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Genetic variation for egg-to-adult survival in *Drosophila melanogaster* in a set of recombinant inbred lines reared under heat stress in a natural thermal environment

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Abstract

Quantitative trait loci (QTL) for thermotolerance were previously identified for adult flies in several mapping populations of *Drosophila melanogaster* Meigen (Diptera: Drosophilidae) in the laboratory. However, laboratory assays may not necessarily reflect the performance under heat stress in the field. For instance, do the heat-resistance QTL regions in the field match the QTL for thermotolerance in laboratory studies? To address this and related questions we used a set of recombinant inbred lines (RIL), which were originally used to identify QTL in the laboratory. We tested egg-to-adult survival (EAS) QTL in a field experiment under naturally varying heat-stress temperatures in fly cultures reared on a rotting fruit (banana) in summer. EAS under heat stress was found to be 3–6× lower (depending on RIL) in the field than in the corresponding control at benign temperature (25 °C). Five QTL for EAS were significant in the field experiment under heat stress, four of them co-located with plasticity QTL, and none of the QTL was significant at control temperature. All significant QTL overlapped (co-localized) with thermotolerance QTL previously identified in the laboratory. A previously found QTL in the middle of chromosome 2 explained near 30% of the phenotypic variance in EAS under heat stress in previous studies in the laboratory, but this QTL explained only 8% of the EAS variation in our field assay. The largest effect on EAS was found for an X-linked QTL (cytological range 7B3-10C3) in the heat-stress field experiment, explaining a high percentage (14–45%) of the phenotypic variation in EAS. The ecological relevance of QTL implicated in this study is discussed.

Introduction

The ability to survive from larvae to adulthood is an important fitness component, especially under severe environmental conditions, as in the case of elevated temperature in a scenario of global warming (Kingsolver et al., 2011; Franks & Hoffmann, 2012; Huey et al., 2012; Rebaudo & Rabbi, 2018). Adaptation to thermally stressing environments is possible if thermotolerance phenotypes are genetically variable in populations in all stages of the life cycle (Hoffmann et al., 2003; Hoffmann & Willi, 2008; Levy et al., 2015; Lommen et al., 2017). Experiments in the laboratory allowed the identification of quantitative trait loci (QTL) for resistance to heat stress in adult *Drosophila melanogaster* Meigen (Diptera: Drosophilidae) (e.g., Norry et al., 2004, 2007a,b; Morgan & Mackay, 2006; Rand et al., 2010). In this insect model, artificial selection on heat-stress resistance changed both expression level of many genes and a subset of the constitutive proteome in adult flies in the laboratory (e.g., Sørensen et al., 2007, 2017). QTL were also identified for survival after heat stress in the pre-adult stage of the life cycle under standardized laboratory conditions

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(Sambucetti et al., 2013). However, studies on thermotolerance conducted under standardized laboratory conditions may not necessarily reflect the performance at varying temperatures of stress in the field (e.g., Kristensen et al., 2007; Loeschcke & Hoffmann, 2007).

Drosophila melanogaster is found over wide geographical areas on most continents. In laboratory studies, a high degree of co-localization was found for QTL identified in mapping populations from diverse geographical origin (Norry et al., 2004, 2007a,b, 2008; Morgan & Mackay, 2006; Rand et al., 2010). Although QTL are usually wide regions of the genome (depending on both marker density and the amount of recombination), it is interesting to test which QTL (or QTL genotypes) show major effects in assays under (semi)-natural conditions, as laboratory conditions may not always reflect the variation in wild environments where natural selection acts. A large-effect QTL on chromosome 2 was tested for the performance of adult flies in the wild by using a field release-recapture design (Loeschcke et al., 2011). Adult flies carrying the QTL genotype for heat tolerance were better at locating their resources in field releases under high temperatures (Loeschcke et al., 2011). In the pre-adult stage of the life cycle, however, thermotolerance QTL were identified in the laboratory but remain to be tested in the field (Sambucetti et al., 2013).

