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RESEARCH ARTICLE

Patterns of longevity and fecundity at two temperatures in a set of heat-selected recombinant inbred lines of *Drosophila melanogaster*

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Abstract Quantitative trait loci (QTL) were mapped for longevity and fecundity at two temperatures, 20 and 30 °C, in two sets of recombinant inbred lines (RIL) highly differing in thermotolerance. Early fecundity (EF) and longevity showed a negative association between temperatures. For instance, longevity was higher and fecundity was lower in the RIL panel showing higher life span at 30 °C. One X-linked QTL (7B3-12E) co-localized for longevity and EF at 20 °C, with one QTL allele showing a positive additive effect on longevity and a negative effect on EF. The across-RIL genetic correlation between longevity and EF was not significant within each temperature, and most QTL that affect life span have no effect on EF at each temperature. EF and longevity can mostly be genetically uncoupled in the thermotolerance-divergent RIL within each temperature as opposed to between temperatures. QTL were mostly temperature specific, although some trait-specific QTL showed possible antagonistic effects between temperatures.

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Introduction

Longevity and early fecundity are two traits strongly suggested to be involved in a trade-off. Extended longevity can have a cost in reproduction (reviewed in Rose 1991; Partridge et al. 2005; Le Bourg 2007; Flatt 2011; but see Tarin et al. 2014; Kengeri et al. 2013; Gagnon 2015). At the evolutionary genetic level, it is postulated that survival costs of reproduction arise from alleles with opposite effects on reproduction and survival in wild animals (e.g., Williams 1966; Rose 1991; Leroi et al. 2005; Partridge et al. 2005; Flatt and Promislow 2007). At advanced age, the strength of natural selection is weak resulting in an accumulation of late-acting deleterious mutations (the theory of mutation accumulation, Medawar 1952). Another (not mutually exclusive) postulated mechanism of the evolution of senescence and longevity is provided by the antagonistic pleiotropy theory of aging, suggesting that aging evolves because selection favors alleles with beneficial effects on early reproduction some of which are pleiotropic with deleterious effects at advanced age (Williams 1966). This theory predicts a negative correlation between early and late gene effects, especially between longevity and early reproduction (Williams 1966; Kirkwood 1977). The

disposable soma theory of senescence, which can be considered as a special case of antagonistic pleiotropy, suggests that the genes of the somatic repair systems are fundamental for the mechanisms and evolution of senescence and longevity, with somatic repair having a cost in reproduction (Kirkwood and Austad 2000).

One approach to investigate the genetic base of complex phenotypes such as longevity and fecundity is quantitative trait loci (QTL) mapping. It is one of the widely used techniques used to identify genome regions that contain relevant genes affecting the traits (Lynch and Walsh 1998; Mackay 2001). Previous studies successfully used the QTL-approach to identify genome regions in which the relevant QTL are localized for longevity and fecundity in *Drosophila melanogaster*, revealing that major QTL are sex-specific on all three major chromosomes of this model insect (e.g. Nuzhdin et al. 1997; Leips and Mackay 2000; Curtsinger and Khazaeli 2002; Leips et al. 2006; Defays et al. 2011). Putative candidate genes have been identified and discussed elsewhere (e.g. Pasyukova et al. 2004; Leroi et al. 2005; Partridge et al. 2005; Sørensen et al. 2007; Tower 2011; Remolina et al. 2012; Bergland et al. 2012). Genes whose expression is affected by artificial selection on longevity and fecundity are obvious candidate loci (e.g. Sørensen et al. 2007; Remolina et al. 2012). Several previous studies of experimental evolution have shown that increased selection on late-life performance results in increased life span and late-age reproduction and that this increase is expected to be accompanied by decreased EF (Rose and Charlesworth 1980; Luckinbill et al. 1984; Rose 1984; Service et al. 1988; Zwaan et al. 1995; Scannapieco et al. 2009). This pattern is interpreted as evidence for the involvement of alleles with antagonistic pleiotropic effects in the evolution of aging and reproduction. However, EF and longevity can sometimes be decoupled (Khazaeli and Curtsinger 2013; Wit et al. 2013; Kimber and Chippindale 2013).

Genotype-by-environmental interactions can also maintain quantitative genetic variation in heterogeneous environments (Mackay 2002). Furthermore, environmental temperature dramatically affects both longevity and fecundity in *Drosophila* (e.g. Vieira et al. 2000; Klepsatel et al. 2013). QTL mapping in recombinant inbred lines (RIL) is a useful technique for the genetic analysis of possible interactions of longevity and fecundity with temperature, as RIL can be measured for these traits at diverse temperatures. Many QTL for longevity were found to have temperature-specific effects in *D. melanogaster* (Vieira et al. 2000). The possible effects of temperature on longevity and fecundity are of major interest because both traits are expected to be related in a trade-off (see above). Any possible co-localizations between longevity-QTL and fecundity-QTL at different temperatures are particularly interesting because some QTL could be temperature specific in their effects on the association of both traits. Co-localization of QTL for longevity and fecundity has been previously shown as predicted by the antagonistic pleiotropy theory of aging at benign temperature (Leips et al. 2006).

Here we used an intercontinental set of RIL to identify QTL affecting longevity and fecundity at two thermal conditions, a high temperature of 30 °C and a moderate temperature of 20 °C. These RIL were derived from lines artificially selected for high and low knockdown resistance to high temperature (KRHT), which were previously used to map thermotolerance traits in adult flies (Norry et al. 2008; Arias et al. 2012). Two main aims were addressed in this study. First, we examined whether QTL for longevity co-localize with some QTL for fecundity in an antagonistic way as these two traits are often negatively correlated in a trade-off (Rose 1984, 1991), as predicted by the antagonistic pleiotropy theory (Williams 1966). Second, as environmental temperature has been shown to dramatically affect both longevity and fecundity in *Drosophila* (e.g. Vieira et al. 2000; Norry et al. 2006; Klepsatel et al. 2013), QTL were mapped at moderate (20 °C) as well as at high (30 °C) temperature to answer the question: do the chromosomal locations of longevity and fecundity QTL generally match between temperatures?

