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Mutation is a sufficient and robust predictor of genetic variation for mitotic spindle traits in *C. elegans*

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ABSTRACT

Different types of phenotypic traits consistently exhibit different levels of genetic variation in natural populations. There are two potential explanations: either mutation produces genetic variation at different rates, or natural selection removes or promotes genetic variation at different rates. Whether mutation or selection is of greater general importance is a longstanding unresolved question in evolutionary genetics. We report mutational variances (VM) for 19 traits related to the first mitotic cell division in *C. elegans*, and compare them to the standing genetic variances (VG) for the same suite of traits in a worldwide collection *C. elegans*. Two robust conclusions emerge. First, the mutational process is highly repeatable: the correlation between VM in two independent sets of mutation accumulation lines is ~0.9. Second, VM for a trait is a good predictor of VG for that trait: the correlation between VM and VG is ~0.9. This result is predicted for a population at mutation-selection balance; it is not predicted if balancing selection plays a primary role in maintaining genetic variation.

The question “What are the factors that govern genetic variation in natural populations?” has been central to the field of Evolutionary Genetics ever since its inception (DOBZHANSKY 1937; LEWONTIN 1974; LEWONTIN 1997). Within a group of organisms, seemingly similar or related phenotypic traits can vary considerably, and consistently, in the extent of genetic variation in the trait. For example, in many organisms, resistance to acute heat stress is much less heritable and evolvable than resistance to acute cold stress (HOFFMANN *et al.* 2013). If different traits in the same set of organisms have consistently different levels of genetic variation, there are two potential underlying evolutionary causes: mutation and/or selection. Traits may differ in the mutational target they present, i.e., the number and/or types of loci that potentially affect the trait, or in the rate at which those loci mutate. Traits may also differ in the average effect that mutations have on the trait, i.e., they may be differently robust to the effects of mutation. Alternatively, traits may be subject to differing strengths and/or kinds of selection.

In quantitative genetics, a few empirical conclusions seem fairly certain. First, traits that are direct components of fitness – life history traits – are typically more genetically variable than other classes of traits (HOULE 1992; LYNCH *et al.* 1999). Second, when scaled relative to the trait mean, life history traits experience greater input of genetic variation from mutation than other classes of traits (HALLIGAN and KEIGHTLEY 2009; HOULE *et al.* 1996). Third, life history traits appear to be under stronger purifying selection than other classes of traits (HOULE *et al.* 1996; LYNCH *et al.* 1999; MCGUIGAN *et al.* 2015).

A longstanding related, but unanswered, question is the relative influence of balancing selection on the maintenance of genetic variation (CHARLESWORTH 2015;

DOBZHANSKY 1955; LEWONTIN 1974). Presumably, a large fraction of mutations are unconditionally deleterious and are removed more or less efficiently by selection. However, if the mutation rate is high and selection is not too efficient, deleterious alleles segregating at mutation-selection balance (MSB) may represent a large fraction of the genetic variation. Alternatively, it is likely that some alleles are subject to balancing selection, and even if mutations subject to balancing selection are rare, they may in aggregate explain a substantial fraction of the standing genetic variation (BARTON 1990).

HOULE (1998) investigated the relationship between the standing additive genetic variance (VA) and the per-generation input of genetic variation by mutation (the mutational variance, VM) for eight life history and morphological traits in *Drosophila melanogaster* and found a strong positive association between VM and VA (Spearman's $r = 0.95$, $P<0.001$; see Figure 1 in HOULE 1998). That result has a clear interpretation: variation in mutation explains 90% of the variance in standing additive genetic variance *among traits*. Similar analysis of the genotypic variance (VG) and VM for nine morphological and life history traits in *Daphnia pulex* also reveals a strong positive correlation ($r = 0.76$, $P<0.02$; data in Tables 1 and 3 of LYNCH *et al.* 1998). A positive association between the mutational and standing genetic variance is predicted if most genetic variation is due to deleterious alleles at MSB, because the equilibrium frequency of a deleterious allele has a linear dependence on the mutation rate ($\hat{q} = \frac{\mu}{s}$, where $\hat{q}$ represents the equilibrium frequency of the deleterious allele, μ is the mutation rate, and s is the selection coefficient). Conversely, a relationship between VM and VG is not predicted if genetic variance is maintained by balancing selection, because the equilibrium frequencies of the segregating alleles do not depend on the mutation rate

(CHARLESWORTH and HUGHES 2000; HOULE *et al.* 1996). However, CHARLESWORTH (2015) recently analyzed five decades of quantitative genetic and molecular data from *D. melanogaster* and reached the conclusion that MSB cannot be a sufficient explanation for genetic variation for fitness in that species, hence there must be a significant contribution from balancing selection.

The biological particulars of Drosophila and Daphnia are quite different, except that in both species the relevant selection against deleterious mutations is in the heterozygous state. The population genetic milieu of *C. elegans* is very different from that of Drosophila or Daphnia. *C. elegans* reproduces predominantly by self-fertilization (ROCKMAN and KRUGLYAK 2009), so the relevant selection against mutations is in the homozygous state. Moreover, *C. elegans* appears to have undergone one or more global selective sweep(s) within the past 600-1200 generations, resulting in (among other things) linkage disequilibrium that extends over entire chromosomes, little geographic substructure, a large excess of rare alleles (as measured by Achaz' Y statistic) and a global effective population size on the order of 10^4 (ANDERSEN *et al.* 2012). Here we report an analysis of mutational and standing genetic variation in 19 traits related to the first mitotic cell division in the nematode *Caenorhabditis elegans*, chosen on the basis of their relevance to cell biology.

Our primary question of interest is: What is the relationship between VG and VM? Because of the recent history of strong global selection in *C. elegans*, we can imagine two plausible alternative scenarios. First, since genetic variation was recently purged, mutation may predominate: the only genetic variation present is that introduced by new mutations since the recent purge. If so, we should see a strong positive

association between VG and VM, and further, VG should consistently be no more than a few hundreds of generations of VM. Alternatively, because LD is so strong and also because the purge of genetic variation was not complete (THOMPSON *et al.* 2015), idiosyncratic selection at linked loci may predominate, leading to a more or less random association between VG and VM.

Our data permit us to address an additional fundamental question: How consistent is the mutational process? For example, if trait X accumulates mutational variance ten times faster than trait Y in one set of mutation accumulation (MA) lines derived from ancestor A, how closely does that relationship hold in an independent set of MA lines derived from ancestor B? Knowing the answer to that question will provide insight into the deeper question "What would happen if the tape of life was replayed?" (GOULD 1990)

METHODS AND MATERIALS

Mutation Accumulation (MA) lines: The details of the MA lines are reported in BAER ET AL. (2005). Briefly, sets of 100 replicate lines were initiated from highly homozygous populations of the N2 and PB306 strains of *C. elegans* in March of 2001, and maintained by serial transfer of a single immature hermaphrodite every generation for 250 generations, at which point each MA line was cryopreserved. The common ancestor (G0) of each set of MA lines was cryopreserved at the outset of the experiment. At the time the experiments reported here were initiated, ~70 MA lines from each strain remained extant, the others having been lost. Some lines were too

sickly to collect sufficient animals for these experiments; we report results from 46 N2 MA lines and 47 PB306 MA lines. Ancestral (G0) controls were thawed and 15 "pseudolines" were initiated from resuscitated individuals from each strain. The pseudolines were subsequently treated analogously to the MA lines. The purpose of including the G0 pseudolines is to account for among-line variance resulting from any cause other than mutation accumulation (LYNCH and WALSH 1998, p. 332).

Wild Isolates: We assayed first cell division in a worldwide collection of 97 wild isolates of *C. elegans*. Strain IDs and collection information are reported in Supplementary Table S1 or at www.elegansvariation.org.

Mitotic Cell Division Phenotype Assays: The details of maintenance, microscopy, and image processing of the first mitotic spindle in *C. elegans* embryos are reported in FARHADIFAR and NEEDLEMAN (2014) and FARHADIFAR *et al.* (2015). Briefly, all lines were cultured at 24° C on nematode growth media and fed the OP50 strain of *Escherichia coli*. We dissected and imaged the embryos on a 4% agar pad between a slide and coverslip by differential interference contrast (DIC) microscopy. We developed image-processing software to track the spindle poles in the DIC images (Figure 1A; average sample sizes and trait numbers associated with trait descriptions are given in the first column of Table 1). For each embryo, we measured the pole-to-pole distance and fitted a sigmoid function $l(t) = l_0 + l_1/(1 + \exp(-(t - T_l)/\tau))$ to the data (Figure 1B). We defined Trait 1, l_0, and Trait 2, $l_0 + l_1$, as the initial and final length of the spindle (in *μ*m), respectively (Figure 1B). Trait 3 (the elongation rate of the spindle in *μ*m/minute,

see Figure 1B) and Trait 4 (the duration of spindle elongation in seconds) are defined as $l_1/4\tau$ and τ, respectively. We quantified spindle oscillation by measuring the distance of the posterior and anterior centrosomes from the long axis of the embryo (Figure 1C). We defined Trait 5 (oscillation amplitude of the posterior centrosome in μm) as the largest peak-to-trough distance of the posterior centrosome (Figure 1C), and Trait 6 (oscillation duration of the posterior centrosome in second) as the total duration of the posterior centrosome oscillates (Figure 1C). We defined Traits 7 and 8 for the anterior centrosome similar to the Traits 5 and 6 for the posterior centrosome. Traits 9 and 11 (in seconds) are defined as the time difference between the mid-spindle elongation (T_l, see above) and the maximum oscillation peak for the posterior and anterior centrosomes, respectively. Traits 10 and 12 (in seconds) are defined as the time difference between the first oscillation peak and the mid-spindle elongation time for the posterior and anterior centrosomes, respectively. We defined Traits 13 and 14 as the average frequency of centrosome oscillation (in minutes^{-1}) of the posterior and anterior centrosomes. Traits 15 and 16 are defined as the length and width of the embryo in μm (Figure 1A last panel). We defined Trait 17 as the position of the division plane from the posterior end of the embryo in μm, and Trait 18 as the duration of the first division in minutes. Trait 19 is defined as the average size of the centrosomes for $t > T_l + e\tau$ (see above).

