Supporting Information

Ayroles et al. 10.1073/pnas.1503830112

SI Text

All raw data and analysis scripts are available at lab.debivort.org/genetic-control-of-phenotypic-variability.

Drosophila Stocks. The DGRP was created as a community resource for the genetic mapping of complex traits (1). It consists of a collection of isofemale lines derived from a single field collection from the Raleigh, NC, farmers market, followed by 20 generations of full-sib mating that rendered most loci homozygous within lines (expected $F = 0.986$) (2). As a result, the genetic variation that was present between individual flies in the natural population is now captured between lines in the panel. This property of wild-derived inbred lines allows us to measure any phenotype on a given genotypic background and phenotype the same genotype a large number of times in any environment. Completion of the genome sequencing for all lines combined with *Drosophila*'s generally rapid decay in linkage disequilibrium between polymorphic sites makes the DGRP a powerful tool to identify genetic polymorphisms that affect quantitative phenotypes (3). The DGRP lines are available from the *Drosophila* Stock Center (flystocks.bio.indiana.edu). We used a total of 159 lines in this study (lines with the highest inbreeding coefficient); a list is provided along with data at lab.debivort.org/genetic-control-of-phenotypic-variability. Stocks used for *Ten-a* validation were Berlin-K, *central-body-defect*^{KS96} (4), *Df1-Ten-a* (5), and RNAi TRiP.JF03375 (6). All flies were reared on standard fly media (Scientiis and Harvard University BioLabs Fly Food Facility), in a single 25 °C incubator at 30–40% relative humidity with a 12/12-h light/dark cycle. Before each assay, flies were fully randomized across blocks, lines, Y-maze arrays, and position on the array. At least three strains were assayed simultaneous on each array.

Variance, Variation, Variability. The similarity between concepts of variance, variation, and variability may lead to some confusion. The meanings of these terms are reviewed in Wagner and Altenberg (7). In accordance with their definition, we used the term variance (8, 9) to describe the standard statistical dispersion parameter (σ^2) or estimates of it derived from observations (s^2). Variability refers to the potential of an organism or genotype to vary phenotypically. Variation refers to the realized (observable) differences between individuals or genotypes.

Phenotypic Assay. Studying variance as a trait poses a number of challenges including the large sample size required (precise estimates of variance requires a larger number of observations than needed to estimate means), the experimental design (as to not confound sources of error), and potential measurement error of the phenotype itself (9). It is with these considerations in mind that we developed a high-throughput assay aimed at monitoring the behavior of individual flies placed into individual Y-mazes (10) (Fig. 1*A*). Each experiment examines one array of 120 Y-mazes (refered to as maze-arrray). Mazes were illuminated from below with white LEDs (5500K; LuminousFilm), imaged with 2MP digital cameras (Logitech), and the X-Y positions of each fly's centroids were automatically tracked and recorded with software custom written in LabView (National Instruments). Further details about the assay are provided in ref. 10; the code is available at lab.debivort.org/neuronal-control-of-locomotor-handedness/. Although various statistics can be computed to estimate the degree of variability of a distribution, in this study, we use one the most robust metrics, the MAD (11, 12). It is defined as the median of the absolute deviation from each observation's median: MAD = median $[|X_i - \text{median}(X_i)|]$, where X_i is the phenotypic score of an individual fly within a line. MAD scores were computed for each line for each phenotype. Only females were used in this experiment, and only lines yielding data from a minimum of 75 individuals were included. Before each assay, flies were very lightly anesthetized, rapidly transferred to an individual Y-maze, and given a recovery period of 20 min before the start of the assay. Fly behavior in the mazes was monitored for 2 h. This assay generated four phenotypes. (*i*) The handedness or left/right turning bias in the arms of the maze summed over all left/right decisions. A turning bias score of 0.8 for a given fly would indicate that this individual made left turns 80% of the time at the maze's junction over the 2-h period. This simple phenotype is particularly well suited for this study given that it is measured without error, and the high number of turns for any given fly ensures a robust estimate of the turning bias and it variance for each fly. (*ii*) The number of turns over the 2-h period, an estimate of overall locomotor activity. (*iii*) The switchiness or the mutual left-right information between successive turns right/left turn sequence (e.g., LLLLLRRRRR: low switchiness, high mutual information; LLRLLRRRLR: moderate switchiness, low mutual information; LRLRLRLRLR: high switchiness, high mutual information) defined as $(N_{<L,R>}+N_{<R,L>})/(2N_R N_L/N)$, where $N_{<L,R>}$ is the number of left turns followed by right turns, $N_{<R,L>}$ is the number of right turns followed by left turns, N_R is the number of right turns, N_L is the number of left turns, and N is the total number of turns. (*iv*) The regularity of turn timing: a fly with a high score makes turns uniformly throughout the experiment, whereas a low score would characterize a fly making a small number of dense streaks of turns but is inactive for dozens of minutes at a time. It is defined as MAD(ITIs)/(7,200/N), where ITIs is the vector of interturn intervals in seconds. The left/right turning bias is the main focus of this study; additional traits were measured to illustrate that the degree of variability across traits is not correlated between lines.