Here we performed a QTL analysis for survival from egg to adult in *D. melanogaster* under semi-natural conditions of heat stress, in recombinant inbred lines (RIL) reared on a natural resource (decomposing bananas) in the field. For comparison with previous studies in the laboratory we used two sets of RIL, RIL-D48 and RIL-SH2, originally described by Norry et al. (2008) for a QTL mapping in the laboratory. These lines allowed identification of QTL for diverse thermotolerance traits in adult flies in the laboratory (Norry et al., 2008; Arias et al., 2012), as well as for egg-to-adult survival (EAS) under heat stress in the laboratory (Sambucetti et al., 2013). In addition, RIL are useful resources for QTL mapping of heat-stress resistance in both laboratory and field assays because the nearly homozygous lines can be examined in multiple environments. The RIL in this study segregate high variation in thermotolerance as one of their parental lines was derived from a sample of wild flies collected in Denmark (a population from a relatively cold climate), and subsequently selected for reduced resistance to heat stress in adult flies. The other parental line was derived from an Australian population in Melbourne, and was artificially selected for high knockdown resistance to heat. Four main questions are addressed. First, could major-effect QTL be found for EAS in individuals developing under heat-stress conditions in the field? If so, do the chromosomal locations of pre-adult thermotolerance QTL in the field match (overlap) those previously found for EAS in laboratory-reared flies (Sambucetti et al., 2013)? Third, do the chromosomal locations of pre-adult thermotolerance QTL in the field overlap those found in previous studies for adult flies in the laboratory? Although QTL-overlapping in this study can be the result of either pleiotropy or linkage, the total lack of overlap would indicate not only that pre-adult and adult thermotolerance traits differ in their respective genetic bases but also that the traits are influenced by unlinked genes. Finally, by estimating QTL effects we address a fourth question: what is the order of magnitude of the phenotypic variance explained by QTL for EAS under our (semi)-natural conditions of heat stress?

Materials and methods

Fly stocks

RIL used in this study were described in Norry et al. (2008). Briefly, two highly divergent inbred stocks were used as parental lines. These stocks were derived from Melbourne (SH2 line) and eastern Jutland (D48 line), selected for high (SH2) and low (D48) resistance to heat knockdown before inbreeding (Norry et al., 2004). No cytologically detectable inversions were present in D48 and SH2 lines (Norry et al., 2008), which were chosen as parental stocks from a total of 42 heat-sensitive D inbred lines plus 23 heat-resistant SH inbred lines (Norry et al., 2004). F1-females were backcrossed to D48 males, and the backcross progeny were randomly mated for two subsequent generations. After the last generation of random mating, individual pairs were set up, and their progeny were inbred by full-sib mating for 15 generations to form our 'RIL-D48' panel. This procedure was also followed to obtain RIL-SH2 lines, with the only difference that F1-females were backcrossed to SH males (Norry et al., 2008). RIL from both reciprocal backcrosses rather than from a single backcross (single-way introgression) can increase the statistical power to detect QTL (Norry et al., 2008). Thirty-six microsatellite loci were used as markers, and the genetic map for the three major chromosomes is given in Norry et al. (2008). Briefly, map positions (in cM) – after the chromosome number – and cytological band (in parenthesis) are: 1-0 cM (band 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), and 3-140 (99D6-D9); see Norry et al. (2008) for references of microsatellites used.

Egg-to-adult survival

To collect experimental eggs, flies were reared at 25 °C and, at the age of 4–5 days, 10 males plus 10 females per RIL were transferred to vials containing a small spoon with agar plus yeast paste. Four such vials were replicated per RIL and all vials were kept at 25 ± 1 °C. After 22–24 h, 40 eggs were collected from each spoon and transferred to bananas (20–23 cm long), as standardized laboratory culture media could affect heat-stress resistance patterns when compared to natural feeding and breeding resources for *Drosophila* (e.g., decomposing fruits; Kristensen et al., 2016). In these banana cultures, a section (1 × 18 cm) of the fruit epidermis was removed to allow the transfer of eggs into the fruit. Each banana containing 40 eggs was placed within a net bag (40 cm long, 20 cm diameter) with a plastic support allowing free air circulation. Four replicates of such cultures were prepared per RIL, and all cultures were simultaneously placed on the ground, under the shadow of a small group of trees in a field station at the Faculty of Exact and Natural Sciences, University of Buenos Aires, Argentina (34°32′31.4″S, 58°26′32.9″W), on 7 March 2015, at 11:00 hours. Egg-to-adult survival was successfully scored for four replicates in 49 lines (30 lines RIL-D48 and 19 lines RIL-SH2). Although the number of contrasting RIL can limit our statistical power (i.e., allowing only the detection of large-effect QTL), this issue was partially improved because two reciprocal sets of RIL were used (i.e., from the two reciprocal backcrosses, as mentioned above), and because RIL were constructed from selected populations (Yan et al., 2006).

For each individual RIL, survival was estimated for each sex as the proportion (expressed as %) of flies that emerged from each rotting banana, after considering a 1:1 sex ratio in the egg stage (Sambucetti et al., 2013), and averaged over four replicates (there was high repeatability among replicates, particularly for lines showing either extremely low or high survival). We also estimated EAS over both sexes pooled, and the results for sexes pooled (data not shown) were similar to results for the sex showing a significant source of variation (or QTL). In total, 7 840 individuals were scored for EAS in the field (40 eggs × 4 replicated bananas × 49 RIL). Temperatures ranged from 19 to 33 °C in the experimental field (Table 1). Daily minimum temperature ranged from 19 to 24 °C whereas daily maximum temperature ranged from 26 to 33 °C throughout the experiment (Table 1). Most flies (90%) emerged between 14 and 17 March.

