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RESEARCH ARTICLE

Heat-stress survival in the pre-adult stage of the life cycle in an intercontinental set of recombinant inbred lines of *Drosophila melanogaster*

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SUMMARY

In insects, pre-adult stages of the life cycle are exposed to variation in temperature that may differ from that in adults. However, the genetic basis for adaptation to environmental temperature could be similar between the pre-adult and the adult stages of the life cycle. Here, we tested quantitative trait loci (QTL) for heat-stress survival in larvae of *Drosophila melanogaster*, with and without a mild-heat-stress pre-treatment. Two sets of recombinant inbred lines derived from lines artificially selected for high and low levels of knockdown resistance to high temperature in young flies were used as the mapping population. There was no apparent increase in heat-shock survival between heat-pretreated and non-pretreated larvae. There was a positive correlation between the two experimental conditions of heat-shock survival (with and without a heat pre-treatment) except for males from one set of lines. Several QTL were identified involving all three major chromosomes. Most QTL for larval thermotolerance overlapped with thermotolerance QTL identified in previous studies for adults, indicating that heat-stress resistance is not genetically independent between life cycle stages because of either linkage or pleiotropy. The sign of the effects of some QTL alleles differed both between the sexes and between life stages.

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INTRODUCTION

Temperature has important consequences for fitness. The ability of organisms to cope with thermal stress is important for adaptation to changing thermal environments and global warming (Hoffmann and Parsons, 1991; Hoffmann and Daborn, 2007; Reusch and Wood, 2007; Bowler and Terblanche, 2008; Hoffmann and Willi, 2008). The persistence of natural populations that experience heat stress depends not only on the potential of adults to respond to and survive thermal stress but also on that of all other life cycle stages. Resistance to thermal stress is a trait that exhibits phenotypic plasticity and there is a potential for considerable variation between life cycle stages (Loeschcke and Krebs, 1996; Hercus et al., 2000; Hoffmann et al., 2003). Furthermore, thermotolerance may change with previous exposure to thermal stress (Hoffmann and Parsons, 1991; Hoffmann et al., 2003; Bowler and Terblanche, 2008).

In holometabolous insects such as *Drosophila*, the adult stage is morphologically different and much more mobile than all pre-adult life stages of the life cycle. Adult *Drosophila melanogaster* can move within and between host plant patches, thereby potentially avoiding high temperatures, but larvae are much less mobile. Although larvae can move within food substrates, resistance to thermal stress may be more critical in larvae than in adults because of a rather limited ability for evasion from heat stress compared with the adult fly. Several studies have focused on thermal resistance of pre-adult life stages (e.g. Krebs and Loeschcke, 1995; Loeschcke and Krebs, 1996; Feder et al., 1996; Feder et al., 1997; Krebs and Feder, 1998; Hercus et al., 2000; Gibbs et al., 2003; Takahashi et al., 2011). One way of assessing heat resistance in the pre-adult stage of *Drosophila* is to study survival to heat stress as the ability to develop to adulthood following exposure to a potentially lethal stress of high temperature [see Hoffmann et al. (Hoffmann et al., 2003) for a review on thermal stress traits]. This heat-shock survival assay is widely used to measure thermotolerance in pre-adult *Drosophila* (reviewed in Hoffmann et al., 2003).

Quantitative trait loci (QTL) mapping is a widely used technique to identify genomic regions that contain relevant genes affecting a trait of interest (Lynch and Walsh, 1998; Mackay, 2001). Recent studies have used this technique in adult *D. melanogaster* to identify genomic regions in which relevant loci for adaptive change in thermotolerance are localized, with QTL found on all three major chromosomes (Norry et al., 2004; Morgan and Mackay, 2006; Norry et al., 2007a; Norry et al., 2007b; Norry et al., 2008; Arias et al., 2012). Putative candidate genes have been identified and discussed elsewhere (e.g. Morgan and Mackay, 2006; Sørensen et al., 2007; Rako et al., 2007; Hoffmann and Willi, 2008; Franks and Hoffmann, 2012). Genes whose expression is affected by thermal stress, including genes that code for heat-shock proteins (Hsps), are primary candidate loci for both basal and induced thermotolerance (e.g. McColl et al., 1996; Feder et al., 1996; Feder and Hofmann, 1999; Feder et al., 2000; Bettencourt et al., 2002; Frydenberg et al., 2003; Walser et al., 2006). However, it is still unclear whether the same QTL for thermotolerance in adults are also involved in thermotolerance in the pre-adult stages of the life cycle. Previous studies have shown variation in relative resistance to thermal stress

across life stages (e.g. Krebs and Loeschcke, 1995). Artificial selection experiments showed that thermotolerance selection on the adult fly does not always affect thermotolerance in the pre-adult stage (Loeschcke and Krebs, 1996). Thus, it seems relevant to know whether the genetic basis for adaptive evolution of thermotolerance in the pre-adult stage may involve either the same or different QTL as in the adult fly.

