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Patterns of variation in desiccation resistance in a set of recombinant inbred lines in *Drosophila melanogaster*

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Abstract. Desiccation, resulting from extremely dry environmental conditions, is a serious obstacle to the survival of organisms. Water is vital for the maintenance of intracellular structure and prevents the irreversible formation of aggregates, an occurrence leading to loss of cellular function. To characterize genetic variation in desiccation stress resistance (DSR) in *Drosophila melanogaster* Meigen, an intercontinental set of recombinant inbred lines (RIL) is used. Flies are exposed to a low humidity environment (<10% relative humidity) at a constant temperature of 25 °C. Desiccation stress resistance is higher in RIL derived from a backcross to the parental stock sensitive to heat stress (from Denmark) than in RIL derived from the reciprocal backcross to the heat-stress resistant stock (from Australia). Composite interval mapping reveals significant quantitative trail loci (QTL) for DSR in the set of RIL. Both major and minor effects QTL are detected, suggesting a complex genetic architecture. When compared with a previous investigation performed on the same set of RIL, the present study indicates that not all traits of resistance to environmental stressors are affected in the same direction by segregating co-localized QTL.

Key words. Dehydration stress, environmental stress, thermal stress, small insects.

Introduction

Most organisms, especially animals and plants living in arid environments, experience periods of dryness. Insects, in particular, exhibit a variety of strategies aiming to avoid several forms of abiotic stress such as desiccation. Physiological and behavioural adaptations to endure water scarcity are necessary to maximize survival with respect to a rapid loss of body water.

Evolutionary responses to environmental stresses such as desiccation are extensively studied and characterized in the model insect *Drosophila melanogaster* Meigen (Hoffmann & Parsons, 1993; Sinclair *et al.*, 2007a,b; Kristensen *et al.*, 2008). Desiccation stress resistance (DSR) is a trait of evolutionary, ecological and economic relevance. Most animals and plants do not live long if their cells are allowed contact with dry air because emetabolism is completely impaired in the absence of water. Water supports the structure of intracellular macromolecules and membranes, preventing the irreversible formation of macromolecular aggregates and the loss of organelle functions. Organisms tolerate desiccation mainly by two different mechanisms, either by maintaining a chronic disequilibrium between internal water content and external water availability or by behavioural avoidance strategies. Insects inhabit a wide range of ecological niches and display a number of strategies to withstand desiccation, with the most widespread being the behavioural avoidance strategies of burrowing and nocturnal activity. Also, the conservation of body water is challenged in insects by the high surface area to body mass ratio, and physiological adaptations such as augmented water stores and low water loss rate lead to enhanced dehydration tolerance and, consequently, increased desiccation resistance.

In *D. melanogaster*, survival under conditions of low relative humidity is a complex trait with genetic variability. Studies in natural populations and laboratory lines show consistent

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correlations between desiccation resistance, body water management and cuticle permeability (Gibbs *et al.*, 1997, 2003; Hoffmann & Harshman, 1999). In some *Drosophila* species, increased resistance to starvation, desiccation and oxidative stress is associated with increased longevity (Hoffmann & Harshman, 1999), suggesting a close relationship between life-history traits and environmental stress resistance. Adaptive mechanisms, such as the stress-induced heat-shock proteins (HSPs), are known to be involved in the response to several forms of environmental stress (Sørensen *et al.*, 2003). However, although HSPs indicate the existence of a general response underlying resistance to diverse forms of stress (i.e. a general stress response), heat-shock protein induction alone is unable to completely explain stress resistance, suggesting the involvement of other mechanisms and genes.

Artificial selection for desiccation resistance on adult flies results in altered larval developmental physiology, extending the developmental time of selected flies (Geffen *et al.*, 2006). In addition, desiccation resistance in *D. melanogaster* is associated with postponed ageing, providing further evidence for the correlation between genetically determined ageing and stress resistance in *Drosophila* (Nghiem *et al.*, 2000).

