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RESEARCH ARTICLE

Elevated extension of longevity by cyclically heat stressing a set of recombinant inbred lines of *Drosophila melanogaster* throughout their adult life

Federico H. Gomez · Pablo Sambucetti · Fabian M. Norry

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Abstract An extremely high (about 100 %) increase in longevity is reported for a subset of recombinant inbred lines (RILs) of *Drosophila melanogaster* subjected to a cyclic heat stress throughout the adult life. Previous work showed that both longevity and heat sensitivity highly differed among RILs. The novel heat stress treatment used in this study consisted of 5 min at 38 °C applicated approximately every 125 min throughout the adult life starting at the age of 2 days. In spite of the exceptionally high increase in longevity in a set of RILs, the same heat stress treatment reduced rather than increased longevity in other RILs, suggesting that heat-induced hormesis is dependent on the genotype and/or the genetic background. Further, one quantitative trait locus (QTL) was identified for heat-induced hormesis on chromosome 2 (bands 28A1-34D2) in one RIL panel (RIL-D48) but it was not significant in its reciprocal panel (RIL-SH2). The level of heat-induced hormesis showed a sexual dimorphism, with a higher number of lines exhibiting higher hormesis effects in males than in females. The new heat stress treatment in this study suggests that longevity can be further extended than previously suggested by applying a cyclic and mild stress throughout the life, depending on the genotype.

Keywords Hormesis · Lifespan · Heat-shock stress · Quantitative trait locus (QTL)

Introduction

Stress treatments for extended longevity and slowed senescence have received special attention in model animals, especially because of their implications in human gerontology (Rattan 2008; Calabrese et al. 2015). Hormesis is a beneficial effect of mild stress involving the induction of mechanisms that protect against stress itself. Hormesis in longevity is the increase in life span of an organism resulting from its previous exposure to one moderate environmental stress. For instance, a mild heat stress early in life can increase longevity in about 25 % in *Drosophila* (Lithgow et al. 1995; Khazaeli et al. 1997; Hercus et al. 2003; Le Bourg 2011; Scannapieco et al. 2007).

The expression of heat-shock proteins (Hsps) is almost certainly one system that upon activation can increase both longevity and resistance to several types of stresses (e.g., Olsen et al. 2006). However, the positive correlation between life span and stress resistance is not always

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F. H. Gomez (✉) · P. Sambucetti · F. M. Norry
Departamento de Ecología, Genética y Evolución, Facultad de Ciencias Exactas y Naturales, Universidad de Buenos Aires – IEGEBA (CONICET-UBA), C-1428-EHA Buenos Aires, Argentina
e-mail: fedegz@ege.fcen.uba.ar; fedehgz@hotmail.com

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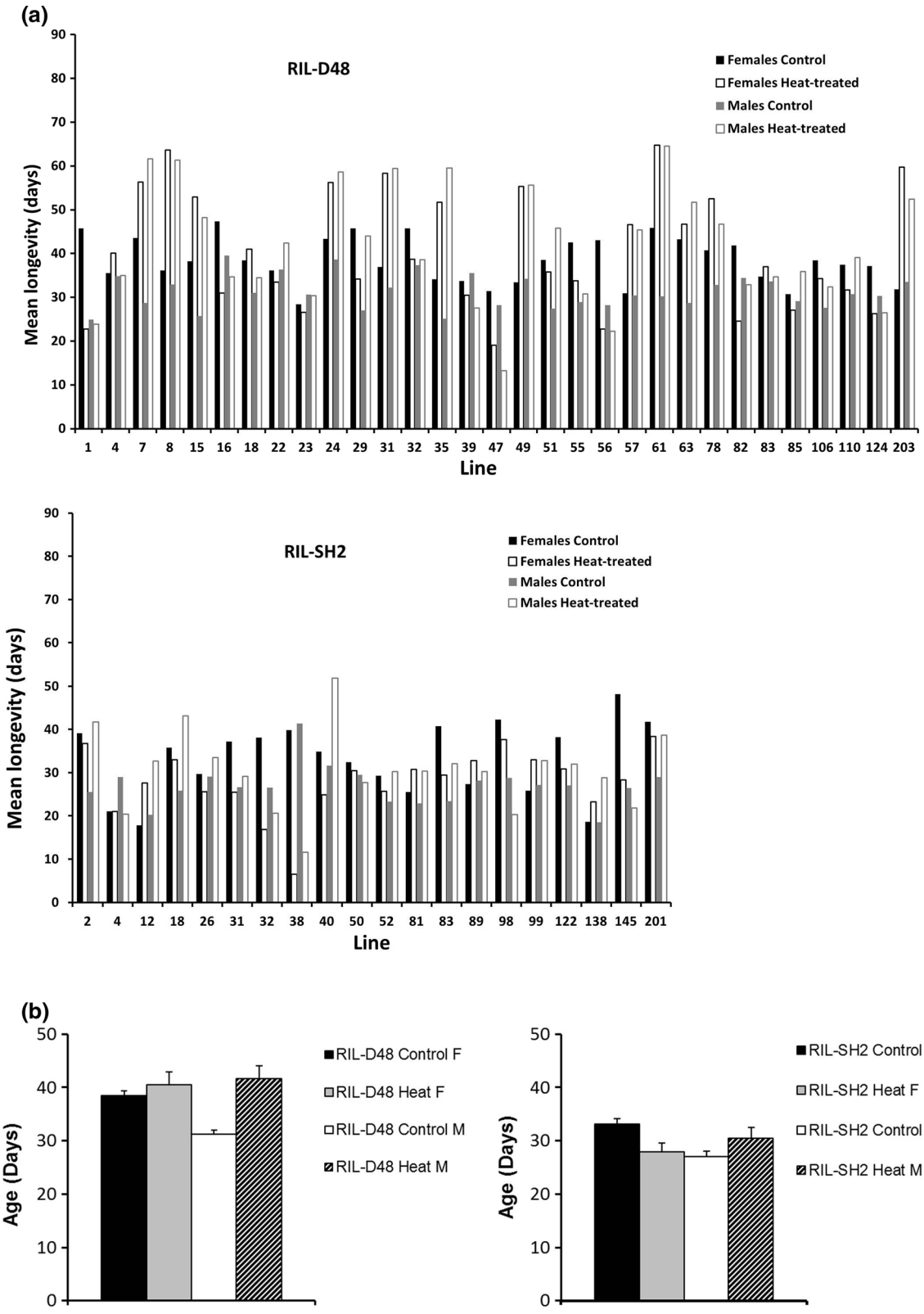
(a)
RIL-D48
Females Control
Females Heat-treated
Males Control
Males Heat-treated
Mean longevity (days)
Line
1 4 7 8 15 16 18 22 23 24 29 31 32 35 39 47 49 51 55 56 57 61 63 78 82 83 85 106 110 124 203
RIL-SH2
Females Control
Females Heat-treated
Males Control
Males Heat-treated
Mean longevity (days)
Line
2 4 12 18 26 31 32 38 40 50 52 81 83 89 98 99 122 138 145 201
(b)
Age (Days)
RIL-D48 Control F
RIL-D48 Heat F
RIL-D48 Control M
RIL-D48 Heat M
Age (Days)
RIL-SH2 Control F
RIL-SH2 Heat F
RIL-SH2 Control M
RIL-SH2 Heat M

