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Consistent effects of a major QTL for thermal resistance in field-released *Drosophila melanogaster*

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ABSTRACT

Molecular genetic markers can be used to identify quantitative trait loci (QTL) for thermal resistance and this has allowed characterization of a major QTL for knockdown resistance to high temperature in *Drosophila melanogaster*. The QTL showed trade-off associations with cold resistance under laboratory conditions. However, assays of thermal tolerance conducted in the laboratory may not necessarily reflect performance at varying temperatures in the field. Here we tested if lines with different genotypes in this QTL show different thermal performance under high and low temperatures in the field using a release recapture assay. We found that lines carrying the QTL genotype for high thermal tolerance were significantly better at locating resources in the field releases under hot temperatures while the QTL line carrying the contrasting genotype were superior at cold temperatures. Further, we studied copulatory success between the different QTL genotypes at different temperatures. We found higher copulatory success in males of the high tolerance QTL genotype under hot temperature conditions, while there was no difference in females at cold temperatures. The results allow relating components of field fitness at different environmental temperatures with genotypic variation in a QTL for thermal tolerance.

1. Introduction

The ability to locate resources in the wild is an important fitness component in insects like *Drosophila* that feed on ephemeral soft fruits. Release-recapture experiments allow testing how well flies can locate food resources under various environmental conditions. Earlier studies have shown that such a release-recapture assay is an efficient tool to assess components of fitness in the wild. Releases have been used to test the importance of heat and cold acclimation in the field (Loeschcke and Hoffmann, 2007; Kristensen et al., 2008a), selection for thermal resistance (Kristensen et al., 2007; Overgaard et al., 2010), inbreeding (Kristensen et al., 2008b) or variation in other traits such as body size and parasitism (Jaenike et al., 1995; Hoffmann and Loeschcke, 2006; Hoffmann et al., 2007). Thus, a field release-recapture assay may be a useful tool to assess to what degree predictions from laboratory assays reflect performance under (semi-)natural conditions (Barker and East, 1980; Hoffmann and O'Donnell, 1992; Kingsolver, 1999; Feder et al., 2000; Kristensen et al., 2007).

Quantitative trait loci (QTL) are functionally variable regions of the genome, with substantial contribution to phenotypic variation in quantitative traits. Recent applications of QTL-mapping for thermal resistance traits across different geographic populations in *Drosophila melanogaster* have found one major QTL for thermal resistance in the middle of chromosome 2. This chromosomal region appears to contain QTL for several traits of thermal resistance in the adult fly, including knockdown resistance to high temperature (KRHT), heat-shock survival and chill-coma recovery (hereafter CCR, an index of cold resistance; (Norry et al., 2004, 2007, 2008, 2009; Morgan and Mackay, 2006)). These genetically variable traits are of ecological and evolutionary relevance for thermal adaptation in small insects like *Drosophila* (e.g. Huey et al., 1992; David et al., 1998; Hoffmann et al., 2002; Sørensen et al., 2005; Sarup et al., 2006; Rako et al., 2007; Rand et al., 2010)

Here we tested genotypes affecting KRHT for their relative performance under field conditions using a field release-recapture design and performing experiments under cold and hot temperature conditions in the field using laboratory lines that differ in genotypes in the above-mentioned QTL.

We tested if performance of these lines in the field can be predicted from QTL thermal resistance genotype determined under laboratory conditions. Further, we tested flies of the alternative genotypes for their copulatory success at low and high temperature in field cages to complement the release data with a behavioral fitness component.

