Genetically distinct behavioral modules underlie natural variation in thermal performance curves

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Supplementary Tables S1, S3, S4, S5, S6 (filenames listed for S2 and S7)

Supplementary Figures S1-S12

Supplementary Tables

Supplementary Table S1. *C. briggsae* **wild isolate strain origin and background.**

Strain name	Phylogeographic group****	Geographic origin
AF16*	Tropical	India
ED3083	Tropical	South Africa
VT847**	Tropical	Hawaii, USA
NIC65***	Tropical	French Guiana
JU1338***	Tropical	India
GXW0023***	Tropical	China
NIC21	Tropical	Taiwan
JU1908***	Tropical	Australia
HK104**	Temperate	Japan
EG4181***	Temperate	Utah, USA
JU439	Temperate	Iceland
QX1547***	Temperate	Tennessee, USA
NIC6	Temperate	Switzerland
PB826	Temperate	Ohio, USA
QR24***	Montreal	Quebec, Canada
QR25***	Montreal	Quebec, Canada
JU1341***	Kerala	India
JU1345	Kerala	India
JU1348***	Kerala	India
ED3092	Nairobi	Kenya
ED3098	Nairobi	Kenya
ED3101***	Nairobi	Kenya
VX0034***	Hubei	China
NIC20***	Taiwan	Taiwan

*genome data in (STEIN *et al.* 2003); ** genome data in (HILLIER *et al.* 2007); *** genome data in (THOMAS *et al.* 2015); **** population structure designations in (FELIX *et al.* 2013)

Supplementary Table S2. RIL strains and genotypes.

SuppTable_S2_CbrQTL_gen_h2na.csv

Supplementary Table S3. NIL markers.

position	6230047	6741889	14167323	14794163
enzyme	DraI	DraI	DraI	DraI
Tm (forward)	55	54.36	55.15	55.29
Sequence (forward)	TGTACCTGAAT TTTCATTGGTA	GAAAATTATGA ACTTTCCGATT	AAAGTGTGATA CGGGAAATAAG	CTTCCTCTTCT GTTGTGAAAAT
Tm (reverse)	55	55.11	54.57	55.99
Sequence (reverse)	AACTTTAGTTT CCGAACCATC	AAAACATTGGT ATGTGTGTCAG	TTTGAAGTTAGC TTTGAGAATTT	CTATCCTGCCA AGAAGATGAT
Size	742	740	753	730
AF16 fragments	344, 377	428, 291	732	473, 236
HK104 fragments	721	719	341, 391	709

Supplementary Table S4. Parameter estimates from three parameter logistic function fit to Locomotion index (LI1) values from pooled wild isolate strains of *C. briggsae*.

	Rearing temperature	Asymptote (α)* (LI1)	Slope (β)*	Inflection point (τ)* (°C)
Lower TPC	16°C	0.344 ± 0.002	0.461 ± 0.008	15.628 ± 0.046
	23°C	0.414 ± 0.002	0.659 ± 0.011	17.623 ± 0.030
	28°C	0.375 ± 0.002	0.787 ± 0.015	18.885 ± 0.030
Higher TPC	16°C	0.345 ± 0.003	-0.854 ± 0.025	30.866 ± 0.043
	23°C	0.402 ± 0.002	-0.788 ± 0.018	32.860 ± 0.036
	28°C	0.376 ± 0.002	-0.683 ± 0.016	33.086 ± 0.044

*Parameter estimates ± 1 standard error

Supplementary Table S5. Position, significance and heritability of QTL peaks from multiple imputation QTL analysis of 'synthetic' principal component traits, function-valued traits, and univariate locomotion index (LI7 at 35.6°C).

Phenotype full name	Chromosome	map position (cM)*	nucleotide position (Mbp)**	Bayes interval L-R marker position (Mbp)	H^2***	LOD	P****
Principal Component 5	1	82.50	13.75	13.06-14.72	0.082	2.83	0.0332
Principal Component 1	2	34.58	6.5	5.36-11.12	0.128	4.54	0.0012
Asymptote (α)	2	39.55	9.91	6.33-11.00	0.192	7.09	<0.0001
Principal Component 6	2	42.59	11.18	0.64-12.47	0.115	4.05	0.002
Principal Component 3	3	37.61	4.09	1.11-14.58	0.082	2.83	0.0452
Asymptote (α)	4	0.30	0.55	0.09-2.42	0.085	2.95	0.0332
Principal Component 3	5	30.50	7.69	3.45-11.62	0.117	4.13	0.0036
Inflection point (τ)	5	32.15	8.08	5.88-12.66	0.146	5.23	<0.0001
Locomotion (LI7) at 35.6°C	5	37.35	11.56	6.19-13.01	0.276	10.74	<0.0001
Principal Component 2	5	37.66	11.62	6.75-14.30	0.147	5.27	<0.0001
Slope (β)	5	37.66	11.62	5.34-13.90	0.203	7.52	<0.0001
Principal Component 3	X	44.36	19.29	16.52-20.38	0.148	5.3	0.0004
Principal Component 4			no significant QTL				
Principal Component 7			no significant QTL				
Principal Component 8			no significant QTL				

* location of the LOD peak on the expanded genetic map of the RILs.

** location of the nearest genotyped marker to the LOD peak.

*** QTL heritability estimated as $1 - 10^{-2\,LOD/n}$ (n = number of RILs).

**** P-value reflects the 5% significance for LOD threshold from 2500 permutations of the data for each phenotype individually (not corrected for multiple hypotheses with Bonferroni correction $0.05/12 = 0.0042$).

Supplementary Table S6. Additive and epistatic interaction effects from two-dimensional QTL models.

