

File S1

Code used for RNAseq analysis.

```
# Sickle (version 1.200)
# Run on each of the four FASTQ files: A3-control (A3C), A3-nicotine (A3N), A4-control (A4C), and A4-nicotine (A4N)

sickle se -f A3C.fastq.gz -t sanger -o A3C.sickle.fastq -q 30 -l 30 -n
gzip A3C.sickle.fastq

# TopHat (version 2.0.9)
# Run on each of the four trimmed FASTQ files

tophat2 -p 12 -G genes.gtf -o ./Assembly_A3C/ --no-novel-juncs --library-type
fr-unstranded genome A3C.sickle.fastq.gz

# Cufflinks (version 2.1.1)
# Run on all four TopHat assemblies simultaneously

cuffdiff -N -o ./CuffDiff_Output/ -b genome.fa -p 12 -u genes.gtf
-L A3C,A3N,A4C,A4N ./Assembly_A3C/accepted_hits.bam ./Assembly_A3N/accepted_hits.bam
./Assembly_A4C/accepted_hits.bam ./Assembly_A4N/accepted_hits.bam
```

Table S1 Raw first-instar larval phenotypes measured on DSPR RILs.

Available for download as a .csv file at <http://www.genetics.org/lookup/suppl/doi:10.1534/genetics.114.162107/-/DC1>

The "Population" column indicates which population (A or B) the line is from. The "PopulationReplicate" column indicates which subpopulation (A1, A2, B1, or B2) the line is from. The "RIL" column is the line number. The "NicotineViability" column is the fraction of 30 first-instar larvae that emerge as adults on nicotine-supplemented media. The "ControlViability" column is the fraction of 30 first-instar larvae that emerge as adults on control, nicotine-free media. There are multiple rows for any given RIL, each row containing the data from a single replicate.

Table S2 Details of QTL mapped for larva-to-adult viability on control food

Name	LOD score	Chr	Peak cM (2-LOD CI) ^a	Peak Mb (2-LOD CI) ^a	Number of genes ^b
i (pA)	8.1	2R	82.8 (82.6–83.4)	13.44 (13.38–13.60)	50
ii (pA) ^c	8.9	2R	88.2 (88.0–89.9)	15.26 (15.19–15.86)	75
iii (pA)	9.2	3R	47.2 (47.2–47.3)	1.30 (1.21–1.47)	50
iv (pB) ^d	8.7	X	32.6 (31.8–33.2)	10.73 (10.52–10.89)	37
v (pB) ^d	7.5	X	39.4 (37.9–40.7)	12.44 (12.10–12.73)	41
vi (pB) ^d	8.4	2L-2R	53.8 (53.7–58.5)	2L:19.76 (2L:19.59–2R:4.44)	733

^a 2-LOD CI indicates the 2-LOD support interval of the QTL.

^b The number of protein-coding genes in the 2-LOD support interval.

^c The location of this control viability QTL overlaps that of Q3 (Table 1), a QTL contributing to viability on nicotine food.

^d Given the relatively small number of RILs phenotyped for population pB, coupled with the low level of within-line replication we recommend caution in interpreting QTL mapped in pB.

Table S3 LOD scores for genomewide QTL scans.

Available for download as a .csv file at <http://www.genetics.org/lookup/suppl/doi:10.1534/genetics.114.162107/-/DC1>

The "Chromosome" column indicates the chromosome arm of the position under test (X, 2L, 2R, 3L, and 3R). The "PhysicalPosition" and "GeneticPosition" columns indicate the physical and genetic positions of the site under test, respectively. The four "LODscore" columns present the LOD scores at each position for the two traits (nicotine resistance and larva-to-adult viability on control food) in each of the two mapping populations (pA and pB). The four "VarianceExplained" columns indicate the fraction of the phenotypic variance in trait/population that is explained by among-line genetic variation at the site under test.