Materials and methods

Recombinant inbred lines

Parental lines and RIL used in this study were described in Norry et al. (2008). Briefly, parental stocks were two nearly homozygous lines derived from Denmark and Australia, denoted D48 and SH2 lines, respectively. These parental lines were selected for low (D48) and high (SH2) heat knockdown resistance in adult flies. Females from the F1 (progeny

of D48 × SH2) were backcrossed to males from each parental stock to construct two panels of RIL, one panel from the D48 backcross (RIL-D48) and another panel from the SH2 backcross (RIL-SH2). The use of both reciprocal backcrosses increases the statistical power to detect QTL in comparison to one design based on a single-way introgression backcross (Norry et al. 2008). All RIL were obtained by full-sib mating for 15 generations. In total, 32 RIL-D48 and 21 RIL-SH2 were used in this study. Microsatellite loci spread throughout all three major chromosomes were used as markers to perform the genetic map associated to these RIL (for bibliographic references of microsatellites used see Norry et al. 2008) with the following map positions: (cM and cytological band): 1-0 (1B8), 1-2 (3A), 1-5 (3C1-C6), 1-15 (4F1-F2), 1-21.7 (7B3), 1-40 (10A1-A2), 1-45 (10C3), 1-54 (12D-E), 1-71 (16F3-F6), 1-85 (19F3-F6), 2-1 (21C3), 2-6.44 (22C), 2-10.98 (23A-E), 2-25 (25F5-26A), 2-37 (28A1-A3), 2-49 (30A3-A6), 2-70(34C4-D2), 2-76 (38E1), 2-80 (42A), 2-97 (49C), 2-100 (50C), 2-115 (54B1-B2), 2-129 (56D11-E6), 2-142 (59A1-A2), 3-0.1 (62A), 3-9 (63D2-F1), 3-17 (64D), 3-34 (66D10-E2), 3-45 (67A), 3-59 (73A1-B7), 3-71 (86E3), 3-84 (90B1-B2), 3-95 (90E-F), 3-125 (95C6-C8), 3-128 (97F), 3-140 (99D6-D9).

Phenotypes measured

All RIL were maintained at 25 ± 1 °C in replicated 95 × 20-mm vials (hereafter referred to as standard vials) containing 6 ml of a culture medium prepared with instant mashed potatoes plus water and nipagin. Longevity in all 53 RIL was measured at 25 °C elsewhere (Defays et al. 2011). Here, we used a mixed-sex environment but otherwise similar conditions as in Defays et al. (2011) to measure longevity at both 20 and 30 °C for each RIL under a 12:12 h light/dark cycle. To obtain the experimental individuals, flies from each RIL were placed in 2-3 standard bottles per RIL with 25 males plus 25 females per bottle. Standard bottles were 125-mL glass bottles containing 40 mL of the above described culture medium. Flies were allowed to lay eggs for 4 days and after that were removed from the bottles. One-day-old flies emerged from these cultures were placed in standard vials to measure longevity. For each RIL, two replicated standard vials each containing 20 males plus 20 females approximately were set up at each of the experimental temperatures. The flies were transferred to fresh vials every 3 days at 20 °C and every 2 days at 30 °C and all vials were examined for dead flies at each transfer until the last flies had died. A 2-day interval to score longevity at 30 °C allowed us to handle the experiment to identify QTL, as in Defays et al. (2011) in the same RIL at 25 °C. As flies live much longer at 20 °C than at 30 °C (Figs. 1, 2), the difference of 1 day longer of the interval used to score longevity at 20 °C may be considered as our scaling to measure longevity in this study. Variation in longevity was tested as a Gamma distribution (best fitted distribution of the data), using analysis of deviance

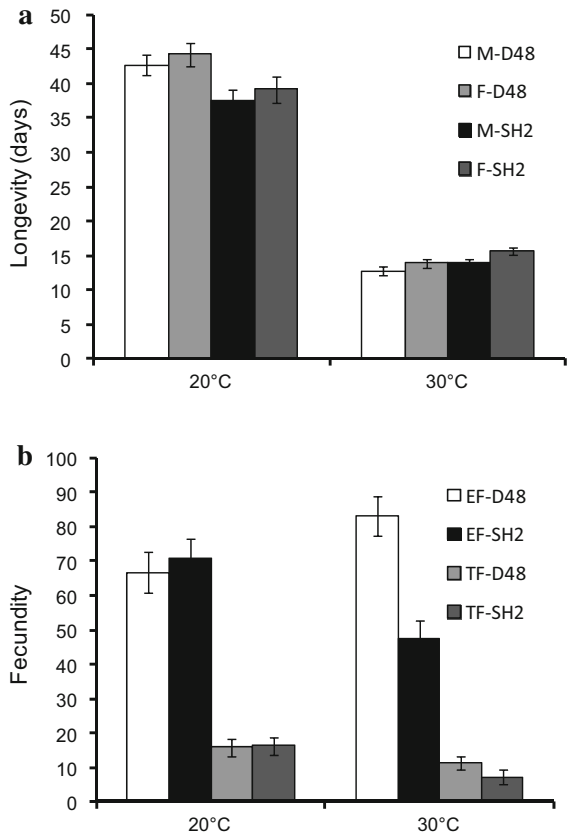

Fig. 1 Mean longevity (**a**) and fecundity (**b**) of flies (±SE) at 20 and 30 °C for each RIL panel, D48 and SH2, used for QTL mapping. *M* males, *F* females, *EF* absolute early fecundity, *TF* relative total fecundity. Longevity was measured for 53 RIL (N = 32 and 21 for RIL-D48 and RIL-SH2, respectively), and the data point for each RIL was obtained by averaging the life span of approximately 40 flies of each sex per RIL at each temperature (fly density was 20 males plus 20 females per vial, with two replicated vials per RIL per temperature). Both EF and TF were averaged on four females per RIL