◀**Fig. 1** **a** Mean longevity of each recombinant inbred line of *Drosophila melanogaster* assayed within the RIL-SH2 and RIL-D48 sets. **b** Mean longevity of the two sets of recombinant inbred lines, RIL-SH2 and RIL-D48. Mean survival of females (F) and males (M) (in days) was scored for all lines and then averaged within each RIL set (i.e. the average of 31 lines for RIL-D48 and 20 lines for RIL-SH2). *Error bars* correspond to the standard error of the mean

apparent (Phelan et al. 2003; Norry and Loeschcke 2003; Bubliy and Loeschcke 2005). Mild heat-stress pre-treatments had a beneficial effect (hormesis) more often in shorter-lived than in longer-lived inbred lines in *Drosophila melanogaster* (Defays et al. 2011). The genetic architecture of longevity is complex and the Hsp system is probably one among several systems that might lead to an extended life (Tower 2009, 2011).

The genetic background was found to dramatically influence the position of the hormetic zone in *Drosophila*, both between artificial selection regimes and among the lines within selection regime, suggesting that in genetically variable populations a life enhancing treatment in one individual could have no effect or even be detrimental in other individuals (Gomez et al. 2009, 2013; Sarup and Loeschcke 2011). However, it is still not sufficiently clear how heat-induced hormesis in longevity is dependent on genotype.

In model systems like recombinant inbred lines (RILs) in *Drosophila melanogaster*, heat-induced hormesis in longevity can be tested for a set of recombinant genotypes to identify genetic differences in the hormetic response to heat-stress treatments. Here we used a set of RILs that greatly segregated genetic variation in heat-stress resistance. We used such a RIL set because the two base lines used to construct it dramatically differed in thermal sensitivity (Norry et al. 2008), and because the lines also substantially differed in longevity (Defays et al. 2011; Sambucetti et al. 2015). This RIL set allowed us to test for between-RILs differences in heat-induced hormesis using a novel treatment of regularly repeated exposure to mild-heat stress along the full time of life in adult flies.

Materials and methods

RIL stocks

Lines in this study were described in Norry et al. (2008), and basic information follows. Two highly-inbred stocks were denoted D48 and SH2, extremely heat-sensitive and heat-resistant, respectively. D48 stock was derived from a wild population in Denmark and artificially selected for reduced resistance to high temperature knockdown in adults (i.e., an ecologically relevant trait of heat-sensitivity) before inbreeding (Norry et al. 2004). SH2 stock was derived from a wild population in Melbourne (Australia) and artificially selected for elevated resistance to heat knockdown in adults (i.e., the heat-resistant line) before inbreeding (McColl et al. 1996; Norry et al. 2004). F1-females obtained from crossing the two stocks were back-crossed to either D48 or SH2 males. The progenies were randomly mated for two more generations, individual pairs were set up, and the offspring of each pair was subsequently inbred by full-sib mating for fifteen consecutive generations to obtain the nearly homozygous RIL stocks. RILs are maintained using a population size of $N > 500$. Both the D48- and SH2-backcross derived lines (RIL-D48 and RIL-SH2 sets, respectively) were used. In total 51 RILs were used, 31 RIL-D48 lines plus 20 RIL-SH2 lines. All RIL were expanded for one generation from our stocks in 125-mL glass bottles containing 40-mL of dehydrated potato-based culture medium with water, nipagin and yeast. Two bottles were set up per RIL, with 20 males plus 20 females. After 48 h all flies were removed from the bottles. Experimental individuals were adult flies that emerged from standard culture bottles at 25 ± 1°C under a 12:12 h light–dark cycle.

