All have been created or discovered in the laboratory and do most probably not represent naturally occurring standing genetic variation. It is not known whether these mutations have fitness effects in natural environments, even if the extended longevity is the product of pleiotropy and not the sole trait affected. Longevity as a trait is situated in the selection shadow and can thus not be directly selected for in nature (Hoekstra 1993). The selection shadow comprises the age range in which natural selection cannot act because individuals are unlikely to live to these ages. This means that no selective pressure in favour of reaching this old age is present. Exception to this rule may be those species where (grand)parental care adds to survival, such as primates. There, the elderly have their use in reproductive success. Yet, also in this case there is a selection shadow, only longer after reproduction has ceased. The added scientific value of long-lived mutant strains at present lies mainly in the identification of pathways that could determine longevity. Research into such pathways should elucidate whether they are relevant to the variation in longevity in natural systems or not (cf. Flatt 2004; Geiger-Thornsberry and Mackay 2004).

The link between the laboratory and nature is important when addressing the evolution of life span using mutant analysis. One example is the dwarf *chico*^{1}-mutant, which affects insulin signalling (Clancy et al. 2001). The associated increase in longevity of this mutation was found to be highly dependent on the food environment (Clancy et al. 2002). A similar scenario applies to the long-lived *Indy* mutant (Rogina et al. 2000), a gene involved in intermediary food metabolism. Under adverse food conditions *Indy* displayed a lower fertility than controls, whereas the differences were reversed when food was present in excess (Marden et al. 2003).

Here, we focus on a mutant of the *methuselah* (*mth*) gene, which has been shown to be involved in longevity in the laboratory. The *mth*^{1} mutant line was originally derived from the *w*^{1118} strain by allowing a transposon (P-element) to randomly insert in genes (Lin et al. 1998). Strikingly, apart from the increased longevity, the *mth*^{1} mutant individuals do seem to functionally age in a similar way as the control flies (Cook-Wiens and Grotewiel 2002). This means that *mth*^{1} individuals are capable of normal behaviour for about as long as *w*^{1118} individuals, but become less active afterwards, when the control strain is already dying off. Thus, when it comes to actual life span, the *methuselah* mutant outlives *w*^{1118}. This may indicate that there is not a real advantage of living longer for the mutant *mth*^{1} individuals. *mth* encodes a G-protein coupled receptor involved in neurotransmitter release (Song et al. 2002). Natural variation in the *mth* gene has been reported to show correlations with life span and body size over a latitudinal gradient (Schmidt et al. 2000), however, no such patterns could be identified in a homolog (Duvernell et al. 2003). This indicates that *mth* is linked to life span and that its homologs do not show similar patterns. The specific selection on the *mth* gene, associated with lifespan, makes *mth* an interesting gene for further study. The fact that natural variation exists for the *mth* gene and that longevity per se is not under selection in natural *Drosophila* populations suggests that the increased life span may be the by-product of selection on other traits, such as body size and reproductive patterns, in specific environments.

In the original paper, the long-lived *mth*^{1} flies were shown to have elevated starvation, paraquat and heat resistance relative to their progenitor strain (Lin et al. 1998). In the light of the earlier comments on laboratory specific physiology, natural variation, and earlier findings in mutant strains the overall superiority of this mutant is investigated here. Lin et al. (1998) tested paraquat and starvation resistance at specific ages. These traits may develop over time and therefore, the traits are measured here at several ages.

Because the P-element interferes with *mth* expression, neural signalling will be lower. In order to function properly, we hypothesise that the mutant requires affluence, i.e. high caloric conditions (cf. Clancy et al. 2002). Notably, our food rich medium contained more yeast, inducing reproduction (Simmons and Bradley, 1997). Therefore, we hypothesised that *mth*^{1} individuals that carry the *mth*^{1} allele will suffer from a negative effect of reproduction, which is often found to negatively correlate with longevity. We tested this hypothesis by assaying lifetime reproductive success of females and examining whether differences could be found in early and late life reproductive success. Such findings would be in line with the disposable soma theory of ageing. This theory states that the body of an individual is not of interest to evolutionary processes, but that the transmission of genetic material to the next generation is. Therefore, when an individual meets conditions in which it is favourable to reproduce it does so on the cost of the body. Here, the results of the tests of both the environmental and temporal specificity hypothesis and the reproductive alteration hypothesis are presented and discussed in the light of evolutionary physiology theory.

The aim of this study was to examine the gene-by-environment interactions that modify the life history of *mth*^{1}-bearing *Drosophila melanogaster*. Our results explain why the information inferred from *mth*^{1}, and longevity mutants in general, should be treated with caution when extrapolating laboratory results to naturally occurring variation in ageing and longevity. They provide insight in the importance of the test environment on life history correlations, especially the trade off between reproduction and lifespan.