Table 1 Minimum, maximum, and mean temperature for each day of March 2015 when the field experiment was carried out in the experimental field at Ciudad Universitaria, University of Buenos Aires, Argentina

	Temperature (°C)		
Day	Minimum	Maximum	Mean
7	23	30	26
8	23	29	27
9	23	31	28
10	24	30	27
11	24	29	26
12	23	31	29
13	23	33	29
14	24	30	27
15	19	27	24
16	22	28	25
17	24	31	28
18	23	30	27
19	22	29	27
20	24	33	29

The same protocol was performed simultaneously in banana-based control cultures at constant 25 ± 1 °C in a transparent-glass-door Lovibond thermal incubator which was allowed to have same photoperiod as in the field experiment (as the incubator was placed in a wide-window laboratory room). Two sets of these control cultures were performed: heat-treated and non-heat-treated larval cultures. For the non-heat-treated set, banana cultures containing 40 larvae (as described above for the field experiment) were kept at 25 ± 1 °C until survival was measured for each sex as the percentage of flies that emerged from each RIL culture, after considering a 1:1 sex ratio in the egg stage (Sambucetti et al., 2013), averaged over four replicates per RIL. For the heat-treated (hardened) set, bananas containing 40 eggs were set up as described above for each RIL and, starting on the next day, all of them were exposed to 29 °C (water bath) for 3 h (13:00 to 16:00) every day during three consecutive days, whereas the rest of the time all cultures remained at 25 ± 1 °C. Egg-to-adult survival in heat-hardened cultures was estimated in % of mean survival for each sex, as described above for the non-heat-treated control. The rationale for also using heat-hardened control cultures was that heat-hardening provided a treatment for possible hormetic effects on egg-to-adult survival at otherwise benign temperature (Parsons, 2001; Costantini et al., 2010).

Variation in EAS was tested with ANOVA using experiment (heat-stress field experiment vs. laboratory controls at 25 °C without or with a heat-hardening treatment), RIL

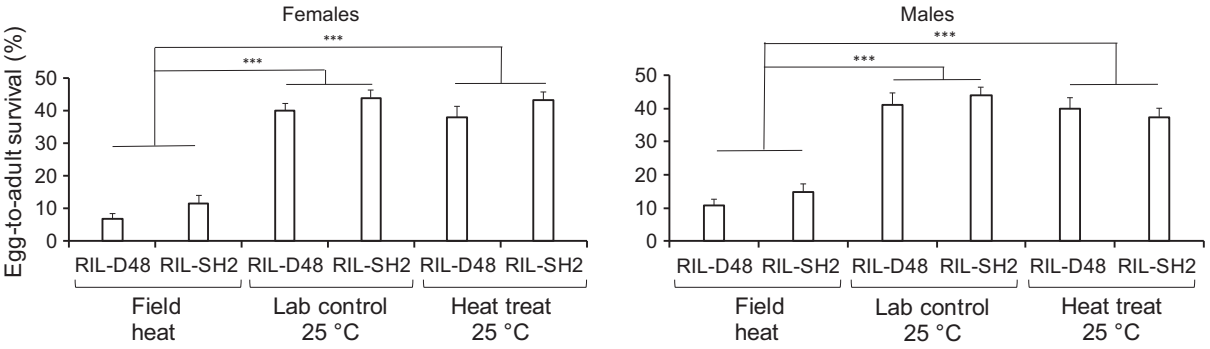

Figure 1 Mean (+ SE) egg-to-adult survival (%) of *Drosophila melanogaster* for a heat-stress field environment, a laboratory experiment at constant 25 °C in non-treated individuals (control), and a laboratory experiment at 25 °C in heat-hardened larvae. Asterisks indicate significant differences (Tukey's tests: P<0.0005).

panel (RIL-D48 vs. RIL-SH2), and sex as fixed factors. As in Norry et al. (2008), we used the mean value of each single RIL as the data point for all RIL in ANOVA. Post-hoc test was carried out with Tukey's honestly significant difference (HSD) test, in the STATISTICA package (StatSoft, 1999). When expressed in %, the difference in EAS was too large between the field experiment and laboratory controls. Therefore, to reduce statistical problems arising from ratio traits in post-hoc tests (Atchley et al., 1976), results for ANOVA and Tukey's HSD tests are reported for ln-transformed data (mean values are in Table S1). We also used generalized linear models (GLM) to test the same effects as in ANOVA, and GLM yielded identical conclusions as reported for ANOVA.