Per RIL, two standard culture vials each one containing 15 males plus 15 females were set up and kept at 25 ± 1 °C, and 48 h later all vials were taken to a thermal treatment box for longevity measurement (30 flies of each line were measured per sex, control and heat-treatment).

Heat-stress treatment for hormesis induction

Experimental flies were placed into standard culture shell vials at 23 ± 1 °C for 48 h. All vials were then transferred to a water bath with a cooling system within a Lovibond thermal incubator (Germany) at 23 ± 1 °C. The water bath containing all experimental vials was connected to a Din Rail digital programmable timer switch and the system was regulated to increase the within-vial temperature from 23 ± 1 to 38 ± 1 °C in a time of 7 ± 1 min. The water bath was automatically switched off after 5 min at 38 ± 1 °C,

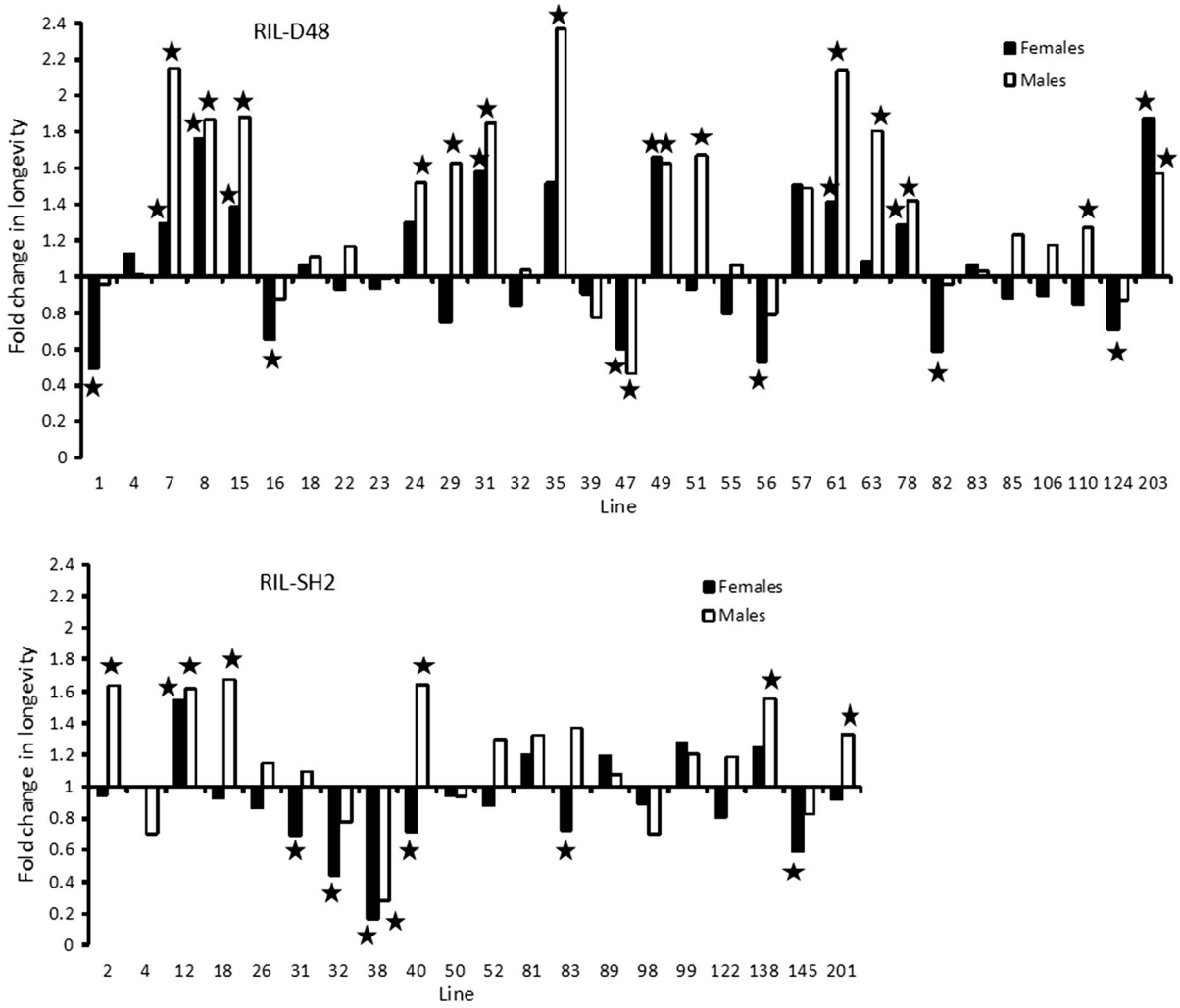

Fig. 2 The ratio of mean longevity in heat-stressed to control flies (i.e., an index of hormesis) is shown for females and males of each RIL-SH2 and RIL-D48 line. Significance was assessed by using the non-parametric Mann–Whitney test (control vs heat-treated flies). *P*-values were corrected for multiple comparisons by using a Bonferroni test. Significant values are indicated by an asterisk ($*P < 0.05$)