Materials and methods

Material

Mth^{1} flies were derived from the *w*^{1118} strain by Lin et al. (1998) through the creation of P-element insertion lines. *Mth*^{1} is genetically different from *w*^{1118} only because it has a P-element (P{LacW}*mth*^{1}) insert in the third intron of the gene (Lin et al. 1998). This mutant is longer lived than its control line at both 29 and 25 ºC (Lin et al. 1998).

Fly rearing

Mth^{1} and *w*^{1118} flies were kindly provided by Professor Seymour Benzer, Caltech University. They were kept in the lab for over 10 generations for acclimation to the local culture conditions. All stock maintenance and experiments took place at 25 ºC, 50% relative humidity and a 12/12 hour day/light regime, unless indicated otherwise.

Flies were maintained in half-pint bottles containing 24 ml of standard medium. Standard medium consisted of 20 g agar, 9 g kalmus (10 parts acidum tartaricum, 4 parts ammonium sulphate, 1 part magnesium sulphate and 3 parts potassium phosphate), 10 ml nipagin (100 g 4-methyl hydroxy benzoate per liter ethanol), 50 g saccharose and 35 g granulated yeast per liter water. For experiments, flies were reared at a controlled density of 100 eggs per vial of 6 ml standard medium.

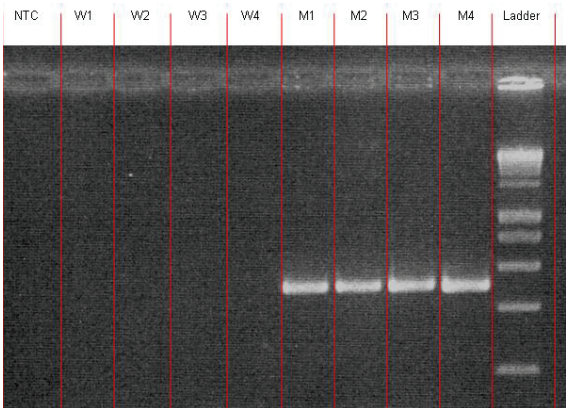

Figure 1. Gel showing the result of the PCR test on the P-element insert in methuselah and its absence in w^{1118}. NTC means No template control; W1-4 mean samples of w^{1118}, M1-4 mean samples of methuselah; Ladder means smart ladder with bands on 200, 400 500 etc. base pairs. Evidently, the no template control and w^{1118} lanes are empty, whereas the methuselah lanes show PCR product. The no template control shows that the PCR product is not produced in the absence of a template. The W-samples demonstrate that in the control strain no P-element insert is present in the *mth* gene. The M-samples demonstrate that the P-element insert is present in the *mth* gene. Therefore, the methuselah and w^{1118} strains we worked with are considered equal to the ones as used by Lin et al. (1998) and Cook-Wiens and Grotewiel (2002).

P-element check

We tested whether the P-element (P{LacW}mth^1) insert was still present in the *methuselah* gene. We isolated DNA from 4 groups of 10 female flies for both the mth^1 and w^{1118} strains using a Qiagen DNeasy minicolumn kit. We used primers that were originally used by Cook-Wiens and Grotewiel (2002);

Mth specific primer: 5'-ggtaattcctgtcaggaccaccg-3'
P-element primer: 5'-cgacgggaccaccttatgttatttcatcatg-3'

In a 25µl PCR reaction we used 2.5µl Qiagen 10* PCR Reaction buffer, 2µl 2.5µM dNTPs, 2.5 u Qiagen Taq DNA polymerase, 1µl 10µM *Mth* specific primer, 1µl 10µM P-element specific primer, 2 µl template (200ng), 16µl H_2O. We used the following PCR programme on a Biometra T-gradient thermocycler; 1. 95ºC 10 minutes, 2. 95ºC 30 seconds, 3. 72ºC 2 minutes 30 seconds, 4. 72ºC 5 minutes, 4ºC ∞, with a 30 time repeat of steps 2 and 3.

The results are shown in figure 1. The no template control shows that the PCR product is not produced in the absence of a template. The W-samples demonstrate that in the control strain no P-element insert is present in the mth^1 gene. The M-samples demonstrate that the P-element insert is present in the mth^1 gene. Therefore, the mth^1 and w^{1118} strains we worked with are considered equal to the ones as used by Lin et al. (1998) and Cook-Wiens and Grotewiel (2002).

Longevity assays

Virgin flies
Flies were sexed after eclosion and maintained as virgins. For both sexes we assayed the life span of 50 animals. In the longevity assays, flies were examined at a density of 1 individual per vial. Each standard vial contained 6 ml of either standard or double medium, the latter having the yeast and sugar concentrations doubled relative to the standard medium (table 1). These experiments were conducted at either 25 or 18 ºC. Mth^1 flies were *a priori* thought to increase their lifespan differences in the presence of more abundant food conditions (cf. Clancy et al 2002). Flies were checked daily for mortality and vials were replaced weekly for ones with fresh medium.