In this study we identified QTL for heat-stress survival in larvae of *D. melanogaster* using a set of recombinant inbred lines (RIL) that were derived from lines artificially selected for high and low knockdown resistance to high temperature (KRHT). These RIL were previously used to map thermotolerance traits in adult flies (Norry et al., 2008; Arias et al., 2012). However, it is not known whether previously identified QTL for KRHT in adults affect also heat-stress survival in larvae or whether different QTL are affecting heat resistance in this pre-adult stage of the life cycle. Recently, Takahashi et al. (Takahashi et al., 2011) used a genome-wide deficiency screen to find deletions affecting heat resistance in *D. melanogaster*. Several significant deficiencies in Takahashi et al. (Takahashi et al., 2011) were included within QTL regions previously found by recombination mapping (Norry et al., 2004; Morgan and Mackay, 2006; Norry et al., 2007a; Norry et al., 2007b; Norry et al., 2008). However, this genome-wide deficiency screen was performed using deletions without any tests for quantitative complementation (Moehring and Mackay, 2004). QTL mapping may use wild-type alleles to QTL-map thermotolerance (e.g. Norry et al., 2004; Morgan and Mackay, 2006; Norry et al., 2007a; Norry et al., 2007b; Norry et al., 2008), as in the present study. Two main aims are addressed in this study. First, we examined whether QTL for heat-stress survival in larvae co-localize with and without a heat-stress pre-treatment. Second, we examined whether chromosomal regions affecting these traits in larvae co-localize with major thermotolerance QTL previously identified in the adult fly (Morgan and Mackay, 2006; Norry et al., 2007a; Norry et al., 2007b; Norry et al., 2008). Specifically, we address the question of to what extent do the QTL regions affecting variation in heat-stress resistance in larvae match those detected in previous studies in adult flies? Co-localized QTL could contain multiple tightly linked stage-specific genes or one or more genes with pleiotropic effects on thermotolerance in both larvae and adults. If such QTL co-localize between larvae and adults, thermal selection in larvae will not respond independently from thermal selection in adult flies because of either linkage or pleiotropy.

MATERIALS AND METHODS

Recombinant inbred lines

Lines of *Drosophila melanogaster* Meigen 1830 used in this study were described in Norry et al. (Norry et al., 2008). In short, parental stocks were D48 and SH2 lines derived from Denmark and Australia, respectively, selected for low (D48) and high (SH2) resistance to heat knockdown in adult flies. F1 females (progeny of D48 × SH2) were backcrossed to males from each parental stock. One set of RIL was constructed from the D48 backcross and another set of RIL was constructed from the SH2 backcross to form our 'RIL-D48' and 'RIL-SH2' stocks. The use of both reciprocal backcrosses increases the statistical power to detect QTL comparing to designs based on 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. The genetic map associated to these RIL was based on microsatellite loci throughout all three major chromosomes [see Norry et al. (Norry et al., 2008) for bibliographic references of microsatellites used], and map positions were (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), 3-1125 (95C6-C8), 3-128 (97F), 3-140 (99D6-D9).

Phenotypes measured

Heat-stress survival was measured in the pre-adult stage with and without heat-shock pre-treatment, hereafter referred to as HT and NT, respectively. Forty first-instar larvae were transferred from small spoons containing agar and yeast to standard vials (80×20 mm) containing 5 ml of a culture medium (instant mashed potatoes, dry yeast, sugar, nipagin and water). All cultures were placed in a temperature-controlled room at 25±1°C, with five to eight vials per line for each pre-treatment (HT and NT). Non-heat-treated larvae were kept at 25±1°C until heat-stress survival was measured. Heat pre-treatment consisted of exposing first-instar larvae cultures to 29°C (water bath) for 3 h (13:00 to 16:00 h) every day during three consecutive days at 25±1°C. Heat-stress survival was measured by exposing third-instar larvae cultures to 33°C (water bath) for 3 h (13:00 to 16:00 h). Survival was scored as the proportion of flies of each sex that emerged from each culture. Variation in heat-stress survival was tested by a three-way ANOVA using RIL panel (RIL-D48 *versus* RIL-SH2), pre-treatment (heat-treated *versus* non-heat-treated) and sex (males *versus* females) as fixed factors. The same protocol described above was repeated but without the heat-stress survival test as control for viability for each RIL, also with (HTC) and without (NTC) the heat pre-treatment.