Table S4 Viability measures for founder lines A3 and A4 used for RNAseq

Founder	Replicate ^a	Nicotine Viability ^b	Control Viability ^c
A3	1	0.233	0.367
A3	2	0	0.633
A3	3	0	0.6
A3	4	0	0.733
A3	5	0	0.867
A3	6	0	0.6
A3	7	0	0.833
A3	8	0	0.767
A3	9	0	0.867
A3	10	0.267	0.667
A3	11	0.533	0.733
		mean = 0.09	mean = 0.70
		SD = 0.177	SD = 0.147
A4	1	0.233	0.133
A4	2	0.3	0.9
A4	3	0.767	0.833
A4	4	0.833	0.8
A4	5	0.6	0.767
A4	6	0.5	0.7
A4	7	0.467	0.6
A4	8	0.533	0.5
A4	9	0.567	0.767
A4	10	0.767	0.733
A4	11	0.333	0.8
		mean = 0.54	mean = 0.68
		SD = 0.199	SD = 0.214

^a Each replicate assay involved 30 first-instar larvae.

^b The fraction of first-instar larvae emerging as adults on nicotine-supplemented media.

^c The fraction of first-instar larvae emerging as adults on control, nicotine-free media.

Table S5 All nominally significant ($p < 0.05$) differentially-expressed genes identified by RNAseq.

Available for download as a .csv file at <http://www.genetics.org/lookup/suppl/doi:10.1534/genetics.114.162107/-/DC1>

The data is taken directly from the "gene_exp.diff" Cuffdiff output file, and simply trimmed to remove genes with per-contrast p -values above 5%. The "Gene" column is the symbol for the gene under test. The "Chromosome", "GeneStart", and "GeneStop" columns give the position of the gene in the *D. melanogaster* reference genome (NCBI build 5.3). The "Sample1" and "Sample2" columns give the names of the two samples being compared, with four contrasts possible: A3-control *versus* A4-control, A3-nicotine *versus* A4-nicotine, A3-control *versus* A3-nicotine, and A4-control *versus* A4-nicotine. The columns "Sample1.FPKM" and "Sample2.FPKM" give the FPKM (Fragments Per Kilobase of exon model per Million mapped fragments) values for each sample. The "Log2.FoldChange" column gives the \log_2 fold change in expression (Sample2 divided by Sample1). The "TestStatistic" column gives the test statistic used by Cuffdiff to compute the significance of the observed change in FPKM between samples, the "Pvalue" column provides the uncorrected p -value of the test statistic, and the "Qvalue" column provides the Benjamini-Hochberg FDR-adjusted p -value of the test statistic.

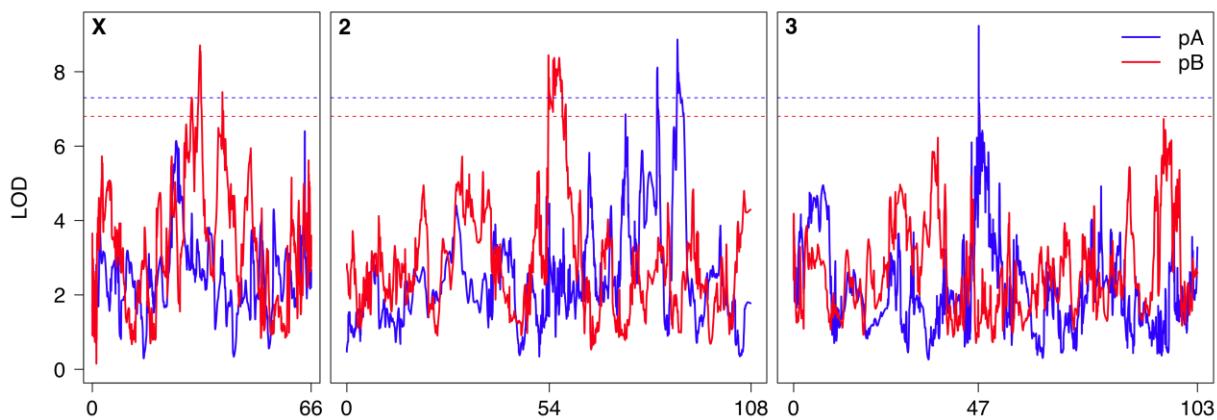


Figure S1 Genome scan for QTL contributing to larva-to-adult viability on control media. Solid curves are the scans for the pA (blue) and pB (red) data, and the horizontal dotted lines represent the genome-wide 5% permutation threshold (LOD = 7.3 for pA and 6.8 for pB). Genetic distances along the chromosomes are indicated along the x-axis. The centromeres are at positions 54 and 47 on chromosomes 2 and 3, respectively. QTL identified in this scan are described in Table S2.

