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Research article

Quantitative Trait Loci and Antagonistic Associations for Two Developmentally Related Traits in the *Drosophila* Head

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Abstract

In insects, some developmentally related traits are negatively correlated. Here, we mapped Quantitative Trait Loci (QTL) for traits of eye size and head capsule, in an intercontinental set of recombinant inbred lines (RILs) of *Drosophila melanogaster*. Composite interval mapping identified QTL on all major chromosomes. Two negatively correlated traits (size of eyes and between-eyes distance) were influenced by one QTL that appeared to be antagonistic between the traits (QTL cytological range is 25F5–30A6), consistent with a negative genetic correlation between these traits of the head capsule. Comparisons of QTL across traits indicated a nonrandom distribution over the genome, with a considerable overlap between some QTL across traits. Developmentally-related traits were influenced by QTL in a pattern that is consistent both with 1) the sign of the genetic correlation between the traits and 2) a constraint in the micro-evolutionary differentiation in the traits.

Key words: developmental constraint, morphological trait; negative genetic correlation; phenotypic differentiation; Quantitative Trait Loci

In traits related to size of complex organs, genetic differences exist between individuals within populations as well as between populations within species. In *Drosophila melanogaster*, Quantitative Trait Loci (QTL) of morphometric traits have been identified for thorax and wing traits (e.g. Weber et al. 1999, 2001; Zimmerman et al. 2000; Gockel et al. 2002; Calboli et al. 2003; Debat and Peronnet 2013), as well as for genitalia morphology (e.g. McNeil et al 2011; see also Liu et al. 1996, Tanaka et al. 2015), and only recently other body parts related in development have been QTL-mapped, but rather for inter-specific differences (e.g. Arif et al. 2013).

One small body part which is interesting to explore using a genetic approach is the head capsule in the adult fly (Cowley and Atchley 1990, Norry and Vilardi 1996, Norry et al. 2000, Hurley et al. 2001, Posnien et al. 2012, Arif et al. 2013). This body part is largely produced by a single pair of imaginal discs, the "eye-antennal" discs (Morota and Lawrence 1979), and two traits derived from this developmental precursor, face width (FW) and eye width (EW), are both negatively and pleiotropically correlated in *D. melanogaster* (Cowley and Atchley 1990). QTL for these traits were recently identified for inter-specific differences between *Drosophila simulans* and *Drosophila mauritiana* (Arif et al. 2013). Mapping also the genetic basis of the intra-specific variation in FW and EW represents an opportunity to better understand the micro-evolutionary changes of these traits FW and EW within species (Arif et al. 2013). Variation in size of compound eyes is likely to influence vision. As a developmental constraint (Wagner 1988), an increase in EW is associated with a reduction in the adjacent face cuticle affecting the head capsule in *Drosophila* (Cowley and Atchley 1990, Norry et al. 2000, Posnien et al. 2012).

QTL mapping can also be used as one starting point for characterizing where some of the relevant loci may be found for negative genetic correlations between FW and EW on the basis of a full genome chromosomal scan in *D. melanogaster*. QTL for head and eye size remain to be studied in *D. melanogaster*, a cosmopolitan species of Afrotropical origin. QTL for FW and EW might segregate in intercontinental crosses of *D. melanogaster*.

Here we present a QTL-based scan for size traits of head and eyes in a set of intercontinental recombinant inbred line (RIL) previously described in Norry et al. (2008). As parental lines strongly differed in both FW and EW (with no significant difference in body size traits such as thorax length [TL]), derived RIL are useful to find QTL that explain at least part of the genetic variation in head morphology between some populations. In this context, QTL-mapping should find genome regions (QTLs) linked to genes that determine the differences in FW and EW between the parental lines (Lynch and Walsh 1998). One parental line was derived from a sample of wild flies collected in Denmark. The other parental line was derived from

an Australian population in Melbourne (Norry et al. 2004). Several aims are addressed. First, we examine whether or not FW and EW are genetically negatively correlated across RIL. Second, we examine whether the size of head traits, including FW and EW, are influenced by any major QTL on all major chromosomes. Third, we test the correlation between traits and examine co-localization of QTL between correlated traits such as FW and EW. Fourth, by comparing two sets of RILs and parental lines we also address the hypothesis that FW and EW evolve in opposite directions as previously suggested (Norry et al. 2000, Posnien et al. 2012, Arif et al. 2013). Finally, we address the hypothesis that developmentally-related traits are influenced by at least one QTL in a pattern that is consistent with the sign of the genetic correlation between the traits.