Data Analysis: With 19 traits, there are many more elements in the covariance matrix (190) than there are MA lines (46 or 47), which precludes formal matrix comparisons in the absence of some sort of data reduction. Because our primary interests relate to the

organismal phenotype *per se*, we restrict the analyses to the univariate case, except for an exploratory Principal Components analysis intended to investigate the overall structure of phenotypic variation (explained in the Results).

Data were analyzed for each set of MA lines (N2 and PB306) separately except as noted.

Trait standardization: Meaningful comparisons among traits require that the traits be standardized on a common scale. Traits can be standardized either by the trait mean, in which case the mean-scaled genetic variance is the squared genetic coefficient of variation, or by the phenotypic standard deviation (SD), in which case the SD-scaled genetic variance is the heritability. The squared genetic CV is naturally related to the evolvability of a trait (HANSEN *et al.* 2011; HOULE 1992), which is the "opportunity for selection" when the trait is fitness (CROW 1958). In some cases, however, mean standardization is not appropriate, e.g., when the trait value can be either positive or negative. Of the 19 traits, four (Traits 9-12) cannot be meaningfully mean standardized. We report results for raw (unstandardized) data and SD standardized traits from the full data set and results for mean standardized traits from the reduced set of 15 traits. MA lines were mean standardized by dividing each data point by the mean of the G0 ancestor and SD standardized by dividing each data point by the square-root of the within-line (environmental) variance averaged over all lines (G0 and MA). Wild isolates were mean standardized by the global mean of the wild isolates and SD standardized by the square-root of the within-line variance of the wild isolates. In all cases, the important conclusions are qualitatively similar for the two different standardizations.

206 *Evolution of Trait Means in MA lines (ΔM)*: A directional change in the trait mean over
207 the course of a MA experiment indicates a mutational bias. The per-generation change
208 in the trait mean (*ΔM*) was determined from the slope of the regression of the
209 (standardized) trait mean against generations of MA, i.e., $\Delta M = \frac{\bar{z}_{MA} - \bar{z}_0}{\bar{z}_0 t}$ where $\bar{z}_{MA}$ and $\bar{z}_0$
210 are the means of the MA lines and the G0 ancestors, respectively, and t is the number
211 of generations of MA. Regression slopes were calculated from the general linear model
212 $z_{ijk} = \mu + t_k + l_{i|k} + e_{ij|k}$
213 where z_i is the standardized trait value of individual (= replicate) *j* of line *i* in treatment
214 group *k* (G0 ancestor or MA), μ is the overall trait mean (defined = 0 in the G0
215 ancestor), *t* represents the number of generations of mutation accumulation (0 in the G0
216 ancestor, 250 in the MA lines), $l_{j|k}$ is the random effect of line (or pseudoline) *i* and $e_{ij|k}$ is
217 the random residual associated with individual *j* of line *i*. Random effects are denoted
218 conditioned on treatment group *k* because variance components of those effects were
219 estimated independently for each treatment group. Analyses were performed using the
220 MIXED procedure in SAS v.9.4. Variance components of the random effects were
221 estimated by Restricted Maximum Likelihood (REML) separately for each treatment
222 group using the GROUP= option in the RANDOM and REPEATED statements of the
223 MIXED procedure (FRY 2004). Degrees of freedom were determined by the Kenward-
224 Roger method (KENWARD and ROGER 1997). Statistical significance of the regression
225 slope was determined by F-test with Type III sums of squares. Estimation of the
226 regression slope from standardized traits fails to account for sampling variance of the
227 G0 controls, but with the sample sizes in this study (hundreds of control individuals) the
228 bias is negligible, and the empirical 95% confidence intervals calculated by

bootstrapping over lines (data not shown) are very close to those calculated from the linear model.

Mutational Variance (VM): The mutational variance is half the difference in the among-line component of variance between the MA lines and the G0 pseudolines, divided by the number of generations of MA, i.e, $VM = \frac{V_{L,MA} - V_{L,G0}}{2t}$, where $V_{L,MA}$ is the variance among MA lines, $V_{L,G0}$ is the variance among the G0 pseudolines, and *t* is the number of generations of MA (LYNCH and WALSH 1998, p. 330). To estimate VM, we first estimated variance components from the linear model in (*ii*), and then estimated the among-line variance from the model

$z_{ijk} = \mu + t_k + l_{ik} + e_{ij|k}$,

the difference between the two models being that in the second model there is only a single among-line component of variance estimated, whereas in the first model the among-line variance is estimated separately for the G0 and the MA groups. Statistical significance of VM is determined by Likelihood Ratio Test (LRT) of the model with separate among-line variances of G0 and MA compared to the model with a single among-line variance. The models are nested and differ by a single parameter, so the likelihood ratio is asymptotically chi-square distributed with a single degree of freedom. There are 38 significance tests (19 traits in two sets of MA lines), so the approximate experiment-wide 5% level of significance is $P < 0.05/38$.

Genetic variance of wild isolates (VG): The inferred rate of outcrossing among *C. elegans* in nature is very low (ROCKMAN and KRUGLYAK 2009), so we treat the wild isolates as if they are homozygous lines. The genetic variance among a set of homozygous lines is half the among-line component of variance (FALCONER 1989, p.

265). Variance components were estimated from the linear model $z_{ij} = l_i + e_{ij}$, where l_i represents the random effect of wild isolate *i* and e_{ij} is the random residual of individual *j* of wild isolate *i*. Significance of the among-line component of variance was assessed by LRT comparison of models with and without the Line term included.

Data Availability: Data (raw trait values) are deposited in Dryad (http://dx.doi.org/10.5061/dryad.js880).

RESULTS

Multivariate Trait Architecture: Significant correlations exist between at least some of these traits (FARHADIFAR *et al.* 2015), so the possibility exists that the 19 traits are essentially only one or a few traits. To address that possibility, we carried out a principal component analysis (PCA) on the phenotypic correlation matrix (i.e., on SD standardized traits) of the MA lines, as implemented in the PRINCOMP procedure of SAS v.9.4. The resulting eigenvalues of the principal components are depicted in Supplementary Figure S1 and the eigenvectors are given in Supplementary Table S2. For N2, PC1 explains approximately 18% of the phenotypic variance (the expectation for uncorrelated traits is ~6%), PC2 explains another ~14% of the variance, and the first 9 PCs collectively explain ~80% of the variance. Those values are almost exactly the same for PB306. Thus, although the traits are clearly correlated beyond the random expectation (Supplementary Figure S4), there is also considerable scope for independent evolution of these traits. This conclusion is reinforced by inspection of the pairwise phenotypic correlations (Supplementary Table S3). In N2, only six of the 171

pairwise absolute correlations are greater than 0.5; in PB306 nine are. The pairwise correlations are almost identical in the two sets of MA lines ($r_{N2,PB}$ = 0.96, df= 169, P<0.0001).

Evolution of Trait Means in MA lines (ΔM): Trait means evolved very little over the course of the MA experiment (Table 1). For the N2 lines, the median absolute change in the trait mean for the 15 traits that could be mean-standardized was 0.0034% per-generation; in no case was the change significant at the Bonferroni-corrected experiment-wide 5% level (0.05/(2*15), P<0.0017). For the PB306 lines, the median absolute change was 0.0073% per-generation, and only two traits (1 and 4) changed significantly at the experiment-wide 5% level. *ΔM* was not significantly correlated between the two sets of MA lines (r = 0.15, P>0.60). Of the 19 traits, nine changed in the same direction in both sets of lines and ten changed in opposite directions, exactly as predicted if the direction of change was random. Moreover, these results are consistent with the traits being under some degree of stabilizing selection (perhaps collectively; FARHADIFAR *et al.* 2015), because deleterious mutations do not have consistently directional effects. For a trait with a consistent mutational bias, at equilibrium selection must exactly counteract the mutational bias. Otherwise the trait would evolve without bound.

The *ΔM*s for these traits can be compared to *ΔM* for other traits expected to be under directional selection. For example, in these same sets of lines lifetime reproduction weighted by probability of survival ("Total fitness") decreased by about 0.1% per-generation (BAER *et al.* 2006) and body volume at maturity decreased by

about 0.07% per generation (OSTROW *et al.* 2007); in each case the change was highly significant and consistent between the two sets of lines.