and it was again switched on after 125 min from the last 5-min interval at 38 °C. The time to return to 23 ± 1 °C was 100 ± 5 min within each vial in the water bath, with the cooling system switched on until achieving 25 °C within the first 20 min. This cyclic treatment of 5 min at 38 ± 1 °C followed by a 100 ± 5 min cooling period until return to 23 ± 1 °C for 25 min was applied throughout the experiment until the death of each fly. In short, the cyclic regime to which 2 days-old flies were subjected throughout the rest of their life was a repetition of the following: 5 min at 38 ± 1 °C followed by a longer cooling period of 100 ± 5 min until return to 23 ± 1 °C followed by 25 min at 23 ± 1 °C (i.e., flies received the 5-min heat treatment at 38 °C 11 times per day, every 125 min, until the death of each fly). Control flies received no heat-stress treatment but they were otherwise subjected to similar conditions as heat-stressed flies, kept in the same Lovibond incubator at 23 ± 1 °C without a cycling water bath.

RILs mean lifespan and hormesis induction

Hormesis in longevity was explored in a three-way ANOVA for longevity (in days) with [1] RIL panel (RIL-D48 vs RIL-SH2), [2] treatment (control vs heat-

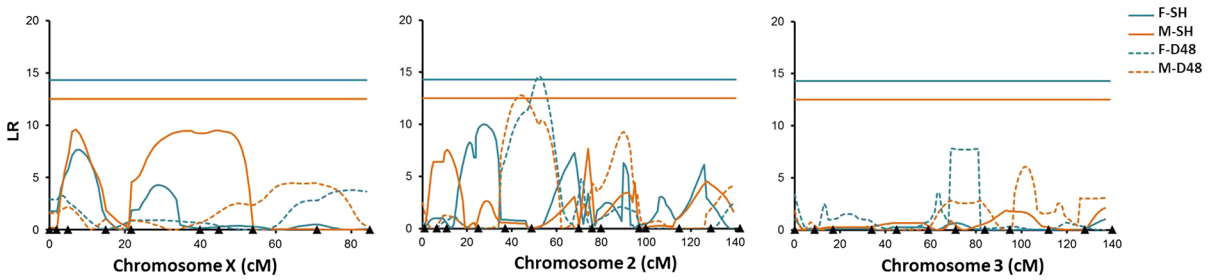

Fig. 3 Likelihood ratio (LR) as a function of the chromosome location (in cM). Composite interval mapping was performed on the ratio of mean longevity in heat-stressed to control flies (i.e., an index of hormesis, as in Fig. 2) in RIL-D48 and RIL-SH2 lines. A significant quantitative trail loci (QTL) peak was detected on chromosome 2. Significance thresholds were determined by 1000 random permutations ($P < 0.05$). Microsatellite marker positions are represented by *solid black triangles* on the horizontal axis. *F* females, *M* males, *D* RIL-D48 panel, *SH* RIL-SH2 panel

stressed flies) and [3] sex (male vs female) as fixed factors. In this ANOVA we used mean longevity of each RIL as dependent variable, which was normally distributed by averaging longevity over 30 individuals scored per RIL, sex and treatment. Hormesis was further tested for each line by using the non-parametric, two-tailed Mann–Whitney U test, which allowed us to test for longevity differences between control and heat-treated flies without making the assumption that longevity is normally distributed within each line. Both ANOVA and Mann–Whitney test were run using the Statistica package (StatSoft 1999). All *P*-values for hormesis tests were corrected for multiple comparisons by using a Bonferroni test (i.e., dividing *P*-values by the number of comparisons, e.g., 0.05/31 = 0.001613 for RIL-D48; 0.05/20 = 0.0025 for RIL-SH2). Additionally, we also performed a Log-rank test for differences in survival curves between control and heat-treated flies in each RIL (see supplementary Table S2), using online statistics calculators by Miller (2013). However, in several cases Log-rank test was significant not because mean longevity differed between control and heat-treated flies but because of variation in the survival curve only.

QTL analysis

The genetic map associated to the RIL-D48 and RIL-SH2 is based on 36 microsatellite markers (supplementary Fig. S2), as described in Norry et al. (2008). Marker genotypes were the number of SH2-alleles (0 or 2), as in Norry et al. (2008). QTL analysis for hormesis was performed on a ratio trait: the ratio between longevity in heat-stressed flies to longevity in control flies (i.e., the fold change in longevity induced by heat stress). Additionally, we also tested QTL for longevity (in days) in both control and heat-stressed flies (see supplementary Fig. S1). To test the hypothesis that an interval flanked by two microsatellite markers contains a QTL, a composite interval mapping procedure was implemented (Zeng 1994). The test was performed using the sib-mated RIL design (Ri2 design) under model 6 in the QTL-Cartographer Version 2.5 software for Windows (Wang et al. 2010), with 10 cM as window size and 5 control markers. Mildly altering the initial combination of mapping parameters yielded no difference, with significant QTL showing consistency across a range of parameter combinations. Significance thresholds were determined by 1000 random permutations ($P < 0.05$).

Pairwise epistatic interactions were tested by using a linear model of $y = m_x + m_y + m_x m_y + e$, where m_x and m_y are the genotypes of markers x and y (Morgan and Mackay 2006).