Materials and Methods

Fly Strains and Traits

The RIL and associated marker map have been described elsewhere (Norry et al. 2008). Briefly, two nearly homozygous stocks from Norry et al. (2004) were denoted D48 and SH2 and used as parental lines. These parental lines were derived from eastern Jutland (Denmark) -D48-, and Melbourne (Australia) -SH2-, respectively, selected for low (D48) and high (SH2) knockdown resistance to high temperature, and subsequently inbred. Lines D48 and SH2 differ widely for FW and EW. F1-females (progeny of D48 × SH2) were backcrossed to D48 males, and the backcross progeny were randomly mated for another 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 consecutive generations to form our "RIL-D48" stocks. This procedure was also used to obtain RIL-SH2 lines, with the only difference that F1-females (progeny of D48 × SH2) were backcrossed to SH2 males (Norry et al. 2008). RIL from both reciprocal backcrosses (two-way introgression) rather than from a single backcross (single-way introgression) can increase the statistical power to detect QTL (Norry et al. 2008). Microsatellite loci were used as markers, resulting in a genetic map with markers throughout all major chromosomes (Supp Fig. 1 [online only]).

All stocks (RIL) were maintained at 25 ± 1 °C in replicated vials containing a culture medium prepared with instant mashed potatoes plus water. In this study, we used 50 out of 54 currently available genotyped RIL that were successfully reared (i.e. free of bacterial growth contamination) in all our experimental cultures (30 RIL-D48 and 20 RIL-SH2). Experimental individuals of 30 RIL-D48 plus 20 RIL-SH2 were simultaneously reared under standardized conditions to reduce the environmental variance at 25 °C, with 30 1–2-h old larvae per standard culture vial and 5 replicate vials per line, under a photoperiod of 12:12 (L:D) h cycle. Standard culture vials are 95 × 20-mm shell vials containing 6 ml of Carolina culture medium (Biological Supply, Burlington, NC, USA), hereafter referred to as standard vials. Twenty individuals (four flies from each of five replicated vials) were measured per sex and RIL (20 flies × 2 sexes × 50 RIL). Measurement of more individuals did not affect the estimation of the mean value of each trait scored per RIL when we examined several RIL for a larger number (40) of flies measured in this study.

Head traits were measured as in Norry et al. (2000), by using a binocular microscope fitted with an ocular micrometer. The head was removed, placed on agar 1.5% into a small Petri dish in the position to be observed from the front for measurements by one of us (F.M.N.) at 80× magnification. All measurements were performed with a binocular microscope fitted with an ocular micrometer (1 mm = 50 ocular units). Head width (HW) is the distance between the left and the right side of the head capsule, and FW is the smallest distance between the eyes (see Fig. 1). EW is the difference between HW and FW (Cowley and Atchley 1990, Norry et al. 2000). Right eye length (EL) was measured from the front view (Fig. 1). Additionally, we measured TL (an index of body size) in the parental lines D48 and SH2, as the distance between the anterior margin of the thorax to the posterior tip of the scutellum at 50× magnification (Norry and Loeschcke 2002).