QTL analysis

Marker genotypes were the number of SH2 alleles (0 or 2) for both RIL-D48 and RIL-SH2 as described in Norry et al. (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 test was performed 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 using a window size of 10 cM and five control markers were consistent across a wide range of parameter combinations. Significance thresholds were determined by 1000 random permutations. For significant QTL, confidence intervals were estimated using 1.5 LOD (6.9 LR) for confidence >95% [where LOD is logarithm (base 10) of odds and LR is likelihood ratio], according to Dupuis and Siegmund (Dupuis and Siegmund, 1999). In order to identify QTL for heat-stress survival rather than QTL for viability itself, QTL mapping was performed separately for both HT and NT larvae as well as for their respective controls (HTC and NTC).

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

RESULTS

Mean survival to heat stress is shown in Fig. 1 for each RIL panel and sex. RIL-SH2 was more resistant to heat stress than RIL-D48 and there was no difference between the sexes (Fig. 1, Table 1). Heat-

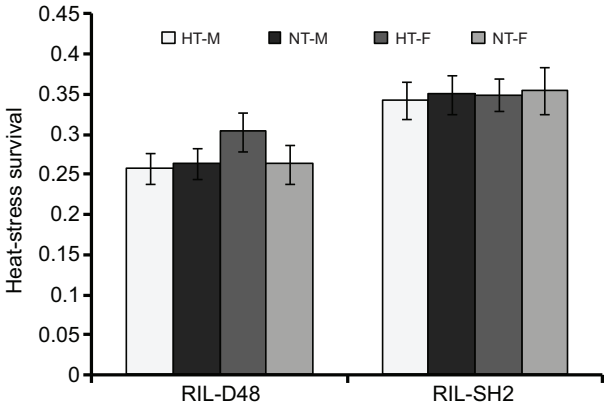

Fig. 1. Mean (±s.e.m.) proportion of *Drosophila melanogaster* flies emerged from heat-stress survival (HS) in heat pre-treated (HT) and non-treated (NT) larvae for each recombinant inbred line (RIL) panel, D48 and SH2, used for quantitative trait loci (QTL) mapping.

stress survival was lower than survival in the respective controls in both RIL-D48 and RIL-SH2 (supplementary material Fig. S1, Table S1). Heat-stress survival showed a considerable variation among lines in their respective responses to our stressful heat pre-treatment. However, only few lines showed significant differences between NT and HT (i.e. non-treated and heat-pre-treated larval cultures), as was apparent from two-tailed *t*-tests for each line (three lines showed an increased resistance in RIL-D48 females and two lines showed a decreased resistance in RIL-SH2 males; supplementary material Table S2). There was a positive correlation between NT and HT in all cases except for SH2 males (Spearman's rank correlation: r_S=0.47** for D48 males; r_S=0.48** for D48 females; r_S=0.28 for SH2 males; r_S=0.54* for SH2 females; *P<0.05; **P<0.01; Fig. 2).

Composite interval mapping for heat-stress survival in NT larvae revealed multiple QTL for the pre-adult stage of the life cycle (Fig. 3). X-linked QTL were significant in RIL-D48 females (cytological range, 3C1-4F1) and RIL-SH2 males (12D-19F6; Fig. 3), with positive and negative additive effects, respectively (Table 2). Further, five autosomal QTL were significant (Fig. 3), including two QTL on chromosome 2 and three QTL on chromosome 3 (Fig. 3). One well-known QTL region included the pericentromeric region of chromosome 2 (cytological bands, 42A-49C) in RIL-SH2 females (Fig. 3, Table 2). One QTL was found in females from both RIL panels on the left arm of the chromosome 3 (66D10-73B7) but with opposite additive effects (positive for RIL-D48 and negative for RIL-SH2; Table 2). Two other QTL were localized on the right arm of chromosome 3 in RIL-SH2 males (62A-63F1 and 90B1-95C8), with additive effects that were opposite in sign between these QTL (Table 2). Both the 3C1-4F1 and 12D-19F6 QTL mentioned above co-localized with QTL for viability in RIL-SH2 (3C1-4F1 in HTC females and 12D-19F6 in NTC males; supplementary material Fig. S2).