Table 1. Yeast and sugar quantities in grams per litre of agar medium used in relevant experimental systems with *D. melanogaster*. The column 'Extra' comprises additional nutrients, not including antibiotics and agar.

Author and medium	**Yeast**	**Sugar**	**Extra**
Baldal et al. (in prep) standard medium	35	50	-
Baldal et al. (in prep.) double medium	70	100	-
Lin et al. (1998) medium (pers. comm. G. Carvalho)	15	75	83 cornmeal
Cook-Wiens and Grotewiel (2002) medium (pers. comm.)	10	50	33 cornmeal
Mair et al. (2005) normal medium	150	150	-
Mair et al. (2005) yeast restricted medium	65	150	-

Mated female flies
Longevity of mated females of both lines was determined by daily observation of the 50 vials, each containing a single female fly together with two males of the same genotype. Vials contained standard medium and yeast granules and were changed on a daily basis during the first five days following eclosion, and from then onwards with an interval of 3 days until the death of the female. Dead males were replaced with live ones from the stocks of the appropriate genotype.

Metabolic rate, paraquat- and starvation resistance

To assess superior performance of *mth*^{1} in our laboratory, we assayed the mutant flies and their controls for metabolic rate, paraquat and starvation resistance using the standard protocols of our own laboratory. Paraquat and starvation resistance were measured in groups of 100 individuals per sex and per line, 5 flies per vial, for each time point. All flies were virgins and maintained in vials containing standard medium until being assayed on the time points of 0, 3, 5 and 10 days from eclosion. Prior to the experiments, adults were kept at an adult density between 10 and 20 per vial (except for the experiment on starvation resistance, where besides the normal slightly varying density also some flies from a controlled adult density, 10 flies per vial, were tested).

Metabolic rate was measured for 35 flies divided into 7 groups of 5 individuals for each time point and genotype, before which they were anaesthetized on ice and weighed. The flies were then assayed in a 16-channel respirometer (Li-6251 CO_2 analyzer of Li-Cor) containing a small piece of agar medium to prevent the flies from dehydrating. Two channels were left empty as independent controls in each experiment. For each channel, 5 runs of respirometry were performed. The first 2 of these runs were discarded in each case because they generally showed elevated CO_2 levels due to the experimenter's exhaling when the flies were put into the channels by aspiration. The data thus comprise 3 separate runs of 7 groups of 5 flies per sex per line per feeding condition. Data were acquired and analyzed using the program Sable. The amount of CO_2 produced in the experiments was divided by the wet weight determined just before the experiment to get an estimate of the mass specific metabolic rate.

Paraquat resistance was measured in virgin flies at a density of 5 flies per vial. Flies were given agar plugs (20gr agar per liter water) to provide moisture and 500µl of a 5% sucrose, 30mM paraquat (methyl viologen, M2254, Sigma Aldrich) solution on 5 filtration papers (1 by 1 cm, as in (Vermeulen et al. 2006)) and the flies were checked for survival twice a day. Please note that where Lin et al. (1998) used 20mM of paraquat and looked at mortality percentages after certain hours we used 30mM and used all of the mortality data available.

Starvation resistance was measured in virgin flies at an adult density of 5 flies per vial, by putting them in vials containing containing 6 ml of agar medium (20 gr. agar, 9 gr. kalmus, 5 cc. nipagin per liter water). Flies were checked daily for survival.

Reproductive output

Reproductive output was measured for each female used in the mated-longevity experiment. We examined the number of adult progeny by scoring the number of eclosed adult offspring that was produced. During the period from the second to the fifth day from eclosion we examined the total number of adult progeny for each day. From the fifth day onwards, progeny output was scored for intervals of three days. After 4 weeks, the adult progeny output was measured in alternating intervals of 3 and 4 days.

Statistics

All data were analysed using JMP 5.0.1. Longevity data were analysed using Cox Proportional hazards analysis. The starvation and paraquat resistance data as well as the metabolic rate data and those on reproductive output were analysed using full factorial ANOVA. Fixed factors always included treatment (e.g. age of testing, mated vs. unmated longevity, early vs. late reproduction) and line, and in most cases the factor sex. *Post hoc* analysis was performed using a Tukey test in order to examine which groups differed from one another in the different experiments. Replicate vials were always nested into the factor sex and treated as a random factor and did not prove to significantly contribute to the observed variation. Different time points in starvation resistance, paraquat resistance and metabolic rate were analysed as category variables.