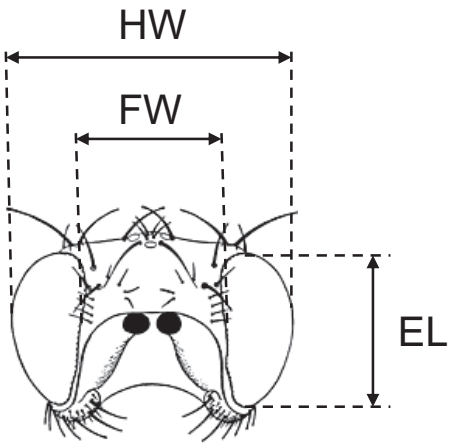

Fig. 1. Traits measured in *D. melanogaster*. HW, head with; FW, face width; EL, eye length. The difference between HW and FW is EW. Scheme of head is as in FlyBase Consortium (2003).

Differences between sets of lines were estimated for each trait using: $DZ = X_{RIL\text{-}D48} - X_{RIL\text{-}SH2}$, where $X_{RIL\text{-}D48}$ is the over-RIL mean value in the RIL-D48 set and $X_{RIL\text{-}SH2}$ is the over-RIL mean value in the RIL-SH2 set (in mm). On average, RIL-D48 flies are estimated to have 75% of D48 genes whereas RIL-SH2 are estimated to have 75% of SH2 genes (Norry et al. 2008), and DZ should represent the difference in trait means between RIL panels. These DZ-values were consistent with DZ-values between the parental lines, D48 and SH2 (see Supp Tables 1–3 [online only] for mean values and ANOVAs). Body size (as indexed by TL) does not differ between the parental lines D48 and SH2 (see Supp Tables 1 and 3 [online only]), and heat traits were thus not transformed for body size adjustments. Transgressive segregation was apparent for some traits including FW and EW (results not shown). Significance for DZ was tested by three-way ANOVA with either RIL panel or parental line and sex as fixed factors (Supp Tables 2 and 3 [online only]), from which a single effect analysis was run to test for differences between RIL panels for each sex.

QTL Analysis

Marker genotypes were the number of SH2-alleles (0 or 2) for both RIL-D48 and RIL-SH2. For each trait, the mean values per RIL were used in QTL analysis (i.e. for each sex, the phenotypic value of each trait for each RIL was the mean value of the 20 flies measured per RIL). 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 Windows Version 2.5 (Wang et al. 2010), as well as the 1.3 version of this software, 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

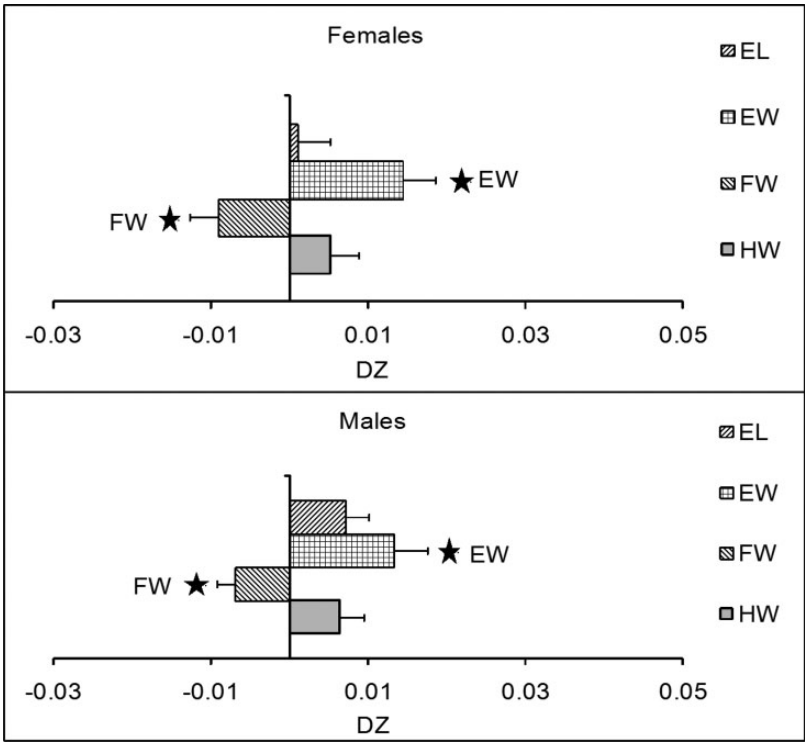

Fig. 2. The difference in the mean value (DZ) between averaged RIL-D48 lines ($N = 30$) and averaged RIL-SH2 lines ($N = 20$) is shown for each trait (in mm). DZ-values are significant for cases where the name of the trait is indicated for each bar ($^*P < 0.05$), as determined from two-tailed *t*-tests as well as from an analysis of simple effects from ANOVA for each trait (results not shown based on ANOVA in Supp Table S2 [online only]). Abbreviations of the traits are as in Table 1.