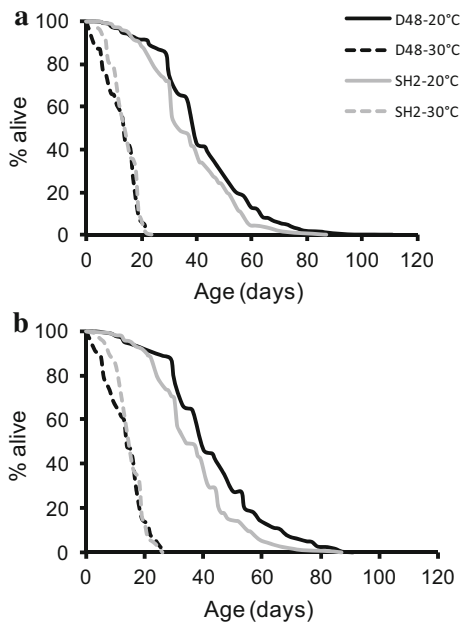

Fig. 2 Survival curves of males (**a**) and females (**b**) are shown for each pooled RIL panel used for QTL mapping (D48 and SH2) at 20 and 30 °C. Number of RIL (N) as in Fig. 1

and inverse link function in a generalized linear model (GLM), with RIL panel (RIL-D48 vs. RIL-SH2), temperature (20 vs. 30 °C) and sex (males vs. females) as fixed factors. The fitted model contained all possible interactions. Analyses were performed using InfoStat software (Di Rienzo et al. 2014). This software implements an interface of the R platform (version 3.1.2; R Core Team 2014) to estimate generalized linear models through GLM and GLMER procedures from the stats and lme4 libraries (Bates et al. 2013). Reported GLM results yield identical conclusions as an Analysis of Variance (ANOVA) on non-transformed longevity (in days), using RIL panel, temperature and sex as fixed factors (results not shown). For QTL analysis, mean longevity (in days) was averaged over replicates for each RIL.

Fecundity was scored at the same experimental conditions as for longevity (i.e. at 20 and 30 °C under a 12:12 h light/dark cycle) in each RIL. All experimental individuals were obtained as described above for longevity assays. Vials containing a small spoon with agar plus a food colouring and yeast paste were set up for each RIL, with two males plus one female of 1 day of age and four vials per RIL. Food colouring was a green colour additive for human food (Fleibor S.R.L. Laboratory, Tablada, Buenos Aires), allowing us to easily see all eggs and egg shells in the scoring by using a stereo microscope. Number of eggs was scored from spoons every 2 days when flies were transferred to new vials with fresh spoons. This procedure was repeated until the death of the flies. Males that occasionally were dead were replaced by new ones of the same age from the same RIL. As longevity is highly variable among RIL, we used relative instead of absolute total fecundity (TF). Therefore, TF (hereafter referred to as TF) was estimated for each female as the total number of eggs laid during its lifetime relative to the age of death of the fly (i.e., total number of eggs/death age of the fly, in days). Early fecundity (hereafter referred to as EF) was estimated as the absolute number of eggs laid within the first 5 days of age (Huey et al. 1995; Sambucetti et al. 2005). Variation in TF and EF was tested using RIL panel (RIL-D48 vs. RIL-SH2) and temperature (20 vs. 30 °C) as fixed factors in a GLM analysis as above described for longevity. A two-way ANOVA using RIL panel and temperature as fixed factors on non-transformed data of fecundity yielded identical conclusions as GLM analysis. For QTL analyses, TF as well as EF were averaged over four scores for each RIL.

QTL analysis

The number of SH2 alleles (0 or 2) was used as genotypic information for each marker in both RIL-D48 and RIL-SH2, as in Norry et al. (2008). Composite interval mapping (Zeng 1994) was used to test the hypothesis that an interval flanked by two adjacent markers contains a QTL. This was done using model 6 in QTL-Cartographer for Windows, version 2.5 (Wang et al. 2010), for Ri2 design, with five markers and a window size of 10 cM. The effects of altering this initial combination of parameters were also explored and QTL positions that were significant by using 10 cM as window size and five control markers were consistent across a wide range of parameter combinations. Mean longevity was ln-transformed to perform composite interval mapping. This transformation did not modify the number and position of QTL previously identified on non-transformed data. Significance thresholds were determined by 1000 random permutations. For significant QTL, confidence intervals were estimated by using 1.5 LOD