Results

Mean longevity is shown for both heat-treated and control flies in Fig. 1. There was high variation among RIL lines in their longevity responses to our heat treatment, with an exceptionally elevated extension of longevity in some lines, a reduction of lifespan in other lines, and one QTL on chromosome 2 (Figs. 1, 2, 3). This effect was highly significant when tested by ANOVA interaction terms involving heat treatment (three-way ANOVA with [1] RIL panel, RIL-D48 vs

Table 1 Candidate genes mapping within the QTL region

QTL Range	Some candidate genes within the QTL region
28A1–34D2	[a]*Pvr, CG14277, Tsp29Fb, CG18511, CG18619, CG17124, Ast2, CG16826, Nckx30C, bru-2, Rh5, Pen, CG5384, CG6579, Acer,*
	[b]*Glt, Uro, CG7953, CG7916, CG13095, CG9466, CG9468, CG17633, CG5390, CG17108, CG 16743, hgo, CG14935, CG9928, CG5945, Cyp4d21, CG7300, CG17124*
	[c]*hsp60D, CG13133*

Candidate genes are genes that changed their expression levels in previous studies, either by heat stressing flies ([a]Sørensen et al. 2005) or by artificially selecting for both longevity and heat-stress resistance in lines sharing the same genetic background as in one of the parental lines (D48) of the RILs used in this study ([b]Sørensen et al. 2007). In addition, candidates hsp60D and CG13133 are genes with known or inferred Hsp function (cAttrill et al.; the 23 Flybase Consortium). The proportion of the phenotypic variance explained by the QTL ranged from 14 to 20 %

RIL-SH2; [2] treatment, control vs heat-stressed; and [3] sex, male vs female, as fixed factors, with the following ANOVA results, [1]: $F_{1,\ 196} = 39.9$***; [2]: $F_{1,\ 196} = 4.04$*; [3]: $F_{1,\ 196} = 3.38$; [1] x [2]: $F_{1,\ 196} = 7.30$**; [1] x [3]: $F_{1,\ 196} = 0.214$; [2] x [3]: $F_{1,\ 196} = 10.5$**; [1] x [2] x [3]: $F_{1,\ 196} = 0.008$. *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$). A two-way ANOVA for the RIL-D48 set, confirmed that heat-induced hormesis was highly significant in RIL-D48 flies, with [1] heat treatment (control vs heat-stressed) and [2] sex (male vs female) as fixed factors ([1]: $F_{1,\ 120} = 11.45$***, [2]: $F_{1,\ 120} = 2.83$, [1] x [2]: $F_{1,\ 120} = 5.33$*; *$P < 0.05$, ***$P < 0.001$). A similar two-way ANOVA was performed for the RIL-SH2 set revealing a significant sex-by-treatment interaction ([1]: $F_{1,\ 76} = 0.28$, [2]: $F_{1,\ 76} = 1.13$, [1] x [2]: $F_{1,\ 76} = 6.61$*; *$P < 0.05$).

As all three above-mentioned ANOVAs revealed significant interactions, hormesis was further tested for each individual RIL, as some specific genetic recombinations might allow an elevated hormetic response and others do not. This analysis is shown in Fig. 2, where either hormetic or detrimental responses to our heat-stress treatment were significant in 20 out of 31 RIL-D48 lines as well as in 11 out of 20 RIL-SH2 lines (Figs. 1, 2). These responses were often sex-specific, consistent with a significant sex-by-treatment interaction in the above mentioned two-way ANOVAs. The level of heat-induced hormesis showed some sexual dimorphism, with a higher number of lines exhibiting higher hormesis effects in males (Fig. 2, see supplementary Table S1 for further information on mean longevities).

The number of lines exhibiting significant hormesis was 8 in females RIL-D48, 14 in males RIL-D48, 1 in females RIL-SH2, and 6 in males RIL-SH2 (after correcting *P*-values for multiple comparisons; Fig. 2). The number of lines exhibiting a negative effect of our heat treatment was 6 in females RIL-D48, 1 in males RIL-D48, 6 in females RIL-SH2, and 1 in males RIL-SH2 (Fig. 2). The rest of the lines showed no heat-induced significant change in longevity (17 lines in females RIL-D48, 14 lines in males RIL-D48, 12 lines in females RIL-SH2, and 13 lines in males RIL-SH2).

There was a negative across-RIL correlation between mean longevity in control flies and hormesis in RIL-SH2 but not in RIL-D48 (as in Fig. 2, hormesis was indexed as the ratio of mean longevity in heat-stressed to control flies. Pearson correlation, $r = -0.33$ in females RIL-D48, $r = -0.28$ in males RIL-D48, $r = -0.74$** in females RIL-SH2, $r = -0.65$* in males RIL-SH2. *$P < 0.05$; **$P < 0.01$), indicating that hormesis was higher in shorter-lived lines from the RIL-SH2 panel than in longer-lived lines from the RIL-SH2 panel.

The QTL mapping results for the three major chromosomes are shown in Fig. 3 and Table 1. The phenotypic trait used for QTL mapping of hormesis was the ratio between longevity in heat-stressed flies to longevity in control flies (i.e., the fold change in longevity induced by heat stress). A significant QTL was detected on chromosome 2 in both females and males in RIL-D48 only, indicating that hormesis induction is a variable trait under genetic control (Fig. 3). Furthermore, this QTL was also consistently significant for longevity in heat-stressed RIL-D48 flies and not for longevity in control flies, as expected if the QTL for longevity was the result of our heat-stress treatment (supplementary Fig. S1). Additionally, other QTL were significant only for longevity in control

flies, which co-localized with previously found longevity QTL in Defays et al. (2011; Supplementary Fig. S1).