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. 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 and Mackay 2006).

For each QTL region found in this study we subsequently explored the FlyBase gene ontology database (FlyBase Consortium 2003) for possible candidate genes with known or inferred functions on eye, wing, or body size phenotypes. Besides, candidate loci were also genes for which information on their implication in the development of the head capsule is known (Bessa and Casares 2001, Posnien et al. 2012, Neto et al. 2016).

Results

Two traits of the head capsule, FW and EW, substantially differed between RIL panels as well as between parental lines (mean values and ANOVAs are given in Supp Tables 1–3 [online only]), as their respective DZ-values were significant (Fig. 2). EW showed an interesting variation in opposite direction to FW, as *DZ*-values differed in sign between these traits (Fig. 2). Total HW did not differ between RIL-D48 and RIL-SH2, as an increase in EW was associated to a decrease in FW (Fig. 2). Females were larger than males in all traits and there was no significant interaction between RIL panel and sex (see Supp Tables 1 and 2 [online only]).

The genetic correlation between traits, as estimated from the among-RIL covariance between traits, was computed by pooling both RIL panels. Significant correlations were negative between FW and EW in males, and positive between HW and EW (Table 1). The

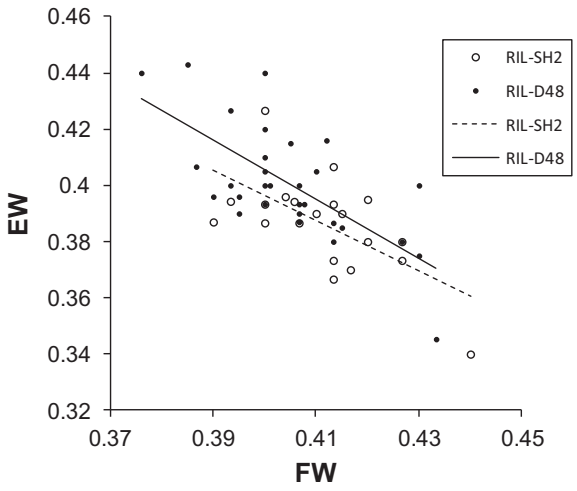

Fig. 3. Two negatively correlated traits of the head capsule. EW (in mm) is plotted against FW (in mm) in males from RIL-D48 and RIL-SH2. Data points represent the mean values of each individual RIL for each trait in 30 lines RIL-D48 and 20 lines RIL-SH2.

antagonistic association between FW and EW is also shown in Figure 3. In contrast to EW, EL was non-significantly correlated with other traits in this study (Table 1).

Head morphology showed an interesting genetic basis. The two negatively correlated traits, FW and EW, have diverged in opposite directions: On average, RIL-D48 flies exhibited heads with more expanded eyes (increased EW) but shorter distance between the eyes (reduced FW) than RIL-SH2 flies (Fig. 2).

Table 1. Across-RIL correlations between traits are shown for females (below diagonal) and males (above diagonal) reared at 25 °C

Trait	HW	FW	EW	EL
HW		−0.11	**0.80**	0.20
FW	0.39		**−0.69**	−0.04
EW	**0.70**	−0.34		0.16
EL	0.43	0.28	0.17	

Results are shown for data pooled across RIL-D48 and RIL-SH2 lines. Abbreviations of traits are: head width (HW), face width (FW), eye width (EW), eye length (EL). Boldface values indicate significant correlations after correction for multiple comparisons by using a sequential Bonferroni test ($P < 0.05$).