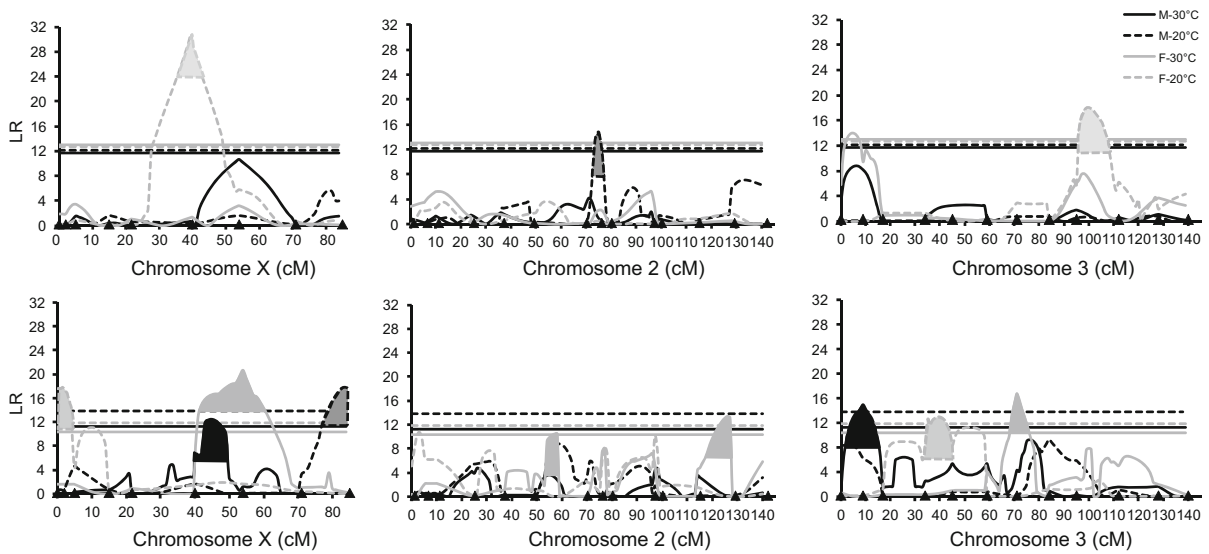

Fig. 3 Plot of likelihood ratio (LR) scores against map position (in cM) from composite interval mapping for longevity at 20 and 30 °C in *D. melanogaster* from RIL-D48 (*upper panel*) and RIL-SH2 (*lower panel*) populations. Significance thresholds were determined by 1000 random permutations (*horizontal lines*). *Triangles on the x-axis* correspond to location of markers used in composite interval mapping. Number of RIL (N) as in Fig. 1. Confidence intervals for a level higher than 95 % are shown for significant QTL (maximum width of marked QTL-peak), by using 1.5 LOD = 6.9 LR (Dupuis and Siegmund 1999)

(6.9 LR) for confidence >95 %, according to Dupuis and Siegmund (1999).

Pairwise epistatic interactions were evaluated 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, respectively (Morgan and Mackay 2006).

Results

Mean longevity and the corresponding mortality curves are shown in Figs. 1 and 2, respectively. Longevity was higher at 20 °C than 30 °C (Figs. 1a, 2). There were no significant longevity differences between the sexes (Fig. 1a; GLM with (1) RIL-D48 vs. RIL-SH2, (2) 20 vs. 30 °C and (3) males vs. females as fixed factors: $\chi^2_1 = 10.77$** for (1); $\chi^2_1 = 1041.66$*** for (2); $\chi^2_1 = 0.426$ for (3); $\chi^2_1 = 10.03$** for (1) × (2); $\chi^2_1 = 0.12$ for (1) × (3); $\chi^2_1 = 1.97$ for (2) × (3); $\chi^2_1 = 0.004$ for (1) × (2) × (3); $^{**}P < 0.01$; $^{***}P < 0.001$). There was a significant two-way interaction between RIL panel and temperature (see GLM described above). At 20 °C, mean longevity was higher in RIL-D48 than RIL-SH2 (Fig. 1; GLM with (1) RIL-D48 vs. RIL-SH2 and (2) males vs. females as fixed factors: $\chi^2_1 = 10.15$*** for (1); $\chi^2_1 = 0.09$ for (2); $\chi^2_1 = 0.12$ for (1) × (2); $^{***}P < 0.001$). The opposite pattern was observed at 30 °C, with mean longevity being higher in RIL-SH2 than RIL-D48 (Fig. 1; same GLM as above: $\chi^2_1 = 5.3$* for (1); $\chi^2_1 = 2.3$ for (2); $\chi^2_1 = 0.004$ for (1) × (2); $^{*}P < 0.05$). In all 53 RIL, longevity at 20 °C (present study) was longer than longevity at 25 °C measured in Defays et al. (2011), the difference being highly significant in a two-tailed sign test: $P < 0.005$), as expected in *Drosophila* and many other ectotherms in which longevity decreases with environmental temperature.

There was no significant genetic correlation between longevity and EF across each set of RIL at each experimental temperature (Spearman rank correlations: $r_s = 0.16$; $P = 0.48$ and $r_s = 0.02$; $P = 0.94$ for RIL-D48 and RIL-SH2, respectively, at 20 °C. $r_s = 0.33$; $P = 0.08$ and $r_s = 0.03$; $P = 0.89$ for RIL-D48 and RIL-SH2, respectively, at 30 °C. Similar results were obtained by estimating Pearson correlations). Similarly, no significant correlation was found between longevity and EF when pooling the two sets of RILs ($r_s = 0.06$; $P = 0.74$ and $r_s = 0.11$; $P = 0.47$ at 20 and 30 °C, respectively).

Table 1 QTL for longevity, total fecundity and early fecundity identified by composite interval mapping in RIL-D48 and RIL-SH2 at 20 and 30 °C