Possible epistatic interactions were tested between all pairwise combinations of markers (see supplementary Fig. S2). Before correcting for multiple comparisons, epistatic effects were significant between AC005889 (band 30A3-30A6) and AC009392 (band 23A-23-E) in females ($P = 0.009$) and males ($P = 0.02$), between AC005889 and 3L-5235154gt (band 64D) in females ($P = 0.02$), between AC005889 and AC008198 (66D10-66E2) in females ($P = 0.02$), as well as between DRONINAC (28A1-28A3) and SU(Z)2 (49C) in females ($P = 0.01$). All these interactions involved always either marker within the QTL range (AC005889 or DRONINAC), although these apparent interactions were non-significant after correcting for multiple comparisons by using a sequential Bonferroni test (Rice 1989).

Discussion

Longevity was exceptionally increased by our heat-stress treatment in several RILs (with about 100 % of longevity extension). Hormesis induction was strongly dependent on the genotype and the genetic background because the hormesis-QTL was significant in RIL-D48 only and because the results show an exceptionally strong effect of hormesis in several RIL-D48 lines, but also a negative effect in the form of strongly reduced longevity in some other RILs (Fig. 2).

Previous work showed that several forms of mild stress extend longevity. The process is currently not fully understood, but it is hypothesized that more than one mechanism play a role in the extension of longevity by hormesis (Tower 2009; Moskalev et al. 2011; Le Bourg et al. 2012). In this study we report a novel heat-stress treatment which was successful in extremely increasing longevity in several fly lines. In fact, the heat-shock induced increase in longevity was about a 100 %, which is an increase much higher than in previous studies where the heat treatment was limited to rather one or a few exposures to a heat stress (e.g., Lithgow et al. 1995; Khazaeli et al. 1997; Hercus et al. 2003; Gomez et al. 2009; Le Bourg 2011; Scannapieco et al. 2007; Defays et al. 2011). In the same RILs as in the present study, a negative correlation was found between longevity at 25 °C and hormesis induced by a single heat shock in young flies (Defays et al. 2011). This negative association was also significant in RIL-SH2 in the present study, where a very different thermal treatment was applied. However, only 30 % of the lines that exhibited hormesis in Defays et al. (2011) were found to also exhibit hormesis in the current study, suggesting that the hormetic response to very different forms of mild-heat stress differs genetically. In addition, none of the homesis-QTL in Defays et al. (2011) co-localized with the hormesis-QTL in the present study, even though both studies used the same sets of RILs and widely differed only in the heat-induction treatment. The heat-sensitive genetic background in this study (RIL-D48) responded to our chronically cyclical heat-stress treatment to a considerably higher degree than did the heat-resistant RIL-SH2 panel (Fig. 2). Presumably, the genetic background of SH2 flies could "buffer" them from the impact of being exposed to high temperature several times every day whereas the converse might be the case for RIL-D48, derived from a heat-sensitive line. Another possible evidence for an effect of the genetic background are possible epistatic interactions (see Results), though these interactions were non-significant after correcting for multiple comparisons. In addition, the proportion of the phenotypic variance in hormesis explained by the only significant QTL was not more than 14–20 %, suggesting that epistatic effects in addition to environmental variance could be involved in the hormesis response level.

The genetic architecture of longevity is complex and more work is needed in order to increase our understanding of the mechanisms contributing to lifespan extension. The two RIL panels in this study differentially responded to our heat-stress treatment, with heat-induced hormesis being higher in RIL-D48 (Figs. 1, 2). Further, one hormesis-QTL was identified on chromosome 2, which was significant in RIL-D48 but not in RIL-SH2 (Fig. 3). A number of studies suggest that the housekeeping function of some Hsps plays a significant role in prolonging life and reducing the disruption of cell homeostasis and the deleterious effects of ageing, but other systems could also be implicated (e.g., Olsen et al. 2006; reviewed in Tower 2009, 2011). The parental lines used to construct RILs significantly differed in the heat-induced Hsp70 levels (Norry et al. 2004), but no hormesis-QTL was found in

the genome region of this Hsp gene (Fig. 3). However, although important to the genetic architecture of longevity, the Hsp system is only one among several others that might lead to an extended life (Tower 2009, 2011).

In Sørensen et al. (2007), one SH2-inbred line (from the same base population as one of the parental of all RILs in this study) was crossed to other fly stocks to set up a base population on which several selection regimes were applied to obtain five replicated lines of selection (Bubliy and Loeschcke 2005). Many genes within our QTL region (Table 1) changed their expression levels by artificial selection on either longevity or heat-stress resistance in Sørensen et al. (2007), and other genes were either up- or down-regulated by heat stress in Sørensen et al. (2005). Many candidates have still unknown functions, whereas inferred functions of other candidate genes include cellular response to insulin stimulus (*Pvr,* Tran et al. 2013), regulation of heart rate (*Ast2*, Merte and Nichols 2002), sodium ion transport (*Nckx30C*, Haug-Collet et al. 1999), circadian clock (*Rh5*, *Acer*, Szular et al. 2012; Carhan et al. 2011), mating (*Hgo*, Fujii et al. 2008), and determination of adult lifespan (*tam*, Mandavilli et al. 2002).