Composite interval mapping revealed a nonrandom distribution of QTL over the genome, as some of the QTL showed considerable overlapping across traits (Table 2; Fig. 4). QTL ranges are delimited by the following markers (see Supp Fig. 1 [online only]): X2297267gt–DMU56661 (Q1), DMTROPINI–AF017777 (Q2), DROGPDHA–AC005889 (Q3), AC009392–DRONINAC (Q4), DMRHOb–AC004658 (Q5), AC005889–AC004759 (Q6), DROSEV–AC010705 (Q7). One of the co-localized QTL (Q3) had additive effects that differed in sign between FW and EW in males (Table 2), indicating antagonistic effects of the QTL on FW and EW in the same sex where the two traits were negatively correlated (Table 1). In this antagonistic QTL, SH2-alleles (from the Melbourne line) not only increased FW but also decreased EW. In contrast, EL was controlled by one different QTL on chromosome X (Table 2; Fig. 4).

Most QTLs included candidate genes (Table 2). Epistasis was not apparent after correction for multiple comparisons for each trait, as tested by the interaction term in the linear model indicated in Materials and Methods ($P > 0.05$).

Discussion

In *Drosophila*, the developmentally related traits FW and EW are negatively correlated and we found at least one QTL (Q3) for this antagonistic association in *D. melanogaster*. The traits FW and EW differed in opposite directions between RIL panels (and parental lines) in this study, as expected if the negatively correlated traits change in opposite directions (Fig. 2).

The two parts of the head examined, FW and EW, are derived from a common precursor (the eye-antennal discs), and were antagonistically affected by Q3, as indicated by the difference in sign of additive effects of the QTL (Tables 1 and 2). This QTL (Q3) was significant in males only (Table 2), the only sex exhibiting the significant and negative correlation between FW and EW in this study (Table 1). This apparently pleiotropic QTL can be defined as a genomic region that influences the morphological scaling of the head. Although pleiotropic QTL could contain multiple tightly linked trait-specific genes or a single gene with pleiotropic effects on the different traits, the antagonist QTL such as Q3 for FW and EW can at least partially explain why these traits are negatively correlated in males (Tables 1 and 2). These two traits can evolve in opposite directions in diverse *Drosophila* species, presumably involving a genetic trade-off during development (Cowley and Atchley 1990, Norry et al. 2000, Posnien et al. 2012). FW and EW also have evolved in opposite directions in geographical populations of *D. melanogaster* (e.g. compare the DZ-sign between the traits in Fig. 2). It is suggested that negative correlations between FW and EW reflect subdivision of the imaginal disc cells into a population that forms the multifaceted eye and a population that forms tissue that is not destined to form eye (Norry et al. 2000, Posnien et al. 2012). In this study, the developmentally-related traits (FW and EW) were influenced by some co-localized QTL in a pattern that is consistent with the sign of the genetic correlation between the traits. Candidate genes in the regions of all QTL include many loci with either known or inferred functions in eye development and compound eye morphogenesis (e.g. *N, upd1, dome, Hs3st-B, Mer, eya, wg, bchs, CSN8, Mad*; *ecd, sty, Awh, pie, pelo, SCAR, ifx, Limk1, hop, Amun*, and several other of the genes listed in Table 2), but to find the genes that are responsible of each QTL requires future studies of fine-scale mapping (e.g. deletion mapping in each QTL region). As noted earlier, the approach in this study was not to find the individual genes affecting each trait but rather to find QTLs that influence the variation between strains that differ for the traits examined. Other mapping populations might reveal other additional QTL, as all QTL studies depend on the differences in the putative alleles between the parental populations (Lynch and Walsh 1998). Nevertheless, it is interesting that some QTL in this study were found to be trait specific whereas at least one QTL (Q3) was found to have antagonistic associations on FW and EW.