Phenotype	Locus 1*	Locus 2*	Model**	lod.fv1	p-value	lod.av1	p-value	lod.int	p-value
Asymptote (α)	II:39.5	III:60	Additive	3.49	0.31	**3.47**	**0.008**	0.025	1
Asymptote (α)	II:39.5	IV:0.5	Additive	3	0.609	**3**	**0.022**	0.006	1
PC1	II:39.5	III:0	Additive	3.55	0.254	**3.45**	**0.007**	0.104	1
PC1	II:34.5	V:77.5	Additive	3.63	0.223	**3.57**	**0.005**	0.057	1
PC2	II:22.5***	V:38	Additive	**4.5**	**0.037**	4.16	**0**	0.338	1
PC3	III:37.5	V:23.5***	Additive	**6.18**	**0.001**	3.65	**0.005**	2.528	0.4256
PC3	IV:46.5	V:31.5	Additive	3.04	0.546	**2.99**	**0.019**	0.051	1
PC3	V:28.5	X:44.5***	Additive	**4.89**	**0.018**	**4**	**0.002**	0.889	0.9998
PC5	III:37***	IV:48***	Interaction	**5.73**	**0.027**	1.33	0.847	**4.396**	**0.0302**
Slope (β)			NA						
Inflection point (τ)			NA						
PC4			NA						
PC6			NA						
LI7 at 35.6°C			NA						

* Chromosome map position (cM) on the expanded RIL genetic map.

** Interaction model type determined from a significant full model (2-dimensional QTL scan in r/qtl) allowing for interactions (lod.fv1) when additive model (lod.av1) was not; additive model type obtained from significant lod.av1 and non-significant lod.fv1, or by a non-significant interaction between them (lod.int); significant model statistics shown in bold.

*** Change in position by >5cM between models for lod.fv1 and lod.av1.

Supplementary Table S7. Genes with coding differences between Tropical and Temperate clade *C. briggsae* strains (from Thomas et al. 2015) in the Chromosome V QTL region.

SuppTable_S7 - Subset of genes with nonsynonymous changes and GO terms.xlsx
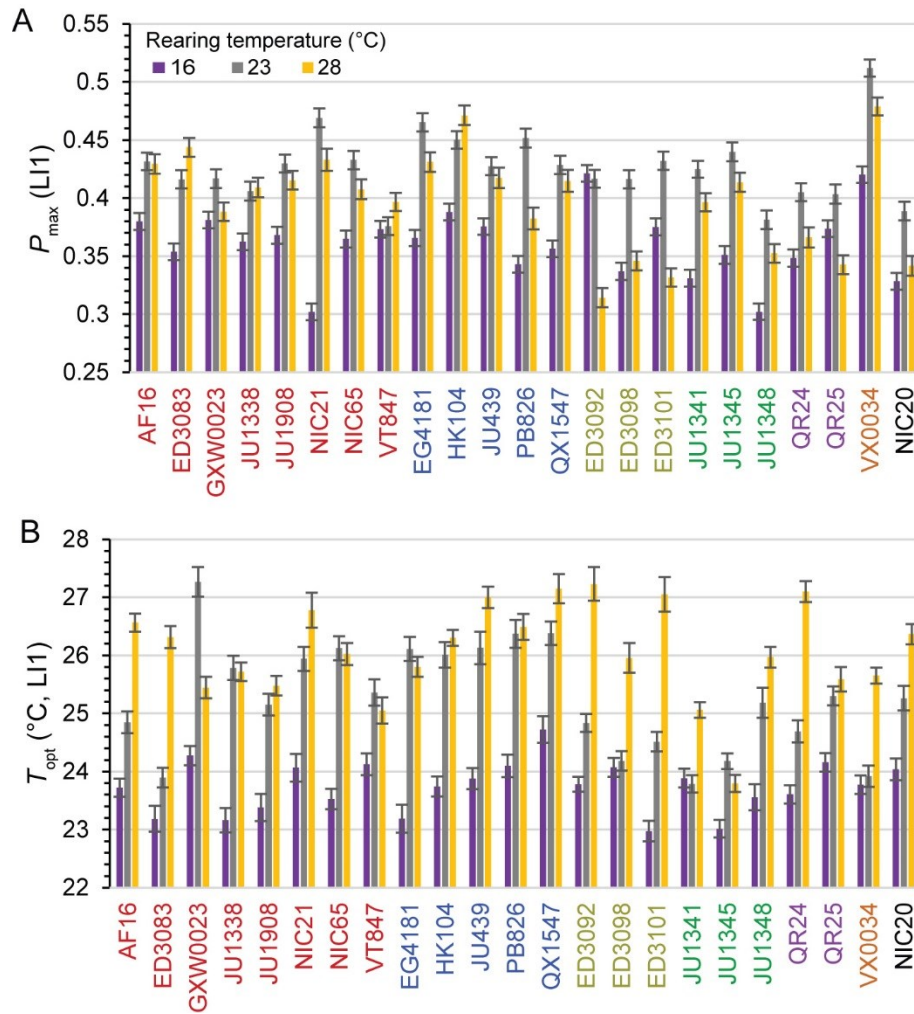
Supplementary figures
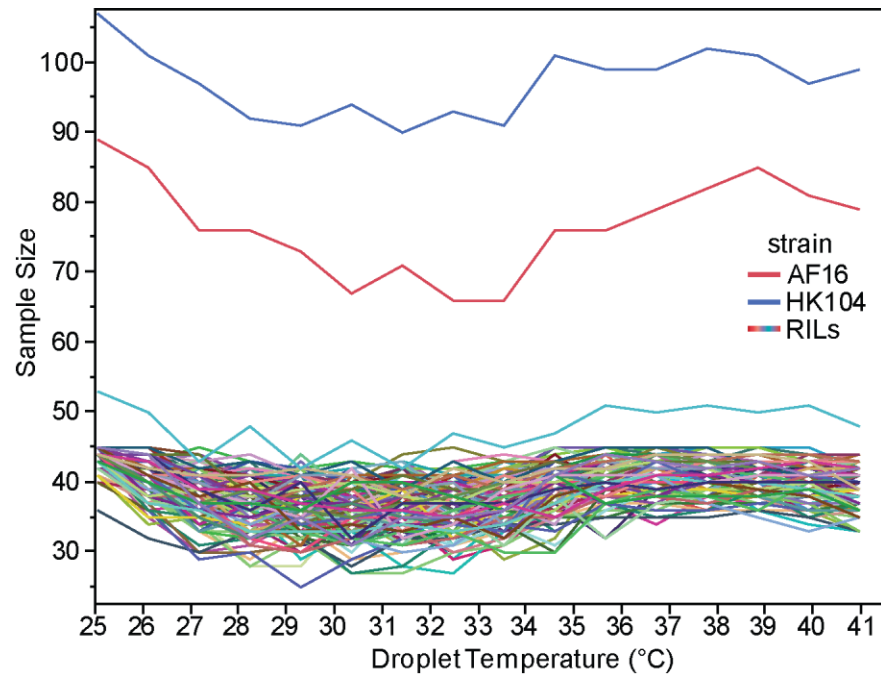
Supplementary Figure S1. Number of worms per strain passing quality filters at each temperature. All strains tested ≥45 individuals (except n=36 for PB1209). A minimum of 25 individuals were included in all means for a given temperature.
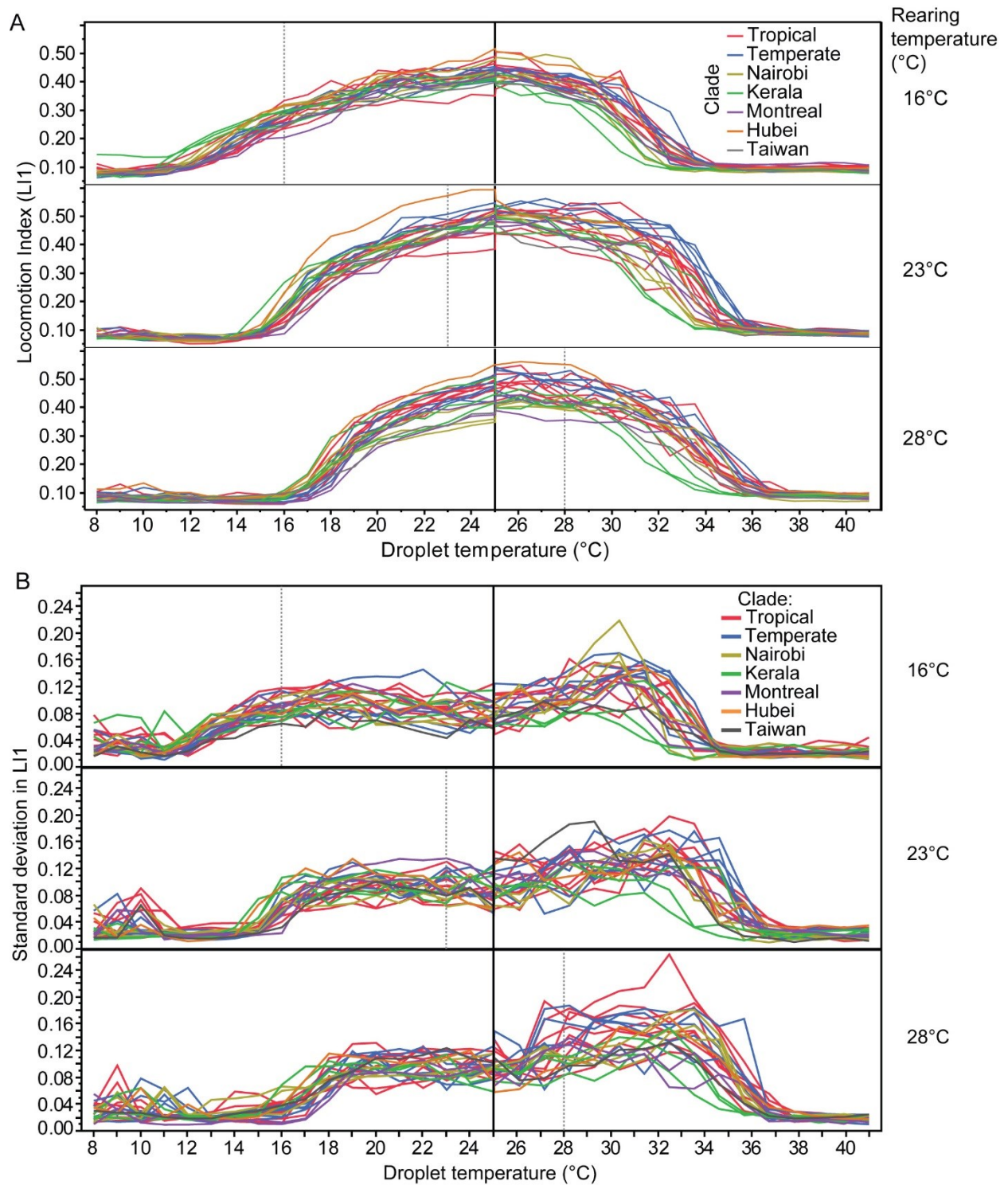
Supplementary Figure S2. Effect of rearing temperature on locomotory performance for each wild isolate strain of *C. briggsae*. (**A**) Thermal performance curves (TPCs) for mean LI1 locomotion index for each of 23 strains at three rearing temperatures (16°C, 23°C, 28°C; dashed vertical lines). (**B**) The standard deviation across individuals in LI1 for each strain. Elevated