Results

Longevity of virgins

We examined whether there was a difference between the lines for each medium and temperature. On standard medium, in general the *methuselah* mutant lived significantly longer than w^{1118} (χ^2_1=46, P<0.0001), but on double medium this was reversed (χ^2_1=11, P=0.0007, see figure 2). In the overall analysis, the effects of the different alleles was dependent on the sex of the individuals (line*sex interaction χ^2_1=5.4, P=0.02), therefore, the sexes were analysed separately. On standard medium, female mth^1 flies were longer-lived than w^{1118} flies at both 18 and 25 ºC, whereas for males there were no significant differences. On double medium, we found that w^{1118} males at both temperatures lived longer than the experimental group, whereas w^{1118} females only lived longer at 25 ºC, but the pattern at 18. Females on double medium at 18 ºC was the same (see figure 2).

Thus, under standard conditions we confirmed the longer life span of the mth^1 mutant, but we could not on double medium. Also, the sexes revealed strong differences in their life span response as a result of the different alleles.

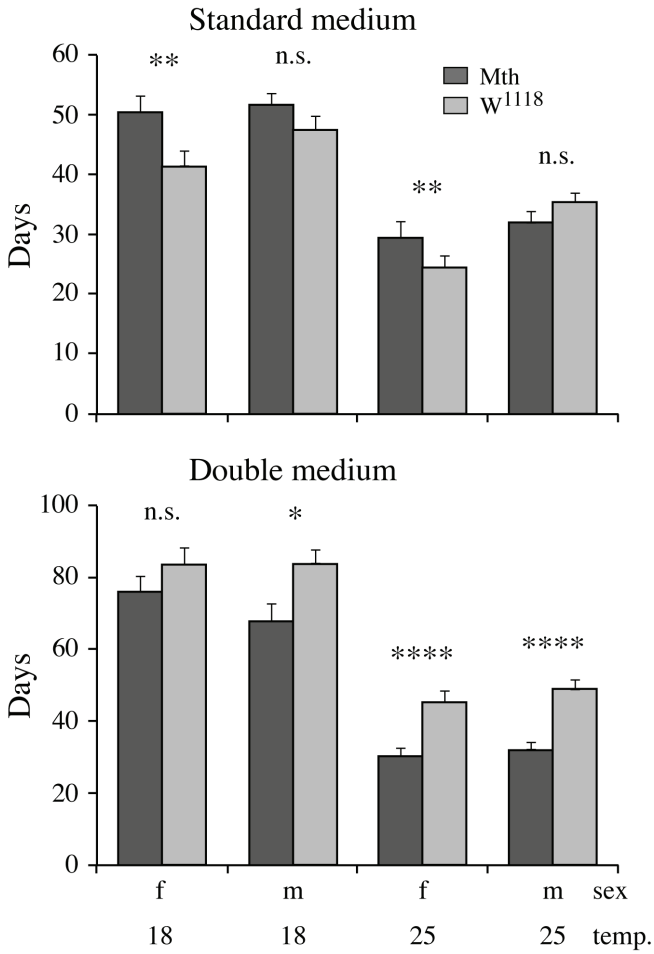

Figure 2. Longevity of both lines and both sexes with standard error bars on standard or double medium at 18 and 25ºC. n.s. = not significant, * <0.05, ** <0.01, **** <0.0001.

Longevity of mated versus virgin females at 25ºC

The overall analysis showed a significant effect of mating status (χ^2_1=5.25, P=0.022). Examining the effect of mating status per line (figure 3) reveals a significant negative effect of mating in *mth*^{1} females (χ^2_1=7.4, P=0.0064). In *w*^{1118} females a similar trend was observed, only the difference was not significant (χ^2_1=0.29, P=0.59). Overall, there was no significant effect of genotype on life span (χ^2_1=2.7, P=0.10). The apparent difference in longevity between the genotypes in virgins was not found in mated individuals. Thus, it was concluded that when mated, *mth*^{1} individuals lose their long-lived phenotype.

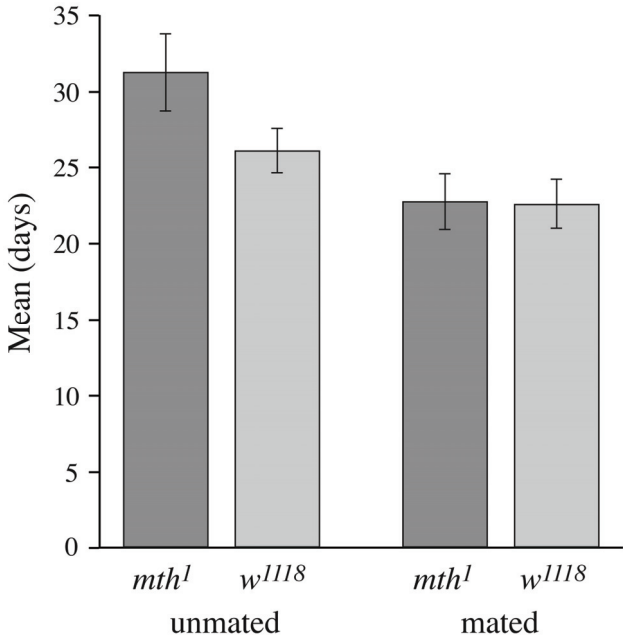

Figure 3. Average life span of unmated and mated females of lines *mth*^{1} and *w*^{1118} with standard error bars.