QTL analysis

As in Norry et al. (2008), marker genotypes were the number of SH2-alleles (0 or 2) for both RIL-D48 and RIL-SH2. Composite interval mapping 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 v.2.5 (Wang et al., 2010), for Ri2 design (RIL, sib mated), initially with five control markers and a window size of 10 cM. We explored the effects of altering this initial combination of parameters. QTL positions that were found by using 10 cM as window size and five control markers were consistent across a wide range of parameter combinations. Significance thresholds were determined by 1 000 random permutations for each experiment. Results are reported for non-transformed data (i.e., EAS in %) but QTL positions as well as the relative magnitude of additive effects of each QTL were similar in ln-transformed data (not shown) as for non-transformed EAS. Additionally, QTL mapping was also performed on the difference in the mean survival between the non-hardened control at 25 °C and the field (heat) experiment, as well as between the heat-treated (hardened) control at 25 °C and the field experiment. This is an analysis of phenotypic plasticity to test for QTL-by-environment interactions (Tétard-Jones et al., 2011). For significant QTL, confidence intervals were estimated by using 1.5 LOD (6.9 likelihood ratio, LR) for confidence >95%, according to Dupuis & Siegmund (1999). Pairwise epistatic interactions were evaluated by using a linear model, with $y = m_x + m_y + m_x m_y + e$, where m_x and m_y are the genotypes of markers x and y (Morgan & Mackay, 2006).

Results

Egg-to-adult survival (EAS, in %) under heat stress in the field was 3–6× lower than survival in the control laboratory environment at 25 °C (Figure 1). The treatment effect (control vs. heat-hardened control vs. field) was highly significant, and the interaction between treatment and sex was significant (Table 2). Post-hoc comparisons also revealed a highly significant difference in EAS for both sexes, between our heat-stressing natural environment vs. the laboratory control environments at 25 °C (Figure 1). Temperature was the main difference between our heat-stressing natural environment and the laboratory control environment at 25 °C, as maximum daily temperature ranged from 27 to 33 °C in the field, with a maximum daily mean temperature of 29.7 °C throughout the experiment (Table 1).

There was no difference in survival between the RIL-D48 and RIL-SH2 sets in laboratory experiments at 25 °C (Tukey's HSD tests: P>0.15; Figure 1). In the field assay under heat stress, the RIL-SH2 panel displayed higher survival than RIL-D48, as expected from considering that the RIL-SH2 set is derived from a backcross to the heat-resistant parental line, SH2 (Tukey's test for an across-sex contrast between RIL-D48 and RIL-SH2: P = 0.0013;

Table 2 ANOVA on ln-transformed data of egg-to-adult survival rate of *Drosophila melanogaster* performed to test for effects of treatment (heat-stress field experiment vs. laboratory control at 25 °C without any heat-treatments vs. laboratory control at 25 °C with a heat-hardening treatment), recombinant inbred lines (RIL) panel (RIL-D48 vs. RIL-SH2), and sex in RIL lines used in this study

Source of variation	d.f.	MS	F	P
Treatment (T)	2	86.34	134.85	<0.001
RIL panel (R)	1	5.00	7.82	<0.01
Sex (S)	1	1.81	2.82	>0.05
T*R	2	1.29	2.02	>0.05
T*S	2	2.30	3.59	<0.05
R*S	1	0.38	0.59	>0.05
T*R*S	2	0.05	0.08	>0.05
Error		282	0.64	>0.05

Figures 1 and 2). The difference between RIL panels in the heat-stress field experiment was also significant in an ANOVA on non-transformed data, with RIL panel (D48 vs. SH2) ($F_{1,94}$ = 4.98, P<0.05) and sex ($F_{1,94}$ = 3.58, P<0.05) as fixed effects, but not their interaction ($F_{1,94}$ = 0.02, P>0.05). Egg-to-adult survival in the field was correlated between the sexes in both RIL panels (Figure 2).

Composite interval mapping revealed a multigenic basis of variation for heat-stress survival from egg to adult in the field with at least four significant autosomal QTL in females plus one X-linked QTL in both sexes (Figure 3). Comparison with other studies revealed a non-random distribution of QTL over the genome, as all QTL overlapped with previously identified QTL for heat resistance in laboratory conditions, indicating either a common genetic base or tightly linked QTL (Table 3).