values at temperatures below 12°C reflect condensation artifacts in the image analysis. Plots in A and B show results of two experiments, one decreasing and one increasing temperature from an initial assay temperature of 25°C (black vertical line). Strains are color coded according to phylogeographic group: red – Tropical (n=8 strains), blue – Temperate (n=5), yellow – Nairobi (n=3), green – Kerala (n=3), purple – Montreal (n=2), orange – Hubei (n=1), black – Taiwan (n=1).

Supplementary Figure S3. Estimates of (A) P_{max} and (B) T_{opt} for each wild isolate strain of *C. briggsae* when reared under three temperature conditions. Error bars indicate ±1 standard error.
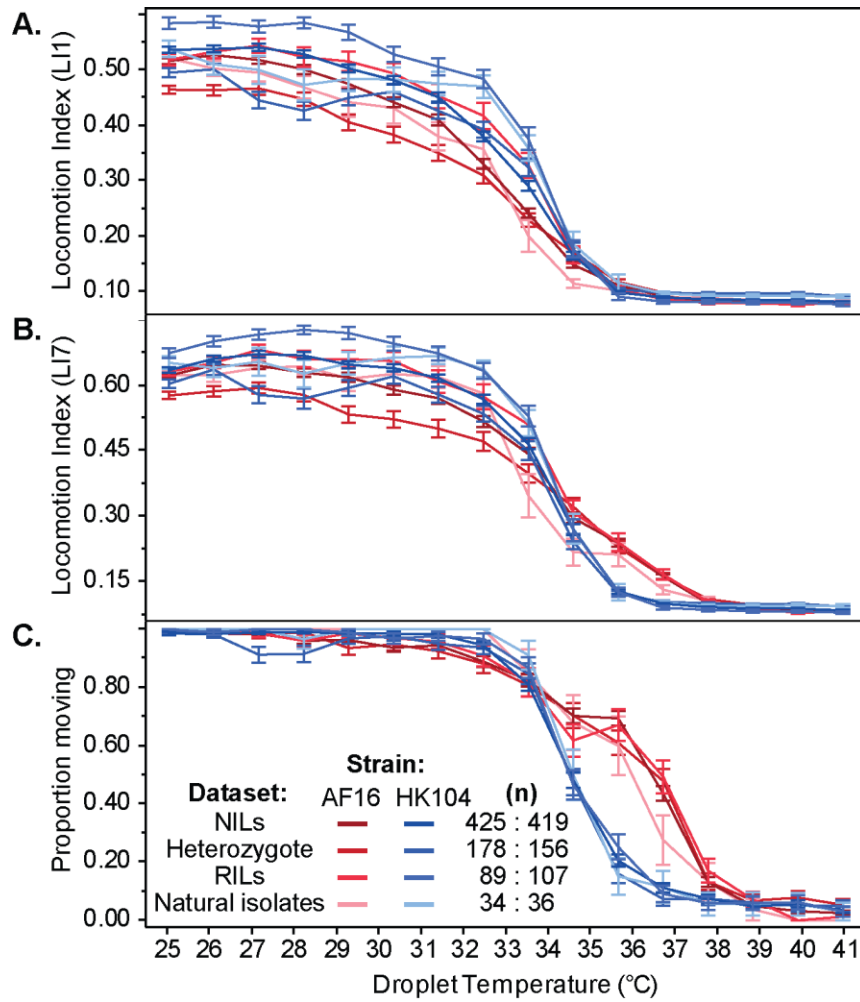
Supplementary Figure S4. Repeatability of multiple metrics of thermal performance for AF16 (red lines) and HK104 (blue lines) strains across four independent experiments: (**A**) LI1 locomotion index, (**B**) LI7 locomotion index, (**C**) proportion of animals moving. All animals reared at 23°C. Error bars indicate ±1SEM; darker lines indicate higher sample size for the corresponding experiment.
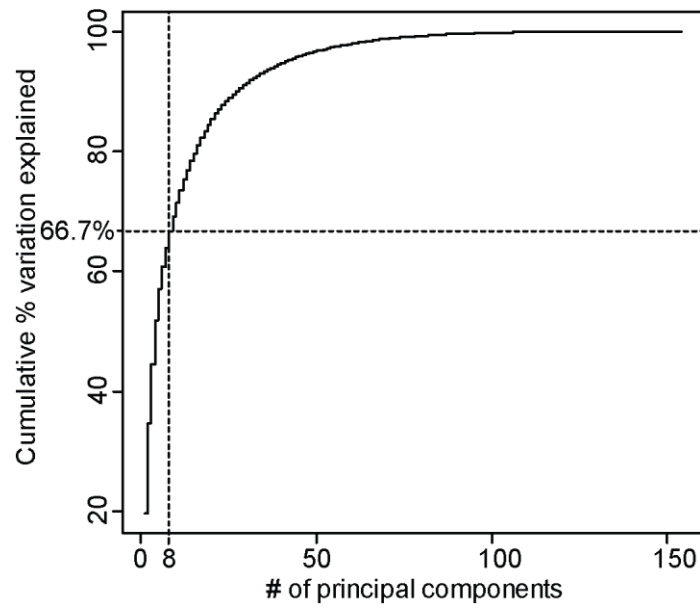
Supplementary Figure S5. Cumulative fraction of variation in 167 trait metrics explained by principal component axes. Horizontal and vertical dashed lines indicate the 8th principal component (PC8), which corresponds to ~66.7% of total variation explained by PC1 through PC8.

Figure (PCA loadings, PC1–PC8). Each panel lists feature names with their absolute weight values.

A. PC1

Feature	Weight
disp7_31	0.147
LI_31	0.147
disp_32	0.146
LI_32	0.145
disp_31	0.145
disp7_32	0.145
disp_30	0.141
disp_33	0.141
disp7_33	0.141
LI_30	0.140
LI_28	0.140
disp7_30	0.139
LI_33	0.138
LI_29	0.136
disp_28	0.134
disp7_29	0.134
disp7_28	0.130
disp_29	0.129
LI_27	0.128
LI_26	0.128
std_disp7_34	0.126
disp7_26	0.126
LI_34	0.126
LI_25	0.125
std_LI_34	0.124
disp7_34	0.124
LI7_33	0.123
disp_34	0.122
disp7_25	0.122
LI_25	0.122
disp7_27	0.121

B. PC2

Feature	Weight
LI_37	0.147
disp7_37	0.147
LI7_38	0.146
disp_36	0.145
SE_InflectionPt	0.145
disp_37	0.145
LI_36	0.141
disp_40	0.141
disp_39	0.141
LI7_39	0.140
LI7_37	0.140
LI_38	0.139
disp_38	0.138
std_moving_37	0.136
LI7_40	0.134
LI_39	0.134
disp7_39	0.130
LI_40	0.129
std_LI_37	0.128
disp7_38	0.128
disp7_36	0.126
std_LI_40	0.126
disp_35	0.126
moving_37	0.125
std_LI_38	0.124
std_LI7_39	0.124
disp7_40	0.123
std_LI7_30	0.122
std_LI7_38	0.122
std_LI7_40	0.121

C. PC3

Feature	Weight
std_disp7_32	0.171
std_disp_31	0.162
std_disp7_31	0.162
std_LI_32	0.160
std_disp_32	0.156
std_disp7_28	0.152
std_disp_29	0.150
disp_25	0.144
disp_26	0.143
std_disp_28	0.140
std_LI_31	0.139
std_disp7_30	0.138
std_disp7_29	0.135
std_LI7_32	0.134
std_disp_30	0.133
disp_27	0.129
InflectionPt	0.128
std_LI_29	0.122
LI7_35	0.120
std_LI_28	0.117
std_LI_30	0.117
std_moving_32	0.117
std_disp_27	0.115
std_LI7_31	0.114
disp7_35	0.112
std_LI7_29	0.109
LI7_34	0.108
moving_34	0.106
disp7_25	0.105
std_disp7_27	0.104