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2. Materials and methods

2.1. Drosophila stocks

Two lines denoted KD− and KD+ were constructed from crosses among seventeen recombinant inbred lines (RIL), as described below. When assessed in the laboratory KD− and KD+ differ dramatically in KRHT with KD− being sensitive and KD+ resistant, respectively. RIL used to construct KD− and KD+ were a subset of lines described elsewhere (Norry et al., 2008; Supporting information Tables S1 and S2). Briefly, the parental lines (SH and D48) were dramatically divergent for KRHT, as SH2 was selected for increased KRHT whilst D48 was selected for decreased KRHT (McColl et al., 1996; Norry et al., 2004). F1-females (progeny of D48 × SH2) were backcrossed to D48 males, and the backcross progeny were randomly mated for other two 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". Similarly, F1-females (progeny of D48 × SH2) were backcrossed to SH2 males, and the backcross progeny were randomly mated for two generations. Individual pairs were set up from the last generation of random mating, and their progeny were inbred by full-sib mating to form "RIL-SH2". Thirty-six microsatellite loci were used as markers for QTL mapping on chromosomes X, 2 and 3 in Norry et al. (2008), and AC004759 (bands 38E1–38E9) was used as marker for the genotype of the QTL investigated in the present study (Supporting information Tables S1 and S2).

2.2. Release lines, KD− and KD+

The KD− line was set up by crossing nine RIL (Supporting information Table S2): 4 RIL-SH2 (38; 99; 122; 300) × 5 RIL-D48 (1; 4; 49; 78; 106). The KD+ line was set up by crossing eight other such RIL (Supporting information Table S2): 4 RIL-SH2 (12; 32; 44; 98) × 4 RIL-D48 (31; 35; 39; 83). The KD− line resulting from these crosses was fixed for the AC004759 marker allele from the D48 line of low KRHT (corresponding to the low-KRHT allele of the major QTL between bands 34C–42F in Norry et al. (2008, 2009)). The KD+ line was fixed for the high-KRHT allele of the same QTL region (Fig. 1, Supporting information Tables S1 and S2). Sizes of alleles for the AC004759 marker are 224 bp for SH2-allele and 227 bp for D48-allele. For further details on construction of the lines used, see Supporting information (Tables S1 and S2).

Each KD− and KD+ line was started with 10 males and 10 virgin females from each one of the above mentioned RIL in two replicated standard bottles (10 virgin flies × 2 sexes × 8 or 9 RIL × 2 replicated bottles). Standard bottles were 125 mL bottles containing 40 mL of culture instant medium. KD− and KD+ were maintained in bottles allowing random mating for 15 generations before performing the experiments in the present study. All cultures were maintained at 25 ± 1 °C under a 12 h light/12 h dark cycle, with four replicated standard bottles per line. Flies were mixed among replicated culture bottles within the KD− and the KD+ line in every generation for the first 10 generations of the make-up of the lines. This procedure allowed extensive recombination between D48 and SH2 genotypes except for fixed alleles within the 34C–42F QTL region, with the rest of the genome being randomized for any other possible thermotolerance QTL in both KD− and KD+ lines. In addition, as all RIL were genotyped for 36 markers covering all three major chromosomes in Norry et al. (2008), the subset of RIL used in this study was chosen to allow extensive randomization of the rest of the genome in both KD− and KD+ lines.

2.3. Releases

All releases were performed in areas where there were no natural food resources, i.e. where no *D. melanogaster* occurred naturally, with released flies not being able to survive except in the buckets with mashed bananas that we placed around the release point. Three releases at cold temperatures were performed in Denmark, ca. 50 km west of Aarhus (56°3ǵ E, 9°3ǵ N), releasing 3000 flies (with 1:1 sex ratio) per genotype per release. The wind was mild and average temperatures during the recapture periods were 16.0, 16.4 and 16.9 °C for cold releases 1, 2 and 3, respectively. Minimum temperatures observed during the recapture periods in the three releases varied between 12.0 and 13.2 °C and maximum temperatures varied between 18.5 and 19.5 °C. Flies were brought to the field in foam boxes where the temperature was kept at 25 °C (normal rearing temperature in the lab), and then transferred to new vials with 2 mg of fluorescent dust per 200 flies, and lightly shaken just before release (for details of the methods used in the releases see Loeschcke and Hoffmann, 2007). Capture points in these cold releases were 5 m apart and extending up to 25 m away from the release point in two directions (east and west). Releases were done at 10 a.m. Capture started 1 h after the release and was repeated each hour for 4 h.

