

Figure S1 A) Heritabilities of the sixty-six behavioral components in the wild isolate and the RIAIL population in response to $\Delta T \sim 0.4^{\circ}$ C. **B**) Correlation of six representative components of the avoidance behavior among wild isolates. We analyzed 66 traits for pattern of correlation in the RIAIL population. For the traits that are speed at different time points there were two uncorrelated groups. The speeds between 0.72 and 1.96 seconds were uncorrelated to the speeds at the other time points which were strongly correlated. For simplicity, representative speeds are included in the plot: one at early time point (sp0.18s), one at late time point (sp8.1s) and one in the 0.72-1.96 range (sp1.61s). The behavioral components are: sp0.18s: speed at 0.18 seconds, sp1.61s: speed at 1.61 seconds, sp8.1s: speed at 8.1 seconds after start of the assay, pFR: probability of responding by reversals, W=number of body bends during reversals, pRO: probability of inducing omega turns. The magnitude of significant Pearson correlation coefficients (p< 0.01) among these behavioral metrics are shown for wild isolates in circles. Absence of circles represents an absence of significant correlations. The phenotypes are hierarchically clustered and cut into two clusters bordered by red lines. **C,D**) Common naturally occurring variation on chromosomes IV and X contribute to natural variation in speed at early time points (C) and acceleration (D) during escape behavior. Plots of the results of genome wide association mapping for acceleration and speed at 0.2 seconds. Significantly associated markers are indicated by red lines.





Figure S2 Linkage analysis of various escape behavioral components related to centroid speed. Logarithm of the odds (LOD) scores plotted against marker positions. Each facet represents a chromosome depicted as I through V and X. The traits that are mapped are shown on the title of the corresponding figure. The horizontal dashed line represents the 5% genomewide significance threshold obtained after 1000 permutations. A-D) LOD profiles for centroid speed at particular time points. E and F) LOD profiles for centroid speed at every 0.18 seconds for chromosomes IV (E) and X(F) respectively. Each facet represents the mapping results of speed at a single time point (depicted as sp*.**s in red). The red horizontal line represents the 5% genomewide significance thresholds. The gray vertical line in (E) represent the peak marker position of the significant QTL. The blue vertical line in (F) represents the position of *npr-1* gene. Asterisks represent the speed at time points where a significant QTL was detected on chromosome IV. G-J) QTL analysis results of the speed related escape behavior components. The axes of the plots are same as in Fig S2A. The traits mapped are in the title of each plot.



Figure S3 Linkage analysis of various escape behavioral components related to reversals and omega turns. The axes of the plots are same as in Fig S2A. The traits mapped are in the title of each plot (A-E).



Figure S4 Overlay of the speed (black) and the probability of responding by reversals (red) QTL on chromosome X. The horizontal dashed lines represent genomewide significance thresholds. The vertical dashed lines represent the peak of the respective QTL. **B**) The breakpoints on the X-chromosomes (X-axis:physical position in basepairs) of introgression lines (Y-axis) as determined by whole genome sequencing. Blue indicates Hawaii markers whereas orange indicates Bristol markers. The vertical red-dashed line represents the position of *npr-1*. The black and the white vertical dashed lines represent the proximal and distal QTL intervals respectively.

Files S1-S5

Available for download at www.genetics.org/lookup/suppl/doi:10.1534/genetics.115.178491/-/DC1

Videos:

File S1: Avoidance behavior of Bristol animal in response to $\Delta T \sim 0.4^{\circ}$ C File S2: Avoidance behavior of Hawaiian animal in response to $\Delta T \sim 0.4^{\circ}$ C File S3: Avoidance behavior of Bristol animal in response to $\Delta T \sim 9.1^{\circ}$ C File S4: Avoidance behavior of Hawaiian animal in response to $\Delta T \sim 9.1^{\circ}$ C

File S5: .xlsx file with genotype and phenotype details for strains used in the study and QTL information.

Strain	Fraction responding by reversal
Bristol	0.9 (n=154) A x
Hawaii	0.6 (n=149)
N2 male x CB4856 herm	0.5 (n=34)
CB4856 male x N2 herm	0.4 (n=29)
npr-1(ad609)	0.9 (n=21)
QG1 (N2 > CB4856, npr-1 CB4856)	1.0 (n=29)
kyIR9 (N2>CB4856, N2 npr-1)	0.5 (n=30)

Table S1 *npr-1* does not contribute to probability or responding by reversals

Table S2ANOVA results: Effects of genetic background, *npr-1* and distal QTL on
chromosome X (QTL_Xb) on speed.