Mutational Variance (VM): In 37/38 cases (19 traits in two sets of MA lines) the SD-standardized among-line variance of the MA lines is greater than the among-line variance of the G0 controls, trait 13 in the PB306 lines being the sole exception (Supplementary Table S4). However, in only two cases (traits 3 and 5 in the PB306 lines) is VM significant at the experiment-wide 5% level ($P<0.0013$). To assess the overall significance of the differences in among-line variance in MA lines relative to the G0 pseudolines, we performed paired t-tests on the N2 and PB306 lines, with each trait constituting a paired sample and a hypothesized difference of zero. In both cases, there was a highly significant overall increase in the SD-standardized among-line variance in the MA lines (N2, two-tailed $t = -6.23$, $df=18$, $P<0.0001$; PB306, two-tailed $t = -4.79$, $df=18$, $P<0.0002$). Thus, we proceed under the assumption that the point estimates of VM represent a reasonable approximation of the truth, even though they do not reach experiment-wide significance at the 5% level in most cases.

Averaged over the two sets of MA lines, mean-standardized VM varies by slightly under two orders of magnitude, from 3.3×10^{-7}/generation for trait 14 to 2.6×10^{-5}/generation for trait 7 (Table 2). These values can be put into context by comparison to a set of life history traits measured in these same sets of MA lines (Supplementary Table S5). The average VM for the traits reported here (mean = 6×10^{-6}/gen, median = 2×10^{-6}/gen) is substantially smaller than that for the life history traits (mean = 9×10^{-5}/gen, median = 8×10^{-5}/gen), although the ranges of variability overlap.

In contrast to the *ΔM*s, which are uncorrelated between the two sets of MA lines, the mutational variances are highly correlated between the N2 and the PB306 lines. For the full data set of 19 traits, the correlation between the raw (unstandardized) VMs in the two strains is 0.95 (P<0.00001; Figure 2) and the correlation for the subset of 15 mean-standardized traits is 0.89 (P<0.0001; Supplementary Figure S2). The correlation between the mutational heritabilities in the two strains is smaller, although still significantly positive (r = 0.56, P<0.02).

Genetic variance of wild isolates (VG): For all traits the among-line component of variance (raw and mean-standardized) among the wild isolates is highly significantly different from zero (P<0.0001 in all cases), as is the broad-sense heritability, H^2 (Table 3). However, the potentially non-zero among-line variance of the G0 ancestors of the MA lines introduces the possibility that some fraction of the among-line variance of the wild isolates is not true genetic variance. To address that possibility, we subtracted the average of the two estimates of the among-line variance of the G0 controls from the estimate of the among-line variance of the wild isolates before calculating VG; we refer to the corrected estimate of VG as VG* (Table 3). On average, VG* is reduced by about 20-30% relative to the uncorrected VG (mean reduction = 27%; median reduction = 20%). For the full set of 19 unstandardized traits, the correlation between the average mutational variance VM and the genetic variance VG* is nearly perfect (r = 0.99, P<0.0001; Supplementary Figure S3); the correlation is essentially the same for the 15 mean-standardized traits (r = 0.95, P<0.0001; Figure 3). The correlation between the mutational heritability and H^2 is somewhat smaller but remains highly significant (r = 0.69, P<0.002).

342 The ratio VG/VM has several interpretations, depending on the context. First, in
343 an infinite population at MSB, it represents the persistence time (t_P) of a new mutation,
344 i.e., the expected number of generations before the mutant allele is removed by
345 selection (GARCIA-DORADO *et al.* 2003). The stronger selection is, the shorter the
346 persistence time. Second, for a neutral trait in a finite population, VG = 2*Ne*VM at
347 mutation-drift equilibrium (LYNCH and HILL 1986), so VG/VM is equal to 2*Ne* (4*Ne* in the
348 case of obligate self-fertilization, which is approximately the case with *C. elegans*).
349 Finally, VG/VM represents the number of generations of mutation required to produce a
350 given amount of genetic variance, irrespective of other evolutionary forces.
351 For almost all of the traits in this study, the ratio VG*/VM (called t_P* in Table 3)
352 falls within the relatively narrow window of 300-800. Two traits are obvious outliers:
353 Embryo size (Trait 15; $t_P^* \approx 160$) and Centrosome size (Trait 19; $t_P^* > 1100$). Embryo
354 size has been previously inferred to be under long-term stabilizing selection
355 (FARHADIFAR *et al.* 2015) and the reduced t_P is consistent with stronger selection on that
356 trait than on the other traits. We have no intuition about why Centrosome size is a high
357 outlier. Balancing selection for some unknown reason is possible, although random
358 chance seems equally plausible.
359
360 ## DISCUSSION
361 Two robust conclusions emerge from this study, which inform several longstanding
362 issues in evolutionary genetics. First, for this relatively large set of functionally-related
363 but (on average) only modestly correlated traits, the mutational process is highly

repeatable: the correlation between estimates of trait-specific VM in two independent sets of MA lines derived from different ancestors is ~ 0.9.

The high repeatability of the mutational process was hardly a foregone conclusion. To put this result in perspective, consider the contrast with fitness-related traits in *Drosophila melanogaster*, which are notoriously noisy and inconsistent (KEIGHTLEY and EYRE-WALKER 1999). There are potentially several factors that underlie the differences between our results and those from Drosophila, including the demonstrable genetic variation for mutation rate in *D. melanogaster* (SCHRIDER *et al.* 2013). Although we have yet to exhaustively characterize the mutational process in these two strains of *C. elegans* for all categories of molecular mutations, the base-substitution (DENVER *et al.* 2012; F. BESNARD and M-A. FELIX, personal communication) and short-tandem repeat (PHILLIPS *et al.* 2009) mutation rates are quite similar in the two strains.

The simplest explanation for the consistency of the results of this study (*contra* the Drosophila oeuvre) is consistency in the experiments. The mutation accumulation lines were maintained in the same lab at the same time under the same conditions, and the phenotypic assays were done in the same lab by the same person at the same time under the same conditions. Further, the level of replication in these experiments is substantially greater (~25 replicates per line) than in many, albeit not all, phenotypic assays of MA lines. This high replication is especially important because the mutational heritabilities for these traits are actually quite low ($VM/VE \approx 10^{-4}$; Supplementary Table S4; compare to values in Table 1 of HOULE *et al.* 1996).

It is certainly possible that life-history traits are somehow qualitatively different than the traits in this study. Our traits are restricted to a single, narrow window of time in development, so the phenotype, and thus the phenotypic variance, is not integrated over a long period. We have previously assayed lifetime productivity and size at maturity in these same lines. Averaged over six assays at two temperatures, VM for G0 mean-standardized lifetime productivity varies by less than threefold between the two strains (data from Table 2 of BAER *et al.* 2006); size at maturity varies by 1.5-fold (data from Table 2 of OSTROW *et al.* 2007). Those values are well within the range of variation between the two strains for single traits in this study. In contrast, VM for egg-to-adult viability in *D. melanogaster* varies by at least 27-fold across studies, and VM for abdominal bristle number varies by at least 130-fold (data from Table 1 of HOULE *et al.* 1996). Thus, the difference in repeatability between this study and the Drosophila oeuvre does not seem to be due to a qualitative difference between categories of traits.

The second robust result is that VM almost perfectly predicts VG for these traits (Figure 3, Supplementary Figure S3). Again, this was not a foregone conclusion (CHARLESWORTH 2015). This finding is not without precedent, however, as evinced by Figure 1 of HOULE (1998). HOULE calculated a correlation between VG and VM of 0.95 for eight life-history and morphological traits in *D. melanogaster*. LYNCH *et al.* (1998) reported similar data for nine life-history and morphological traits in *Daphnia pulex*, although they did not explicitly calculate the correlation between VG and VM (r = 0.75, reanalysis of data in their Tables 1 and 3). An analogous relationship between VM and between-species divergence was reported for a set of several thousand gene-expression traits in *D. melanogaster* (RIFKIN *et al.* 2005); the correlation between VM

409 and between-species divergence ranged between 0.25 and 0.4 (P<0.0001) for three
410 species pairs. Similar data exist for other sets of traits and in other organisms, and we
411 predict the correlation between mean-standardized VM and VG will generally be large
412 and positive.

413 Two potentially interrelated underlying evolutionary mechanisms predict a
414 positive correlation between VG and VM. The first is the interplay between mutation
415 and random genetic drift. For a neutral trait at mutation-drift equilibrium (MDE) in a
416 selfing organism, VG = $4N_e$VM (LYNCH and HILL 1986). Global N_e of *C. elegans* has
417 been estimated from the standing nucleotide polymorphism (θ) to be on the order of 10^4
418 (ANDERSEN *et al.* 2012). In no case does VG of any of the traits investigated here come
419 close to the value of 40,000VM predicted for a neutral trait at MDE (the dashed line in
420 Figure 3); the average is about 550VM. However, *C. elegans* is almost certainly far
421 from global MDE, so it seems intuitively obvious that VG should be well below the value
422 predicted at MDE, even for a neutral trait. However, both VG and θ increase at a rate
423 proportional to the mutation rate and decrease by drift at a rate inversely proportional to
424 N_e. It is definitely possible that the loci that underlie most quantitative genetic variation
425 mutate 70-fold more slowly than do single nucleotides. It seems less likely that N_e
426 differs consistently by that much between the two categories of loci.