There was no significant QTL for EAS in non-hardened controls at 25 °C in the laboratory (Figure 3), indicating that all of the QTL identified in the heat-stress field environment are relevant for resistance to elevated temperature instead of developmental survival itself. Only in heat-hardened larvae cultures there were two marginally significant QTL, and none of these QTL were found to exactly co-localize with QTL in the heat-stressing field experiment (Figure 3).

QTL ranges for Q1, Q2, Q3, and Q4 were also significant in a plasticity analysis on the difference in mean survival between the controls at 25 °C and the heat-stressing field experiment, further supporting these QTL and their QTL-by-temperature interactions (Figure S1, Table 3). All plasticity QTL overlapped with main effect QTL (i.e., QTL for the direct association between phenotype and loci), except for Q5 where no plasticity QTL was found

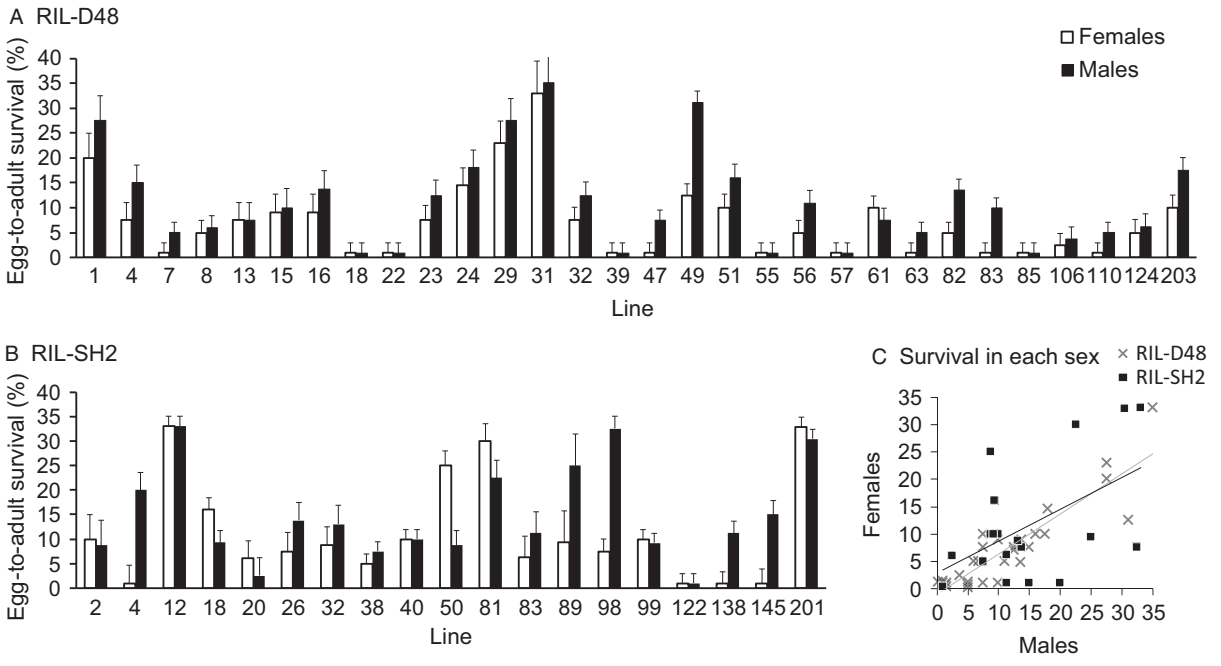

Figure 2 Mean (+ SE) egg-to-adult survival (%) for each *Drosophila melanogaster* line from (A) RIL-D48 and (B) RIL-SH2 sets in our field assay under heat stress. (C) Pearson correlation of egg-to-adult survival in each RIL set between the sexes (RIL-D48: r = 0.91, P<0.005; RIL-SH2: r = 0.52, P<0.05).