In the present study, a quantitative trait loci (QTL) approach is employed to explore the genetic basis of DSR using a set of *D. melanogaster* recombinant inbred lines (RIL) previously obtained from parental stocks highly divergent for heat-stress resistance (Norry *et al.*, 2008). The RIL are derived from reciprocal backcrosses to a heat knockdown resistant line (SH2, derived from Australia) selected for high heat resistance and a heat knockdown sensitive line (D48, derived from Denmark) selected for low resistance to heat knockdown. First, the level of genetic variation between sets of RIL derived from each backcross is tested, revealing genetic variation in DSR between parental lines. Because SH2-derived RIL are more resistant to heat, cold, starvation and senescence than D48-derived RIL (Norry *et al.*, 2008; Gomez *et al.*, 2009; Defays *et al.*, 2011; Sambucetti *et al.*, 2013), the present study investigates whether or not SH2-derived RIL have higher DSR than D48-derived RIL because this can reveal an interesting across-RIL genetic correlation between DSR and other previously studied stress resistance traits. Second, QTL for DSR are identified on the three major chromosomes in *D. melanogaster.* In addition, the possible QTL-co-localization (i.e. overlapping of genome regions) with other studies based on totally different mapping populations is considered. Third, the present study considers any possible QTL-co-localizations with other traits (e.g. resistance to heat, cold, starvation, ultraviolet-C radiation and senescence) that may be evident from previous studies performed on the same set of RIL.

Materials and methods

RIL

Lines used in the present study were constructed as described by Norry *et al.* (2008). Briefly, two almost homozygous stocks that are extremely divergent for heat-stress resistance, namely D48 and SH2 (heat-sensitive and heat-resistant, respectively), were used as parental lines. F1 females from a D48 × SH2 cross were backcrossed to either D48 or SH2 males, and the progenies were randomly mated for two more generations. The next step consisted of the set-up of individual pairs and subsequent inbreeding by full-sib mating for 15 generations to obtain the RIL stocks. Both the D48- and SH2-backcross derived lines (hereafter referred to as RIL-D48 and RIL-SH2 sets, respectively) were used. The RIL were expanded for one generation from the resultant stocks in 125-mL glass bottles containing 40 mL of dehydrated potato-based culture medium with water, nipagin (methylparaben) and yeast. Two bottles were set up per RIL, with 20 males and 20 females. After 48 h, all flies were removed from the bottles. Experimental individuals were adult flies yielded by standard bottles were maintained under a LD 12 : 12 h photocycle at 25 ± 1 °C.

Desiccation resistance assessment

The survival of males and females (approximately 1 : 1 sex ratio) of each RIL was scored under 5–10% relative humidity, as achieved by placing 1 g of silica gel into 2 × 10-cm^2 plastic vials. Silica gel was added to the bottom of the vials to maintain low humidity, with a thin stopper separating it from the upper part of the vial, where the flies were later transferred. All vials were placed into a thermostatic cabinet (Lovibond, Germany) at a constant temperature of 25 ± 0.5 °C. Mortality in low humidity vials was scored every 6 h, after which number and sex of dead flies were scored. The experiment was performed under a LD 12 : 12 h photocycle. The experiment lasted between 25 and 40 h for most of the RIL.

QTL analysis

The composite interval mapping procedure (Zeng, 1994) was used to test the hypothesis that an interval flanked by two markers contains a QTL. The test was performed using model 6 and the Ri2 design (sib-mated RIL design) in QTL-CARTOGRAPHER, version 2.5 (Wang *et al.*, 2010). For the mapping, a genetic map of 36 microsatellite marker loci spread throughout the *D. melanogaster* X, two and three chromosomes was used. Details on the genetic map associated with these RIL are provided in Norry *et al.* (2008). Marker genotypes comprised the number of SH2-alleles (0 or 2), as in Norry *et al.* (2008), Gomez *et al.* (2009) and Defays *et al.* (2011).

The effects of altering the initial combination of mapping parameters were assessed and significant QTL peaks showed consistency across a range of parameter combinations. The QTL profiles reported in the present study are those found when using 10 cM as window size and five control markers. All composite interval mapping significance thresholds were determined by 1000 random permutations ($P < 0.05$). Ninety-five percent confidence intervals were added for significant QTL in accordance with the procedure suggested by Dupuis & Siegmund (1999). The RIL sets (RIL-D48 and RIL-SH2) were analyzed separately and jointly (RIL-P or pooled set).