Quantitative Genetic Analysis.

Analysis of means. To determine whether there was genetic variation segregating in the DGRP affecting the mean turning bias, we partitioned the variance for line means using the ANOVA model $Y = \mu + L_{random} + B_{random} + L \times B_{random} + A + X + A \times X + e$, where Y is turning bias score of each fly; L is the effect of line treated as random, B is the effect of block treated as random, X is the box effect, A is the maze-array effect, and e is the error variance (Table S1). ANOVA was implemented using PROC MIXED in SAS 9.3 (13).

Variance heterogeneity. We used several statistical approaches to estimate heterogeneity of variance for turning bias between lines (Table S1). (*i*) The Brown–Forsythe test, which is based on a one-way ANOVA and relies the absolute deviation from the median (8). (*ii*) Nonparametric bootstrapping in which we first pooled all of the turn bias scores for all individual flies across lines and then resampled each line experimental group from this pool, matching the sample size. Lines in which the MAD of the resampled group was closer to the MAD of the pooled data in fewer than 10 of 10,000 resamples were taken as significant. This analysis tests the null hypothesis that each group is drawn from an identical distribution of observations, using MAD as a test statistic. (*iii*) A nonparametric version of the ANOMV (14, 15). This approach compares the group means of the MAD to the overall mean MAD under the null hypotheses that the group MAD means equals each line specific MAD (results in Table S1), implemented

in SAS 9.3 (13, 15). (*iv*) Finally, we used the same ANOVA model described above for the analysis of mean but used the absolute deviation from the median (11, 16) as a measure for each fly as the dependent variable, implemented using PROC MIXED in SAS 9.3 (13).

Phenotypic correlation between traits. We assessed four traits as measured in this study and four additional traits gathered from the literature (SD for starvation, startle response, chill coma recovery, coefficient of environmental variation for night sleep). Data are from refs. 2 and 17. The phenotypic correlation between traits was computed as the Pearson product-moment correlation (implemented using PROC GLM in SAS 9.3). P values are not corrected for multiple comparison (18).

High and Low Variance Lines Intercrosses. To confirm that variability was heritable, we crossed high variability lines 45 and 105 together and low variability lines 796 and 535 together. Ten females and five males were used for each cross. Flies were reared and phenotyped using the same protocoled described above. Note that parental behavior was remeasured concurrently with F_1 behavior following a corresponding self-cross (e.g., 45 × 45). We assessed statistical significance between parental lines and their progeny using the Brown–Forsythe test and a bootstrapping two-tailed z-test (with $n = 10{,}000$ resamples). We resampled the turn bias of the parents and for each iteration calculated the MAD of turning bias and then compared the MAD for the F_1 progeny to their parents.

Genome-Wide Association Mapping. GWAS was performed using the code and approach described in ref. 2 (dgrp2.gnets.ncsu.edu). In a first step, phenotypic stores were adjusted for the potential effect of *Wolbachia* and known large inversions segregating in this panel [namely: *In(2L)t*, *In(2R)NS*, *In(3R)P*, *In(3R)K*, and *In (3R)Mo*]; none of them were associated with variability turning bias. We then fitted a series of loci-specific mixed linear model using the model: $Y = \mu + Sb + Iu + e$, where Y is the MAD of turning bias of each DGRP lines, S is the design matrix for the fixed SNP effect b, I is the incidence matrix for the random polygenic effect u, and e is the residual (2). A total of 1,931,250 SNPs and indels were used in these analyses with the minor alleles present in at least seven DGRP lines, using only biallelic sites. Polymorphisms segregating within lines were discarded and for each SNP at least 60 DGRP lines had to have been genotyped to be analyzed. Given the number lines available in the DGRP, GWAS will generally be underpowered (19); however, our goal is not to describe the overall genetic architecture of each of these phenotypes but rather to identify interesting candidate genes that would provide some insight into the genetic basis of variance control. For this reason, we used a liberal threshold of $P < 10^{-6}$. The analysis for tissue enrichment was based on FlyAtlas data, which are publically available (20). For each tissue, we used FlyAtlas AffyCalls (21) to determine which genes were expressed in which tissue (using a conservative filter of four of four present calls). To determine significance, we used Fisher's exact test comparing the expected number of gene expressed in each tissue across the entire genome to the observed number of gene expressed in each tissue in our gene list.

