Supplementary Text S1 – Ingredients and brief protocol for Macdonald lab cornmeal-yeast-molasses fly media.

28.5-liters water
280-g agar (Genesee Scientific; 66-111. This is based on a gel strength of 1,090 g/cm^2, and will change depending the batch)
        Add water to steam kettle, turn on electric mixer, and slowly add agar
        Bring mix to a boil

3,200-ml molasses (Genesee Scientific; 62-117)
        Reduce the kettle pressure to reduce the heat slightly
        Add molasses, and bring mix back to a boil

4-liters water
1,460-g inactive dry yeast (Genesee Scientific; 62-107)
        Mix in bucket using paint-stirring drill attachment

4-liters water
2,600-g yellow cornmeal (Genesee Scientific; 62-101)
        Mix in bucket using paint-stirring drill attachment
        Add both the water/yeast and water/cornmeal mixes to the steam kettle
        Bring mix back to boil, and simmer for ~15-min
        Release pressure from steam kettle, but continue to stir with electric mixer

330-ml water
259-ml propionic acid (ThermoFisher; A258-500)
31-ml phosphoric acid (85%; ThermoFisher; A242-500)
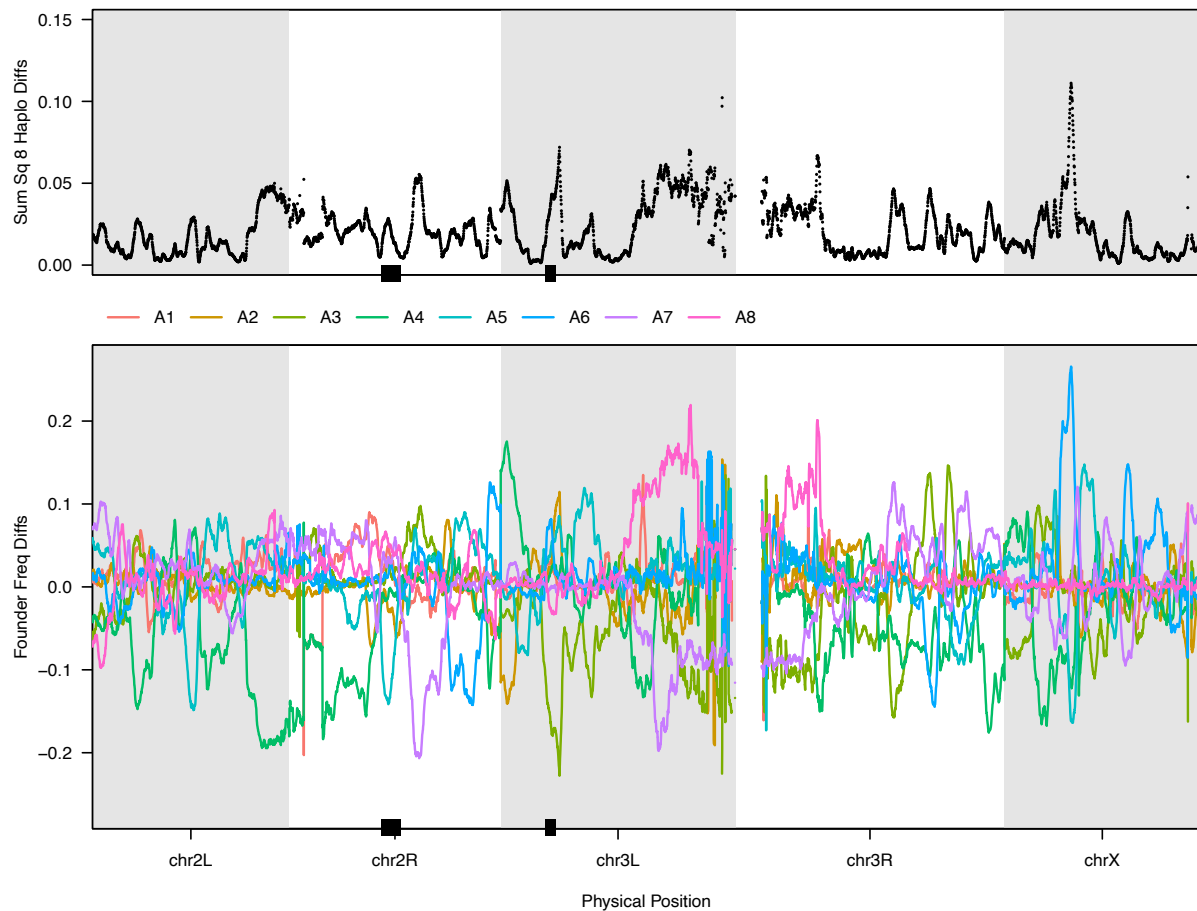   Pour mix into kettle