Q1 pA (2L:4990000..5250000)

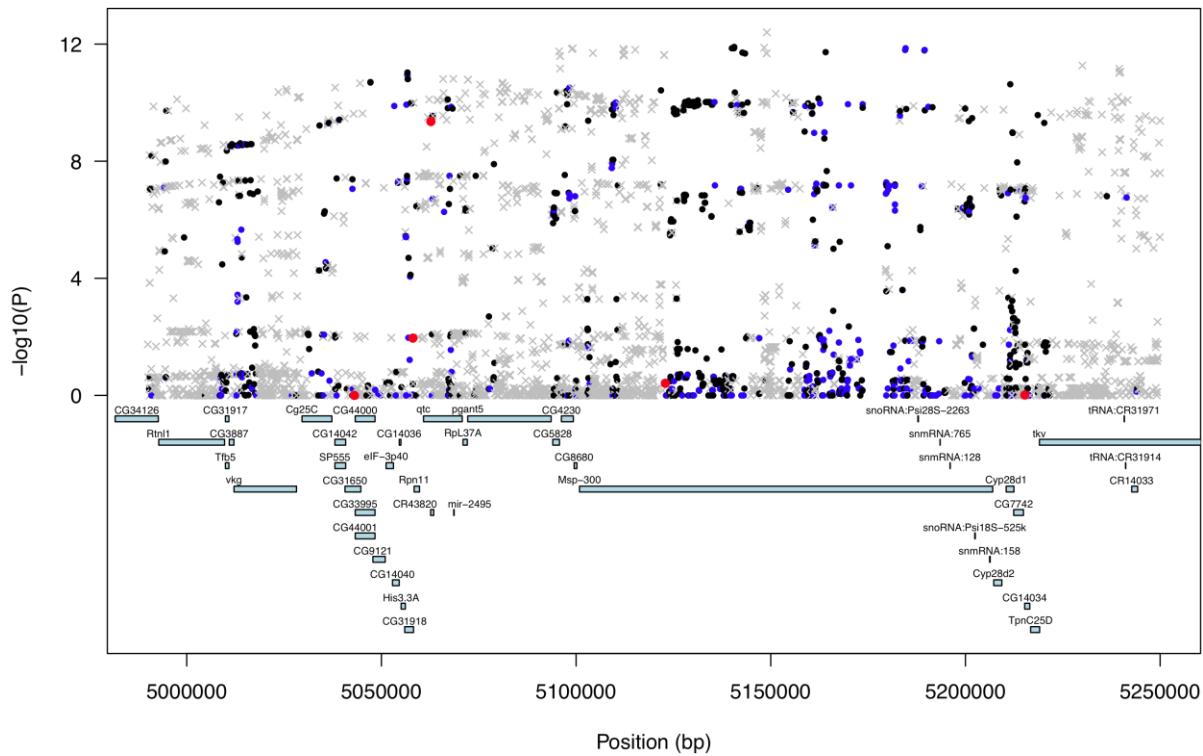


Figure S2 Association tests at SNPs residing beneath all four mapped QTL for nicotine resistance (Q1, Q2, Q3, and Q4 mapped in pA, and Q4 only mapped in pB). SNP genotypes in RILs were inferred from the mosaic founder haplotype structure of each RIL. The $-\log_{10}(p)$ value is plotted for each segregating site, with the plot symbol reflecting the impact of the variant: Red filled circles (loss of a start codon, loss or gain of a stop codon, changes in splice sites), blue filled circles (nonsynonymous changes), black filled circles (synonymous changes), and gray crosses (all other variants). The position of each gene within each QTL is shown as a light blue box, with the width of each box showing the distance from the start to the end of the gene model. Note that due to the number of genes under Q3, no gene names are provided for this plot. Note also the absence of tested variants at \sim 15,600-kb in the Q3 plot; This is due to a large array of 5S rRNA genes that likely precluded high-quality SNP identification in this region.