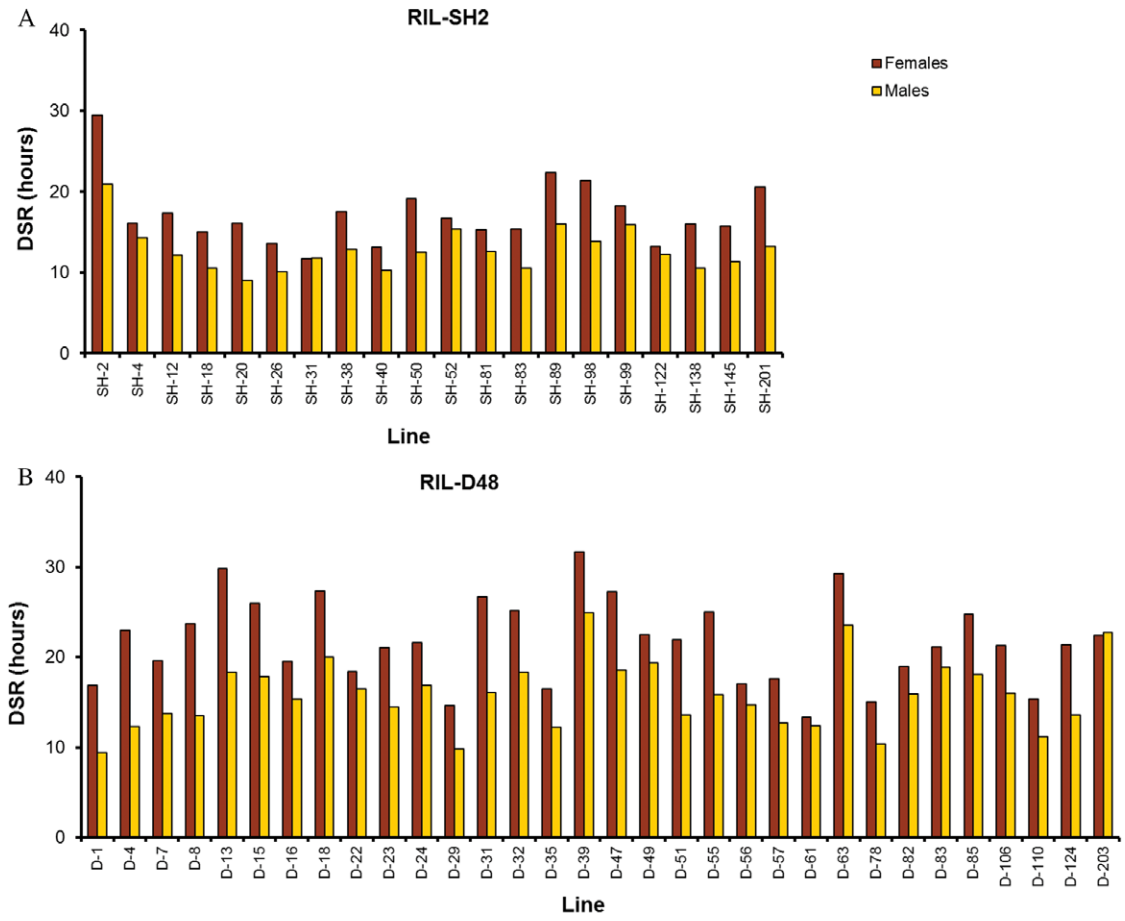

Fig. 1. Desiccation stress resistance (DSR) of two sets of recombinant inbred lines [(A) RIL-SH2 and (B) RIL-D48] in *Drosophila melanogaster*. For phenotypic measurements, mean survival of individuals (in hours) was scored for each RIL. Mean DSR of males and females of all lines from both sets are also shown. Averaging all 52 RIL, DSR values were 19.9 ± 4.94 h for females and 14.7 ± 3.75 h for males.