400-ml 95% ethanol
1.5-g tegosept (Genesee Scientific; 20-258)
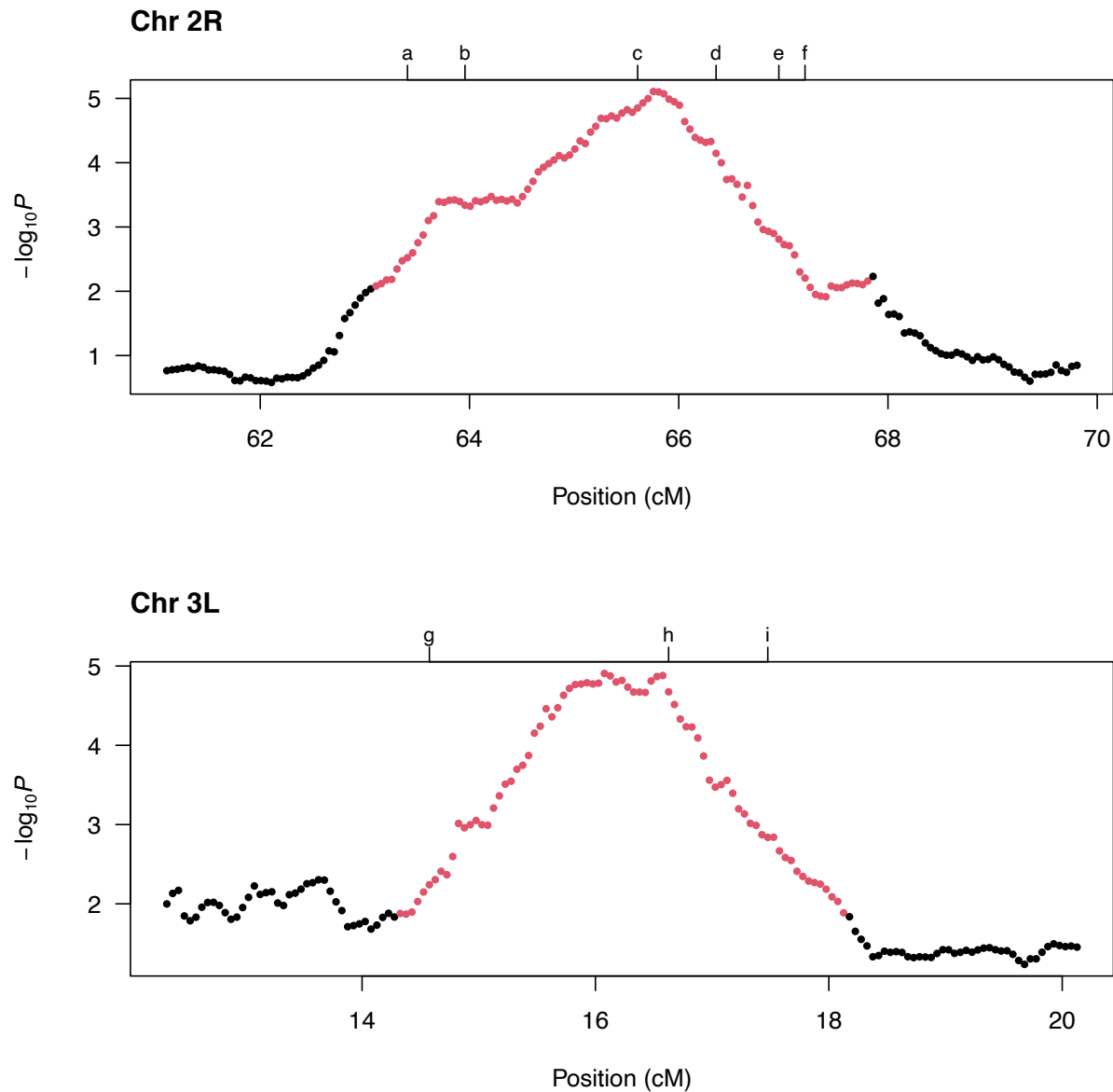   Dissolve tegosept in ethanol
        Pour mix into kettle
        Fill vials/bottles