Three releases at hot temperatures were done in Argentina, around Villa Numancia–Glew (34°9ź S, 58°41' W), province of Buenos Aires. Here, approximately 2200 flies (with 1:1 sex ratio) of each genotype were released in each of the three replicate releases. Under none of the releases the wind was strong. Capture points were chosen 10 m apart extending to 50 m in two directions (east and west). From previous releases (e.g. Loeschcke and Hoffmann, 2007; Kristensen et al., 2008a), we know that flies fly longer distances at hot temperatures and therefore we placed buckets further apart at hot compared to at cold temperatures. We also generally caught less flies at hot temperatures, which we presume

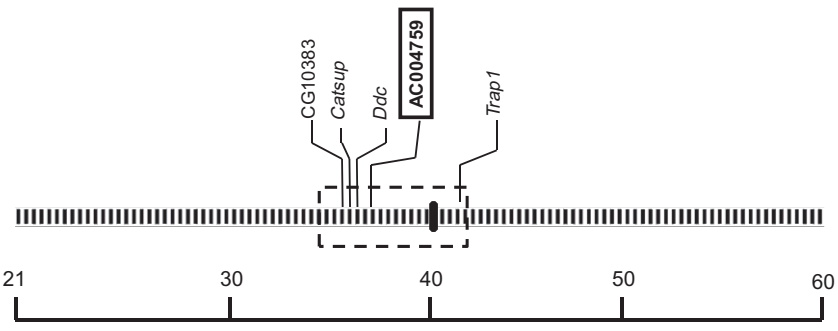

Fig. 1. Scheme of chromosome 2 of *D. melanogaster* showing some of the candidate genes and marker AC004759 within the QTL region that differs between KD− and KD+ genotypes of flies released in the field. This QTL interval (dashed line) was also significant with respect to both heat and cold resistance in laboratory assays with adult flies (e.g. Norry et al., 2007, 2008). Numbers correspond to the cytological map.

is, partly due to that flies die much faster – within a few hours – at hot temperatures if they do not find a resource reasonably fast. We observed that many flies fail in migrating from the vials at hot temperatures whereas all flies succeed in doing that at cold temperatures (we did not quantitate this observation). Flies were brought to the field in boxes (mortality was <2%), and dust colored as described above. Average temperatures during the recapture periods were 33.2, 34.4, and 32.4 °C for hot releases 1, 2 and 3, respectively, with minimum temperatures being 32.1, 33.5, and 30.4 °C and maximum temperatures being 34.2, 36.2, and 34.3 °C, respectively. Capture started at 1 p.m., 1 h after flies were released, and was repeated each hour for 5 h.

2.4. Mating tests

All experimental flies were reared in standard culture vials (40 mL), with 7 mL medium and a controlled larval density (50 first-instar larvae per vial) and at 25 ± 1 °C under a 12 h light/12 h dark cycle. Flies emerging from the vials were sexed under CO_2 anesthesia within 8 h (as virgins) and placed in standard vials with fresh food.

For each of the three replicates performed at low and benign (control) temperatures, 30 flies of each sex and line (120 individuals in total) were aged to three days, and released within a 15 × 20 × 30 cm field cage, using a thin cloth-constructed net as lid. For the two replicates performed at high temperature, 90 flies of each sex and line (360 individuals in total) were aged to 3 days, and released within mating cages of similar dimensions placed in the field. Virgin flies were released within the cages between 11 a.m. and 1 p.m. Just before the release, groups of 60 flies were transferred into vials with 0.15 mg of fluorescent micronized dust and lightly shaken. Dust colors were randomly assigned to the different lines, and changed between replications of the experiment. During 3 h, when mating was observed the copulating pairs were collected from the experimental cages using an aspirating tube, and with minimum disturbance of the surrounding flies. Collected pairs were immediately placed in an empty vial and frozen at −20 °C before scoring the color of each fly. These mating experiments were performed at each of three thermal environments: high (min. and max. temperatures: 31.8 and 32.8 °C), benign (min. and max. temperatures: 24.5 and 25.6 °C), and low temperatures (min. and max. temperatures: 15.3 and 18.8 °C).