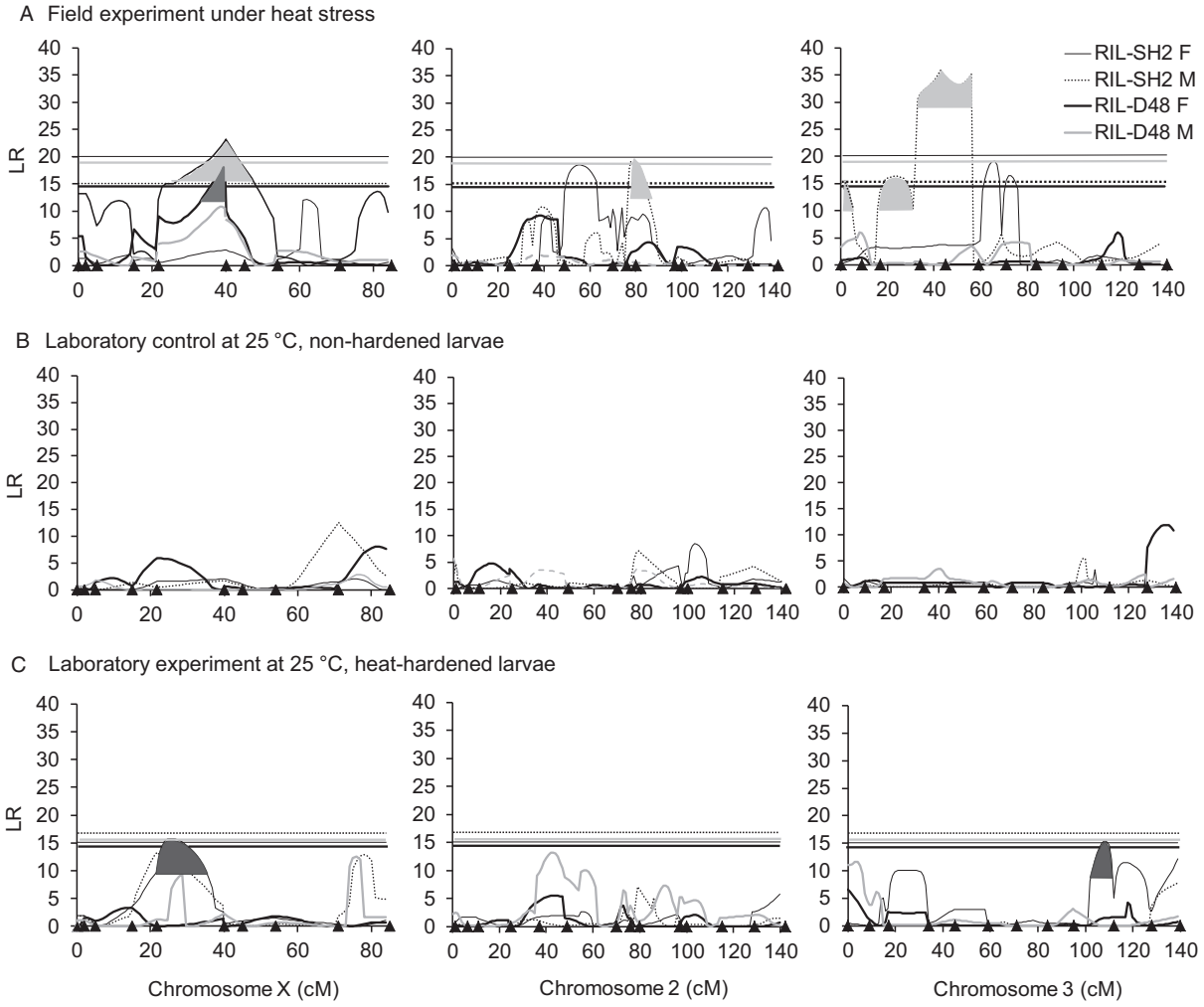

Figure 3 Plot of likelihood ratio (LR) scores against map position (in cM) from composite interval mapping for egg-to-adult survival in *Drosophila melanogaster* females (F) and males (M) from RIL-D48 and RIL-SH2 panels, based on experiments (A) in the field, in a heat-stress environment, and in the laboratory, at constant 25 °C, (B) with non-heat-treated larvae (control), or (C) with heat-hardened larvae. Significance thresholds were determined by 1000 random permutations (horizontal lines, shown for significant cases only). 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), by using 1.5 LOD = 6.9 LR (Dupuis & Siegmund, 1999).

(Figure S1, Table 3). Epistatic interactions were tested between marker pairs but no such interactions were significant after correction for multiple comparisons.

Discussion

Egg-to-adult survival is a fitness-related trait which was genetically variable across our set of RIL under semi-natural conditions of heat stress in the field. Significant QTL effects were found for this fitness-related trait under heat-stress in the field but not in the control cultures at 25 °C, indicating that QTL in this study are attributable to elevated temperature experienced in the field. On average, survival rate in our field experiment was about 4.5× lower than at the constant 25 °C in the laboratory experiment. No QTL was significant in the control flies reared at constant 25 °C without any heat treatments, indicating that there is no substantial genetic variation for EAS in our RIL lines at benign temperature in our experimental conditions.