QTL range	Trait	Temp. (°C)	RIL/sex	*a*	%Vp
1B8-3C6	Longevity	20	SH2-F	0.16	38
	Total fecundity	30	SH2	−2.5	2.4
3C6-4F2	Total fecundity	30	SH2	−2.5	1.1
7B3-12E	Longevity	20	D48-F	0.28	51
	Total fecundity	20	SH2	−3.3	11
	Early fecundity	20	SH2	−15.2	6.6
10A1-12E	Longevity	30	SH2-M	0.15	18
10A1-16F6	Longevity	30	SH2-F	0.10	36
12D-16F6	Total fecundity	20	SH2	2.3	12
16F3-19F6	Longevity	20	SH2-M	0.16	22
	Total fecundity	30	SH2	5.8	31
	Early fecundity	30	SH2	**5.7**	38
23A-28A3	Total fecundity	30	SH2	5.7	17.5
	Early fecundity	30	SH2	8.4	15.6
30A3-34D2	Longevity	30	SH2-F	−0.06	2
	Total fecundity	20	D48	4.0	0.8
	Early fecundity	20	D48	22.9	1.6
	Total fecundity	20	SH2	2.6	4.9
	Early fecundity	20	SH2	12.8	6.8
34C4-42A	Longevity	20	D48-M	0.16	23
38E1-42A	Early fecundity	20	SH2	12.7	6.1
42A-49C	Early fecundity	20	D48	18.2	32
	Total fecundity	20	SH2	−2.5	2.3
	Early fecundity	20	SH2	−17.1	6.1
54B1-56E6	Longevity	30	SH2-F	0.09	8
54B1-59A2	Total fecundity	20	SH2	−3.6	1.1
62A-64D	Longevity	30	SH2-M	0.18	36
	Longevity	30	D48-F	−0.15	21
	Total fecundity	20	D48	−4.9	6
	Early fecundity	20	D48	−30.6	4.5
66D10-67A	Longevity	20	SH2-F	−0.11	17
86E3-90B2	Longevity	30	SH2-F	0.17	37
90B1-90F	Early fecundity	20	SH2	11.9	12.7
90E-95C8	Longevity	20	D48-F	0.12	6
	Total fecundity	20	D48	5.3	11
95C6-97F	Early fecundity	20	D48	30.6	8.4
	Total fecundity	20	SH2	3.4	34.3
	Early fecundity	20	SH2	26.1	42.5

M males, *F* females

QTL ranges are based on the closest markers. % Vp is percentage of total phenotypic variance explained by the QTL. *a* is the additive effect of the QTL

Composite interval mapping revealed thirteen QTL for longevity involving all three major chromosomes (Fig. 3; Table 1). All QTL were temperature specific. At 20 °C, two X-linked QTL were localized in females explaining 51 % (RIL-SH2) and 38 % (RIL-D48) of the phenotypic variance, both of them having a positive additive effect of the SH2-allele(s) of the line of high resistance to heat (Fig. 3; Table 1). Another X-linked QTL was significant in males from RIL-SH2, also with a positive additive effect of the SH2-allele(s), explaining 22 % of the phenotypic variance. Three autosomal QTL were significant also at 20 °C explaining 6–22 %

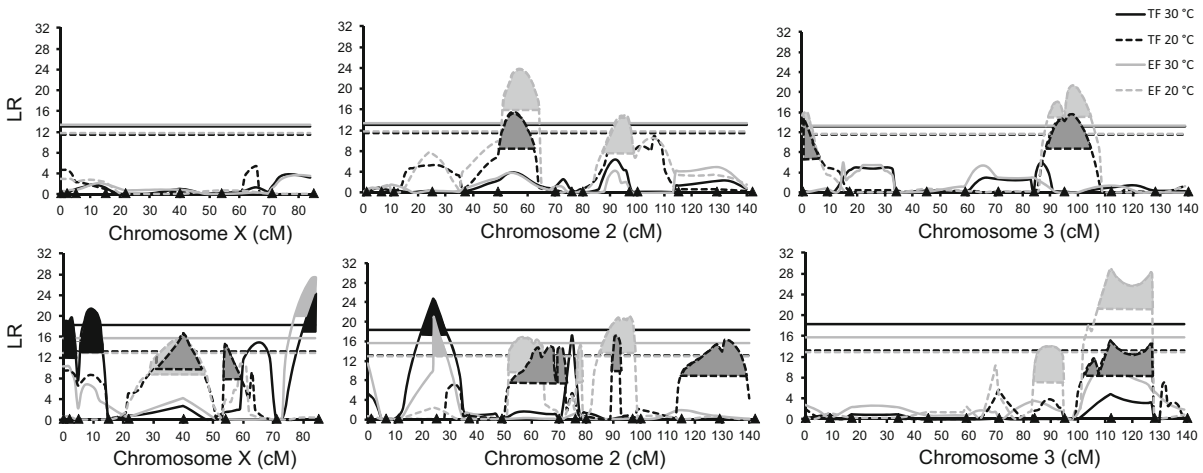

Fig. 4 Plot of likelihood ratio (LR) scores against map position (in cM) from composite interval mapping for total fecundity (TF) and early fecundity (EF) at 20 and 30 °C in *D. melanogaster* from RIL-D48 (*upper panel*) and RIL-SH2 (*lower panel*) populations. Significance thresholds were determined by 1000 random permutations (*horizontal lines*). *Triangles on the x-axis* correspond to location of markers used in composite interval mapping. Number of RIL (N) as in Fig. 1. Confidence intervals for a level higher than 95 % are shown for significant QTL (maximum width of marked QTL-peak), by using 1.5 LOD = 6.9 LR (Dupuis and Siegmund 1999)

of the phenotypic variance (Fig. 3; Table 1). One of these QTL was found in the middle of chromosome 2 in RIL-D48 males, with a positive additive effect of the SH2-allele (Table 1), and two other QTL were found on chromosome 3 in females with positive and negative additive effects of SH2-alleles in RIL-D48 and RIL-SH2, respectively (Table 1). Two other partially overlapping X-linked QTL were significant in males and females of RIL-SH2 at 30 °C (Fig. 3; Table 1), with positive effects of SH2-alleles (Table 1). Further, five autosomal QTL were also significant at 30 °C (Fig. 3; Table 1). Two QTL were significant on chromosome 2 in RIL-SH2 females, with opposite additive effects of SH2-alleles, explaining 2–8 % of the phenotypic variance (Table 1). There were three QTL on chromosome 3 explaining 21–37 % of the phenotypic variance (Table 1).