Eye and the adjacent face cuticle originate from the same pool of cells, involving an antagonistic subdivision of the imaginal disc cells into a population that forms the multifaceted eye and a population that forms head tissue that is not destined to form eye (e.g. Bessa and Casares 2001, Posnien et al. 2012). In this study, Q3 partly explained the antagonistic association between FW and EW in males (Table 2; Fig. 4). Q3 includes one of the candidate genes (*wg*) of two antagonistic signaling pathways which could be implicated in shaping negative genetic correlations between FW and EW, as wingless (*wg*) promotes head while decapentaplegic (*dpp*) promotes eye (Bessa and Casares 2001, Neto et al. 2016). In addition, *dpp* (bands 22F1–22F3) is closely linked to Q3. However, all QTL include many candidate genes (Table 2). Each QTL region can include 250 (Q1) to 570 (Q3) genes, and many of them are loci of still unknown functions. Therefore, the present results should be interpreted not as indicating that *wg* and/or other genes listed in Table 2 are responsible for negative correlations between FW and EW but rather as evidence that one QTL for these traits (Q3) is closely linked to these candidate genes.

The genetic correlation between FW and EW is negative in diverse *Drosophila* species (Cowley and Atchley 1990, Norry et al. 2000), indicating a pleiotropic association between these traits at the intra-specific level of variation. It is evident that the developmentally related traits, FW and EW, evolve typically in opposite directions (Norry et al. 2000, Arif et al. 2013), implying some constraint on the pattern of inter-specific differentiation. The response to selection depends not only on the heritability of the traits but also on genetic correlations between traits (Lande 1979), and the negative genetic correlation- or constraint- between FW and EW is therefore predicted to affect any adaptive change in these traits (Table 1). Nevertheless, inter-specific differentiation in FW and EW might also depend on a sub-set of QTL that are not pleiotropic on these traits (Arif et al. 2013). Although FW may be correlated with mating success, these traits could be influenced not only by development but also by functional effects (e.g. Norry and Vilardi 1996, Arif et al. 2013). The well-known case of *Drosophila heteroneura* and *Drosophila silvestris*, where FW extremely changed between species without an equivalent change in EW might suggest that constrained patterns in these traits can sometimes disappear by environmental stress (Rutherford and Lindquist 1998), and/or change by sexual