Metabolic rate

Mth^{1} flies of both sexes had a lower metabolic rate than *w*^{1118} flies at all ages measured (figure 4). In both sexes, at all ages, and time points, except for day 3 males, these differences were statistically significant. There was a remarkable increase in metabolic rate in *w*^{1118} males on day 5 just after the peak of female reproductive activity ($F_{3,160}$=5.0, P=0.0025, figure 5). Relative to the metabolic rate at other ages, the metabolic rate of *w*^{1118} males on day 5 proved to be significantly different from the other days when examined with *post hoc* Tukey testing. In females a similar trend was observed, but no significant differences were identified.

Paraquat resistance

There is a dramatic decrease in paraquat resistance with increasing age in both *mth*^{1} and *w*^{1118} flies (Figure 4). Males showed a higher average paraquat resistance than females ($F_{1,1583}$=18.8, P<0.0001). There is also a significantly higher paraquat resistance directly after eclosion in females of *w*^{1118} flies compared to *mth* females. Male *mth*^{1} flies remain more paraquat resistant than *w*^{1118} flies until day 10. In both sexes *mth*^{1} is significantly more paraquat resistant at days 3 and 5. The observed differences do not approach the extent of those found by Lin et al. (1998); especially the poor performance of the *w*^{1118} genotype could not be replicated.

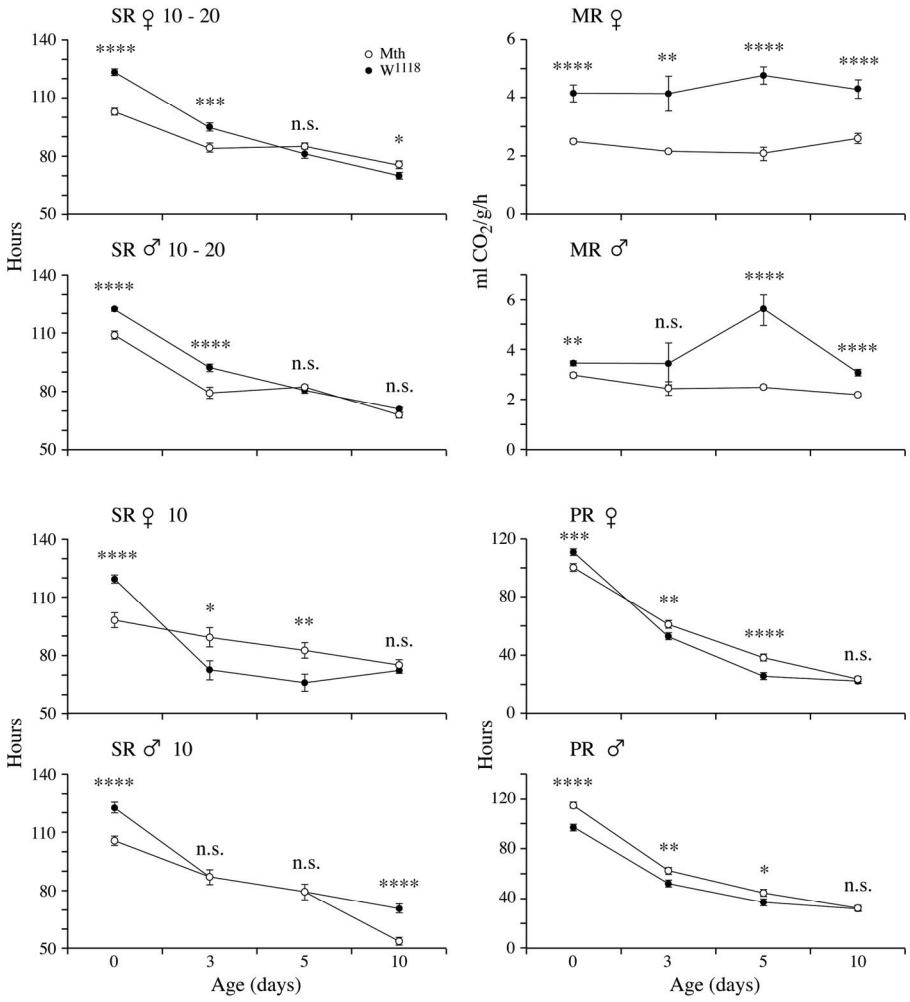

Figure 4. Patterns of traits on days 0, 3, 5 and 10 for both sexes of each line, all means ± s.e. Starvation resistance in hours (SR) at adult densities of 10 and 10-20 individuals per vial, mass specific metabolic rate in ml CO2/g/h (MR), paraquat resistance (PR) in hours. n.s. = not significant, * <0.05, ** <0.01, *** <0.001, **** <0.0001.