D. PC4

Feature	Weight
std_LI7_26	0.150
std_LI_26	0.147
std_LI7_27	0.139
std_disp_26	0.132
std_LI_27	0.130
moving_29	0.124
std_moving_27	0.124
LI7_40	0.118
LI7_27	0.116
InflectionPt	0.115
LI7_26	0.115
moving_27	0.115
disp7_40	0.114
std_disp7_26	0.113
std_LI7_25	0.113
std_LI_25	0.113
disp7_38	0.112
LI7_38	0.111
std_LI7_29	0.111
moving_28	0.109
moving_26	0.109
LI7_39	0.109
disp_40	0.108
std_disp7_29	0.107
LI_40	0.106
std_moving_26	0.106
std_LI7_40	0.104
std_LI_29	0.104
std_moving_29	0.104
LI7_29	0.104

E. PC5

Feature	Weight
SE_GrowthRate	0.174
GrowthRate	0.173
moving_32	0.149
disp7_35	0.146
SE_Asymptote	0.145
LI7_32	0.141
LI7_36	0.140
LI7_35	0.139
LI_32	0.132
std_LI_31	0.130
LI7_40	0.130
moving_36	0.128
moving_31	0.128
LI_35	0.127
LI_40	0.123
std_LI7_31	0.122
disp7_32	0.122
SE_InflectionPt	0.120
LI7_39	0.119
LI_39	0.117
disp_32	0.116
std_disp7_31	0.112
std_LI7_36	0.111
moving_37	0.111
LI_38	0.109
moving_35	0.109
std_LI7_32	0.109
std_LI_25	0.109
std_moving_32	0.109
std_disp7_35	0.107

F. PC6

Feature	Weight
moving_39	0.172
disp7_36	0.165
std_LI7_39	0.164
std_moving_34	0.163
std_moving_39	0.158
LI7_37	0.155
std_LI7_40	0.150
std_moving_40	0.148
LI7_36	0.144
disp7_37	0.137
std_LI_39	0.137
moving_25	0.133
std_moving_25	0.132
LI_36	0.132
std_moving_33	0.132
moving_40	0.130
SE_GrowthRate	0.127
std_LI_40	0.125
std_disp7_36	0.122
std_LI_25	0.120
disp_36	0.119
std_disp_32	0.117
std_LI7_30	0.117
std_LI7_33	0.116
moving_33	0.116
std_LI7_34	0.115
std_LI_38	0.115
disp_38	0.115
moving_38	0.115
std_LI_34	0.113

G. PC7

Feature	Weight
std_disp7_25	0.197
std_disp7_26	0.184
std_LI7_26	0.170
std_LI_26	0.167
std_LI_25	0.166
std_moving_26	0.163
std_LI7_25	0.156
moving_26	0.156
moving_29	0.153
moving_39	0.151
std_LI_37	0.150
std_moving_39	0.143
std_LI_38	0.139
std_LI7_36	0.137
std_moving_29	0.136
std_LI_39	0.133
std_moving_25	0.133
std_LI7_39	0.133
moving_28	0.132
std_disp_29	0.131
std_moving_28	0.130
moving_25	0.129
std_disp_26	0.128
std_LI7_28	0.124
std_LI_40	0.123
std_LI_28	0.122
std_LI_29	0.122
std_LI7_38	0.121
std_LI7_33	0.119
std_disp_25	0.118

H. PC8

Feature	Weight
std_disp7_39	0.256
std_disp7_38	0.208
std_disp_39	0.195
std_disp7_40	0.192
LI_37	0.190
disp7_39	0.190
std_disp7_37	0.186
std_disp_38	0.182
LI_38	0.170
LI_39	0.169
LI_36	0.159
LI_40	0.159
LI7_37	0.146
std_disp_25	0.146
std_disp_40	0.146
LI7_38	0.137
std_LI7_25	0.135
disp7_40	0.135
disp7_38	0.133
LI7_39	0.132
std_disp7_36	0.125
std_LI_25	0.125
std_moving_29	0.123
LI7_40	0.121
std_disp7_37	0.118
disp_39	0.117
std_LI_38	0.114
moving_37	0.111
moving_29	0.110
moving_31	0.109

Legend:

LI7	Locomotion index (LI7)
LI	Locomotion index (LI1)
disp	Displacement
disp7	Displacement (7 frame)
moving	Proportion Moving

|	
bold	proportion moving
italicized	Locomotion Index
underlined	measured one frame apart

Slope	Slope parameter estimate
InflectionPt	Inflection Point parameter estimate
Asymptote	Asymptote parameter estimate
std	White text is Standard deviation
SE	or white text is Standard error

Absolute value of weight
Percentage of range in values
0 10 20 30 40 50 60 70 80 90 100
90 Negative weights in magenta

Phenotype Temperature (°C)

| 25.03 | 26.10 | 27.16 | 28.22 | 29.28 | 30.34 | 31.40 | 32.46 | 33.52 | 34.59 | 35.65 | 36.71 | 37.77 | 38.83 | 39.89 | 40.95 |