3. Results

Flies caught at similar distance from the release site on both sides of the release site were combined, and only differences between the two QTL genotypes (KD− and KD+) were compared for each sex, separately. No *D. melanogaster* fly without fluorescent dust was captured. At cold temperatures, KD− was captured significantly more often than KD+ (Table 1). This pattern was revealed for males and females and in all three replicated releases (Table 1). At hot temperatures, the pattern was reversed, as KD+ was in both sexes more often captured than KD− flies (Table 1).

In the mating experiment, no assortative mating was found between KD+ and KD− males and females within each replicate, as inferred from contingency tests with χ^2 values lower than 2 (Supporting information Table S3). QTL effects were less clear at cold temperatures as no differences between KD− and KD+ lines were detected (Table 2). Similarly, at benign temperatures flies from the KD− and KD+ lines did not show differences with respect to engagement in mating (Table 2). Only at hot temperatures we found a significant signal in males (Table 2), indicating that KD+ males engage more often in mating at this temperature.

Table 1
Number of KD− and KD+ flies caught in the three releases performed at cold temperatures in Denmark and the three releases performed at hot temperatures in Argentina. 3000 and 2200 flies, respectively, were released per line in each of the cold and hot releases respectively. Mean temperatures are listed for each release (further details including minimum and maximum temperatures are given in Materials and methods). Chi-square values were computed as a conservative test for differences in the number of flies caught from KD− and KD+ lines.

Temperature	Females			Males		
	KD+	KD−	χ^2	KD+	KD−	χ^2
cold 1, 16.0 °C	34	84	21.18***	16	66	30.49***
cold 2, 16.4 °C	365	598	56.37***	395	488	9.79**
cold 3, 16.9 °C	364	528	30.15***	355	434	7.91**
hot 1, 33.2 °C	23	9	7.84**	19	9	3.57*
hot 2, 34.4 °C	17	8	3.24	16	5	5.76*
hot 3, 32.4 °C	38	14	11.08***	32	11	10.26**

Significance levels for chi-square values (χ^2) are: $^*P < 0.05$; $^{**}P < 0.01$; $^{***}P < 0.001$.

Table 2
Number of KD− and KD+ flies engaged in mating in cage experiments performed at cold, benign ("control") and hot temperature conditions. In the experiments performed at cold and benign temperatures, 30 males and 30 females per line were released into a small cage. In the mating experiments performed at hot temperatures 90 males and 90 females per line were released into a similar sized cage. Flies were colored with dust colors to distinguish flies from the two lines. In all experiments, mating flies were removed and frozen for later identification of colors. Chi-square values were computed to test for differences in the number of flies engaged in mating from the KD+ and KD− lines.

Temperature	Females			Males		
	KD+	KD−	χ^2	KD+	KD−	χ^2
cold 1	16	11	0.92	16	11	0.92
cold 2	15	9	1.50	10	14	0.67
cold 3	12	8	0.80	11	9	0.20
benign 1	11	9	0.20	10	10	0.00
benign 2	9	11	0.20	9	11	0.20
benign 3	13	10	0.39	11	12	0.01
hot 1	20	14	1.06	25	9	7.53**
hot 2	41	38	0.11	47	32	2.85
hot pooled	61	52	0.72	72	41	8.50**

Significance levels for chi-square values (χ^2) are: $^{**}P < 0.01$.