Q2 pA (2R:14510000..14730000)

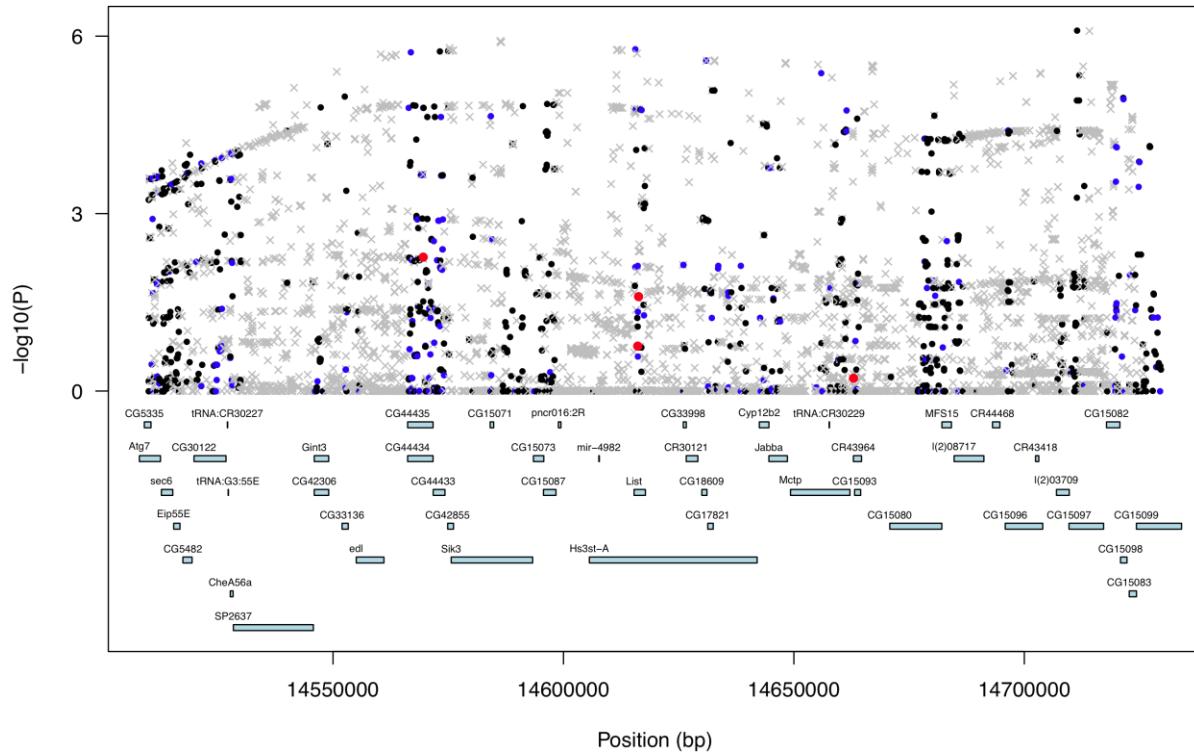


Figure S2 Continued.

Q3 pA (2R:15030000..16420000)