427 More importantly, it seems unlikely to us that these traits are neutral over the
428 entire range of phenotypic space. An alternative, more reasonable possibility is that the
429 traits are not neutral, but rather are subject to some degree of purifying selection, which
430 probably manifests itself as stabilizing selection, either real or apparent (KONDRASHOV
431 and TURELLI 1992). If so, the observed positive relationship between VG and VM is

predicted at MSB. In an infinite population at MSB, $VG \propto \frac{VM}{S}$, where *S* is the mean strength of selection against a new mutation; the proportionality becomes equality if the average selective effects are assumed to be uniform (BARTON 1990; BULMER 1989). If the average selective effects are not uniform (and surely they are not), $VG = \frac{VM}{S(1+C^2)}$ where *C* is the coefficient of variation of mutational effects on fitness (CHARLESWORTH 2015), but unless *C* is highly variable among traits, VM/VG provides a reasonable approximation of the relative strength of selection. Thus, if genetic variation is maintained by MSB, we expect a positive correlation between VG and VM unless the average strength of selection is different between traits and/or the CVs of the mutational effects are different. For example, if VM varies by two orders of magnitude, as it does here, selection would have to vary by nearly that much in order to remove the relationship between VG and VM.

The strong relationship between VG and VM implies that, perhaps with a couple of exceptions, selection must be remarkably uniform across this set of traits. Why might that be? One possibility is that the traits themselves are all highly genetically correlated, even though the phenotypic correlations are modest. If so, direct selection on one trait might lead to sufficient indirect selection on the other traits to produce the pattern. There are too few degrees of freedom to calculate the full set of quadratic selection gradients for these traits (LANDE and ARNOLD 1983). However, a previous analysis of a subset of six of these traits (Traits 1, 2, 3, 15, 17, and 19) revealed that stabilizing selection on Embryo size (Trait 15) of strength $V_S = VM/(VG)^2$ is sufficient to explain the observed standing genetic covariance matrix **G** for those traits, with no need to invoke selection on the other traits (FARHADIFAR *et al.* 2015). In that study, Embryo size was

chosen *a priori* as the likely target of stabilizing selection, for three reasons. First, because from direct measurement of fecundity, we observed that embryo size showed the largest association with fecundity. Second, studies with many organisms have demonstrated that embryo size and size at birth are subject to stabilizing selection. And third, because it showed the largest deviation from the neutral expectation. The results of this study reinforce the previous finding: t_P of Embryo size is about half that of the next smallest t_P of the other 18 traits.

Any claim that direct selection on a favored trait(s) can explain the evolution of a set of correlated traits must be greeted skeptically, because all traits must be correlated with something, and one can never be certain one has accounted for all the relevant variables. Given the strong positive correlation between VM and VG for this particular set of traits, we can ask: where do other traits fall out in VM-VG space? Might it be that *any* arbitrary trait falls out more or less on the same line, and if so, why?

VG and VM have been previously quantified for four other traits in *C. elegans*: Lifetime reproduction weighted by survival measured under the MA conditions (*W20*), Lifetime reproduction measured in a high-throughput "worm-sorter" assay (W_{SORT}), median lifespan when exposed to the pathogenic bacteria *Pseudomonas aeruginosa* (*LT50Pa*) and body volume at maturity (*Size*) (Figure 3; Supplementary Table S5; ETIENNE *et al.* 2015). Inspection of Figure 3 shows that fecundity (W_{20}, W_{SORT}), body volume, and embryo size appear to fall farther below the line than the other traits, consistent with these traits being under stronger purifying selection than the others. Of the four traits, VG for W_{SORT} and *LT50Pa* were measured on nearly the same set of wild isolates as those reported in this study, so the values of VG and t_P are directly

comparable with those reported here. Persistence time for W_{SORT} (166 generations) is almost identical to that of Embryo size (163 generations), and t_P of *LT50Pa* (335 generations) is on the low end of the spindle trait values. Persistence times for *W20* and *Size* are substantially smaller, but VG for those traits was measured on a smaller subset of wild isolates, some of which may be very closely related. Unfortunately, only 11 isolates are common to the two datasets; for those 11 isolates VG and t_P for W_{SORT}, *W20*, and *Size* are both more similar to each other and closer to the common line, but the confidence limits are so large as to make the interpretation tenuous if not meaningless.

It is certainly within the realm of possibility that t_P for more or less any trait measured in this set of wild isolates falls within the relatively narrow range observed here. We can think of at least two possible reasons why that might be. First, since *C. elegans* apparently experienced at least one hard, global, more or less genome-wide selective sweep within the recent past (~600-1250 generations; ANDERSEN *et al.* 2012), selection at linked loci must necessarily have been very inefficient immediately following the sweep, in which case the standing genetic variation may mostly represent a few hundred generations of input of effectively neutral mutations. The average persistence time of ~500 generations is consistent with that scenario. However, the two traits most clearly under selection on *a priori* grounds - Embryo size and lifetime reproduction - fall farthest below the line, which suggests, unsurprisingly, that some mutations are sufficiently deleterious as to have been effectively purged by selection.

A second possibility is that the predominantly self-fertilizing life history of *C. elegans*, combined with relatively restricted recombination within gene-rich regions of

501 the genome (ROCKMAN and KRUGLYAK 2009) means that most traits experience
502 approximately the same overall level of background selection, although again, certain
503 traits clearly experience atypically strong (or weak) selection.

504 The peculiar population genetic features of *C. elegans* invite comparison of the
505 persistence times reported here with those of other taxa, particularly Drosophila. On
506 average, t_P for life history traits in *D. melanogaster* is on the order of 50 generations,
507 and about twice that for morphological traits (HOULE *et al.* 1996). Fruit flies are obligate
508 outcrossers, so selection against new mutations occurs primarily in heterozygotes,
509 which implies that selection against mutant homozygotes must be substantially
510 stronger. Persistence times of heterozygous mutations affecting life history traits in
511 Daphnia are similar to those in *D. melanogaster*, on the order of 40 generations (LYNCH
512 *et al.* 1998).

513 Probably the most comparable data to ours in terms of the types of traits are from
514 *Drosophila serrata*, in which MCGUIGAN ET AL. (2015) report VG and VM for eight
515 cuticular hydrocarbons and ten wing dimensions. The median t_P was ~125 generations,
516 again suggestive of substantially stronger selection against homozygotes. It appears
517 that selection against homozygous mutations for these traits must be substantially
518 weaker than selection against homozygous mutations in Drosophila. One possible
519 explanation is that VG reported here is calculated from a global collection of wild
520 isolates, whereas the Drosophila VG data are based on samples from small
521 populations. However, the population genetic features of *C. elegans* suggest massive
522 (i.e., global) recent gene flow (ANDERSEN *et al.* 2012), so our estimate of VG would
523 probably not be wildly different with a different sampling scheme (SALOMON *et al.* 2009).

Another possibility is that sexual selection is likely to be much more important in flies than in self-fertilizing nematodes.

For the traits reported in this study, the mean-standardized VM varies over nearly two orders of magnitude (~80X). STEARNS and KAWECKI (1994) proposed that VM provides a measure of the robustness of a trait to the perturbing effects of new mutations, such that 1/VM is a meaningful estimate of mutational robustness. HOULE (1998) dissented on the grounds that VM cannot provide an unambiguous measure of mutational robustness because different numbers of loci may affect different traits. GIBSON AND WAGNER (2000) argued that "Comparing variabilities in `fitness' with `wing shape' is like comparing apples with oranges." However, the "number of loci" argument in its essence comes down the distribution of mutational effects (DME), because in an infinitely large population all mutations at all loci in the genome will affect all traits, however small the effect, because all chemical reactions in an organism are ultimately coupled to some extent, however small. By the same logic, comparison of ΔM between traits provides an unambiguous measure of the average mutational effect on a trait, because all traits are subject to the effects of the same set of mutations. To take an opposing viewpoint from that of GIBSON AND WAGNER, perhaps 'fitness' is a uniquely non-robust phenotype precisely because so many mutations at so many loci have effects that are not vanishingly small.

A distinct but related issue concerning mutational robustness is the effect of genetic background, i.e., are different genotypes differently robust to the effects of mutational perturbation? We can address that question with our data by pairwise comparison of VM for the same set of traits in the two genetic backgrounds, provided

we are willing to make the assumption that the number of accumulated mutations does not differ significantly between the two stocks (as noted above, there is no reason to think it does). A paired t-test shows no significant difference in pairwise mean-standardized VM between the N2 and PB306 backgrounds (t= -1.33, df=14, two-tailed P>0.20).

A second issue relating to mutational robustness concerns the relationship between genetic robustness (i.e., VM) and robustness to the effects of random environmental noise (variation due to the effects of unique environment, VE in the terminology of quantitative genetics). The extent to which genetic and environmental robustness have similar underlying mechanisms is an open empirical question (e.g., FRASER and SCHADT 2010). Averaged over both sets of MA lines, there is a strong positive correlation between VM and VE ($r \approx 0.95$). This result is commonly observed, and is consistent with the idea that genetic variation and environmental variation have a common biochemical and/or physiological basis (MEIKLEJOHN and HARTL 2002).