Results

Desiccation stress resistance was consistently higher in RIL-D48 than in RIL-SH2, and the trait was sexually dimorphic, which was tested by two-way analysis of variance with [1] line (RIL-D48 versus RIL-SH2) and [2] sex (Male versus Female) as fixed factors (Figs 1 and 2). Females exhibit higher DSR than males in the two RIL panels: (1) $F_{1,100} = 22.2$ ($P < 0.01$) and (2) $F_{1,100} = 40.8$ ($P < 0.01$); (1) × (2): $F_{1,100} = 0.86$. This result reveals an interesting across-RIL negative genetic correlation between DSR and previously studied life-history traits in these same experimental lines because RIL-SH2 are more resistant to heat, cold, starvation and senescence than RIL-D48 (Norry *et al.*, 2008; Gomez *et al.*, 2009; Defays *et al.*, 2011; Sambucetti *et al.*, 2013).

All three major chromosomes were tested by composite interval mapping (CIM). The mapping results are summarized in Table 1. A major QTL was evident on the X-chromosome in RIL-D48 (Q1). Additionally, six other significant QTL were apparent across all three chromosomes in the RIL-SH2 (Q2–Q7). Q1 accounted for 24% of the phenotypic variance for DSR in RIL-D48 (Table 1). Significant QTL in RIL-SH2 (Q2–Q7) explained almost 2–30% of the phenotypic variance in DSR (Table 1), suggesting that desiccation tolerance is under complex genetic control in these experimental lines, with both major and minor effect QTL contributing to the genetic architecture of the trait. Additionally, mapping populations were jointly analyzed by pooling RIL-D48 and RIL-SH2 (referred to as RIL-P). CIM analysis showed that Q1 consistently co-localized in the RIL-D48 and RIL-P line sets (Fig. 3).

Discussion

Desiccation stress resistance is a genetically variable trait in the set of intercontinental RIL of *D. melanogaster* that is constructed for the present study. This trait is consistently higher in RIL-D48 than RIL-SH2 lines, which is particularly interesting because all previously studied stress traits show the opposite pattern in these RIL, with RIL-SH2 comprising the more stress-resistant and long-lived lines (Norry *et al.*, 2008; Gomez *et al.*, 2009; Defays *et al.*, 2011; Arias *et al.*, 2012). Three major QTL are identified. A large-effect QTL (Q1) is consistently revealed by CIM on the X-chromosome

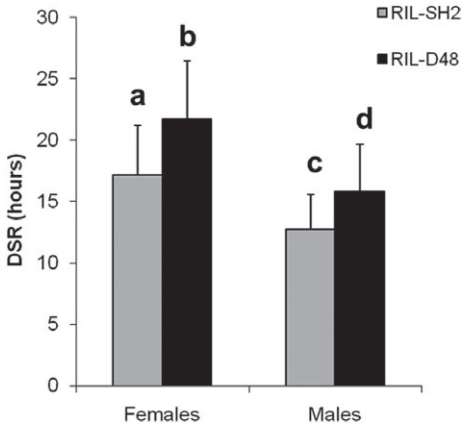

Fig. 2. Mean desiccation stress resistance (DSR) across all lines in males and females of the RIL-D48 and RIL-SH2 sets for recombinant inbred *Drosophila melanogaster*. Error bars given represent the SD. Different lowercase letters above each bar indicate that all means are statistically different from one another (i.e. the analysis of variance specified in the Results, with nonsignificant interaction, indicated that all means are significantly different, $P < 0.01$).

Table 1. Quantitative trait loci (QTL) for survival under low humidity identified by composite interval mapping in *Drosophila melanogaster*.