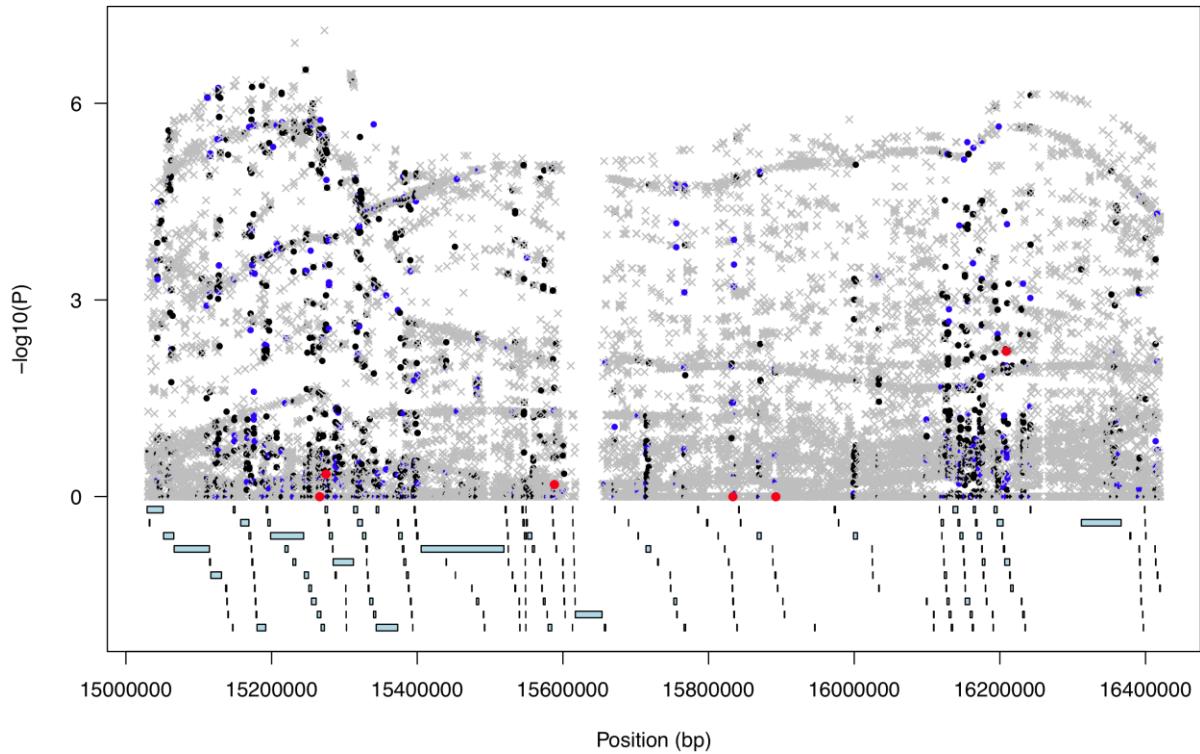


Figure S2 Continued.

Q4 pA (3R:6830000..7060000)

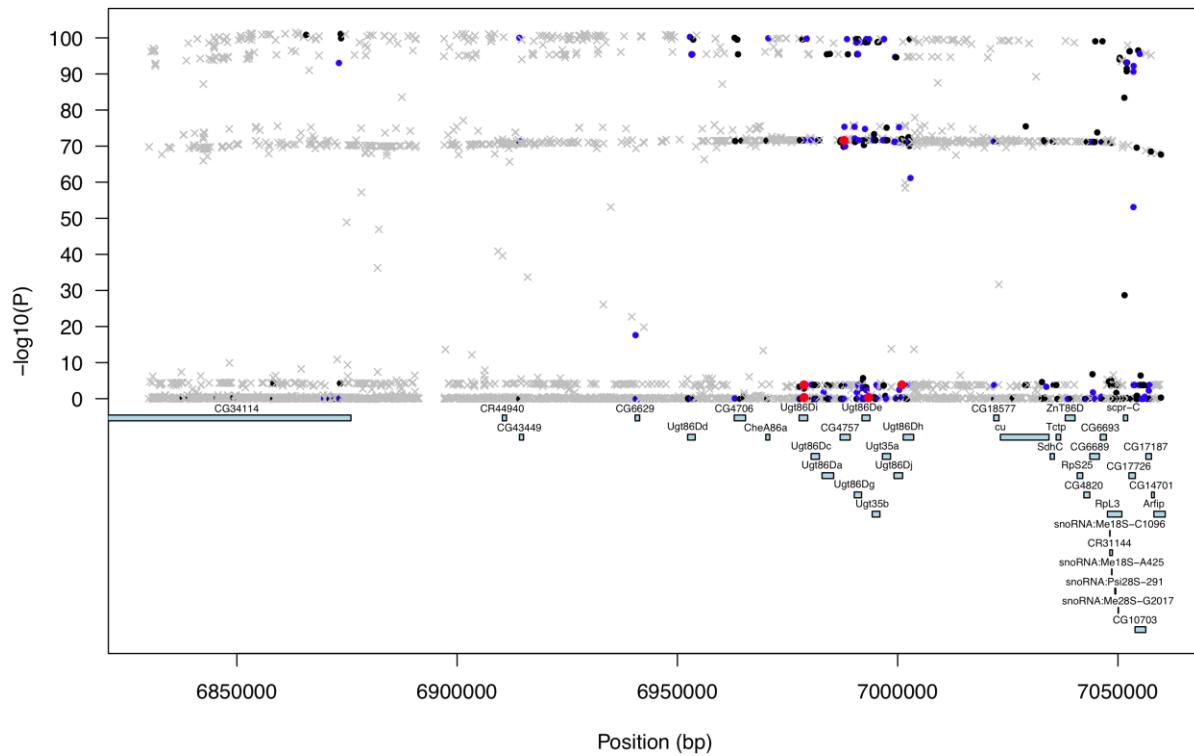


Figure S2 Continued.