Egg-to-adult survival at elevated temperature is expected to be an important fitness component, particularly under a possible global warming scenario. One major aim in the present study was to test for QTL effects under

Table 3 Quantitative trait loci (QTL) for egg-to-adult survival of *Drosophila melanogaster* in a heat-stressing field environment (HSFE) identified by composite interval mapping in RIL-D48 and RIL-SH2. QTL mapping was performed on individuals reared on banana in an experimental field station in summer. QTL range is given as cytological bands. '% var' is the percentage of the phenotypic variance explained for each main effect QTL. In a plasticity analysis (QTL profiles are shown in Figure S1), only four QTL were significant and all of them either co-located or overlapped with four main effect QTL (Q1, Q2, Q3, Q4), as indicated by superscript letters on QTL ranges for the difference between (a) heat-hardened control at 25 °C vs. HSFE in RIL-SH2 males, (b) heat-hardened control at 25 °C vs. HSFE in RIL-SH2 females, (c) non-hardened control at 25 °C and HSFE in RIL-SH2 males, and (d) heat-hardened control at 25 °C vs. HSFE in RIL-D48 males

QTL	RIL	Sex	QTL range	% var	References for laboratory studies showing overlap of QTL in our field assays
Q1	RIL-D48	Female	7B3-10C3[a,b]	45	Norry et al. (2007b, 2008); Rand et al. (2010); Arias et al. (2012)
	RIL-SH2	Male	7B3-12D-E[a,b]	14	Norry et al. (2007b, 2008); Rand et al. (2010); Arias et al. (2012)
Q2	RIL-SH2	Male	38E1-49C[a,b,c]	8	Norry et al. (2004, 2007a, 2008); Morgan & Mackay (2006); Rand et al. (2010); Arias et al. (2012); Sambucetti et al. (2013)
Q3	RIL-SH2	Male	60A-62A[d]	4	Morgan & Mackay (2006)
Q4	RIL-SH2	Male	64D-66D10[d]	34	Morgan & Mackay (2006); Norry et al. (2008); Sambucetti et al. (2013)
Q5	RIL-SH2	Male	66D10-73B7	34	Norry et al. (2004, 2008); Sambucetti et al. (2013)

natural variation of high temperature and humidity in the field (Stazione et al., 2017). Several thermotolerance QTL were previously found in laboratory studies both in larvae and adults, and most of them co-localized with QTL in the present field study. However, as QTL regions are wide, QTL overlapping should best be interpreted as a result of either a common genetic base (pleitropy) or tightly linked QTL rather than pleitropy itself (Rönnberg-Wästljung et al., 2006). In insects, it is not clear that a developing individual experiences heat stress in the same way that adult individuals do, but there is evidence for some generalized stress response with a partially shared genetic basis (Sørensen et al., 2017). In addition, the across-RIL correlation between the heat knockdown time in adult flies (as measured in Norry et al., 2008) vs. EAS under heat stress in the field (in this study) is significant in females (Pearson correlation: $r = 0.44$, $P<0.01$).

Adaptation to elevated temperature environments requires genetic variation for thermotolerance phenotypes in all stages of the life cycle (Hoffmann et al., 2003; Hoffmann & Willi, 2008; Levy et al., 2015; Lommen et al., 2017). Our present results for EAS in a field experiment are consistent with a field release-recapture study based on adult flies where one thermotolerance QTL in the laboratory was also significant in adult flies released at elevated temperatures in the field (Loeschcke et al., 2011). The QTL in the middle of chromosome 2 (Q2) was tested for field-released adult flies, and flies carrying the QTL genotype for heat-stress resistance were better at locating resources in field releases under high temperatures (Loeschcke et al., 2011). In addition, this QTL on chromosome 2 was also significant for EAS under heat stress in a laboratory experiment (Sambucetti et al., 2013), as in the present field study. An extensible list of many candidate genes was provided for Q2 in previous studies (Morgan & Mackay, 2006; Norry et al., 2007a, 2008). This QTL could be the result of a large number of multiple co-expressed genes with relatively small individual effects (Norry et al., 2009).

The X-linked QTL in this study (Q1) was also significant in previous laboratory studies. This QTL was significant in several mapping populations. Although our QTL region is wide, it is interesting that expression levels of *hsc70-3* and *hsp60* (within Q1) were associated to heat knockdown resistance in adult flies (Norry et al., 2009; see also Sørensen et al., 2017). Another candidate locus within Q1 is *dLg1*, which clinally co-varied with latitude (Božičević et al., 2016). Although these previous results are not a direct evidence for the involvement of *hsc70-3*, *hsp60*, and *dLg1* in thermotolerance, the X-linked QTL (Q1) is interesting for further analysis as this QTL showed a large effect in the field assay.