QTL	Sex	RIL	QTL range	*a*	% Variance
Q1	Males	D48, P	3C6-7B3	−2.05 (P)[a (1)]	22
				−2.94 (D)	24
Q2	Females	SH2	12E-16F3	−2.04	7
Q3	Females	SH2	34C4-38E	−2.06[a (2)]	1.8
Q4	Males	SH2	42A-50C	1.93	29
Q5	Males	SH2	54B1-56E6	−2.20	11
Q6	Females	SH2	66D10-73B7	−2.79	21
Q7	Females	SH2	90E-95C6	5.46	16

[a]Co-localized QTL in previous studies on resistance to other environmental stressors that differed in sign for QTL mean effect: (1) Sambucetti *et al.* (2013); (2) Norry *et al.* (2008). The QTL range was determined by the nearest microsatellite markers flanking QTL peaks. % Variance is the phenotypic variance accounted for by the QTL. The value *a* is the additive effect (in hours) for each QTL.

in both RIL-D48 and RIL-P. This major QTL for survival to desiccation overlaps with genome regions important for desiccation resistance (see Supporting information, Table S1; Sørensen *et al.*, 2007; Telonis-Scott *et al.*, 2012). Although some QTL are sometimes nonsignificant in one of the sexes because of either sex-specific environmental variation or the statistical power to detect QTL, the overall pattern of the results suggests that DSR-QTLs are frequently sex-specific in the RIL used in the present study (Table 1).

Significant QTL in the present study contain at least 81 candidate genes with altered expression levels for desiccation resistance resulting from artificial selection acting on a base population that includes both D48 and SH2 alleles in its genetic background (Sørensen *et al.*, 2007). In another study, Telonis-Scott *et al.* (2012) identify natural molecular variants associated with survival to low humidity that also map