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Supplementary Figure S6. Diagrammatic representations of phenotype weights of principal component axes PC1 to PC8 (**A-H**). Each pair of columns lists the top 30 phenotypes that contribute to a principal component axis and their absolute value weights. Short-form phenotype names explained in legend. The colour of the first column is graded according the temperature of the phenotypes (blue 25°C to red 41°C); function fit parameter phenotypes are coloured cyan, magenta or yellow; font style differences correspond to phenotype categories (see legend). White text in trait column indicates a "variation" phenotype (standard deviation for locomotion metrics, or standard error for function fit parameters); magenta text in weight column indicates a negative weight (see legend).
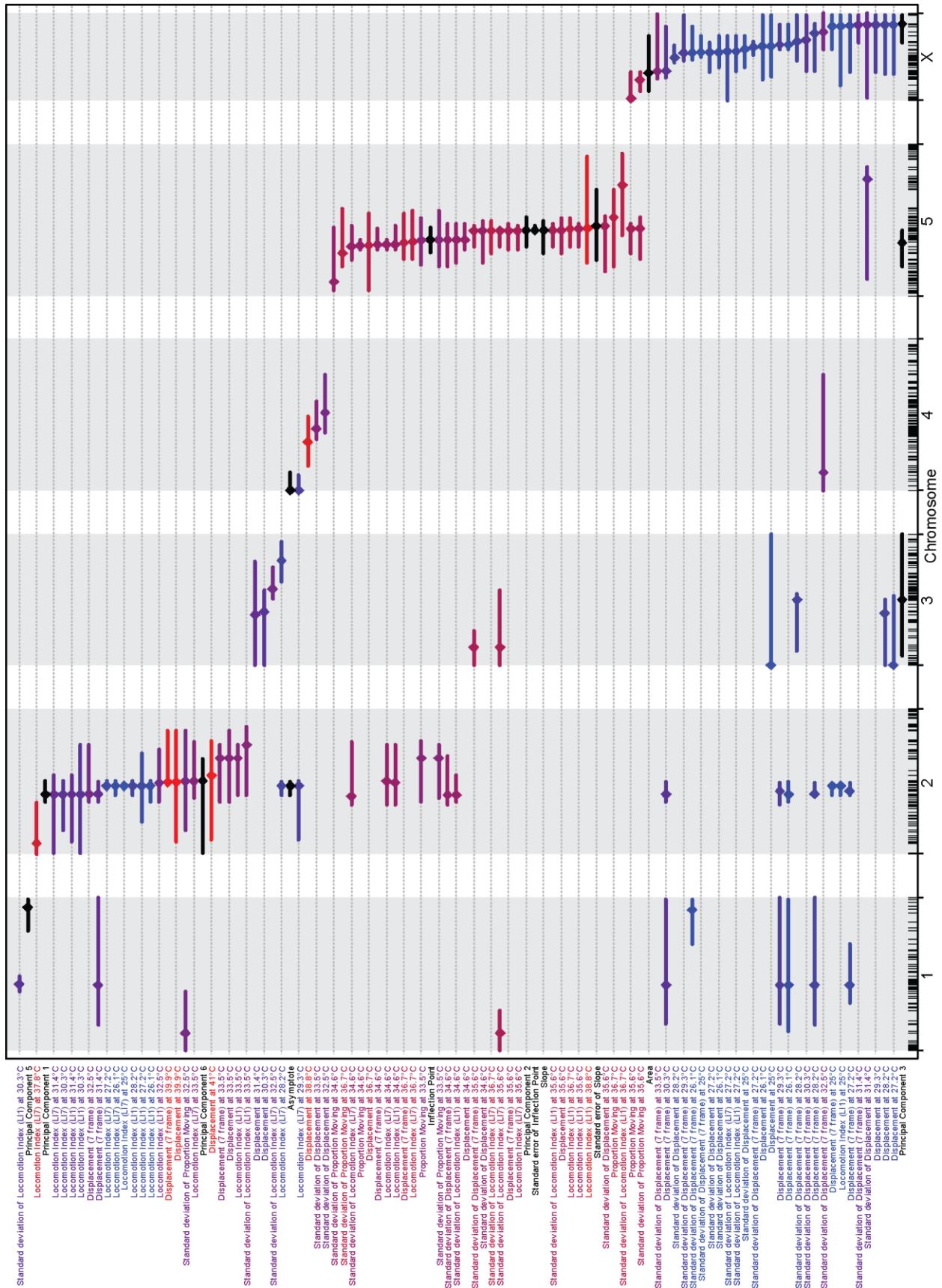
Supplementary Figure S7. Exploratory QTL mapping across all chromosomes for univariate trait metrics, function-valued trait phenotypes, and synthetic PC phenotypes. QTL LOD peak location