In *Drosophila*, heat-induced hormesis has been extensively supported by previous results on mild heat stress applied at young ages (e.g., Khazaeli et al. 1997; Hercus et al. 2003; Scannapieco et al. 2007; Gomez et al. 2009; Le Bourg 2011), where either single or periodic exposure to mild stress improved the organism's longevity. The body of evidence in favor of hormetic effects leading to longevity extension suggests that a mild-heat stress is often beneficial (Cypser and Johnson 2002). The results here discussed present newfound evidence for hormesis in longevity following a treatment consisting of repeated and very short-time exposures to mild-heat stress every day throughout the full life, starting the exposures after 2-days of age of the adult fly. This treatment is different from previous studies in that, generally, in order to induce hormesis, individuals in previous studies were exposed to one or two hours of mild stress either a single or repeated times in early ages and no more than one time per treatment day. It is interesting that the hormesis effect can be extended using recurring peaks of a mild heat stress throughout almost each day of the life of adult flies. The results strongly suggest that the level of induced hormesis is determined by the genotype, since not all the assessed lines benefited from increased lifespan. In fact, rather the opposite was observed in some lines, mainly those from the RIL-SH2 set, where lifespan was often decreased in comparison to controls. Thus, the level of heat-induced longevity was much higher in heat-sensitive lines than in heat-resistant lines of *D. melanogaster* flies. Interestingly, hormesis induction was stronger in males than in females (Fig. 2), with hormesis being significant for 20 RIL in males and 9 RIL in females, suggesting that hormesis is a trait that can present a sexual dimorphism. Perhaps, cyclic treatments of heat stress could be more successful in inducing hormesis if applied at young ages only (Le Bourg et al. 2000; Olsen et al. 2006).

The observation that the chronic, yet moderate, heat stress regime could markedly increase longevity in some lines and severely decrease longevity in other lines is the most interesting finding in this study. Hormesis was especially pronounced in the RILs that predominantly had a D48 genetic composition. It is possible that lines selected for heat-sensitivity are more responsive to heat stress, as the lines selected for heat resistance may be "buffered" against reacting to thermal stress (Norry and Loeschcke 2003). In addition, the genetic background was found to dramatically influence hormesis in previous studies, both between artificial selection regimes and among the lines within selection regime, suggesting that in genetically variable populations a life enhancing treatment in one individual could have no effect or even be detrimental in other individuals (Gomez et al. 2009; Sarup and Loeschcke 2011). However, it is still not fully clear how heat-induced hormesis in longevity is dependent on genotype. In model systems like recombinant inbred lines (RILs) of *Drosophila melanogaster*, heat-induced hormesis can be tested for a set of recombinant genotypes to identify genetic differences in the hormetic response to heat-stress treatments. Here we used RILs that greatly segregated genetic variation in heat-stress sensitivity. This RIL set allowed us to indentify large between-RILs differences and a QTL for heat-induced hormesis using a novel treatment of short but cyclic exposures to a moderate heat stress throughout the adult life. To delineate the possible limits of hormesis induction by cyclic treatments of heat stress, it would be interesting to test their hormetic effects at diverse ages using strains recently derived from natural populations.

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References

Bubliy OA, Loeschcke V (2005) Correlated responses to selection for stress resistance and longevity in a laboratory population of *Drosophila melanogaster*. J Evol Biol 18:789–803

Calabrese EJ, Dhawan G, Kapoor R, Iavicoli I, Calabrese V (2015) What is hormesis and its relevance to healthy aging and longevity? Biogerontology 16:693–707

Carhan A, Tang K, Shirras CA, Shirras AD, Isaac RE (2011) Loss of Angiotensin-converting enzyme-related (ACER) peptidase disrupts night-time sleep in adult *Drosophila melanogaster*. J Exp Biol 214:680–686

Cypser JR, Johnson TE (2002) Multiple stressors in *Caenorhabditis elegans* induce stress hormesis and extended longevity. J Gerontol A 57:B109–B114

Defays R, Gómez FH, Sambucetti P, Scannapieco AC, Loeschcke V, Norry FM (2011) Quantitative trait loci for longevity in heat-stressed *Drosophila melanogaster*. Exp Gerontol 46:819–826

Fujii S, Toyama A, Amrein H (2008) A male-specific fatty acid omega-hydroxylase, SXE1, is necessary for efficient male mating in *Drosophila melanogaster*. Genetics 180:179–190

Gomez FH, Bertoli CI, Sambucetti P, Scannapieco AC, Norry FM (2009) Heat-induced hormesis in longevity as correlated response to thermal-stress selection in *Drosophila buzzatii*. J Therm Biol 34:17–22

Gomez FH, Sambucetti P, Norry FM (2013) Effects of dietary composition on life span of *Drosophila buzzatii* and its short-lived sibling species *D. koepferae*. Biogerontology 14:423–429

Haug-Collet K, Pearson B, Webel R, Szerencsei RT, Winkfein RJ, Schnetkamp PPM, Colley NJ (1999) Cloning and characterization of a potassium-dependent sodium/calcium exchanger in *Drosophila*. J Cell Biol 147:659–670

Hercus MJ, Loeschcke V, Rattan SIS (2003) Lifespan extension of *Drosophila melanogaster* through hormesis by repeated mild heat stress. Biogerontology 4:149–156