	Df	Sum Sq	Mean Sq	F value	Pr(>F)
npr1	1	12.86	12.86	10.04	0.0039
bkg	1	0.06	0.06	0.05	0.8248
QTLXb	1	0.00	0.00	0.00	0.9768
bkg:QTLXb	1	0.22	0.22	0.17	0.6835
npr1:QTLXb	1	0.17	0.17	0.14	0.7154
npr1:bkg	1	0.01	0.01	0.01	0.9425
Residuals	26	33.30	1.28		

ANOVA Model with Predictors: Background, npr-1,QTLXb and their interactions

	Df	Sum Sq	Mean Sq	F value	Pr(>F)
npr1	1	0.01	0.01	0.34	0.5633
bkg	1	0.30	0.30	16.44	0.0004
QTLXb	1	0.18	0.18	10.10	0.0038
bkg:QTLXb	1	0.00	0.00	0.05	0.8186
npr1:QTLXb	1	0.00	0.00	0.22	0.6444
npr1:bkg	1	0.01	0.01	0.57	0.4563
Residuals	26	0.47	0.02		

Table S3ANOVA results: Effects of genetic background, *npr-1* and distal QTL on
chromosome X (QTL_Xb) on probability of responding by reversals

ANOVA Model with Predictors: Background, npr-1,QTLXb and their interactions

	df	Type III SS	LOD	%var	F value	Pvalue(Chi2)	Pvalue(F)
X@76.6	2.0000	0.7496	6.7660	17.6137	16.9716	0.0000	0.0000
X@190.0	2.0000	0.3015	2.9079	7.0859	6.8276	0.0012	0.0015
X@76.6:X@190.0	1.0000	0.0595	0.5965	1.3980	2.6940	0.0974	0.1031

Table S4Summary of multiple QTL analysis of variation in probability ofresponding by reversals

Summary of Multiple QTL Model with Reversal Response Probability

	df	Type III SS	LOD	%var	F value	Pvalue(Chi2)	Pvalue(F)
V@171.7	4.0000	5828.0643	2.5009	5.2248	2.8288	0.0213	0.0273
X@69.0	4.0000	16689.1963	6.6687	14.9616	8.1005	0.0000	0.0000
X@174.7	4.0000	6758.1974	2.8815	6.0586	3.2803	0.0100	0.0134
V@171.7:X@69.0	2.0000	331.6153	0.1480	0.2973	0.3219	0.7111	0.7253
V@171.7:X@174.7	2.0000	315.6435	0.1409	0.2830	0.3064	0.7229	0.7366
X@69.0:X@174.7	2.0000	1240.6088	0.5501	1.1122	1.2043	0.2817	0.3032
V@171.7:X@69.0:X@174.7	1.0000	52.7602	0.0236	0.0473	0.1024	0.7416	0.7494

 Table S5
 Summary of multiple QTL analysis of variation in mean reversal duration

Summary of Multiple QTL Model with Mean Reversal Duration

	df	Type III SS	LOD	%var	F value	Pvalue(Chi2)	Pvalue(F)
III@140.0	4.0000	0.5186	4.9341	11.8678	5.8171	0.0001	0.0002
IV@199.0	4.0000	0.5362	5.0879	12.2701	6.0143	0.0001	0.0002
V@164.0	4.0000	0.3403	3.3276	7.7872	3.8169	0.0041	0.0057
III@140.0:IV@199.0	2.0000	0.0095	0.0986	0.2185	0.2142	0.7969	0.8074
III@140.0:V@164.0	2.0000	0.1041	1.0581	2.3831	2.3362	0.0875	0.1007
IV@199.0:V@164.0	2.0000	0.0293	0.3015	0.6705	0.6573	0.4994	0.5199
III@140.0:IV@199.0:V@164.0	1.0000	0.0038	0.0389	0.0862	0.1690	0.6720	0.6817

Table S6Summary of multiple QTL analysis of variation in probability of responding byomega turns.

Summary of Multiple QTL Model with Reversal to Omega transition Probability

Table S7Summary of multiple QTL analysis of variation in number of body bends duringreversal.

	df	Type III SS	LOD	%var	F value	Pvalue(Chi2)	Pvalue(F)
IV@195.6	2.0000	5.6736	3.5485	9.8662	8.4226	0.0003	0.0004
V@165.8	2.0000	4.1708	2.6487	7.2529	6.1917	0.0022	0.0027
IV@195.6:V@165.8	1.0000	0.0047	0.0031	0.0081	0.0139	0.9047	0.9063

Summary of Multiple QTL Model with Mean number of body bends during reversals