Conclusions and Future Directions:

1. For this set of 19 functionally related traits, the mutational process is very repeatable. There are several other organisms for which there are extant MA lines from multiple starting genotypes, among them *Chlamydomonas reinhardtii* (NESS *et al.* 2016); *Caenorhabditis briggsae* and *Oscheius myriophila* (BAER *et al.* 2005), *Arabidopsis thaliana* (C. FENSTER, personal communication); *Daphnia pulex* (S. SCHAACK, personal communication) and probably others. Similar studies including suites of different types of traits will help establish the boundaries of repeatability and idiosyncrasy in the mutational process.

2. For these traits in this species, mean-standardized VM almost perfectly predicts VG. This result has been previously documented, in *D. melanogaster* (HOULE 1998) and to a lesser extent in *D. pulex* (reanalysis of data in LYNCH *et al.* 1998, above). It is the predicted result if genetic variation is predominantly due to mutation-selection balance or the interplay between mutation and drift. It is not predicted if balancing selection of primary importance in the maintenance of genetic variation.

C. elegans is, *prima facie*, an unlikely target for balancing selection because of the strong evidence for a recent episode of global strong directional selection (ANDERSEN *et al.* 2012). However, recent evidence suggests that balancing selection may have maintained variation in numerous regions throughout the *C. elegans* genome (THOMPSON *et al.* 2015).

3. Looking farther afield, it has been convincingly argued that VM is not a sufficient predictor of VG for life-history traits in Drosophila, and that there must be a significant contribution to VG from balancing selection (CHARLESWORTH 2015). If so, the effect of balancing selection would be to move the line of relationship between VG and VM (depicted in Figure 3) upwards, i.e. to increase the intercept. If balancing selection contributes more to VG for life history traits than for other classes of traits, it implies that, all else equal, the slope of the relationship between VG and VM will be steeper than the line of neutrality, with persistence times of high-VM life history traits falling closer to the line of neutrality. All else is not equal, however; persistence times for life history traits in *D. melanogaster* and other taxa are, on average, less than half those for morphological or other traits (HOULE 1998; HOULE *et al.* 1996; LYNCH *et al.* 1999). The discrepancy between the clear, compelling relationship between VM and VA and the

apparent insufficiency of MSB to explain the standing genetic variation in *D. melanogaster* is an important unresolved issue in evolutionary genetics.

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Trait	**Mean (G0)**		**Mean (MA250)**		**ΔM (x 10^4)**		
	N2	**PB306**	**N2**	**PB306**	**N2**	**PB306**	**Ave**
1 - Initial spindle length [μm] $\bar{n} = 23.6$	11.62 (0.059)	11.36 (0.102)	11.75 (0.040)	10.77 (0.068)	0.41 (0.24)	-2.10***** (0.43)	-0.85 (0.25)
2 - Final spindle length [μm] $\bar{n} = 23.6$	24.20 (0.094)	25.61 (0.123)	24.19 (0.077)	25.75 (0.116)	-0.05 (0.21)	0.21 (0.26)	0.08 (0.17)
3 - Elongation rate [μm/min] $\bar{n} = 23.6$	5.62 (0.061)	6.17 (0.048)	5.55 (0.064)	6.16 (0.079)	-0.40 (0.61)	-0.10 (0.58)	-0.25 (0.42)
4 - Elongation time [s] $\bar{n} = 23.6$	34.38 (0.540)	35.37 (0.395)	34.59 (0.421)	37.50 (0.396)	0.34 (0.78)	2.36***** (0.63)	1.35 (0.50)
5 – Posterior centrosome, oscillation amplitude [μm], $\bar{n} = 22.6$	6.911 (0.064)	6.82 (0.060)	6.64 (0.084)	6.93 (0.079)	-1.60 (0.62)	0.68 (0.57)	-0.46 (0.41)
6 – Posterior centrosome, oscillation duration [s], $\bar{n} = 22.6$	128.07 (1.867)	119.55 (1.716)	124.88 (1.40)	122.19 (1.540)	-1.00 (0.73)	0.92 (0.77)	-0.04 (0.53)
7 - Anterior centrosome, oscillation amplitude [μm], $\bar{n} = 22.6$	3.13 (0.071)	2.84 (0.076)	2.99 (0.073)	2.90 (0.065)	-1.80 (1.30)	0.67 (1.38)	-0.57 (0.95)

Trait	Mean (G0)		Mean (MA250)		ΔM (x 10^4)		
	N2	PB306	N2	PB306	N2	PB306	Ave
8 - Anterior centrosome, oscillation duration [s], $\bar{n} = 22.6$	97.33 (1.979)	94.17 (2.221)	96.25 (1.903)	95.88 (2.120)	-0.40 (1.13)	0.73 (1.30)	0.17 (0.86)
9 - Posterior centrosome, mid-elongation to maximum oscillation peak [s], $\bar{n} = 21.8$	-11.38 (0.878)	-17.22 (0.742)	-11.77 (0.715)	-14.14 (0.574)	-1.10 (3.32)	11.41 (3.48)	5.16 (2.40)
10 – Posterior centrosome, first oscillation peak to mid-elongation [s], $\bar{n} = 21.8$	36.52 (1.075)	33.91 (0.955)	36.16 (0.923)	36.28 (0.755)	-0.80 (3.25)	5.39 (2.77)	2.30 (3.03)
11 - Anterior centrosome, mid-elongation to maximum oscillation peak [s], $\bar{n} = 21.9$	-29.28 (0.848)	-29.81 (0.945)	-26.81 (0.780)	-27.25 (0.839)	5.52 (2.57)	5.91 (2.77)	5.72 (1.89)
12 - Anterior centrosome, first oscillation peak to mid-elongation [s], $\bar{n} = 21.9$	15.78 (1.273)	13.64 (1.003)	18.27 (0.917)	15.81 (0.873)	5.36 (3.38)	4.99 (3.05)	5.18 (2.28)
13 - Posterior centrosome, oscillation frequency [min^{-1}], $\bar{n} = 22.6$	2.66 (0.017)	2.79 (0.024)	2.66 (0.014)	2.873 (0.011)	-0.02 (0.34)	1.24 (0.38)	0.61 (0.25)
14 - Anterior centrosome, oscillation frequency [min^{-1}], $\bar{n} = 22.6$	2.56 (0.017)	2.650 (0.018)	2.57 (0.012)	2.72 (0.011)	0.09 (0.29)	1.01 (0.30)	0.55 (0.21)

Trait	**Mean (G0)**		**Mean (MA250)**		**ΔM (x 10^4)**		
	N2	**PB306**	**N2**	**PB306**	**N2**	**PB306**	**Ave**
15 - Embryo size [µm] $\bar{n} = 24.4$	50.78 (0.166)	52.63 (0.207)	50.40 (0.152)	52.19 (0.239)	-0.30 (0.19)	-0.30 (0.24)	-0.30 (0.15)
16 - Embryo Width [µm] $\bar{n} = 24.4$	33.93 (0.166)	33.72 (0.195)	34.05 (0.126)	34.40 (0.146)	0.09 (0.24)	0.79 (0.29)	0.44 (0.19)
17 - Division plane position [µm] $\bar{n} = 24.4$	22.31 (0.065)	22.89 (0.097)	22.24 (0.075)	22.54 (0.099)	-0.10 (0.18)	-0.60 (0.24)	-0.35 (0.15)
18 - Division duration [min] $\bar{n} = 24.5$	5.07 (0.051)	4.74 (0.045)	5.08 (0.039)	4.84 (0.039)	0.07 (0.51)	0.93 (0.49)	0.50 (0.35)
19 - Centrosome size [μm^2] $\bar{n} = 24.9$	37.59 (0.303)	35.82 (0.422)	37.97 (0.316)	35.81 (0.325)	0.46 (0.47)	-0.03 (0.59)	0.21 (0.38)
Mean					0.05 (0.44)	1.80 (1.09)	1.02 (0.47)
Mean(Abs)					1.07 (0.26)	2.12 (0.66)	1.32 (0.44)
Median					-0.05	0.58	0.21

Trait	Mean (G0)		Mean (MA250)		ΔM (x 10^4)		
	N2	PB306	N2	PB306	N2	PB306	Ave
Median(Abs)					0.61	0.92	0.50

Table 1. Trait Means, standard errors in parentheses; $\bar{n}$ is the mean number of individuals measured per line. Column headings are: *Trait*, see Figure 1 for descriptions of traits; *Mean (G0)*, mean trait value of the ancestral G0 control; *Mean (MA250)*, mean of the MA lines; *ΔM*, % per-generation change in the trait mean. *ΔM* for traits 9-13 (gray rows) are standardized by the environmental standard deviation rather than the by the mean, thus the per-generation change is given in units of phenotypic standard deviations rather than as a fraction of the mean. Values of *ΔM* marked by * are significantly different from 0 at the experiment-wide P<0.05.