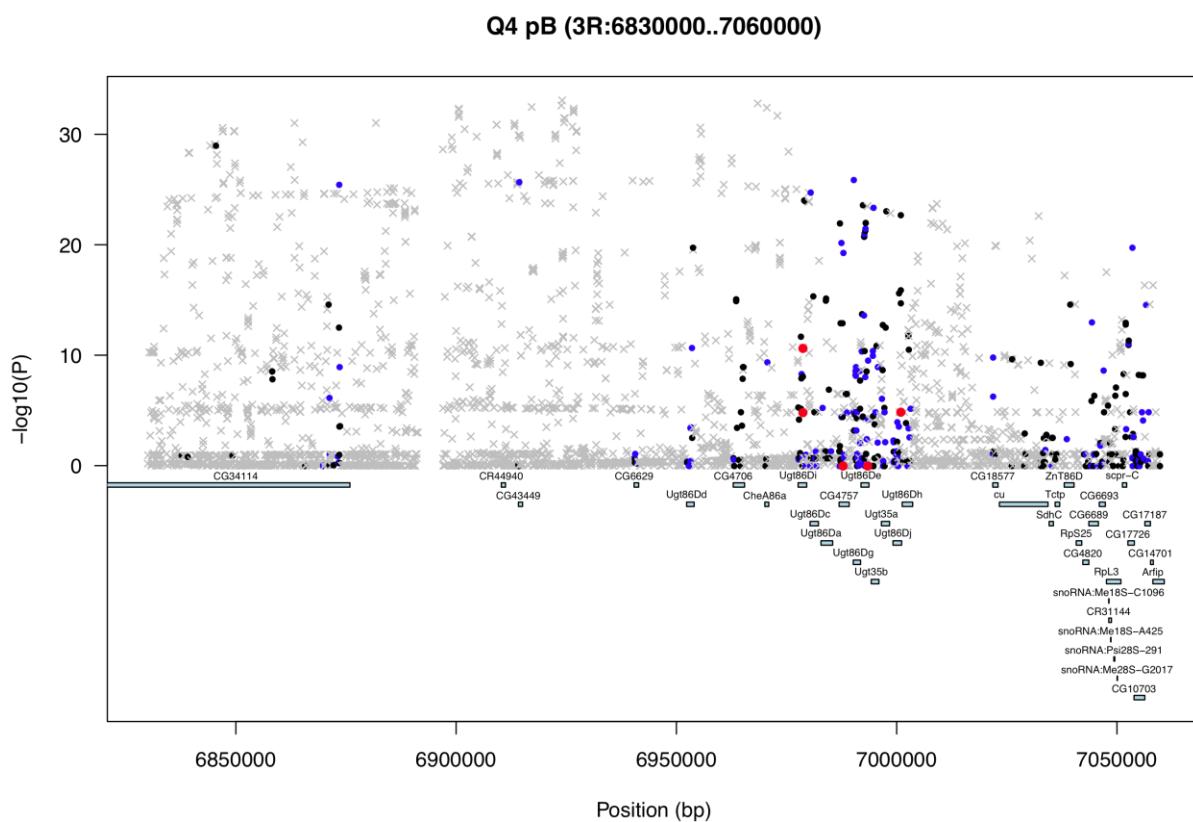


Figure S2 Continued.