Validation of *Ten-a* Effect on Variability.

Ten-a null and deficency. The turning bias and MAD turning bias of homozygotes of both the null allele *Ten-a*$^{cbd\text{-}KS96}$ (5) and deficiency overlapping *Ten-a Df(1)Ten-a* (10) were compared with heterozygous animals over their genetic background, Berlin-K.

Time course knockdown of Ten-a RNAi. Ten adult *Ptub-Gal80ts;Ptub-Gal4/Sb* females were crossed to three *UAS-Ten-a RNAi y1,v1; P(TRiP.JF03375)attP2* males for RNAi induction. Flies were allowed to mate for 24 h at 20 °C, at which point the parents were passaged out, and the bottles containing F_1 eggs were returned to 20 °C until the beginning of their heat shock window. Flies were exposed for 72 h to 30 °C temperature, in a sliding window each day over 14 windows (Fig. 3*A*). All flies assayed were between 3 and 5 d after eclosion. In parallel, each day, developing flies of the same genotype were examined and counted to determine the fraction of flies in each developmental stage at the time of RNAi induction (Fig. 3*B*). Stages containing larval animals were microwaved to melt the media and poured through a sieve, and larval carcasses were counted under a dissecting scope. Controls were performed using *Ptub-Gal80ts;Ptub-Gal4/Sb* females crossed to Canton-S males and Canton-S females crossed to *UAS-Ten-a RNAi y1,v1;P(TRiP.JF03375)attP2* males (Fig. 3*D*); otherwise, they were treated identically. Data for *Ten*-a expression over developmental time (Fig. 3*C*) were downloaded from FlyBase (22) and derived from ModEncode (23) (modENCODE DDC ids: modENCODE_4433, _4435 and _4439 through _4462). These data reflect animals synchronized by developmental stage to within 2 h. To make these data comparable to our experimental groups, in which egg laying occurred over 24 h, we corresponded the developmental stages of the FlyBase data to our developmental stage time course (Fig. 3*B*), linearly interpolated the expression values, and applied a 24-h sliding window average to the interpolated data, mimicking the dispersion effects of our longer egg collection window.

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Fig. S1. Turn bias variability is correlated neither with the mean of turning bias nor residual genetic variation within line. (*A*) Distribution of the mean turning bias for each line, ranked by the MAD of turning bias (gray dots, scale on the right axis). Tick marks represent a 99.9% CI around the mean. There is no significant difference between lines in mean turning bias. Each lines are on average unhanded, making equal portions of left and right turns. ANOVA, $P < 0.87$. (*B*) No relationship between the mean turning bias and intragenotypic variability ($r^2 = 0.0004$, $P = 0.80$). (*C*) No relationship between intragenotypic variability and residual genetic variation segregating within line ($r^2 = 0.0005$, $P = 0.78$).

Fig. S2. No genetic variation segregating within lines is associated with turning bias. Crosses between extreme left turning parents (*Left*) and crosses between extreme right turning parents (*Right*) produce unbiased F_1 with a 50/50 left-right turning bias. Data modified from ref. 4.