Table 1. Results of ANOVA performed to test for differences in heat-stress survival in recombinant inbred lines of *Drosophila melonogaster* used for quantitative trait loci mapping

Source of variation	d.f.	MS	*F*
(1) RIL-D48 *vs* RIL-SH2	1	2.1	45.36***
(2) Heat-treated *vs* non-treated	1	0.005	0.107
(3) Males *vs* females	1	0.077	1.67
(1) × (2)	1	0.12	2.59
(1) × (3)	1	0.01	0.21
(2) × (3)	1	0.054	1.17
(1) × (2) × (3)	1	0.039	0.85
Error	1260	0.046	

***P<0.001.

In HT larvae, three autosomal QTL were detected (Fig. 3). One QTL was significant on chromosome 2 (cytological range, 30A6-38E9) in RIL-SH2 males, with a negative additive effect (Table 2). Two other QTL were found on the right arm of chromosome 3 (ranges 90E-95C8 and 95C6-99D9, both in females from RIL-SH2 and RIL-D48, respectively), which differed in the sign of their additive effects (Table 2). The 90E-95C8 QTL overlapped with a QTL for viability identified in HTC females for RIL-SH2 (supplementary material Fig. S2).

We also tested for possible epistatic interactions between all pairwise combinations of markers used for heat-stress survival QTL mapping [see Norry et al. (Norry et al., 2008) for references of the markers]. Putative epistatic interactions were detected involving markers DMU56661 (4F1-F2), AC006302 (34C4-D2) and DROTG121 (42A). Interactions between DROTG121 and DS00361 (54B1-B2) as well as between DROTG121 and AC004307 (56D11-E6) were apparent across pre-treatment (NT and HT) and sex (ANOVA: DROTG121 × DS00361 interaction: $F_{1,49}$=5.7, P=0.021 for HT males; $F_{1,49}$=4.2, P=0.046 for NT males; $F_{1,49}$=8.8, P=0.004 for HT females; $F_{1,49}$=6.1, P=0.018 for NT females; DROTG121 × AC004307 interaction: $F_{1,49}$=9.9, P=0.003 for HT males; $F_{1,49}$=6.6, P=0.013 for NT males; $F_{1,49}$=8.9, P=0.004 for HT females; $F_{1,49}$=5.9, P=0.018 for NT females). Interaction between DMU661 and AC006302 was also apparent (ANOVA: $F_{1,49}$=10.9, P=0.0018 for HT males; $F_{1,49}$=9.7, P=0.0031 for NT males; $F_{1,49}$=5.1, P=0.029 for NT females). These significant interactions involved markers included in QTL regions where many candidate genes map (Table 2). However, all these hypothetical interactions were non-significant after correction for multiple tests.

DISCUSSION

QTL were identified for larval survival to heat stress in a set of RIL that was previously used to identify thermotolerance QTL in the adult stage of the life cycle. Only three of the 11 QTL detected for heat-stress survival were also identified as QTL for viability in our controls. The main result of this study was that heat-stress QTL in larvae generally overlapped with QTL for heat resistance in adult flies previously found using not only the same set of RIL as in Norry et al. (Norry et al., 2008) but also other mapping populations (Morgan and Mackay, 2006; Norry et al., 2007a). This main result is largely consistent with that of Takahashi (Takahashi et al., 2011), where QTL in larvae generally co-localized with previously detected QTL in adults. However, a new QTL was also detected for heat resistance that is exclusive for the larval stage, involving chromosome 3 in NT larvae (Table 2). Although survival to heat-stress did not differ between HT and NT larvae, we identified a few QTL that were exclusive for HT larvae. It is well known that environmentally induced effects such as acclimation and heat-hardening can trigger the expression of many genes (e.g. Sørensen et al., 2005). Changes in gene expression and/or regulation of gene networks and epistatic interactions could be responsible for the differences in the genetic architecture between NT and HT larvae, even when no NT-*versus*-HT differences are found at the phenotypic level. The mild-heat-stress pre-treatment decreased the number of significant QTL, and QTL did not overlap between NT and HT larvae (Table 2). This reduction in the number of significant QTL