and 95% Bayes intervals shown for 102 of 175 traits for each chromosome for all phenotypes that showed LOD scores above significance threshold derived from 2500 permutations of the data (P=0.05, not corrected for multiple testing). The intervals are mapped to the nearest genotyped marker (tick marks along the chromosome axis). Intervals and univariate phenotype labels are colour-coded according to temperature (blue for cooler temperatures, red for hotter temperatures).
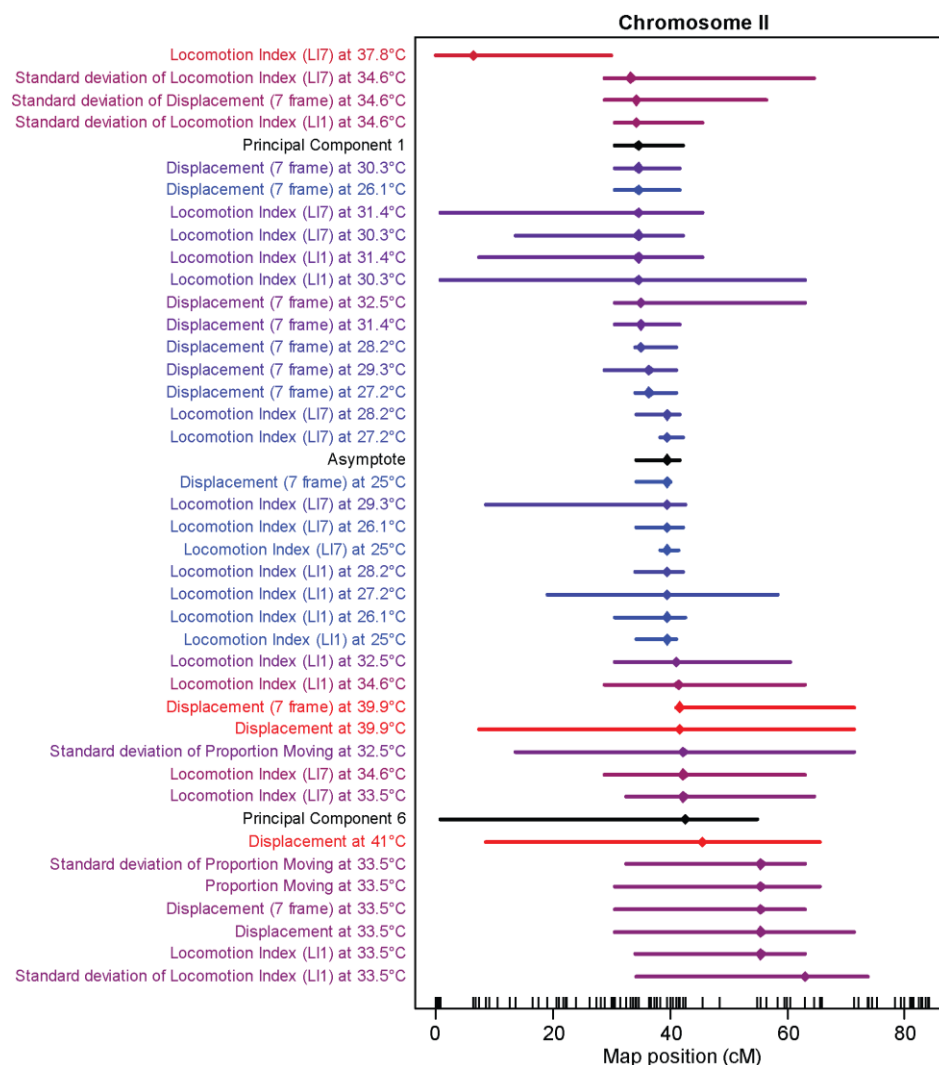
Supplementary Figure S8. QTL LOD peak location and 95% Bayes credible interval width for phenotypes with significant QTL on Chromosome II (from Supplementary Figure S7). Significance threshold determined by 2500 permutations of the data (P=0.05 per trait, not corrected for multiple tests). Intervals and phenotype labels are colour-coded according to temperature when relevant (blue is cooler and red is hotter). Some phenotypes also had peaks on other chromosomes (see Supplementary Figure S7).
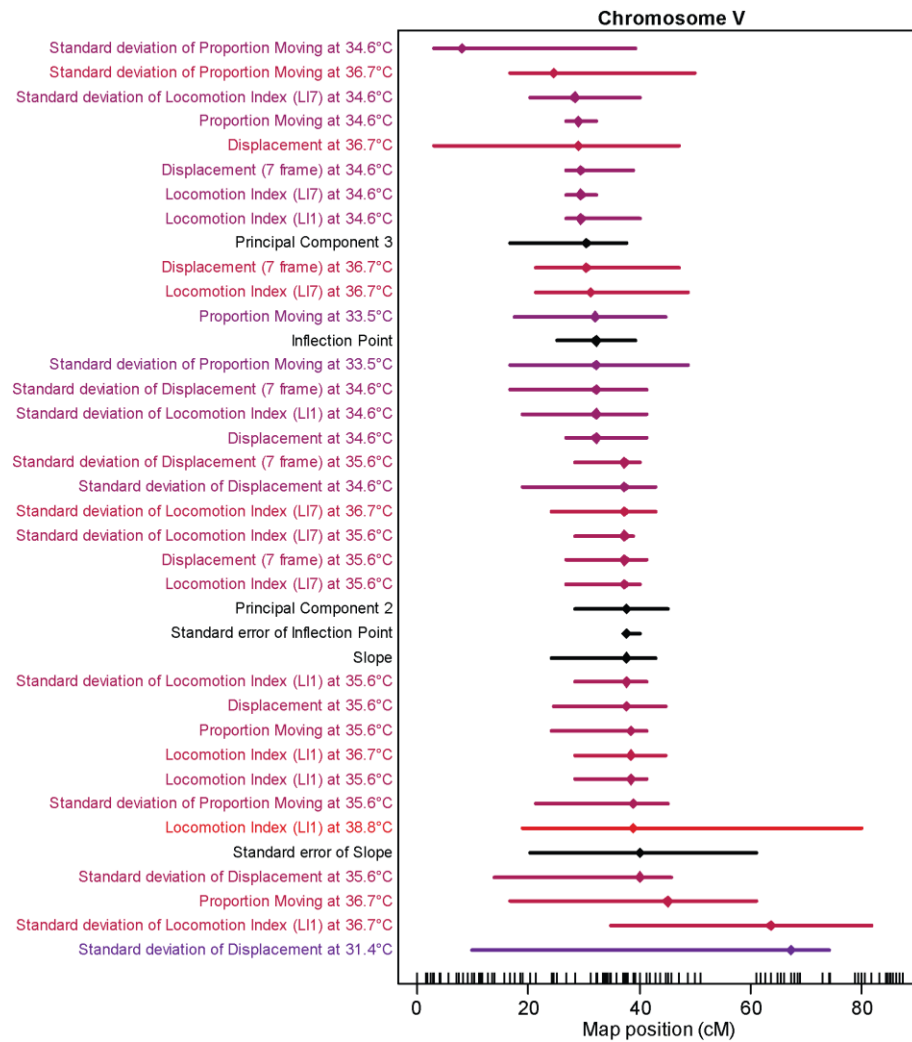