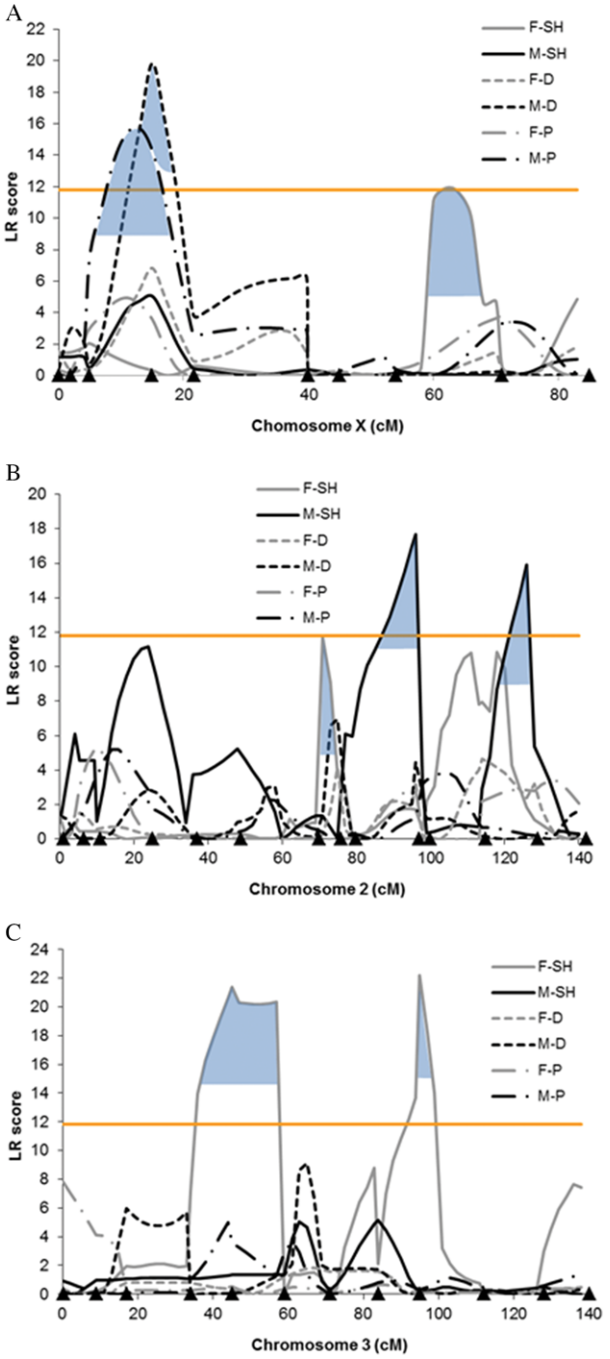

Fig. 3. Likelihood ratio (LR) scores as a function of chromosomal location (cM). Composite interval mapping of desiccation stress resistance (DSR) in *Drosophila melanogaster* was performed in RIL-D, RIL-SH and the pooled RIL-P sets. Significant quantitative trail loci (QTL) peaks were detected on all three analyzed chromosomes (A: chromosome 1 map, B: chromosome 2 map, C: chromosome 3 map), with significance thresholds determined by 1000 random permutations. Microsatellite marker positions are represented by solid black triangles on the horizontal axis. The light-shaded areas under all significant QTL peaks indicate a higher than 95% confidence interval for peak width, based on the 1.5 LOD = 6.9 LR method suggested by Dupuis & Siegmund (1999). F, females; M, males; D, RIL-D set; SH, RIL-SH set; P, RIL-P set.

within the currently established QTL. Nine out of 13 genes identified by Telonis-Scott *et al.* (2012) as containing single feature polymorphisms (SFPs) respond substantially to artificial selection for increased desiccation resistance and are also included within QTL in the mapping population established here (see Supporting information, Table S1). Such genes should either affect DSR or are closely-linked to other loci affecting survival to low humidity. The SFPs detected by Telonis-Scott *et al.* (2012) share specific clusters according to gene ontology, including genes involved in ion transport and respiratory system development. Several genes within the current QTL regions fall under these categories in the study by Telonis-Scott *et al.* (2012), providing likely candidates for evolutionary changes involving excretory and respiratory water balance. In insects, primary osmoregulation occurs in the Malpighian tubules, and ion homeostasis is coupled to water balance as a result of an open circulatory system (Coast *et al.*, 2002). Malpighian tubules secrete urine rich in NaCl or KCl that is isosmotic to the haemolymph, whereas water, ions and necessary metabolites drain into the hindgut, where they are selectively reabsorbed. The QTL ranges in the present study include several genes encoding ion channels (*KCNQ*, *pHCl*, *trpl*, *nAChRα5*, *GluClα*) and symporters (*para*, *CG7720*), providing plausible candidates for the control of hydric balance. Genes *KCNQ* and *trpl* are expressed in Malpighian tubules and localize within Q4, a major effect QTL that explains 29% of the variation in desiccation resistance in RIL-SH2 males (Fig. 3). In addition, chloride ion channels *GluClα* and *pHCl* localize within other major QTL in RIL-SH2 females, Q6 and Q7, respectively (Fig. 3). *Nos*, a major signal transducer, adds to the list of candidate genes expressed in Malpighian tubules and mapping within the Q7 region. Telonis-Scott *et al.* (2012) confirm, in a natural population of *D. melanogaster*, that the *Dys* candidate gene, also included in region Q7 in the present study, presents an association with the desiccation resistant phenotype of artificially selected flies. Another candidate gene in this Q7 region, *SNF4Aγ*, is associated with adaptive evolution for desiccation resistance by both Sørensen *et al.* (2007) and Telonis-Scott *et al.* (2012). Expression of SNF4Aγ is significantly down-regulated in high desiccation resistant *D. melanogaster* (Sørensen *et al.*, 2007). This pattern of down-regulation is observable in most of the 282 genes for which expression is altered significantly by artificial selection for high desiccation tolerance (Sørensen *et al.*, 2007). A total of 81 of those 282 genes are localized within the currently established QTL regions (see Supporting information, Table S1), with 79 out of 81 genes showing down-regulation and only two showing up-regulation in desiccation-selected flies (Sørensen *et al.*, 2007). The list of candidates expressed in the tubules also includes transcription factor *cad* and signal transducers *dnc* and *sl* (see Supporting information, Table S1). The expression of *dnc* in desiccation-selected *D. melanogaster* is significantly different from controls in the gene expression profile performed by Sørensen *et al.* (2007).

An important way to maintain hydric balance in *Drosophila* spp., as in other insects, is via the neuroendocrine peptides that increase or decrease water loss by adjusting tubule secretion and ion and water reabsorption in the hindgut. The gene encoding neuropeptide *sNPF*, which may be released during desiccation stress (Kahsai *et al.*, 2010), is included within the Q3 region (RIL-SH2 females, Fig. 3). In the present study, Q3 accounts for only a small fraction of the phenotypic variance in desiccation resistance.