Starvation resistance

Starvation resistance showed a decline over lifetime as described earlier by Vermeulen et al. (2005). In adults kept at a density of 10-20 individuals, we found a noteworthy difference in starvation resistance in the first few days from eclosion when w^{1118} was highly resistant in both sexes relative to both mth^{1} and to its own resistance at older ages

(see Figure 4). From day 5 onwards, the difference became negligible and even switched to a slight but significant starvation resistance advantage for *mth*^{1} females at day 10 (Figure 4). In flies with controlled adult density, we observed a different pattern, where *mth*^{1} female flies at days 3 and 5 were significantly more resistant (Figure 4). Again, we were not able to reproduce the large differences and advantages of the P-element insert as observed by Lin et al. (1998). Our results do however suggest that at eclosion *w*^{1118} has a superior starvation resistance compared to *mth*^{1}. This result is consistent for the density groups.

Reproductive investment

The total number of live adult progeny was highly dependent on age ($F_{10,738}$=163, P<0.0001) and showed a significant age*line interaction ($F_{10,738}$=12.1, P<0.0001). Figure 5 shows that on day 2, and at a relatively old age (from day 23 on) the *methuselah* mutant flies exhibit a higher reproductive output whereas *w*^{1118} flies produce more offspring early in life (days 3,4 and days 9 to 12). On days 2 to 12 there was a significantly higher number of adult progeny output in *w*^{1118} ($F_{1,464}$=7.65, P=0.0059), whereas the reverse occurred on days 23-33 ($F_{1,92}$=8.56, P=0.0043). It is intriguing to notice that reproduction between days 0-2 is significantly lower in *w*^{1118} females compared to *mth*^{1} females. This may relate to the higher starvation resistance of the *w*^{1118} females, because starvation resistance and reproduction have been shown to phenotypically (Chippindale et al. 1993) and genetically (Zwaan et al. 1995a) trade off.

In summary, lifetime reproductive output of females of *methuselah* mutants and *w*^{1118} was equal, but *w*^{1118} displays higher early fecundity over the first 12 days, whereas *mth*^{1} had higher late life fecundity.

Discussion

General

We show here that the long-lived phenotype of the mutant *methuselah* is strongly dependent on the adult environment. We have presented evidence that implies that the earlier findings (Lin et al. 1998) on longevity and stress resistance must be treated with caution when extrapolated to other environments. Starvation- and paraquat resistance showed differences that are not consistent with earlier findings. In some cases *mth*^{1} flies outperformed their progenitor strain. However, in no case, was this increase in resistance consistently present over time. These differences may have to be attributed to differences in methodology and approach, but are reminiscent of how dependent these resistances are on test conditions. Furthermore, a novel finding is that the metabolism of *mth* mutants at 25ºC is always lower than that of *w*^{1118} individuals. We confirm the earlier findings concerning longevity of Lin et al. (1998) only in certain environments where reproductive output is low. Our data on the reproductive output suggest that the increased life span of the *methuselah* mutant may be the result of reduced early fecundity. Moreover, mated *mth*^{1} females were significantly shorter-lived than virgins, whereas the difference in *w*^{1118} females was less extreme, indicating an increased cost of reproduction in *mth*^{1} females. Maximal reproductive output at young age is probably

necessary to achieve high fitness in most environments in nature. The reduced early fecundity, together with the loss of longevity as a result of mating suggests that the particular *methuselah* mutant under study will not have superior fitness in such natural populations.

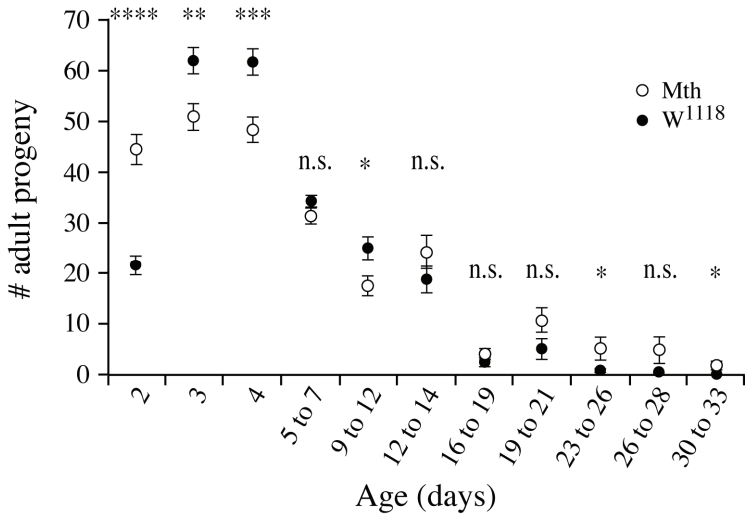

Figure 5. The number of adult progeny per day of female egg laying for each line. In the periods over more than one day, the number of individuals that eclosed were averaged to the number per day. n.s. = not significant, * <0.05, ** <0.01, *** <0.001, **** <0.0001.