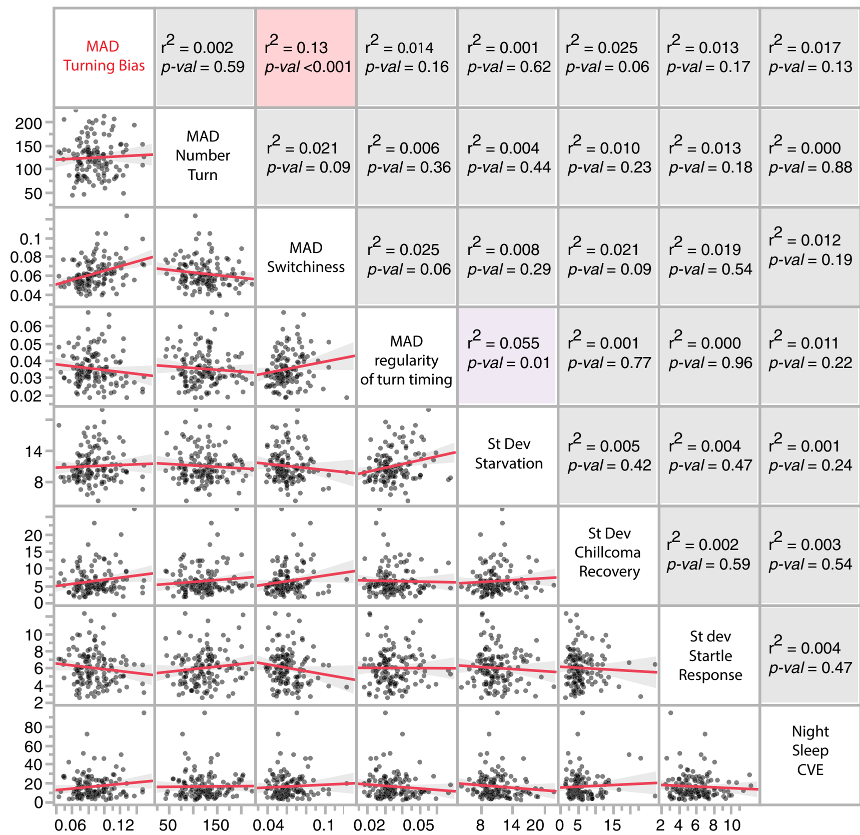

Fig. S3. Intragenotypic variability in various phenotypes is predominantly uncorrelated. Scatter plots of pairs of measures of intragenotypic variability. Points are DGRP lines. Red line is linear fit with 95% CI in gray. SDs for starvation resistance, chill coma recovery, and startle response were calculated based on data from ref. 22. CVE for night sleep data from ref. 23. For starvation resistance, *n* per line = 40; chill coma, *n* per line = 50; startle response, *n* per line = 40; night sleep, *n* per line = 32.

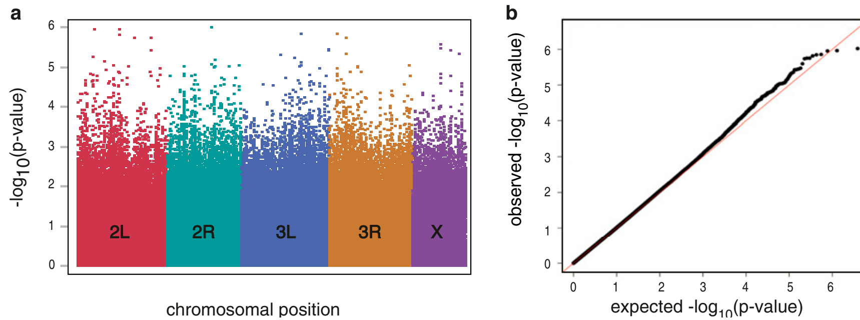

Fig. S4. GWAS *P* value distributions. (*A*) $-\log_{10}$(*P* value) plotted along each chromosomal position for all SNPs. Colors and letters indicate chromosome arms. (*B*) QQ plot comparing observed *P* value to a uniform distribution of expected *P*- value ($\lambda = 1.07$).

Table S1. Statistics for analysis of mean and variance across DGRP lines for turning bias