4. Discussion

We intended to test the performance of lines that differed in the genotype of a major QTL for a thermal resistance trait, knockdown resistance to heat, under different thermal environments and under semi-natural conditions, at sites without naturally occurring *D. melanogaster*, i.e. at places where released flies are presumed to die if they do not localize the food resource (buckets with banana). QTL effects have been shown often to be environment and/or sex-specific (Vieira et al., 2000) or dependent on the genetic background (Leips and Mackay, 2000). The QTL tested here has been shown to map in a region of the 2nd chromosome in flies with very different genetic backgrounds (Norry et al., 2004, 2007, 2009; Morgan and Mackay, 2006) and to harbor a QTL for heat and one for cold tolerance (Morgan and Mackay, 2006; Norry et al., 2007). We found that our proxy for fitness, the ability to locate a food resource in the field, which also can serve as a breeding site, was associated with the KD− and KD+ genotypes at cold and hot temperatures, respectively, and that this was true in both sexes. Thus, this result relates a genomic region (QTL) to a thermal resistance phenotype and its performance in the field.

Behavioral traits are important for coping with extreme temperatures and copulatory success may be dependent on thermal resistance phenotype and QTL genotype. We aimed at complementing the results from the release-recapture experiments by a test of copulatory behavior in outdoor population cages. At benign

and cold temperatures, we did not find any differences among KD− and KD+ females and males with respect to copulatory success but at hot temperatures we found that KD+ males engaged more often in mating as compared with KD− males. Males are the active sex in competing for access to females, whereas females just "sit and wait". This may cause the sex specific QTL effect observed at hot temperatures. Testing males from KD+ and KD− lines with females from a standard line and females from KD+ and KD− lines with males from a standard line would enable more insight into possible interactions between sex, temperature and line. However this design was not used here.

The ability to find food resources at low and high temperatures is expected to be important for coping with temperature extremes in adult insects. Feeding resources are also used for mating and egg laying in *D. melanogaster* and other insect species and thus these resources are more than just a source for food. One major aim in the present study was to test for possible trade-off associations between heat and cold tolerance in field-released *D. melanogaster* carrying different QTL genotypes. Although heat and cold resistance were previously associated in a possible trade-off in laboratory assays for the QTL region in the middle of chromosome 2 (Morgan and Mackay, 2006; Norry et al., 2007, 2008), performances in laboratory assays may not predict field performance. In this regard, the most interesting result from our field releases was the finding of trade-off associations between heat and cold tolerance for ecologically relevant phenotypes of thermotolerance associated to the QTL. This was a clear-cut result from testing the ability to find feeding and breeding resources at low and high temperatures, confirming that the field-tested QTL is a general QTL for thermotolerance in adult *D. melanogaster*, as previously suggested from laboratory assays (Norry et al., 2004, 2007, 2008; Morgan and Mackay, 2006). Future work on this QTL will aim at establishing if the apparent trade-off between cold and heat resistance is due to either the same alleles having antagonistic effects (pleiotropy) or multiple, tightly linked trait-specific genes (linkage). The KD+ and KD− lines used here were recently investigated for expression levels in putative candidate genes within the QTL region. Multiple co-expressed genes (including *Catsup*, *Ddc* and *Trap1*), appeared to jointly contribute to the QTL effects but these associations have not yet been tested for cold resistance (Norry et al., 2009). As several candidate genes are included within the QTL region examined in this study, this QTL might be composed of a large number of genes with relatively small or moderate effects on thermal resistance rather than a small number of large effect genes. Fine scale mapping within this QTL, using complementation analyses of candidate gene mutants, will probably be informative to distinguish a truly pleiotropic locus from tightly linked loci affecting thermotolerance. Some attempts to test correlated responses to artificial selection have failed to find negative genetic correlations between heat and cold tolerance in adult *Drosophila* (e.g. Bubliy and Loeschcke, 2005; Mori and Kimura, 2008; Sambucetti et al., 2010). In other studies, negative rather than positive correlations were detected between heat and cold tolerance in field-cage experiments (Overgaard and Sørensen, 2008).

QTL mapping in laboratory assays can successfully identify large-effect QTL for thermotolerance in *Drosophila*. Field releases further support the hypothesis that genotypes of the QTL in the present study affect thermotolerance phenotypes related to fitness of flies under stressful temperatures in the wild.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.jinsphys.2011.05.013.

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