Mean fecundity is shown in Fig. 1b for each RIL panel and temperature. For TF, there was a significant two-way interaction between RIL panel and temperature (Fig. 1b; GLM with (1) RIL-D48 vs. RIL-SH2, (2) 20 °C vs. 30 °C as fixed factors: $\chi^2_1 = 1.89$ for (1); $\chi^2_1 = 20.12$*** for (2); $\chi^2_1 = 6.48$* for (1) × (2); *$P <$ 0.05; ***$P < 0.001$). In average, there were no significant differences in TF at 20 °C between RIL-D48 and RIL-SH2 (Fig. 1b; GLM for RIL panel at 20 °C: $\chi^2_1 = 0.003$; $P = 0.98$). At 30 °C, TF was higher in RIL-D48 than RIL-SH2 (Fig. 1b; GLM for RIL panel at 30 °C: $\chi^2_1 = 8.37$**; $P < 0.01$). Similar results were observed for EF (Fig. 1b; GLM for RIL panel: $\chi^2_1 = 0.09$; $P = 0.76$ at 20 °C; $\chi^2_1 = 8.37$**; $P < 0.01$ at 30 °C). TF was higher at 20 °C than 30 °C for both RIL panels (Fig. 1b; GLM for temperature in RIL-panels: $\chi^2_1 = 5.18$*; $P < 0.05$ for RIL-D48; $\chi^2_1 = 21.33$***; $P < 0.001$ for RIL-SH2). EF was higher at 20 °C than 30 °C for RIL-SH2 only (Fig. 1b; GLM for temperature in RIL-panels: $\chi^2_1 = 4.83$*; $P < 0.05$ for RIL-SH2; $\chi^2_1 = 3.82$; $P = 0.05$ for RIL-D48). Within each RIL panel (RIL-D48; RIL-SH2), we verified that variation among RIL was significant in both EF and TF at each temperature ($P < 0.001$, GLM statistics not shown).

All QTL identified for fecundity were temperature specific (Fig. 4; Table 1). Ten QTL were identified at 20 °C (Fig. 4; Table 1). Six out of the ten identified QTL co-localized for TF and EF (Fig. 4). One QTL is localized on chromosome X with negative additive effects in RIL-SH2. The other five are autosomal QTL. A consistent QTL with positive effect was localized on the left arm of chromosome 2 in both RIL panels explaining only between 0.8 and 6.8 % of the phenotypic variance (Table 1). Another autosomal QTL localized on chromosome 2 in both RIL panels but with opposite additive effects between them (Table 1). The other three autosomal QTL localized on chromosome 3, one identified on the left arm for RIL-D48 with negative additive effects and the other two on the

right arm (one for RIL-D48 and the other one for RIL-SH2), both with positive effects (Table 1). Further, four QTL were localized that were identified for TF or EF exclusively, all for RIL-SH2 (Fig. 4). One X-linked and one autosomal (chromosome 2) QTL for TF were identified with positive and negative additive effects, respectively, explaining between 1.1 and 12 % of the phenotypic variance (Table 1). For EF, two autosomal QTL with positive effects were identified on chromosomes 2 and 3, explaining between 6.1 and 12.7 % of the phenotypic variance (Table 1).

A total of four fecundity QTL were identified at 30 °C by composite interval mapping (Fig. 3). Two out of these four QTL were identified for both TF and EF in RIL-SH2 (Table 1). One is an X-linked QTL with a positive additive effect that explains between 31 and 38 % of the phenotypic variance (Table 1). The second is localized on the left arm of the chromosome 2 also with positive effects and explaining between 15.6 and 17.5 % of the variance (Table 1). Further, two X-linked QTL with negative effects on TF were identified which explain only between 1.1 and 2.4 % of the phenotypic variance (Table 1).

Possible epistatic interactions were tested between all pair-wise combinations of markers (see Norry et al. 2008, for references of the markers). Putative epistatic interactions between markers included in QTL regions for longevity and fecundity were detected involving several markers. These significant interactions involved markers included in QTL regions where many candidate genes map (Table 1). However, all these hypothetical interactions were non-significant after correction for multiple tests.

Discussion

Longevity at 30 °C was higher in RIL-SH2 (derived from a backcross to the heat-resistant line) than in RIL-D48 (derived from a backcross to the heat-sensitive line). The opposite pattern was found at the lower temperature of 20 °C, with RIL-D48 being the longer lived RIL, as expected if there is a genetic correlation between thermotolerance and longevity at either temperature. In addition, longevity was also higher in RIL-D48 than in RIL-SH2 in a previous study performed at 25 °C (Defays et al. 2011), as opposite to longevity at 30 °C (present study), In contrast to longevity, there were no significant difference in TF at 20 °C between RIL-D48 and RIL-SH2.

At high temperature (30 °C), EF was higher in the shorted-lived RIL panel (RIL-D48; Fig. 1), a negative association as expected from the disposable soma theory of aging. In addition, there was a significant interaction between RIL panel and temperature for EF.

All QTL were temperature specific both for longevity and fecundity. Longevity QTL generally co-localized with significant QTL ranges in previous studies (Nuzhdin et al. 1997, 2005; Leips and Mackay 2000; Vieira et al. 2000; Curtsinger and Khazaeli 2002; Defays et al. 2011; Bergland et al. 2012; Khazaeli and Curtsinger 2013). For instance, three out of five QTL segregating in the mapping population used in Nuzhdin et al. (1997) were significant in this study. However, as QTL ranges are wide and longevity QTL are abundant and widespread over all of the genome, we do not analyze across-studies co-localization of QTL in details. Regarding co-localizations between longevity and themotolerance QTL from previous studies, some longevity QTL co-localized with thermotolerance QTL in the same set of RIL used in the present study (Norry et al. 2008; Sambucetti et al. 2013). Interestingly, the 10A1-12E QTL for longevity detected at high temperature (30 °C) in this study co-localized with a thermotolerance QTL identified in Norry et al. (2008) with positive additive effects for both traits, indicating that the QTL conferring high KRHT also increased longevity at high temperature.