Trait	*Strain*	*VL,G0* (x 10^4)	*VL,MA* (x 10^4)	*VE,G0* (x 10^4)	*VE,MA* (x 10^4)	*VM* (x 10^6)	*ave VM* (x 10^6)
1	N2	2.52 (1.44)	3.30 (1.13)	48.52 (3.15)	47.03 (2.13)	0.16 (0.37)	0.42 (0.62)
	PB	9.73 (4.73)	13.16 (3.51)	60.28 (4.50)	72.59 (3.29)	0.69 (1.18)	
2	N2	2.47 (1.18)	3.60 (1.02)	24.24 (1.57)	23.26 (1.06)	0.23 (0.31)	0.64 (0.28)
	PB	2.28 (1.25)	7.56 (1.99)	23.31 (1.74)	35.47 (1.61)	1.06 (0.47)	
3	N2	9.48 (6.20)	50.45 (12.89)	229.00 (14.88)	234.90 (10.66)	8.19 (2.86)	10.18 (2.13)
	PB	0	60.86 (15.85)	209.80 (15.34)	260.50 (11.85)	12.17 (3.17)	
4	N2	30.67 (15.01)	47.83 (13.77)	346.30 (22.50)	372.90 (16.93)	3.43 (4.07)	5.26 (2.55)
	PB	5.50 (7.56)	40.98 (13.35)	327.40 (24.43)	372.20 (16.95)	7.10 (3.07)	
5	N2	5.63 (5.57)	52.44 (14.28)	238.90 (18.75)	299.40 (13.94)	9.36 (3.07)	8.99 (1.95)
	PB	0	43.04 (12.06)	265.00 (20.17)	308.40 (13.98)	8.61 (2.41)	
6	N2	22.83 (13.42)	36.39 (12.18)	405.90 (26.81)	410.20 (19.11)	2.71 (3.62)	5.96 (2.85)
	PB	11.11 (12.34)	57.13 (18.22)	451.70 (35.19)	437.90 (19.95)	9.20 (4.40)	
7	N2	60.82 (31.62)	207.70 (52.44)	825.50 (54.43)	822.60 (38.26)	29.38 (12.25)	25.97 (8.79)
	PB	68.62 (40.18)	181.40 (48.66)	875.20 (68.00)	914.60 (41.52)	22.56 (12.62)	
8	N2	42.13 (25.68)	130.10 (37.58)	850.40 (56.08)	939.00 (43.69)	17.59 (9.10)	22.99 (7.60)
	PB	41.14 (30.68)	183.10 (52.51)	964.40 (74.87)	1115.0 (50.63)	28.39 (12.16)	
13	N2	5.48 (2.56)	11.28 (2.97)	49.77 (3.29)	54.92 (2.56)	1.16 (0.78)	0.58 (0.60)

Trait	***Strain***	***VL,G0* (x 10^4)**	***VL,MA* (x 10^4)**	***VE,G0* (x 10^4)**	***VE,MA* (x 10^4)**	***VM* (x 10^6)**	***ave VM* (x 10^6)**
	PB	8.83 (4.34)	3.82 (1.43)	57.95 (4.51)	57.76 (2.62)	0.00 (0.91)	
14	N2	3.47 (2.05)	6.25 (2.15)	61.49 (4.06)	74.01 (3.46)	0.56 (0.59)	0.33 (0.41)
	PB	2.93 (2.36)	3.46 (1.52)	70.58 (5.50)	73.93 (3.36)	0.11 (0.56)	
15	N2	1.59 (0.80)	3.27 (0.90)	20.25 (1.29)	21.30 (0.95)	0.34 (0.24)	0.81 (0.25)
	PB	1.62 (0.88)	8.07 (1.97)	17.85 (1.31)	30.63 (1.33)	1.29 (0.43)	
16	N2	2.37 (1.41)	4.50 (1.39)	50.36 (3.22)	44.81 (2.00)	0.43 (0.40)	0.59 (0.33)
	PB	2.59 (1.85)	6.35 (1.82)	61.88 (4.55)	51.78 (2.24)	0.75 (0.52)	
17	N2	0.31 (0.60)	3.38 (1.12)	39.57 (2.53)	134.70 (5.99)	0.61 (0.25)	0.81 (0.24)
	PB	1.44 (1.05)	6.51 (1.80)	32.49 (2.39)	46.32 (2.00)	1.01 (0.42)	
18	N2	15.63 (6.40)	21.57 (5.81)	82.81 (5.28)	134.70 (5.99)	1.19 (1.73)	2.28 (1.18)
	PB	7.77 (4.74)	24.64 (6.42)	118.10 (8.61)	131.10 (5.73)	3.37 (1.60)	
19	N2	5.38 (4.23)	24.84 (7.05)	191.80 (12.13)	179.20 (7.92)	3.89 (1.64)	3.54 (1.42)
	PB	11.23 (8.04)	27.20 (8.25)	255.60 (18.39)	253.70 (10.91)	3.19 (2.30)	
Mean	N2					5.28 (2.20)	5.96 (2.19)
	PB					6.60 (2.30)	
Median	N2					1.19	2.28
	PB					3.19	

Table 2. Variances of G0 mean-standardized traits (= squared coefficient of variation). Standard errors in parentheses. Column headings are: *VL,G0*, among-line variance of G0 pseudolines; *VL,MA*, among-line variance of MA lines; *VE,G0*, within-line variance of G0 pseudolines; *VE,MA*, within-line variance of MA lines; *VM*, mutational variance (x 10^6); *ave VM*, average VM of the two strains. Standard errors of VM for individual traits are calculated from the square-root of the sum of the sampling variances of the G0 pseudolines and MA lines. Standard errors of the among-trait mean VM are calculated as the among-trait variance divided by the square-root of the number of traits.

Trait	*Mean*	*VG (raw)*	*VE (raw)*	H^2	*VG* (std)* $(x\ 10^3)$	t_p* *(raw)*	t_P* *(std)*
1	11.61 (0.04)	0.06 (0.01)	0.74 (0.02)	0.07 (0.01)	0.17 (0.24)	419 (641)	394 (611)
2	24.89 (0.08)	0.25 (0.04)	1.40 (0.04)	0.13 (0.02)	0.28 (0.13)	414 (189)	441 (219)
3	5.76 (0.07)	0.20 (0.03)	0.80 (0.02)	0.17 (0.03)	5.71 (1.24)	544 (150)	561 (149)
4	35.67 (0.38)	6.24 (1.03)	43.56 (1.29)	0.11 (0.02)	3.91 (1.36)	763 (386)	743 (390)
5	7.38 (0.09)	0.40 (0.06)	1.45 (0.04)	0.18 (0.03)	7.16 (1.41)	878 (231)	797 (214)
6	126.4 (1.4)	78.50 (13.27)	629.0 (18.7)	0.10 (0.02)	4.01 (1.47)	781 (432)	674 (351)
7	3.49 (0.08)	0.26 (0.04)	1.03 (0.03)	0.17 (0.03)	18.07 (5.14)	953 (354)	696 (269)
8	105.6 (1.7)	128.8 (21.4)	878.0 (26.1)	0.11 (0.02)	9.54 (3.33)	524 (201)	415 (161)
9	-16.89 (0.60)	13.87 (2.50)	157.6 (4.8)	0.07 (0.01)		864 (529)	
10	34.30 (0.77)	21.70 (4.15)	317.7 (9.7)	0.06 (0.01)		703 (359)	
11	-32.65 (0.62)	11.45 (2.68)	330.8 (10.1)	0.03 (0.01)		448 (210)	
12	15.41 (0.71)	16.59 (3.54)	343.0 (10.5)	0.04 (0.01)		599 (517)	
13	2.75 (0.01)	0.004 (0.001)	0.037 (0.001)	0.09 (0.02)	0.19 (0.27)	404 (533)	335 (384)
14	2.62 (0.01)	0.003 (0.001)	0.046 (0.001)	0.05 (0.01)	0.26 (0.19)	708 (1147)	778 (989)
15	50.96 (0.12)	0.56 (0.10)	5.17 (0.15)	0.09 (0.02)	0.13 (0.08)	165 (65)	163 (66)
16	34.00 (0.10)	0.35 (0.07)	5.81 (0.17)	0.05 (0.01)	0.18 (0.14)	322 (211)	304 (195)

Trait	***Mean***	***VG (raw)***	***VE (raw)***	***H^2***	***VG* (std) ($\times 10^3$)***	***t_p* (raw)***	***t_P* (std)***
17	22.38 (0.06)	0.16 (0.03)	2.10 (0.06)	0.07 (0.01)	0.27 (0.10)	325 (116)	330 (121)
18	4.83 (0.03)	0.04 (0.01)	0.30 (0.01)	0.12 (0.02)	1.31 (0.59)	537 (320)	575 (326)
19	37.17 (0.38)	6.33 (1.00)	31.08 (0.88)	0.14 (0.02)	4.02 (1.00)	1194 (505)	1135 (494)
Mean				0.10 (0.01)	3.68 (1.29)	608 (59)	556 (65)
Median				0.09	2.61	544	561

Table 3. Summary statistics of wild isolates, standard errors in parentheses. Column headings are: *Mean*, trait mean; *VG (raw)*, standing genetic variation calculated from the raw data; *VE (raw)*, environmental (within-strain) variance calculated from the raw data; H^2, broad-sense heritability; *VG**, mean-standardized VG corrected by subtracting the average among-line variance of the MA controls; t_p *(raw)*, expected persistence time of a new mutation (t_p=VG/VM) calculated from raw data corrected by subtracting average among-line variance of MA controls from VG; t_p *(std)* calculated from mean-standardized data.