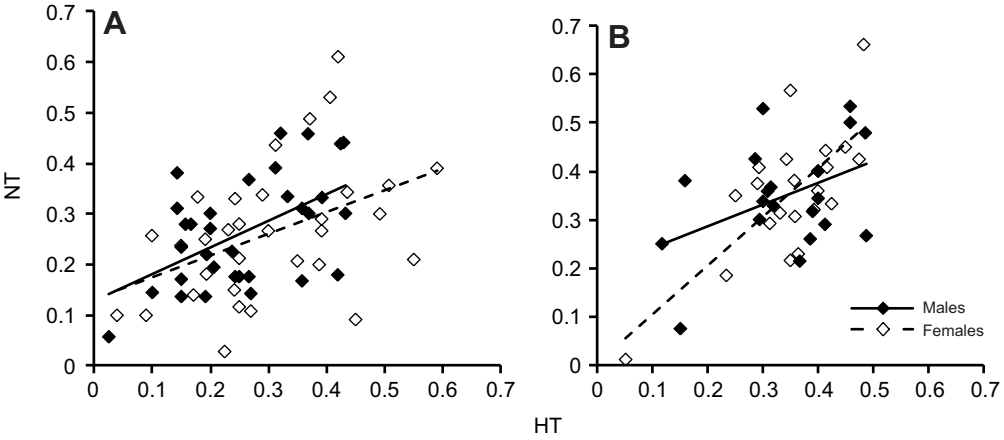

Fig. 2. Mean proportions of *D. melanogaster* flies emerged from heat-stress survival in non-treated (NT) *versus* heat pre-treated (HT) larvae for RIL-D48 (A) and RIL-SH2 (B) panels.

by a sub-lethal heat stress pre-treatment is consistent with previous results where heat-hardening in adult flies decreased the number of significant QTL for heat knockdown resistance (Norry et al., 2008). Besides, QTL for HT in larvae explained only a small proportion of the phenotypic variance (between 5 and 10%; Table 2).

Larvae-to-adult survival to heat stress was positively correlated between NT and HT experimental larvae (Fig. 2). Our heat-stress pre-treatment did not increase heat-stress survival in this study, but milder heat stress in larvae can induce heat-hardening, increasing larvae-to-adult survival to subsequent and potentially lethal heat stress (e.g. Feder et al., 1996; Krebs and Feder, 1998). In contrast to the heat pre-treatment in larvae used in the present study, the heat-hardening treatment used by Norry et al. (Norry et al., 2008) in adult flies substantially increased heat resistance in both RIL-D48 and RIL-SH2.

Two large-effect QTL were found on chromosome 2, each one explaining 18–30% of the phenotypic variance in NT larvae (Table 2). These two QTL partially overlap with thermotolerance QTL previously found for adult flies in the same sex (Morgan and Mackay, 2006; Norry et al., 2007a). Many candidate genes map within these QTL ranges (supplementary material Table S3). All of these candidate genes were either heat upregulated (Leemans et al., 2000; Ekengren and Hultmark, 2001; Sørensen et al., 2005) or differed in their respective expression levels between heat-resistant and heat-sensitive lines in *D. melanogaster* (McKechnie et al., 1998; Nielsen et al., 2006; Sørensen et al., 2007; Norry et al., 2009). Some of these candidate loci are involved in metabolic (e.g. *Gpdh*, *LvpH*, *LvpL*, *Pgi*) or developmental processes (e.g. *Sax*, *anon-23Da*, *Su(var)2-10*) and sensory perception (e.g. *Obp28*, *Obp44a*). One candidate gene included in the 25F5-30A6 QTL range, *slowmo* (*slmo*), is involved in larval behavior, larval peristaltic movements and locomotion (Carhan et al., 2004). Genetic variation at the *slmo* locus could be crucial for resistance to high temperature stress as resistance to heat stress is also related to the ability of avoiding the