Several longevity QTL co-localized with fecundity QTL. The QTL range of 7B3-12E co-localized for longevity and EF at 20 °C, with one QTL allele showing a positive additive effect for longevity and a negative effect for EF (Table 1). The rest of the QTL that co-localized for longevity and fecundity did not differ in the sign of their additive effects, indicating that such QTL were not antagonistic (Table 1). Some QTL showed a between-trait opposite effect across environmental temperatures. For instance, one QTL allele of the 30A3-34D2 range showed a negative effect for longevity at 30 °C and a positive effect for both early and TF at 20 °C. The QTL range of 1B8-3C6 had a positive effect on longevity at 20 °C and a negative effect on fecundity at 30 °C. One QTL allele of the 54B1-59A2 range showed a positive effect on longevity and a negative effect on TF. Other QTL were either trait specific or had the same sign of

additive effects on both traits at either temperature (Table 1). Interestingly, one longevity QTL range of 62A-64D showed opposite effects on longevity of males as compared to females at 30 °C (Table 1).

Negative correlations between EF and longevity are usually considered as consistent with the antagonistic pleiotropy theory. However, genetic correlations are not always significant across RIL in *Drosophila*, even in RIL derived from longevity-selected flies (Khazaeli and Curtsinger 2013). Khazaeli and Curtsinger (2013) showed that the correlation can break down in recombinant genomes. A large-effect QTL that increased adult life span by 20 % had no detectable effect on EF in Khazaeli and Curtsinger (2013). Several recombinant genotypes exhibited both elevated EF and long life (Khazaeli and Curtsinger 2013; present study). As EF was not correlated with lifespan, the results were not fully consistent with a predominant role of negative pleiotropy of the genetic variation of each trait in our RIL set (not selected for EF). Khazaeli and Curtsinger (2013) suggested that the loss or absence of genetic correlations between longevity and EF across RIL might be the result of a mixture of pleiotropic and recombining non-pleiotropic elements. Nevertheless, EF and longevity showed a negative association between temperatures as EF at 30 °C was higher in the short-lived RIL panel at 30 °C (RIL-D48) but not at 20 °C (Fig. 1), and EF was higher at 20 °C than 30 °C for RIL-SH2 whereas the opposite pattern was found for RIL-D48.

The significant interaction between RIL panel and temperature for EF is particularly interesting given that longevity was higher and fecundity lower in the RIL panel showing higher life span at high temperature than in the heat-sensitive RIL-D48. This association pattern between longevity and fecundity is consistent both with the antagonistic pleiotropy theory and the disposable soma theory generally tested at benign temperature only. This pattern of the results is also consistent with apparently pleiotropic QTL co-localizing for EF and longevity (Table 1).

In this study we explored longevity and fecundity patterns at high and moderate temperatures to test for QTL co-localizing for the traits at both temperatures in a set of RIL lines segregating QTL for adult thermotolerance. QTL co-localizing between traits were identified at both experimental temperatures, and such co-localizing QTL differed between temperatures. All QTL were temperature specific and, in addition, one QTL was apparently negative pleiotropic (antagonistic), such as suggested by both the disposable soma and the antagonistic theories of senescence. However, the genetic correlation between longevity and EF was not significant across RIL, indicating that antagonistically acting alleles are not a predominant feature of the genetic variation in longevity and fecundity in our set of RIL. Nevertheless, some QTL alleles showed apparently antagonistic effects on longevity and fecundity across environmental temperatures. These results might reflect the fact that the parental lines of the RIL were dramatically divergent for thermotolerance (but not for EF at benign temperature), and suggest that genotype-by-temperature antagonistic interactions might influence the genetic architecture of longevity and fecundity.

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References

Arias LN, Sambucetti P, Scannapieco AC, Loeschcke V, Norry FM (2012) Survival of heat stress with and without heat hardening in *Drosophila melanogaster*: interactions with larval density. J Exp Biol 215:2220–2225

Bates D, Maechler M, Bolker B, Walker S (2013) lme4: linear mixed-effects models using Eigen and S4. R Package version 1.0-5. http://CRAN.R-project.org/package=lme4

Bergland AO, Chae H, Kim Y, Tatar M (2012) Fine-scale mapping of natural variation in fly fecundity identifies neuronal domain of expression and function of an aquaporin. PLoS Genet 8:4

Curtsinger JW, Khazaeli AA (2002) Lifespan, QTLs, age-specificity, and pleiotropy in *Drosophila*. Mech Ageing Dev 123:81–93

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

Di Rienzo JA, Casanoves F, Balzarini MG, Gonzalez L, Tablada M, Robledo CW (2014) InfoStat versión 2014. Grupo InfoStat, FCA, Universidad Nacional de Córdoba, Argentina. http://www.infostat.com.ar

Dupuis J, Siegmund D (1999) Statistical methods for mapping quantitative trait loci from a dense set of markers. Genetics 151:373–386

Flatt T (2011) Survival costs of reproduction in *Drosophila*. Exp Gerontol 46:369–375

Flatt T, Promislow DEL (2007) Still pondering an age-old question. Science 318:1255–1256

Gagnon A (2015) Natural fertility and longevity. Fertil Steril 103:1109–1116

Huey RB, Wakefield T, Crill WD, Gilchrist GW (1995) Within and between generation effects of temperature on early fecundity of *Drosophila melanogaster*. Heredity 74:216–223

Kengeri SS, Maras AH, Suckow CL, Chiang EC, Waters DJ (2013) Exceptional longevity in female Rottweiler dogs is not encumbered by investment in reproduction. Age 35:2503–2513