Supplementary Figure S1 – Comparison of haplotype frequencies between malathion control populations (from the current study) and caffeine control populations (from Macdonald *et al.* 2021, PMID: 35100395). The sequenced samples for these two studies were harvested from the same mixed DSPR population many generations apart, yet there is little evidence that radical haplotype frequency shifts occured during this period. Founder haplotype frequencies were estimated for every replicate control population for each 200-kb interval across the genome, and averaged over replicates within each study. (*Top panel*) The sum of the squared differences between the 8 founder haplotype frequencies in the control groups for each study. The minimum possible value is 0 (no difference), and the maximum possible value is 2 (controls in the two studies are fixed for alternate haplotypes). The value very rarely rises above 0.1. (*Bottom panel*) The difference in haplotype frequency for each founder between the control groups of each study. The minimum and maximum values possible are –1 and 1, respectively, and the value rarely reaches above l0.2l. The black boxes along the *x*-axes represent the physical positions of the two malathion QTL mapped in the present study.

Supplementary Figure S2 – Zoomed in QTL plots. This is the same information as presented in Figure 2 – i.e., it shows –log$_{10}$(*P*) values comparing haplotype frequencies between control and malathion-selected populations estimated in 1.5-cM windows along the genome – except here only the regions surrounding the pair of mapped QTL are shown. The regions in red reflect the 3-LOD interval for each QTL. The top *x*-axis illustrates the positions of those plausible candidates highlighted in Table 2 as follows: (a) *Cyp12d1-d* and *Cyp12d1-p*, (b) *Sod3*, (c) *Cyp6g1, Cyp6g2*, and *Cyp6t3*, (d) *Cyp301a1*, (e) *Cyp9h1*, (f) *Mdr49*, (g) *Spo*, (h) *CG10226* and *Mdr65*, (i) *sfl*. When individual genes are physically extremely close together, just a single tick/letter is shown per gene "group".

Supplementary Table S1 – Full details of all genes within mapped QTL intervals. (Available as a separate tab-delimited text file.) Presents 407 genes (344 protein-coding) and 178 genes (145 protein-coding) within the Chr2R and Chr3L mapped malathion QTL, respectively. Columns are as follows: Col 1) row number. Col 2) fbgn – FlyBase ID. Col 3) annotation_symbol. Col 4) chr – chromosome arm; since there are only 2 QTL on separate arms, this column distinguishes the QTL intervals. Col 5) gene_loc_max – the maximum (right-most) genomic position of the gene. Col 6) gene_loc_min – the minimum (left-most) genomic position of the gene. Col 7) strand – which strand (+/−) encodes the gene. Col 8) gene_name. Col 9) gene_symbol. Col 10) vocab_hit. Any gene that is a member of one of five specific FlyBase gene groups ("FBgg") or one of two specific gene ontology ("GO") groups is tagged with a number (1-7) indicating this. Numeric codes are: (1) FBgg0000547, ATP-BINDING CASSETTE TRANSPORTERS, (2) FBgg0001222, CYTOCHROME P450, (3) GO:0098754, detoxification, (4) FBgg0000077, GLUTATHIONE-S-TRANSFERASES, (5) FBgg0000797, GT1 FAMILY OF UDP-GLYCOSYLTRANSFERASES, (6) FBgg0001375, OTHER CARBOXYLESTERASES, (7) GO:0017085, response to insecticide. Genes not part of any of these groups are marked with a 0. Col 11) de_hit. Any gene shown to be significantly differentially-expressed in adult female gut tissue in response to malathion treatment by Salces-Ortiz et al. (2020; PMID: 32075557) is marked as such. This paper independently tested 4 strains: (A) SE-Sto, (B) RAL-375, (C) RAL-377, (D) iso-1. For each strain, expression of the gene can be upregulated with treatment ("u") or downregulated ("d"), so genes are marked as "Au", "Ad", "Bu", and so on in the table. Genes not differentially-expressed in any strain are marked with 0. Note that genes determined to be "significant" in the original Salces-Ortiz et al. (2020) expression study are those that survive a strain-specific, Benjamini-Hochberg adjusted p-value threshold of 0.05, and show a fold-change of at least 1.5 between control and malathion treatment.