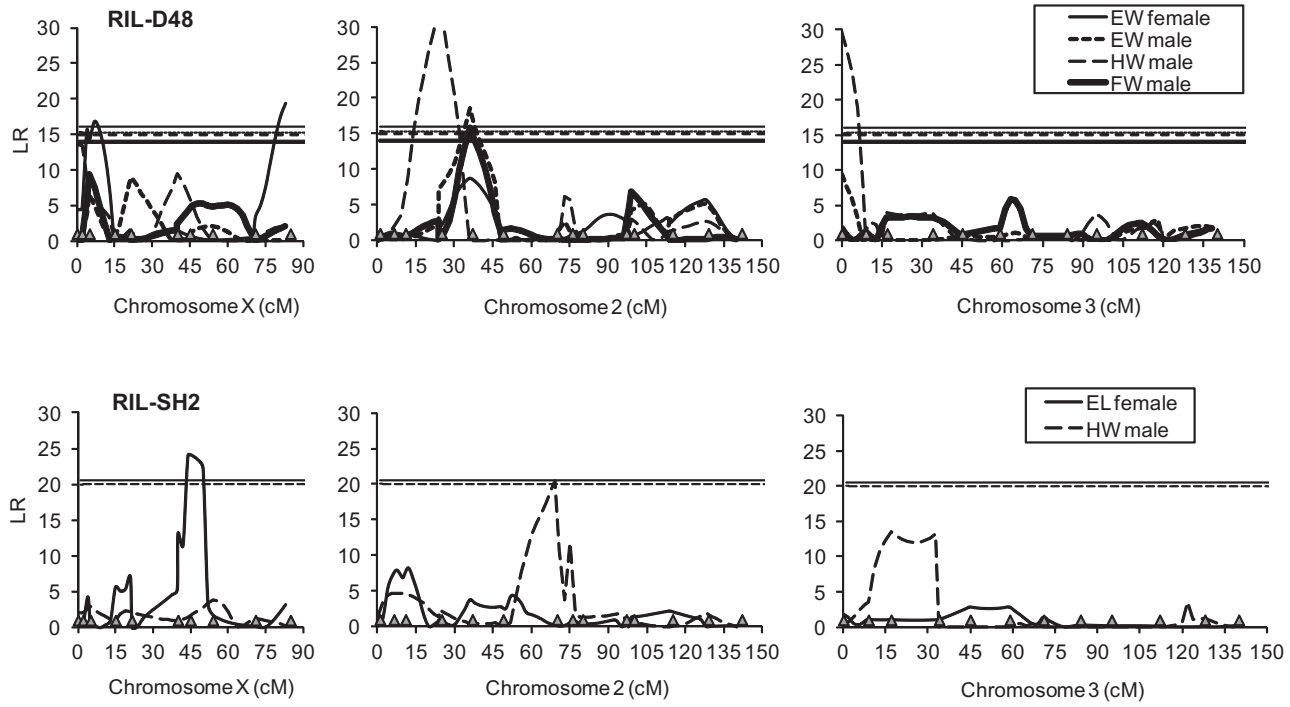

Fig. 4 Likelihood ratio (LR) scores are plotted against map position (in cM) from composite interval mapping for each trait (1 LR = 4.6052 LOD). To avoid verlapping of lines of LR-scores, only cases that were significant for at least one QTL are shown. Significance thresholds were determined by 1,000 random permutations (horizontal line). Triangles on the x-axis correspond to location of markers. Abbreviations of the traits are as in Table 1.

Table 2 QTL for four head capsule traits as identified by composite interval mapping in RIL-D48 and RIL-SH2, in *D. melanogaster* reared at 25 °C

Trait	Sex	RIL panel	QTL range	a	% Var	Name	Qver-Q	Some candidate genes
EW	Female	RIL-D48	3A–4F2	−0.01	10	Q1		*N, ec, Ilp7*
EW	Female	RIL-D48	16F3–19F6	−0.01	21	Q2		*sw, upd1, Hs3st-B, amn, dome, Mer*
EW	Male	RIL-D48	25F5–30A6	−0.02	16	Q3	Q4	*eya, wg, bchs, Rca1, CSN8, d*
FW	Male	RIL-D48	25F5–30A6	0.02	15	Q3	Q4	*eya, wg, bchs, Rca1, Cka,CSN8, d*
HW	Male	RIL-D48	23A–28A3	−0.02	13	Q4	Q3	*eya, wg, slp1,Mad, Hydr2, fred, Rca1*
HW	Male	RIL-D48	62A–63F1	0.02	8	Q5		*Cct1, Bro, dlt, rho, ecd, sty, Awh*
HW	Male	RIL-SH2	30A3–38E9	0.01	39	Q6		*Dac, spi, pie, pelo, chico, Nos, SCAR, Crys, Rab6, nub, ifx, p38b, wb, elB, noc, Su(H), ck, Cul-3, lace, sna, wor, CycE, Gli, Idgf3, Fas3, drl, Pax, Nf-YB, bsh, dia, cad*
EL	Female	RIL-SH2	10A1–12D	−0.04	15	Q7		*Limk1, fw, dsh, hop, bif, Amun, ade5, set2*

Cytological range is given for each QTL. A single name is given for QTL sharing identical range, and substantially overlapping QTL is indicated as "Over-Q". % Var is percentage of phenotypic variance explained by the QTL. Some candidate genes are listed within QTL regions. QTL ranges are based on the closest markers. Additive effects (*a*) are given for traits in mm, which is the additive effect of substituting a SH allele by a D48 allele (marker genotypes were the number of SH2-alleles, 0 or 2, for both RIL-D48 and RIL-SH2). Abbreviations of traits are as in Table 1.

selection (Templenton 1977, Price and Wake 1995). It would be interesting to also test associations between FW and EW in other Diptherians as a constraint that could be not limited to *Drosophila*.

Supplementary Data

Supplementary data are available at *Journal of Insect Science* online.

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