Patterns over time and environments

Starvation resistance was measured at day 2 by Lin et al. (1998). Similar to their findings, in our experiments starvation resistance of animals kept at a density of 10 flies per vial showed that mth^1 females have elevated starvation resistance at day 3. The flies maintained at the density of 10-20 flies per vial showed that w^{1118} is superior to mth^1 flies in both sexes. This confirms advantages of the mth^1 P-element in a certain, relatively favourable environment. In a more stressful environment, such as higher adult density, the *methuselah* mutant is apparently outperformed. Our data also indicate that the relative difference among lines for a trait depends heavily on the age at which the data is collected. That w^{1118} have a higher starvation resistance at eclosion than mth^1 warrants further study which should involve fat allocation patterns.

Food and fecundity

The use of media other than the one used by Lin et al. may be responsible for the differences in survival. We also observed differences in life span on the double medium in the reversed direction (average longevity is generally higher in w^{1118}, see figure 2). The

amount of yeast the animals encountered in the double medium approximates that used by Mair et al. (2005) that facilitates life extension through caloric restriction (see table 1). The double medium yielded longevity values that approach the absolute maximum longevity of the lines on this medium. This is not the same as the maximal difference in longevity between the lines. The food condition of Lin et al (1998) is still the one under which the difference between mth^1 and w^{1118} is relatively the largest. They, like Cook-Wiens and Grotewiel (2002), used a medium where sugar concentrations (including cornmeal) were higher and yeast concentration was less than half that of our standard medium (see table 1).

Mair et al. (2005) found that reducing yeast calories had a disproportionate effect on longevity in relation to the removal of a similar amount of sugar calories from the adult media. This implies that the number of calories in yeast alone does not account for all of the longevity effects that occur with yeast removal. Yeast must therefore trigger additional processes. In *Drosophila*, the presence of yeast induces reproduction (Simmons and Bradley 1997) and is known to reduce longevity (Chippindale et al. 1993; Prowse and Partridge 1997). Here, we speculate that the relatively high proportion of yeast in our medium may reduce life span because of interactions with the reproductive system of the flies. This would be consistent with the reversal of longevity on double medium (see Figure 6). The condition dependent longevity difference between mth^1 and w^{1118} flies should therefore be explained as a classical genotype-by-environment interaction, which may involve differences in gene expression.

That reproductive biology was affected by the mutation was shown by the fact that w^{1118} females had significantly higher early life fecundity and mth^1females had significantly higher late life fecundity. Early life fecundity is often found to be associated with reduced longevity, and late life fecundity with increased longevity (Rose 1984; Leroi et al. 1994a; 1994b; Zwaan et al. 1995a, 1995b; Djawdan et al. 1996; Ackermann et al. 2001; Phelan et al. 2003). Rose's (1984, see his fig. 5) longevity associated late life fecundity seems similar to the combination of increased longevity of mth^1 under standard conditions and their late life fecundity.

Natural conditions and the relativity of superiority in the laboratory

Spencer et al. (2003) noted that there is a considerable demographic laboratory bias in the *Drosophila* populations under study. They argue that laboratory *Drosophila* are relatively short-lived. This could result in the situation where a mutant, whilst relatively 'long-lived' compared to its deficient progenitor strain, only restores a sub-optimal phenotype relative to natural populations. In such light, the superiority of the *mth* mutants is less striking. Nevertheless, the mutation causes a relative increase in life span under certain conditions and is therefore a candidate for further research into mechanisms prolonging life span. Schmidt et al (2000) found a positive correlation between latitude and both mth^1 allele frequency and longevity. They suggested that "in natural populations, selection on mth^1 may involve a pleiotropic trade-off between longevity and other fitness-related traits that are negatively affected". The fact that the *mth* locus carries imprints of past selection in natural populations (Schmidt et al. 2000; Duvernell et al. 2003) makes *mth* an even more important candidate for further study.