Table S2.a. N2

PC	Eigenval	T1	T2	T3	T4	T5	T6	T7	T8	T9	T10	T11	T12	T13	T14	T15	T16	T17	T18	T19
PC1	3.41	0.17	0.23	0.13	-0.04	0.28	0.35	0.32	0.34	0.25	0.37	0.11	0.35	-0.16	-0.17	0.18	0.10	0.08	0.12	0.17
PC2	2.64	0.19	0.33	0.41	-0.24	-0.17	-0.13	-0.12	-0.18	-0.05	-0.11	-0.07	-0.15	0.03	0.08	0.51	-0.06	0.45	0.00	0.15
PC3	2.11	-0.28	0.36	-0.36	0.59	-0.12	-0.05	-0.19	-0.15	0.13	0.04	0.19	0.05	0.02	0.03	0.16	0.10	0.13	0.22	0.27
PC4	1.83	0.10	-0.06	0.06	-0.12	-0.20	-0.03	-0.14	-0.06	0.31	0.30	0.37	0.29	0.49	0.45	-0.04	-0.14	-0.06	-0.09	-0.13
PC5	1.32	-0.52	0.18	0.12	0.16	0.18	0.16	0.16	0.17	-0.09	0.08	-0.31	-0.05	0.29	0.16	0.11	-0.18	0.04	-0.53	-0.01
PC6	1.04	0.21	-0.02	-0.14	0.03	-0.22	0.28	0.01	0.19	-0.35	0.09	-0.38	0.00	0.20	0.26	-0.03	-0.33	-0.10	0.40	0.32
PC7	0.97	-0.02	0.03	0.03	-0.02	-0.19	-0.26	0.17	0.32	-0.20	-0.25	-0.10	0.32	0.08	0.30	0.03	0.66	-0.04	-0.02	0.06
PC8	0.86	0.14	-0.07	0.00	-0.08	0.08	0.10	0.10	-0.41	0.14	0.24	-0.15	-0.32	0.27	-0.05	-0.08	0.44	-0.21	-0.15	0.49
PC9	0.86	-0.09	-0.10	-0.04	0.01	-0.20	0.49	-0.23	-0.06	-0.12	0.30	-0.14	-0.13	-0.02	0.02	0.08	0.40	0.26	0.10	-0.51
PC10	0.72	0.17	-0.13	-0.14	-0.01	0.20	0.20	-0.30	-0.01	-0.51	0.03	0.38	0.14	-0.19	0.14	-0.01	0.02	0.16	-0.42	0.28
PC11	0.62	0.25	-0.19	-0.33	0.07	0.45	-0.13	-0.03	-0.05	0.32	-0.09	-0.37	0.03	-0.05	0.36	0.00	0.00	0.42	-0.01	-0.07
PC12	0.56	-0.24	0.12	0.27	-0.08	0.45	0.19	-0.01	-0.18	-0.07	-0.17	0.19	-0.15	-0.14	0.49	0.00	0.07	-0.26	0.38	-0.05
PC13	0.54	0.04	-0.12	-0.17	0.06	0.11	-0.06	0.61	-0.11	-0.31	-0.02	0.35	-0.19	0.37	-0.07	0.07	-0.02	0.32	0.16	-0.15
PC14	0.50	-0.09	-0.07	0.09	-0.08	0.43	-0.18	-0.47	0.18	-0.15	0.02	-0.05	0.13	0.50	-0.36	0.05	0.09	0.03	0.26	0.02
PC15	0.37	-0.47	-0.51	0.12	-0.19	-0.17	0.15	0.01	0.13	0.23	-0.14	0.11	0.00	-0.05	0.00	-0.09	-0.02	0.37	0.14	0.39
PC16	0.28	0.23	0.10	-0.12	0.04	-0.03	0.27	-0.13	0.50	0.26	-0.38	0.23	-0.50	0.19	0.02	0.06	0.07	-0.07	-0.12	-0.01
PC17	0.19	-0.03	0.15	0.15	0.03	0.01	-0.39	-0.05	0.34	-0.08	0.49	0.07	-0.40	-0.16	0.16	-0.41	0.03	0.21	0.05	0.05
PC18	0.16	0.10	0.37	0.19	0.05	-0.02	0.25	0.00	-0.18	0.02	-0.30	-0.04	0.19	0.16	-0.09	-0.68	0.02	0.30	-0.01	-0.01
PC19	0.02	0.25	-0.37	0.56	0.69	0.00	0.01	0.00	0.01	0.01	-0.02	0.00	0.00	0.01	0.01	0.05	0.00	-0.01	-0.01	-0.01

Table S2.a. Principal Component eigenvectors and the associated Eigenvalues for the phenotypic correlation matrix of the N2 lines.

Trait definitions (T1-T19) are given in Table 1 of the main text. See Methods for details of the PCA.

Table S2b. PB306

PC	Eigenval	T1	T2	T3	T4	T5	T6	T7	T8	T9	T10	T11	T12	T13	T14	T15	T16	T17	T18	T19
PC1	3.64	0.23	0.32	0.23	-0.11	0.23	0.30	0.20	0.27	0.17	0.30	0.05	0.27	-0.20	-0.18	0.32	0.17	0.25	0.13	0.23
PC2	2.76	0.06	0.31	0.27	-0.11	-0.26	-0.25	-0.32	-0.33	-0.04	-0.22	0.03	-0.21	0.08	0.08	0.43	-0.02	0.39	-0.04	0.16
PC3	1.99	0.27	-0.21	0.35	-0.53	-0.11	0.04	-0.02	0.03	0.16	0.19	0.15	0.21	0.33	0.29	-0.05	-0.22	-0.09	-0.18	-0.22
PC4	1.83	-0.14	0.17	-0.26	0.38	-0.18	-0.03	-0.15	-0.12	0.41	0.23	0.44	0.30	0.24	0.26	0.02	0.06	-0.04	0.11	0.10
PC5	1.37	-0.45	0.24	0.11	0.21	0.08	0.23	0.09	0.24	-0.10	0.06	-0.37	0.03	0.27	0.31	0.12	-0.27	0.10	-0.38	0.01
PC6	1.12	0.32	0.04	-0.20	0.06	-0.25	0.20	-0.08	0.12	-0.29	0.07	-0.19	-0.01	0.17	0.07	0.02	-0.48	-0.04	0.55	0.19
PC7	0.98	-0.03	0.03	0.08	-0.03	-0.14	-0.41	0.40	0.35	-0.18	-0.44	0.05	0.35	0.08	0.26	0.01	0.22	0.01	0.19	0.12
PC8	0.82	0.20	-0.03	-0.03	-0.09	0.23	-0.01	0.14	-0.21	0.07	0.05	-0.17	-0.24	0.26	0.22	-0.15	0.23	-0.24	-0.12	0.68
PC9	0.70	-0.17	-0.04	0.12	-0.05	-0.30	0.09	0.11	-0.06	0.22	0.18	-0.43	-0.17	0.31	-0.06	0.03	0.49	0.02	0.37	-0.28
PC10	0.69	0.01	-0.14	-0.09	-0.02	-0.28	0.33	-0.14	0.03	-0.63	0.19	0.23	0.07	0.03	0.10	0.01	0.44	0.11	-0.22	0.07
PC11	0.60	-0.40	0.14	0.41	-0.08	0.14	0.18	0.15	-0.18	-0.21	0.02	0.40	-0.24	-0.08	0.13	0.00	-0.08	-0.32	0.38	0.01
PC12	0.59	0.09	-0.06	-0.08	-0.03	0.43	0.06	-0.39	0.00	0.01	-0.08	-0.18	0.04	-0.26	0.62	0.02	0.19	0.11	0.24	-0.21
PC13	0.55	0.23	0.02	-0.26	0.09	0.30	0.03	0.49	-0.19	-0.11	-0.01	0.24	-0.26	0.32	0.08	0.09	-0.04	0.39	0.02	-0.32
PC14	0.46	-0.06	0.05	0.07	-0.01	0.46	-0.20	-0.41	0.16	-0.22	0.00	0.06	0.16	0.55	-0.37	0.02	0.09	-0.06	0.12	-0.03
PC15	0.32	-0.35	-0.50	0.00	-0.15	0.03	0.16	-0.06	0.17	0.21	-0.13	0.15	-0.10	0.07	-0.04	-0.14	-0.10	0.53	0.17	0.32
PC16	0.27	0.20	0.17	-0.06	0.02	-0.09	0.35	-0.15	0.46	0.24	-0.45	0.22	-0.40	0.13	0.00	0.06	0.13	-0.21	-0.10	-0.09
PC17	0.19	0.01	0.06	0.02	0.02	-0.05	-0.48	-0.05	0.47	-0.04	0.51	0.11	-0.46	-0.11	0.13	-0.10	0.00	0.09	0.00	0.00
PC18	0.11	0.13	0.41	0.25	0.09	-0.04	0.09	-0.06	-0.07	-0.02	-0.08	-0.01	0.05	0.01	-0.02	-0.79	0.02	0.30	0.01	-0.04
PC19	0.03	0.29	-0.41	0.54	0.67	0.01	0.01	0.00	0.01	0.00	-0.01	0.00	-0.02	0.02	0.01	0.08	0.00	0.00	-0.01	0.00

Table S2.b. Principal Component eigenvectors and the associated Eigenvalues for the phenotypic correlation matrix of the PB306 lines. Trait definitions (T1-T19) are given in Table 1 of the main text. See Methods for details of the PCA.