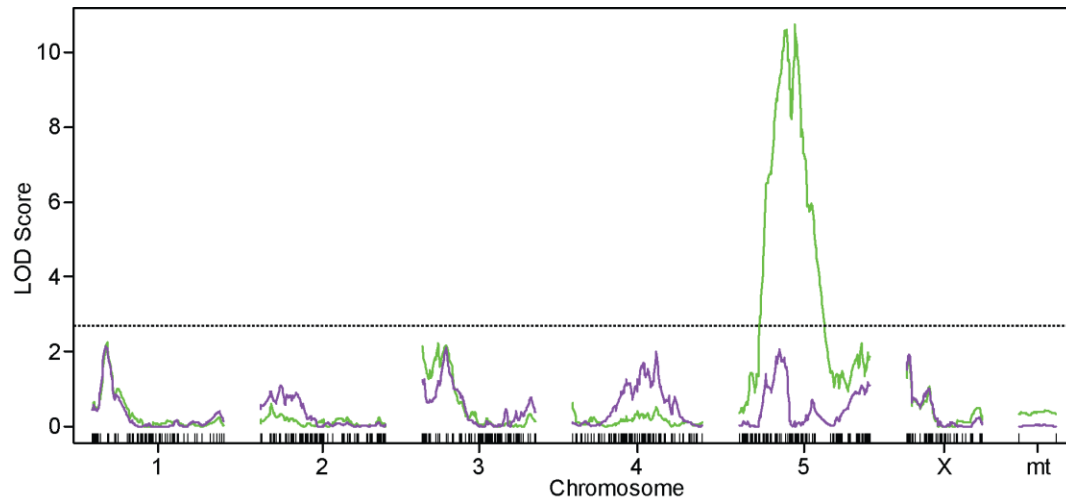**Supplementary Figure S9.** QTL LOD peak location and 95% Bayes credible interval width for phenotypes with significant QTL on Chromosome V (from Supplementary Figure S7). Significance threshold determined by 2500 permutations of the data (P=0.05 per trait, not corrected for multiple tests). Intervals and phenotype labels are colour-coded according to temperature when relevant (blue is cooler and red is hotter). Some phenotypes also had peaks on other chromosomes (see Supplementary Figure S7).
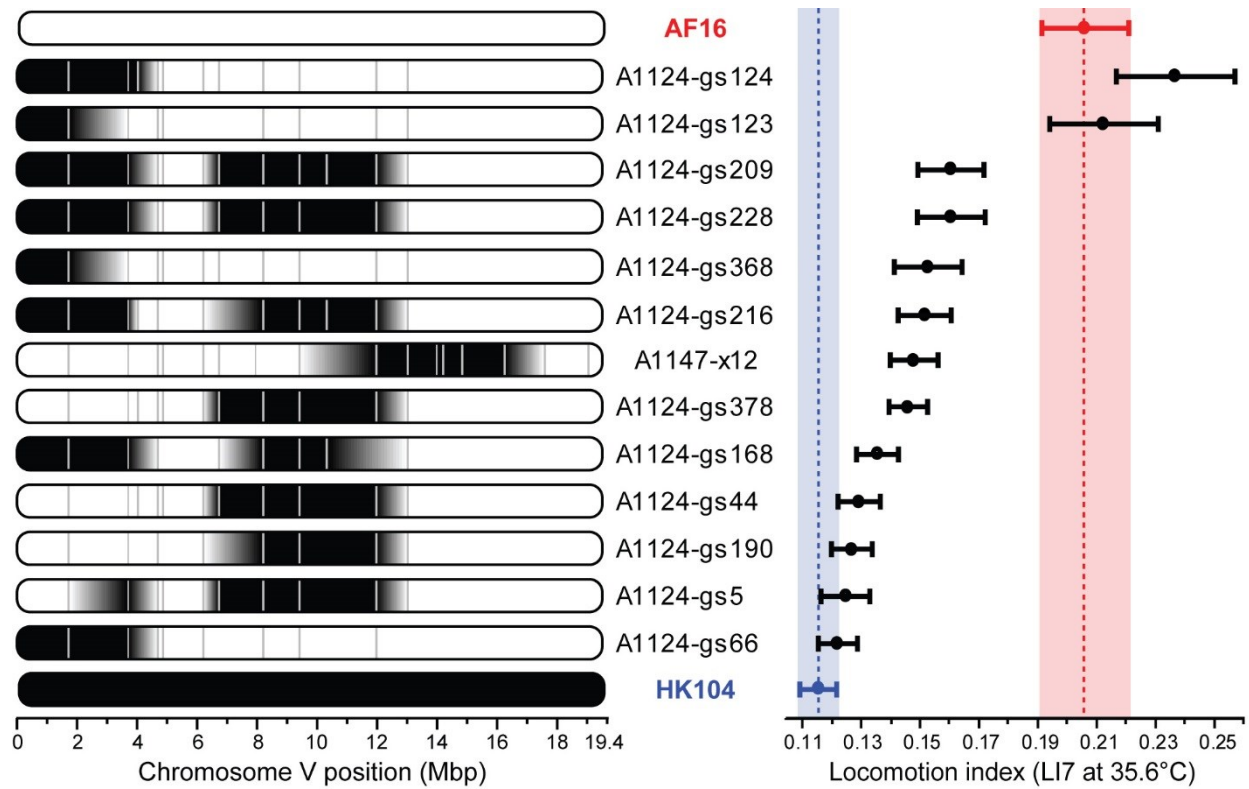
Supplementary Figure S10. Schematic representation of parental strain and near isogenic line (NIL) genotypes for Chromosome V and their corresponding locomotion index (LI7) phenotypes at 35.6°C. Vertical gray lines in on chromosome diagram indicate positions of genotyped markers, with white fill indicating Tropical AF16 sequence and black indicating Temperate HK104 sequence. Gradients indicate region of uncertain breakpoint position between genotyped markers. Error bars indicate ±1 SEM.