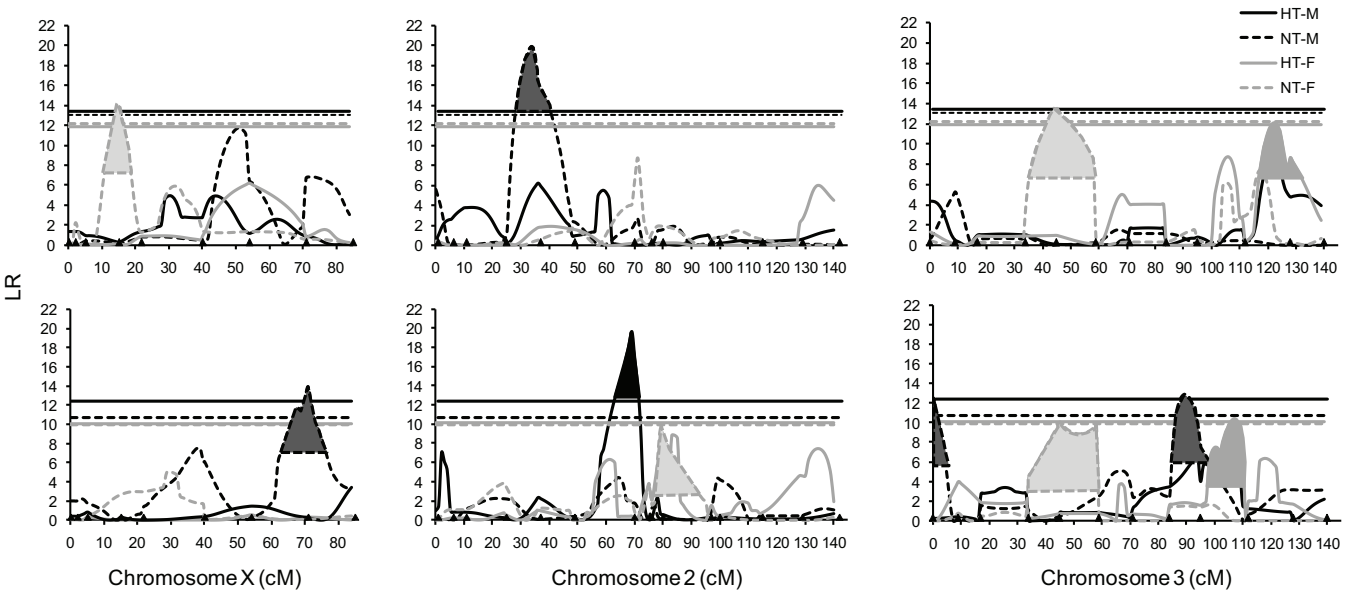

Fig. 3. Plot of likelihood ratio (LR) scores against map position (in cM) from composite interval mapping for heat-stress survival in non-treated (NT) and heat pre-treated (HT) male (M) and female (F) larvae of *D. melanogaster* from RIL-D48 (upper panels) and RIL-SH2 (lower panels) populations. Significance thresholds were determined by 1000 random permutations (horizontal lines). Black triangles on the *x*-axis correspond to location of markers used in composite interval mapping. Confidence intervals for a level higher than 95% are shown for significant QTL (maximum width of marked QTL peak), using 1.5 LOD=6.9 LR (Dupuis and Siegmund, 1999).

Table 2. Quantitative trait loci (QTL) for heat-stress survival in the preadult stage of the life cycle, with and without a heat shock pre-treatment identified by composite interval mapping in RIL-D48 and RIL-SH2

Trait	Sex	RIL	QTL range	*a*	% PV	Reference
NT	F	D48	3C1-7B3	0.10	13	Rand et al., 2010; Arias et al., 2012
NT	M	SH2	12D-19F6	**–0.09**	3	Arias et al., 2012
NT	M	D48	25F5-30A6	0.08	30	Norry et al., 2008; Arias et al., 2012
NT	F	SH2	42A-49C	0.08	30	Morgan and Mackay, 2006; Norry et al., 2007a; Arias et al., 2012
NT	M	SH2	62A-63F1	0.08	8	Morgan and Mackay, 2006; Arias et al., 2012
NT	F	D48	66D10-73B7	0.1	10	Norry et al., 2004; Norry et al., 2008
NT	F	SH2	66D10-73B7	**–0.07**	16	Norry et al., 2004; Norry et al., 2008
NT	M	SH2	90B1-90E	–0.08	0.4	–
HT	M	SH2	30A6-38E9	**–0.07**	5	Norry et al., 2004; Norry et al., 2007a; Norry et al., 2008; Arias et al., 2012
HT	F	SH2	90E-95C8	**–0.08**	5	Norry et al., 2004; Norry et al., 2008; Arias et al., 2012
HT	F	D48	95C8-99D9	0.11	10	Norry et al., 2008; Arias et al., 2012

NT, heat-stress survival without a heat pre-treatment; HT, heat-stress survival with a heat pre-treatment; M, male; F, female. References are given for QTL ranges partially overlapping with QTL for heat resistance identified in previous studies in the adult fly. Bold values indicate a shift in the sign of the additive effect of a QTL allele in larvae (present experiment) as compared with adults [previous studies using the same recombinant inbred lines (RIL) as in this study]. QTL ranges are based on the closest markers. % PV is percentage of total phenotypic variance explained by the QTL. *a* is the additive effect of the QTL.

stress by moving within food substrates. *trap1* is an Hsp-related gene that is involved in the response to various stressors (FlyBase Consortium, 2003). Combined expression of *trap1* with other closely linked candidate genes best predicted thermotolerance phenotypes in adult flies in RIL used in this study (Norry et al., 2009). Another QTL that overlaps with a thermotolerance QTL previously found in the adult fly includes the pericentromeric region of chromosome 2, which was significant in the present study within the 30A3-38E9 cytological interval for heat pre-treated males (Table 2). This autosomal QTL affects heat resistance, exhibiting co-localization across diverse mapping populations (Morgan and Mackay, 2006; Norry et al., 2008). Furthermore, the thermotolerance effect of this QTL was also recently found to be significant in field-released adult flies (Loeschcke et al., 2011).