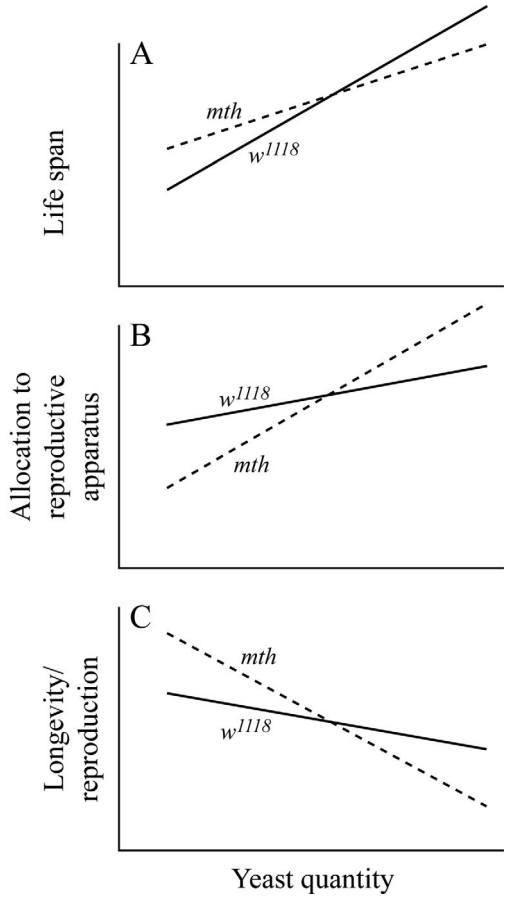

Figure 6. Hypothetical relationships of different traits with yeast quantity. In all figures the dotted line represents *mth* and the solid one *w1118*.
A. Hypothetical relationship between life span and yeast quantity in the medium. B. the hypothetical relationship between resource allocation and yeast quantity. The steeper reaction norm of the *mth* mutant indicates the larger influence of yeast on reproductive output relative to that on *w1118*. C. The relationship between longevity and reproduction as a function of yeast quantity. The steep reaction norm of longevity over reproduction in relation to yeast of *mth* results in higher longevity under poor yeast nutritional conditions.

Our results show that *methuselah* mutants underperform relative to its progenitor strain in most cases under conditions that arguably resemble a more natural situation. *mth* is likely to be under selection in nature (Schmidt et al. 2000; Duvernell et al. 2003), but our findings provide both evolutionary and mechanistic causal explanations as to why a

highly advantageous genotype similar to *mth*^{1} has not been identified in nature. One may argue that the fact that mutants like *mth*^{1} have not been found outside of the laboratory so far does not mean that they are not there. Yet, such advantageous alleles are likely to spread rapidly through the population. It would therefore be very interesting to have a functional comparison among the mutant and some long-lived individuals from natural systems with *mth* alleles with a natural selection imprint as described by Duvernell et al. (2003) and Schmidt et al.(2000). In this way, one could determine whether the selection imprint is acting in the same direction as the laboratory mutation.

Our data underline the importance of including temporal, physiological and environmental components in life history theory in general, and of trade-offs in particular. To identify these important components we will have to expand our knowledge of genetic and physiological systems at different levels. Kaitala (1987; 1991) and Leroi et al. (1994c) demonstrated the plasticity of trade-offs, which complicates investigation in natural systems considerably. Furthermore, the ubiquity of genotype-by-environment interactions makes predicting longevity on the basis of the *mth* genotype in unknown environments difficult.

Mutant analysis in life history theory

In all studies on life span enhancing genetic effects, the mutation takes place in a single gene. This gene may be pleiotropic in its nature and thus affect more than one trait. These effects are likely to induce the expression of a lot of genes. Yet, still a single gene would be responsible for the onset of increased longevity. From earlier experimentation we know that longevity is a quantitative trait (cf. Geiger-Thornsberry and Mackay 2004) and not determined by a single locus. This does not mean that the genes found in mutant research are useless and can be refuted. On the contrary, variation in these genes is likely to contribute to life span. From the wide variety of pathways and processes that are involved in life span determination, we know that even a single mechanism is unlikely to explain all of the life span variation present in nature. Variation at the *mth* locus in nature (Schmidt et al. 2000) is found to be correlated with longevity, which confirms that mth is related to longevity as found by Lin et al. (1998). Therefore, the mth locus is in part responsible for the variation in life span in natural systems, but it is not clear whether the genetic effect induced by the P-element insert is important in nature.. Because there are a lot of these kinds of mechanisms, one should be modest about the implications of finding a longevity associated gene.

When in natural populations only a smaller part of the variation can be explained by the variation on a single locus, the long lived phenotype must also be ultimately dependent on other, genetic or environmental factors. In the era of full genome expression analysis, one should be aware that the genetic background in which a single mutation operates is very important. This may or may not facilitate desirable pleiotropic effects. Even so, as proven here, the environment in which the life span enhancement is found is also of considerable importance, let alone genotype-by-environment interactions.

In the *mth*^{1} example one works with a hampered protein involved in neural transmission. The protein has been selected under natural conditions to be functional and is thus likely to be adaptive in these selective environments. If it does not, no selection imprint could

have been identified on it. A hampered protein loses part of its functionality and will thus not allow an animal to be fully functional in the environment it was selected in.

Though seemingly advantageous under certain laboratory conditions, under natural conditions large disturbances of the homeostasis are seldom beneficial. We, therefore, argue that mutation research is valuable, but only to provide us with candidate mechanisms that may form parts of the longevity puzzle, which, next to genetic components, also involves physiological and environmental aspects and the interactions among all of these. Our work thus contributes to understanding the interplay between genetics and environment in determining the ageing and longevity phenotype.