In insects, desiccation resistance is considered to be genetically correlated with cold-stress resistance. There is some degree of cross-tolerance to both desiccation and cold stress (Sinclair *et al.*, 2007a,b). QTL mapping provides some support for cross-resistance between desiccation and cold stress because some QTL alleles showing increased resistance to desiccation in the present study, such as the Q3 allele, are also shown to consistently increase resistance to cold stress both in the laboratory and in field-released flies (Table 1) (Norry *et al.*, 2007, 2008; Loeschcke *et al.*, 2011). Although an apparently pleiotropic QTL can be defined as a genomic region that affects two or more traits, pleiotropic QTL could contain multiple tightly linked trait-specific genes or a single gene with pleiotropic effects on different traits. In addition, cross-tolerance between cold and desiccation tolerance is limited in *D. melanogaster* (Sinclair *et al.*, 2007a,b), perhaps reflecting the fact that several QTL for desiccation resistance do not co-localize with QTL for cold resistance (Table 1) (Norry *et al.*, 2008). Nevertheless, the finding that at least two QTL alleles have additive effects of increasing both cold and desiccation resistance (Table 1) emphasizes the importance of further investigating QTL for multiple environmental stresses.

In insects, increased surface area to body mass ratio possibly increases the conservation of body water. The establishment of a chronic disequilibrium between internal water and external dry air may pave the way for physiological adaptations leading to low water loss rates. In *Drosophila* spp., consistent correlations are reported between desiccation resistance, body water management and cuticle permeability (Gibbs *et al.*, 1997, 2003; Hoffmann & Harshman, 1999). During the open phase of respiration, epidermal water loss occurs mainly across the spiracles and less predominantly through the cuticle in *D. melanogaster* (Lehmann & Schutzner, 2010). The tracheal respiratory system of *D. melanogaster* is a branched network of epithelial tubes that ramifies throughout the body and transports oxygen to the tissues. Increased water stores and slower water loss rates are likely functionally related to excretory and respiratory system changes. However, analyses of water balance mechanisms between desert and mesic *Drosophila* spp. show that excretory water loss explains a significantly smaller proportion of water loss rates than respiratory water loss (Gibbs *et al.*, 2003). Those genes associated with tracheal system development, as well as those genes involved in other aspects of the response to artificial selection for desiccation stress (Bradley *et al.*, 1999), and mapping within QTL provide more likely candidates that might account for a fraction of the phenotypic variation in desiccation resistance (see Supporting information, Table S1). To identify the genes responsible for QTL effects in the present study, future experiments may include the analysis of transcript levels for candidate genes in desiccation-selected lines, as is reported in previous studies (Sørensen *et al.*, 2007; Telonis-Scott *et al.*, 2012), and further fine-scale mapping by using mutant lines and/or association mapping with sequenced

inbred lines, such as the *D. melanogaster* genetic reference panel (Jordan *et al.*, 2006; Mackay *et al*., 2012). Although overlap is expected by chance to some extent, given that QTL encompass large genomic segments, components of the genetic architecture common to different populations and species yield strong candidates for analysis. Further information about the genetic variation in DSR could be useful in studies on the between-species differences in DSR, improving predictions of climate change effects, as well as providing genetic indicators for DSR-related traits. Resistance to several forms of stress, including heat-knockdown stress, heat-stress survival, chill-coma recovery, starvation resistance and longevity-related traits, is higher in RIL-SH2 than in RIL-D48 (Norry *et al.*, 2008; Gomez *et al.*, 2009; Defays *et al.*, 2011; Sambucetti *et al.*, 2013). It is interesting that RIL-D48 rather than RIL-SH2 are found to be the most desiccation resistant lines in the present study because this result clearly indicates that not all traits of stress resistance are affected in the same direction by either segregating alleles or linked QTL.

Supporting Information

Additional Supporting Information may be found in the online version of this article under the DOI reference: DOI: 10.1111/phen.12103

Table S1. List of candidate genes within quantitative trail loci regions.

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