Four out of five QTL on chromosome 3 (Table 2) overlapped with thermotolerance QTL previously found for adult flies (Norry et al., 2004; Morgan and Mackay, 2006; Norry et al., 2007a; Norry et al., 2008). One QTL explaining 10 to 16% of the phenotypic variance was found on the left arm of chromosome 3 (66D10-73B7) in NT females from both RIL panels, but this QTL exhibited additive effects that were opposite in sign across RIL panels (Table 2). A possible explanation for such a shift in sign across RIL panels is the genetic background, which substantially differs between RIL panels. In any event, further study is necessary to re-test this possible QTL as several candidate genes map within its QTL range (supplementary material Table S3), including the small heat-shock protein genes (*shsp*s). Some *shsp*s were found to show clinal variation with latitude in *D. melanogaster* on the east coast of Australia (Frydenberg et al., 2003). Other heat-shock genes included within the above-mentioned QTL range (66D10-73B7) are *hsp67Ba*, *hsp67Bb* and *hsp67Bc*, with *hsp67Bc* being heat upregulated in a previous study in larvae (Leemans et al., 2000). Hsp83 is another heat-shock protein that was also heat upregulated both in the pre-adult stage of the life cycle (Leemans et al., 2000) and in adult flies (Sørensen et al., 2005). *hsp83* maps within a QTL detected on the left arm of chromosome 3 in NT males. This QTL also co-localized with a QTL detected for heat-stress resistance in adult flies (Morgan and Mackay, 2006). There are also further Hsp genes on the right arm of chromosome 3 that are included within other QTL regions in this study (supplementary material Table S3). For instance, *hsp68* maps within a QTL in HT females that also co-localized with one QTL detected by Norry et al. (Norry et al., 2008) in adults from the same set of RIL. The expression level of *hsp68* has been found to also be heat upregulated in adult flies (Sørensen et al., 2005). This QTL has also been detected as a QTL for viability of control in HTC females. Either pleiotropy or linkage could explain co-localization of this QTL for HT and HTC. *hsr-omega* is another well-known candidate gene for thermotolerance that mapped within a QTL on the right arm of chromosome 3 in HT female larvae (Table 2; supplementary material Table S3). Interestingly, this QTL in pre-adult individuals overlapped with a thermotolerance QTL found by Norry et al. (Norry et al., 2008) in adult flies, showing a shift in the sign of its additive effects, though this QTL in larvae co-localized with a QTL for viability (supplementary material Fig. S2). Previous studies found that *hsr-omega* affects heat resistance in adult flies (e.g. McColl et al., 1996; McKechnie et al., 1998). Anderson et al. (Anderson et al., 2003) found that a polymorphic region of the *hsr-omega*'s promoter exhibits clinal variation with latitude on the east coast of Australia. More recently, Rako et al. (Rako et al., 2007) observed that increased heat resistance was associated with one allele of *hsr-omega*. Other candidate genes are also included on the same QTL region where *hsr-omega* maps (supplementary material Table S3), such as Turandot-related genes, which are known to be involved in the cellular response to heat (Ekengren and Hultmark, 2001). This result supports previous findings showing that the region where *hsr-omega* and other candidate genes map on the right arm of chromosome 3 represents a consistent QTL for thermotolerance not only in the adult fly, but also in the pre-adult stage(s) in *D. melanogaster* (Table 2).

Recently, Takahashi et al. (Takahashi et al., 2011) used a genome-wide deficiency screen to find deletions affecting resistance to high temperature in larvae of *D. melanogaster*. Several significant gene deficiencies in Takahashi et al. (Takahashi et al., 2011) were included within the QTL regions previously found on chromosomes 2 and 3 by recombination mapping in adults (Norry et al., 2004; Morgan and Mackay, 2006; Norry et al., 2007a; Norry et al., 2007b; Norry et al., 2008) and larvae (present study). Partial co-localization of QTL for heat resistance in adult flies (Morgan and Mackay, 2006; Norry et al., 2008) and larvae (Takahashi et al., 2011; present study) indicates that resistance to heat stress is not genetically independent between life cycle stages, although QTL might contribute to different aspects of heat resistance. No significant region on chromosome X was found for heat resistance of larvae in Takahashi et al.'s (Takahashi et al., 2011) study. We detected two X-linked