Supplementary Figure S11. Treemap diagrams of GO term enrichment for Chromosome V QTL region. Treemaps created using REVIGO (SUPEK *et al.* 2011), such that bounding boxes of GO terms are scaled according to frequency of the term in the list of nonsynonymous gene changes within the larger QTL region of interest (3.45-14.30Mb on Chromosome V). Some lower frequency terms are combined according to semantic similarity. Note that 33 of 115 genes lacked GO terms. Overrepresentation analysis using PANTHER (http://www.pantherdb.org/) identified g-protein signaling pathway proteins, membrane proteins, receptors, sensory and neurological genes to be significantly overrepresented relative to the *C. briggsae* genome.

Supplementary Figure S12. Co-factor analysis of Locomotion Index (LI7) at 35.6°C QTL "twin" peak markers finds no statistical support for two separate QTL peaks. LOD values for Locomotion Index (LI7) at 35.6°C phenotype (green) shows significant QTL on Chromosome V, with two nominal LOD score peaks. LOD scores for the same phenotype that includes genotypes at marker cb3903 (37.4cM, right green "peak") as additive covariates (purple) show no QTL (BROMAN AND SEN 2009), indicating that the left "peak" is not statistically separable from the right "peak." Horizontal line indicates the LOD significance threshold from 1000 permutations of the data with the covariate included. We next evaluated a model with the two independent, non-interacting QTLs at the "split" LOD peaks for the LI7 at 35.6°C phenotype from the multiple imputation analysis (positions 37.35cM and 31.5cM on Chromosome V). This model explained 30.8% of the variation in locomotion (LI7) at 35.6°C, with LOD = 12.2 as the likelihood ratio of this full model to the null model of no QTL (P=$1.05*10^{-12}$), and each QTL was significant when dropped from the model one at a time. We then tested for a second additive QTL when starting with a model that contained a QTL at 37.4cM on Chromosome V (using addqtl() from r/qtl (BROMAN AND SEN 2009) for LI7 at 35.6°C), but this analysis just added a QTL on Chromosome III at 16cM with a rather low LOD score of 2.1. When we instead fix the alternate Chromosome V locus at 30.5cM, the analysis does add another QTL on Chromosome V, but at the tip (49.0cM) and with a low LOD = 2.0 (combined two-locus LOD = 12.9, 32.2% of variation explained). Despite this meagre support for a second additive QTL on Chromosome V, the positions are inconsistent with the hypothesis of twin QTL peaks in the center of the chromosome.