Khazaeli AA, Tatar M, Pletcher SD, Curtsinger JW (1997) Heat-induced longevity extension in *Drosophila*. 1. Heat treatment, mortality, and thermotolerance. J Gerontol A 52: B48–B52

Le Bourg E (2011) Using *Drosophila melanogaster* to study the positive effects of mild stress on aging. Exp Gerontol 46:345–348

Le Bourg E, Minois N, Bullens P, Bearet P (2000) A mild stress due to hypergravity exposure at young age increases longevity in *Drosophila melanogaster* males. Biogerontology 1:145–155

Le Bourg E, Malod K, Massou I (2012) The NF-κB-like factor DIF could explain some positive effects of a mild stress on longevity, behavioral aging, and resistance to strong stresses in *Drosophila melanogaster*. Biogerontology 13:445–455

Lithgow GJ, White TM, Melov S, Johnson TE (1995) Thermotolerance and extended life-span conferred by single-gene mutations and induced by thermal-stress. Proc Natl Acad Sci (USA) 92:7540–7544

Mandavilli BS, Santos JH, Van Houten B (2002) Mitochondrial DNA repair and aging. Mutat Res 509:127–151

McColl G, Hoffmann AA, McKechnie SW (1996) Response to two heat shock genes to selection for knockdown heat resistance in *Drosophila melanogaster*. Genetics 143:1615–1627

Merte J, Nichols R (2002) *Drosophila melanogaster* myotropins have unique functions and signaling pathways. Peptides 23:757–763

Miller E (2013) Online statistics calculators, Evan's Awesome A/B Tools. http://www.evanmiller.org/ab-testing/survival-curves.html

Moskalev AA, Plyusnina EN, Shaposhnikov MV (2011) Radiation hormesis and radioadaptive response in *Drosophila melanogaster* flies with different genetic backgrounds: the role of cellular stress-resistance mechanisms. Biogerontology 12:253–263

Norry FM, Loeschcke V (2003) Heat-induced expression of a molecular chaperone decreases by selecting for long-lived individuals. Exp Gerontol 38:673–681

Norry FM, Dahlgaard J, Loeschcke V (2004) Quantitative trait loci affecting knockdown resistance to heat stress in *Drosophila melanogaster*. Mol Ecol 13:3585–3594

Norry FM, Scannapieco AC, Sambucetti P, Bertoli C, Loeschcke V (2008) QTL for the thermotolerance effect of heat hardening, knockdown resistance to heat and chill-coma recovery in an intercontinental set of recombinant inbred lines of *Drosophila melanogaster*. Mol Ecol 17:4570–4581

Olsen A, Vantipalli MC, Lithgow GJ (2006) Lifespan extension of *Caenorhabditis elegans* following repeated mild hormetic heat treatments. Biogerontology 7:221–230

Phelan JP, Archer MA, Beckman KA, Chippindale AK, Nusbaum TJ, Rose MR (2003) Breakdown in correlations during laboratory evolution. I. Comparative analyses of Drosophila populations. Evolution 57:527–535

Rattan SIS (2008) Hormesis in aging. Ageing Res Rev 7:63–78

Rice WR (1989) Analyzing tables of statistical tests. Evolution 43:223–225

Sambucetti PD, Loeschcke V, Norry FM (2015) Patterns of longevity and fecundity at two temperatures in a set of heat-selected recombinant inbred lines of *Drosophila melanogaster*. Biogerontology 16:801–810

Sarup P, Loeschcke V (2011) Life extension and the position of the hormetic zone depend on sex and genetic background in *Drosophila melanogaster*. Biogerontology 12:109–117

Scannapieco AC, Sørensen J, Loeschcke V, Norry FM (2007) Heat-induced hormesis in longevity of two sibling *Drosophila* species. Biogerontology 8:315–325

Sørensen JG, Nielsen MM, Kruhøffer M, Justesen J, Loeschcke V (2005) Full genome gene expression analysis of the heat

stress response *in Drosophila melanogaster*. Cell Stress Chaperon 10:312–328

Sørensen JG, Nielsen MM, Loeschcke V (2007) Gene expression profile analysis of *Drosophila melanogaster* selected for resistance to environmental stressors. J Evol Biol 20:1624–1636

StatSoft (1999) STATISTICA for Windows (Computer Program Manual). StatSoft Inc., Tulsa

Szular J, Sehadova H, Gentile C, Szabo G, Chou WH, Britt SG, Stanewsky R (2012) Rhodopsin 5- and rhodopsin 6-mediated clock synchronization in *Drosophila melanogaster* is independent of retinal phospholipase C-β signaling. J Biol Rhythms 27:25–36

Tower J (2009) Hsp and aging. Trends Endocrinol Metab 20:216–222

Tower J (2011) Heat shock proteins and *Drosophila* aging. Exp Gerontol 46:355–362

Tran TA, Kinch L, Peña-Llopis S, Kockel L, Grishin N, Jiang H, Brugarolas J (2013) Platelet-derived growth factor vascular endothelial growth factor receptor inactivation by sunitinib results in Tsc1/Tsc2-dependent inhibition of TORC1. Mol Cell Biol 33:3762–3779

Wang SC, Basten J, Zeng ZB (2010) Windows QTL Cartographer 2.5. Department of Statistics. North Carolina State University, Raleigh, NC