Khazaeli AA, Curtsinger JW (2013) Pleiotropy and life history evolution in *Drosophila melanogaster:* uncoupling life span and early fecundity. J Gerontol A Biol Sci Med Sci 68:546–553

Kimber CM, Chippindale AK (2013) Mutation, condition, and the maintenance of extended lifespan in *Drosophila*. Curr Biol 23:2283–2287

Kirkwood TB (1977) Evolution of ageing. Nature 270:301–304

Kirkwood TB, Austad SN (2000) Why do we age? Nature 408:233–238

Klepsatel P, Gáliková M, De Maio N, Huber CD, Schlötterer C, Flatt T (2013) Variation in thermal performance and reaction norms among populations of *Drosophila melanogaster*. Evolution 67:3573–3587

Le Bourg E (2007) Does reproduction decrease longevity in human beings? Ageing Res Rev 6:141–149

Leips J, Mackay TFC (2000) Quantitative trait loci for life span in *Drosophila melanogaster*: interactions with genetic background and larval density. Genetics 155:1773–1788

Leips J, Gilligan P, Mackay TFC (2006) Quantitative trait loci with age-specific effects on fecundity in *Drosophila melanogaster*. Genetics 172:1595–1605

Leroi AM, Bartke A, Benedictis GD, Franceschi C, Gartner A, Gonos E, Feder ME, Kivisild T, Lee S, Kartal-Ozer N et al (2005) What evidence is there for the existence of individual genes with antagonistic pleiotropic effects? Mech Ageing Dev 126:421–429

Luckinbill LS, Arking R, Clare MJ, Cirocco WC, Buck SA (1984) Selection for delayed senescence in *Drosophila melanogaster*. Evolution 38:996–1003

Lynch M, Walsh B (1998) Genetics and analysis of quantitative traits. Sinauer Associates Inc, Sunderland

Mackay TFC (2001) Quantitative trait loci in *Drosophila*. Nat Rev Genet 2:11–20

Mackay TFC (2002) The nature of quantitative genetic variation for *Drosophila* longevity. Mech Ageing Dev 123:95–104

Medawar PB (1952) An unsolved problem in biology. Lewis, London

Morgan TJ, Mackay TFC (2006) Quantitative trait loci for thermotolerance phenotypes in *Drosophila melanogaster*. Heredity 96:232–242

Norry FM, Sambucetti P, Scannapieco AC, Loeschcke V (2006) Altitudinal patterns for longevity, fecundity and senescence in *Drosophila buzzatii*. Genetica 128:81–93

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

Nuzhdin SV, Pasyukova EG, Dilda CL, Zeng Z-B, Mackay TFC (1997) Sex-specific quantitative trait loci affecting longevity in *Drosophila melanogaster*. Proc Natl Acad Sci USA 94:9734–9739

Nuzhdin SV, Khazaeli AA, Curtsinger JW (2005) Survival analysis of life span quantitative trait loci in *Drosophila melanogaster*. Genetics 170:718–731

Partridge L, Gems D, Withers DJ (2005) Sex and death: what is the connection? Cell 120:461–472

Pasyukova EG, Roshina NV, Mackay TFC (2004) Shuttle craft: a candidate quantitative trait gene for *Drosophila* lifespan. Aging Cell 5:297–307

R Core Team (2014) R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna

Remolina SC, Chang PL, Leips J, Nuzhdin SV, Hughes KA (2012) Genomic basis of aging and life-history evolution in *Drosophila melanogaster*. Evolution 66:3390–3403

Rose MR (1984) Laboratory evolution of postponed senescence in *Drosophila melanogaster*. Evolution 38:1004–1010

Rose MR (1991) Evolutionary biology of aging. Oxford University Press, New York

Rose MR, Charlesworth B (1980) A test of evolutionary theories of senescence. Nature 287:141–142

Sambucetti P, Sørensen JG, Loeschcke V, Norry FM (2005) Variation in senescence and associated traits between sympatric cactophilic sibling species of *Drosophila*. Evol Ecol Res 7:915–930

Sambucetti P, Scannapieco AC, Loeschcke V, Norry FM (2013) Heat-stress survival in the pre-adult stage of the life cycle in an intercontinental set of recombinant inbred lines of *Drosophila melanogaster*. J Exp Biol 216:2953–2959

Scannapieco A, Sambucetti P, Norry FM (2009) Direct and correlated responses to selection for longevity in *Drosophila buzzatii*. Biol J Linn Soc Lond 97:738–748

Service PM, Hutchison EW, Rose MR (1988) Multiple genetic mechanisms for the evolution of senescence in *Drosophila melanogaster*. Evolution 42:708–716

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

Tarin JJ, Gómez-Piquer V, García-Palomares S, García-Pérez MA, Cano A (2014) Absence of long-term effects of reproduction on longevity in the mouse model. Reprod Biol Endocrinol 12:84

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

Vieira C, Pasyukova EG, Zeng ZB, Hackett JB, Lyman RF, Mackay TFC (2000) Genotype-environment interaction for quantitative trait loci affecting life span in *Drosophila melanogaster*. Genetics 154:213–227

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

Williams GC (1966) Natural selection, the costs of reproduction and a refinement of Lack's principle. Am Nat 100:687–690

Wit J, Sarup P, Lupsa N, Malte H, Frydenberg J, Loeschcke V (2013) Longevity for free? Increased reproduction with limited trade-offs in *Drosophila melanogaster* selected for increased life span. Exp Gerontol 48:349–357

Zeng ZB (1994) Precision mapping of quantitative trait loci. Genetics 136:1457–1468

Zwaan B, Bijlsma R, Hoekstra R (1995) Direct selection on life span in *Drosophila melanogaster*. Evolution 49:649–659