In addition to the above mentioned QTL on chromosomes X and 2, other QTL for pre-adult heat survival in the field were also found to overlap with thermotolerance QTL previously reported for adult flies in laboratory experiments (see Table 3 for references). For instance, Q4 was also significant for adult thermotolerance in several laboratory studies. This QTL (Q4) includes the small heat-shock proteins *hsp22*, *hsp23*, *hsp26*, and *hsp27* as candidate genes (Frydenberg et al., 2003), plus other candidates like 'Chorion protein S19' which responded to artificial selection for heat-stress resistance (Sørensen et al., 2017). Q3 also was found as a QTL region influencing heat-stress resistance in laboratory studies, and this QTL includes several well-known candidates such as *mth* (*Methuselah*), Hsp83, and *DnaJ-1* (Morgan & Mackay, 2006).

The phenotypic variance explained by each QTL in the field experiment is informative of the QTL effect.

The QTL explaining the highest phenotypic variance were Q1 in RIL-D48 females (45%) and Q4-Q5 (34%) in RIL-SH2 males. These results for egg-to-adult viability in the field appear to contrast with previous results for heat resistance in adult flies in the laboratory, where Q2 explained most of the phenotypic variance (27–33%) in heat knockdown resistance (Norry et al., 2008). In addition, Q2 explained about 30% of the phenotypic variance for egg-to-adult viability under heat stress in the laboratory (Sambucetti et al., 2013), contrasting with only 8% of the survival variation explained by this QTL in the present field assay. However, restricted comparisons of the phenotypic variance explained by each QTL should be taken with caution, and best considered as an order of magnitude, as the relative contribution of each QTL might differ in other natural environments under heat stress.

In contrast to control laboratory experiment at 25 °C, where no QTL was found, in heat-hardened larvae two QTL were found for EAS at 25 °C in the laboratory. One of these QTL co-localized with a thermotolerance QTL on chromosome 3 for adult flies in the laboratory studies (Norry et al., 2004; Morgan & Mackay, 2006), in a region of In(3R)Payne (missing inversion in the present study) where diverse candidate genes map (e.g., *hsr-omega*, CG13833, CG6733, and the closely-linked gene *hsp68*; see Rako et al., 2007). Another QTL partially co-localized with the X-linked thermotolerance QTL. These two QTL for pre-adult survival at benign temperature affected EES in heat-hardened larvae, perhaps reflecting genetic variation in hormesis across RIL (Gomez et al., 2016).

The presence or absence of QTL in different environments is a case of QTL-by-environment interaction (Tétard-Jones et al., 2011; Joosen et al., 2013). All five QTL for EAS under heat-stress in the field were absent in non-hardened controls at 25 °C, and four of these QTL co-located with plasticity QTL. The co-location of thermal environment dependent main effect QTL and plasticity QTL provides further support for the influence of such main effect QTL on thermotolerance phenotypes (as discussed in Tétard-Jones et al., 2011, for other phenotypes).

Field experiments are suggested to have a greater ecological relevance than laboratory experiments at constant temperatures as field assays are more representative of natural thermal environments (Hoffmann & Loeschcke, 2006; Loeschcke & Hoffmann, 2007; Angilletta, 2009). As discussed above, comparison with laboratory studies revealed a non-random distribution of QTL over the genome, as all QTL overlapped with previously identified QTL regions for heat resistance in both adults and larvae in laboratory conditions, but the effect of each QTL strongly differed in magnitude between laboratory and field assays. As most of previously found QTL for laboratory thermotolerance in adult flies overlapped with EAS under stressful temperatures in the wild, such QTL will be further studied and considered given their high ecological relevance implicated in this study. It is crucial that QTL effects are large enough to determine phenotypic variation in heat resistance in a natural thermal environment, as in the present study. Fine-scale mapping (several available techniques), single nucleotide polymorphism association mapping, and/or gene expression profile analyses are the tools for further genetic analysis within each QTL region.

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Supporting Information

Additional Supporting Information may be found in the online version of this article:

Figure S1. Plot of LOD scores (LR) against map position (in cM) from composite interval mapping for thermal-induced plasticity of egg-to-adult survival in *Drosophila melanogaster* from RIL-D48 and RIL-SH2 panels. Results are shown for significant cases only, for the difference between (A) heat-hardened control at 25 °C vs. the heat-stressing field experiment (HSFE) in RIL-SH2 males, (B) heat-hardened control at 25 °C and HSFE in RIL-SH2 females, (C) non-hardened control at 25 °C and HSFE in RIL-SH2 males, and (D) heat-hardened control at 25 °C and HSFE in RIL-D48 males. 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.

Tabel S1. Mean (± SE) egg-to-adult survival (%, ln-transformed) in two sets of recombinant inbred lines (RIL) of *Drosophila melanogaster*, RIL-D48 and RIL-SH2, in a heat-stressing field environment and in a constant laboratory environment at the benign temperature of 25 °C with or without a heat-hardening treatment.