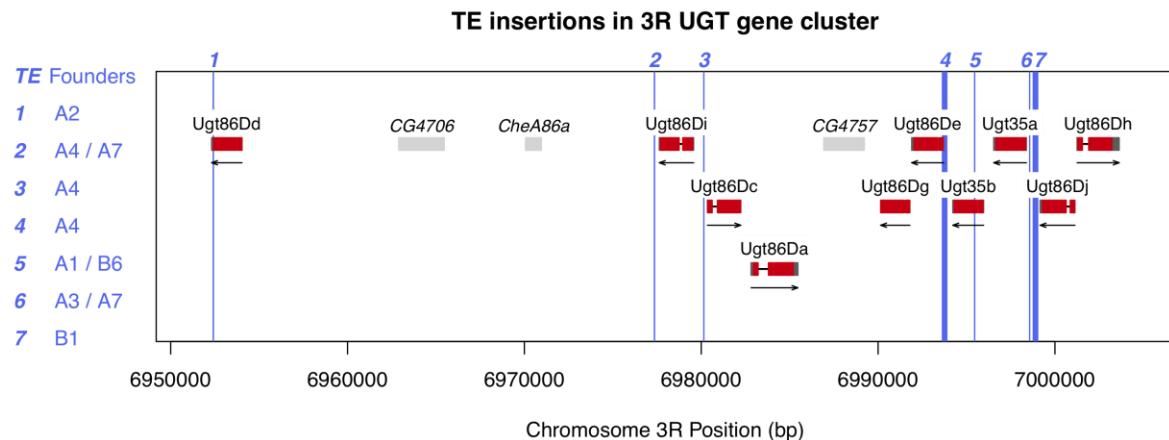


Figure S3 Positions of transposable elements (TEs) in or near the UGT genes implicated in Q4. The gene models for each of the 10 UGT genes are presented as red boxes (protein-coding regions) and dark gray boxes (untranslated regions), with arrows beneath the gene models showing the direction of transcription. The positions of other genes in the region of Q4 harboring UGT genes are presented as light gray boxes. The vertical blue bars are the positions of seven TEs that are either within, or within 1-kb of the start or end of, UGT genes (data taken from Supplementary Table 4 from Cridland *et al.* 2013) with the width of the bars representing ambiguity in the insertion position of the element. To the left of the plot are the founders that the elements are inserted into.

Peak in pB at 2R:7,500,000–8,440,000

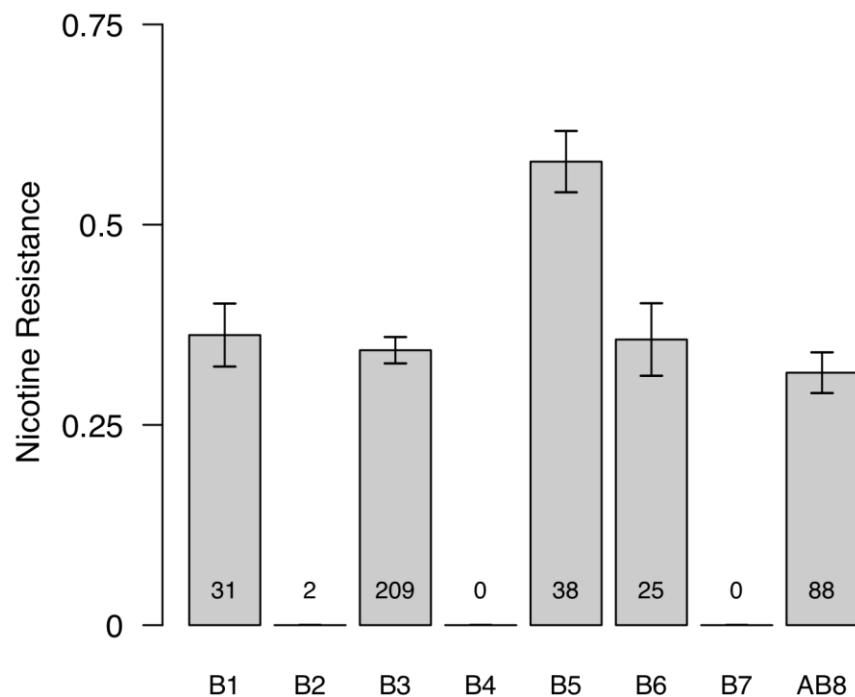


Figure S4 Founder haplotype means and 1-SDs at the pB-specific peak at position 7,500,000-8,440,000 on chromosome 2R. This interval harbors P450 gene *Cyp6g1*. The number of RILs for which we confidently assign a founder genotype (probability > 0.95) is listed at the bottom of each bar, and only founder means associated with at least 5 observations are presented.