Chrs	Position	Variant	Minor allele	Major allele	Minor allele frequency	Minor allele count	Major allele count	Single *P* value	Mixed model *P* val	Flybase ID	Gene ID	Genomic annotation
2R	13909827	SNP	C	T	0.10	15	141	2.63E-06	9.50E-07	FBgn0034289	CG10910	UTR_3_PRIME
2L	9129764	SNP	C	G	0.05	8	145	9.72E-07	1.09E-06	FBgn0052982	CG32982	INTRON
2L	3811210	SNP	T	C	0.11	17	140	1.10E-06	1.11E-06	FBgn0031573	CG3407	SYNONYMOUS_CODING
3R	5804797	DEL	T	TT	0.28	40	105	1.29E-06	1.39E-06	FBgn0263097	Glut4EF	INTRON
3L	13006450	SNP	C	A	0.17	27	129	1.39E-05	1.42E-06	FBgn0036333	MICAL-like	SYNONYMOUS_CODING
2L	9121721	SNP	T	G	0.07	11	146	5.92E-06	1.53E-06			
2L	16392736	SNP	T	C	0.37	52	87	2.68E-06	1.77E-06			
3R	9419254	SNP	C	G	0.22	33	119	1.50E-06	1.77E-06	FBgn0038159	CG14369	UPSTREAM
2L	12447996	SNP	A	T	0.40	56	85	1.13E-06	1.84E-06	FBgn0032434	CG5421	INTRON
X	12153076	SNP	T	A	0.08	12	141	4.61E-06	2.54E-06	FBgn0259240	Ten-a	INTRON
X	12153062	SNP	A	C	0.08	12	143	6.21E-06	3.37E-06	FBgn0259240	Ten-a	INTRON
3L	23001316	SNP	T	C	0.08	12	144	8.36E-07	3.54E-06	FBgn0262509	nrm	INTRON
2L	16392768	SNP	G	A	0.34	49	94	4.64E-06	3.64E-06			
3L	23001309	SNP	A	G	0.08	12	143	9.33E-07	3.74E-06	FBgn0262509	nrm	INTRON
X	16161192	SNP	T	A	0.35	54	100	6.95E-07	3.75E-06	FBgn0040207	kat80	UTR_3_PRIME
X	19449711	SNP	G	A	0.06	9	149	1.44E-05	4.38E-06			
3R	9414756	SNP	G	T	0.25	38	112	9.86E-07	4.58E-06	FBgn0038158	CG14370	DOWNSTREAM
3L	8951894	SNP	C	G	0.22	35	121	2.80E-06	4.77E-06	FBgn0035941	CG13313	UPSTREAM
2L	3572163	SNP	A	T	0.20	30	120	3.06E-05	5.26E-06			
3L	11733450	SNP	A	G	0.20	30	118	1.46E-05	5.81E-06	FBgn0036202	CG6024	INTRON
2R	14651257	DEL	AA	A	0.20	30	122	2.08E-06	6.44E-06	FBgn0034389	Mctp	INTRON
2L	6209462	SNP	A	G	0.05	8	148	6.11E-06	7.03E-06	FBgn0085409	CG34380	UTR_3_PRIME
3L	13006461	SNP	A	C	0.16	25	128	7.03E-05	7.33E-06	FBgn0036333	MICAL-like	NON_SYNONYMOUS_CODING
2R	13909829	SNP	A	G	0.10	16	140	1.87E-05	8.09E-06	FBgn0034289	CG10910	UTR_3_PRIME
2L	8003155	SNP	G	A	0.18	24	110	1.30E-05	8.58E-06	FBgn0031972	Wwox	INTRON
3R	26478454	SNP	C	T	0.46	67	80	2.89E-05	8.69E-06			
3L	12859591	SNP	A	G	0.17	27	128	7.59E-05	8.82E-06			
2R	20092066	SNP	A	T	0.12	19	136	2.54E-05	9.08E-06	FBgn0034990	CG11406	INTRON
3R	13031950	SNP	G	T	0.43	62	83	4.05E-05	9.13E-06			
2R	17929411	SNP	G	A	0.09	14	141	1.36E-06	9.16E-06	FBgn0005778	PpD5	SYNONYMOUS_CODING
2L	10873583	DEL	T	TGA	0.20	29	117	7.57E-06	9.21E-06			
2R	14823241	SNP	A	G	0.07	11	144	6.60E-06	9.26E-06	FBgn0034408	sano	INTRON
3L	12688628	SNP	T	C	0.11	17	141	8.66E-07	9.29E-06	FBgn0014343	mirr	INTRON
2R	7130275	SNP	T	G	0.17	26	129	5.03E-05	9.52E-06	FBgn0033593	Listericin	UPSTREAM
3R	13031960	SNP	C	A	0.42	61	85	3.98E-05	9.85E-06			
2R	14548570	SNP	T	C	0.48	75	81	1.49E-05	9.89E-06	FBgn0259202	CG42306	INTRON

Chrs, chromosome; *F*, *F* ratio statistic; *P*, *P* value for *F* ratio statistic

Table S2. Top GWAS hits for MAD of turning bias

Analysis category	df	*F*	*P*
Analysis of variance for mean turning bias			
$Line_{random}$	158	0.88	0.85
$Block_{random}$	28	1.12	0.29
Line × $block_{random}$	772	1	0.49
Box	5	0.41	0.84
Maze-array	11	0.52	0.88
Box × maze-array	49	1.13	0.26
Analysis of variance for the absolute median deviation of turning bias			
$Line_{random}$	158	4.31	<0.00001
$Block_{random}$	28	0.82	0.74
Line × $block_{random}$	772	1.04	0.2
Box	5	1.76	0.11
Maze-array	11	0.67	0.76
Box × maze-array	49	1.16	0.22
Alternative test for heterogeneity of variance between DGRP lines for turning bias			
O'Brien	158	8.5953	<0.00001
Brown–Forsythe	158	7.567	<0.00001
Levene	158	7.701	<0.00001
Bootstrap		Results in Table S1	
ANOMV		Results in Table S1	

F, *F* ratio statistic; *P*: *P* value for *F* ratio statistic.