QTL that have also previously been found in adults (Table 2). These results suggest X-linked differences between the parental mapping population used by Takahashi and co-workers and in the present study. We performed a QTL mapping using an intercontinental set of recombinant inbred lines where natural genetic variation occurs while Takahashi et al. (Takahashi et al., 2011) performed a genome-wide deficiency screen. Most of the deficiencies on the X chromosome in Takahashi et al. (Takahashi et al., 2011) were lethal in males. Thus, the effect of the X chromosome on heat sensitivity was evaluated only in females. In our study, we evaluated heat resistance in both males and females, and one of the two QTL detected on chromosome X was significant in males only (Table 2). Our results also show that the genetic architecture for larval thermotolerance differs between the sexes (Table 2). The two X-linked QTL were also identified as QTL for viability in the QTL mapping of controls. However, co-localization of these QTL in larvae with QTL for heat resistance in adults (Rand et al., 2010; Arias et al., 2012) suggest that X-linked QTL may affect heat-stress resistance in larvae, because of either linkage or pleiotropic effects on viability and thermotolerance.

The genetic basis for thermal adaptation and evolution in holometabolous insects such as *Drosophila* should depend on the number, effects and chromosomal distribution of QTL across the whole life cycle. The present study was performed on RIL that were set up from two parental lines that were derived from two wild populations in Denmark (D48) and Australia (SH2; see above). Thus, the genetic variation in these RIL resulted from crossings of very different populations. Several selection experiments and population comparison studies suggested that resistance to extreme temperatures is often un-correlated between life cycle stages at the phenotypic level (e.g. Krebs and Loeschcke, 1995; Hercus et al., 2000; for a review, see Hoffmann et al., 2003). Further, we found no phenotypic correlation in heat resistance between larvae and adults in our set of RIL (supplementary material Fig. S3). Although, our results show that most QTL identified in larvae partially overlap with QTL detected in previous studies in adult flies, the sign of the effects of QTL alleles often differed both between the sexes and between life stages (Table 2). This variation in the sign of QTL allele effects could explain the previously mentioned lack of phenotypic correlation in thermotolerance between life cycle stages. Fine-scale mapping might help to test which, if any, thermotolerance QTL are shaped by the same genes in larvae and adults (pleiotropy) and which QTL in larvae overlap with QTL in adults because of linkage.

One new QTL for heat resistance that had not previously been detected in adult flies was found for pre-adult males that received no heat pre-treatment (Table 2). This QTL was found on the right arm of chromosome 3, explaining less than 1% of the phenotypic variance (Table 2). This result suggests that most of the genetic variability responsible for heat resistance in larvae is based on other (large-effect) QTL that co-localize with QTL for basal thermotolerance previously found in the adult fly (see above for references). Additionally, we also found three QTL for thermotolerance that were specific for heat-treated larvae. However, two of these QTL in heat-treated larvae were found to have only a small effect, explaining only 5% of the phenotypic variance (Table 2). This result is consistent with previous work (Norry et al., 2008) in adult *D. melanogaster*, where the number of QTL detected as well as the phenotypic variance explained by each QTL were lower in heat-treated (hardened) than in non-hardened flies. This similar trend in both adults and larvae suggests that heat-hardening effects may generally be related to heat-shock genes that are not functionally variable between individuals or populations. However, our heat-shock pre-treatment on larvae did not substantially induce heat-hardening. This is the first QTL mapping study for thermotolerance in the pre-adult stage of the life cycle in *D. melanogaster*. Most of the large-effect QTL in this experiment for pre-adult *D. melanogaster* co-localized with thermotolerance QTL previously found in the adult fly, suggesting that heat resistance in larvae and adult insects is not genetically independent between life-cycle stages because of either linkage or pleiotropy.

LIST OF ABBREVIATIONS

HT	heat-stress survival with heat-shock pre-treatment
HTC	viability of control with heat-shock pre-treatment
NT	heat-stress survival without heat-shock pre-treatment
NTC	viability of control without heat-shock pre-treatment
QTL	quantitative trait loci
RIL	recombinant inbred line

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AUTHOR CONTRIBUTIONS

P.S. measured thermo-resistance phenotypes, analyzed all data and wrote the first draft of the manuscript. A.C.S. measured thermo-resistance phenotypes and revised the manuscript. Both V.L. and F.M.N. conceived and designed the experiments and analyses, and drafted and revised the final manuscript. All authors contributed in one or more steps of the make up of recombinant inbred lines, which were also used in other studies. All authors contributed in the interpretation of the results, and read and approved the final manuscript.

COMPETING INTERESTS

No competing interests declared.

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