Trait	T1	T2	T3	T4	T5	T6	T7	T8	T9	T10	T11	T12	T13	T14	T15	T16	T17	T18	T19
T1		-0.02	0.26	**-0.57**	0.02	0.08	0.12	0.08	0.09	0.17	0.07	0.16	-0.13	-0.04	0.16	0.00	0.15	0.21	0.12
T2	0.07		0.28	0.27	0.03	0.11	0.08	0.07	0.20	0.17	0.08	0.15	-0.09	-0.05	**0.71**	0.05	0.42	0.16	0.41
T3	0.20	0.34		**-0.77**	0.03	0.03	0.14	0.06	0.03	0.03	-0.10	-0.03	0.02	0.02	0.48	-0.08	0.29	-0.16	-0.01
T4	**-0.55**	0.21	**-0.72**		-0.02	0.00	-0.12	-0.05	0.05	0.01	0.10	0.04	-0.03	-0.04	-0.12	0.09	-0.09	0.15	0.18
T5	0.07	0.08	0.03	-0.01		0.35	0.40	0.35	0.17	0.25	0.00	0.22	-0.27	-0.30	-0.05	0.07	-0.08	-0.07	0.08
T6	0.14	0.15	0.07	-0.04	0.37		0.33	0.42	0.13	**0.61**	0.00	0.26	-0.18	-0.16	0.06	0.05	-0.02	0.11	0.09
T7	0.07	-0.01	0.03	-0.05	0.38	0.31		0.48	0.17	0.27	-0.06	0.28	-0.17	-0.24	0.00	0.12	-0.10	0.00	0.10
T8	0.09	0.06	0.07	-0.05	0.38	0.47	0.52		0.09	0.29	-0.05	**0.57**	-0.18	-0.18	-0.02	0.07	-0.10	0.06	0.02
T9	0.10	0.19	0.07	0.01	0.10	0.08	0.04	0.01		0.45	0.31	0.37	0.04	-0.03	0.08	0.06	0.01	0.10	0.05
T10	0.23	0.16	0.09	-0.07	0.26	**0.66**	0.21	0.30	0.40		0.25	0.48	0.11	-0.07	0.07	0.06	-0.02	0.07	0.11
T11	0.11	0.05	-0.03	0.02	-0.11	-0.08	-0.09	-0.16	0.29	0.17		0.35	0.05	0.06	-0.01	0.04	-0.02	0.09	0.00
T12	0.19	0.14	0.08	-0.04	0.16	0.27	0.27	**0.55**	0.35	0.46	0.31		-0.03	0.05	0.02	0.12	-0.07	0.11	0.08
T13	-0.11	-0.13	0.00	-0.04	-0.34	-0.19	-0.17	-0.19	0.05	-0.01	0.05	-0.02		0.45	-0.04	-0.16	-0.07	-0.18	-0.07
T14	-0.15	-0.09	0.01	-0.01	-0.24	-0.16	-0.18	-0.15	0.04	-0.10	0.09	0.07	0.46		0.00	-0.12	0.02	-0.12	-0.08
T15	0.23	**0.80**	0.52	-0.13	-0.04	0.09	-0.13	-0.04	0.11	0.08	0.03	0.07	-0.12	-0.09		0.03	**0.69**	0.09	0.30
T16	0.02	0.14	0.01	0.07	0.17	0.03	0.17	0.05	0.14	0.10	0.11	0.12	-0.27	-0.21	0.12		0.00	0.08	0.07
T17	0.19	0.56	0.35	-0.10	-0.03	0.03	-0.11	-0.04	0.04	-0.01	-0.01	0.00	-0.13	-0.11	**0.77**	0.14		0.03	0.13
T18	0.23	0.14	-0.13	0.11	-0.02	0.09	0.08	0.11	0.04	0.09	0.07	0.11	-0.16	-0.17	0.09	0.10	0.01		0.16
T19	0.18	0.48	0.09	0.11	0.10	0.11	0.04	0.04	0.06	0.10	0.01	0.06	-0.12	-0.09	0.37	0.19	0.26	0.16	

Table S3. Pairwise phenotypic correlations. Definitions of traits are given in Table 1 in main text. N2 above diagonal, PB306 below diagonal. Absolute correlations greater than 0.6 are highlighted in orange background; absolute correlations between 0.5 and 0.6 are highlighted in yellow background. Highlighted correlations with the same approximate value in each strain are in bold text.

Trait	*Strain*	*VL,G0*	*VL,MA*	*VE,G0*	*VE,MA*	h_m^2 (x 10^4)	ave h_m^2
1	N2	0.038 (0.021)	0.045 (0.015)	0.69 (0.05)	0.65 (0.03)	0.21 (0.79)	0.66 (0.95)
	PB	0.12 (0.06)	0.17 (0.05)	0.81 (0.06)	0.94 (0.04)	1.10 (1.73)	
2	N2	0.10 (0.06)	0.21 (0.06)	1.59 (0.10)	1.39 (0.06)	1.45 (1.10)	2.31 (0.94)
	PB	0.16 (0.09)	0.50 (0.13)	1.65 (0.12)	2.61 (0.12)	3.17 (1.52)	
3	N2	0.04 (0.02)	0.15 (0.04)	0.80 (0.05)	0.82 (0.04)	2.84 (1.16)	3.76 (0.87)
	PB	0	0.24 (0.06)	0.88 (0.06)	1.15 (0.05)	4.67 (1.29)	
4	N2	3.63 (1.81)	6.08 (1.71)	43.65 (2.83)	44.37 (2.01)	1.12 (1.14)	1.52 (0.71)
	PB	0.69 (0.95)	4.99 (1.66)	40.98 (3.06)	48.76 (2.22)	1.92 (0.87)	
5	N2	0.02 (0.03)	0.25 (0.07)	1.42 (0.09)	1.47 (0.07)	3.24 (1.05)	3.21 (0.70)
	PB	0	0.22 (0.06)	1.23 (0.09)	1.54 (0.07)	3.18 (0.92)	
6	N2	37.45 (22.0)	56.57 (19.52)	665.79 (43.98)	688.73 (32.08)	0.56 (0.87)	1.28 (0.66)
	PB	15.87 (17.64)	80.15 (25.81)	645.62 (50.29)	638.75 (29.08)	2.00 (0.99)	
7	N2	0.06 (0.03)	0.20 (0.05)	0.81 (0.05)	0.81 (0.04)	3.57 (1.51)	3.13 (1.04)
	PB	0.06 (0.03)	0.16 (0.04)	0.71 (0.06)	0.79 (0.04)	2.69 (1.43)	
8	N2	39.91 (24.32)	123.25 (35.60)	805.61 (53.13)	889.52 (41.39)	1.97 (1.03)	2.35 (0.79)
	PB	36.48 (27.21)	162.38 (46.56)	855.19 (66.39)	989.14 (44.90)	2.73 (1.19)	

Trait	*Strain*	*VL,G0*	*VL,MA*	*VE,G0*	*VE,MA*	h_m^2 (x 10^4)	ave h_m^2
9	N2	6.79 (4.96)	15.04 (5.07)	179.61 (12.10)	168.31 (7.97)	0.95 (0.82)	0.86 (0.55)
	PB	3.14 (3.13)	8.07 (3.60)	113.64 (8.94)	142.56 (6.77)	0.77 (0.75)	
10	N2	9.07 (7.55)	23.76 (8.56)	296.65 (20.00)	316.58 (15.00)	0.96 (0.75)	0.93 (0.43)
	PB	0	12.95 (5.85)	308.35 (23.76)	266.77 (12.63)	0.90 (0.42)	
11	N2	0.35 (4.29)	10.93 (5.83)	320.95 (21.53)	344.08 (16.26)	0.64 (0.44)	0.77 (0.31)
	PB	0	14.78 (7.27)	301.62 (23.24)	355.12 (16.81)	0.90 (0.45)	
12	N2	16.09 (10.32)	18.31 (8.36)	330.87 (22.26)	408.87 (19.36)	0.12 (0.72)	0.65 (0.49)
	PB	1.58 (5.55)	19.67 (8.11)	302.00 (23.73)	309.44 (14.68)	1.18 (0.65)	
13	N2	0.004 (0.02)	0.008 (0.002)	0.035 (0.002)	0.040 (0.002)	1.93 (1.44)	0.96 (0.77)
	PB	0.007 (0.003)	0.003 (0.001)	0.045 (0.003)	0.046 (0.002)	0	
14	N2	0.003 (0.002)	0.004 (0.001)	0.043 (0.003)	0.057 (0.003)	0.22 (0.91)	0.35 (0.59)
	PB	0.002 (0.002)	0.003 (0.001)	0.064 (0.005)	0.059 (0.003)	0.49 (0.74)	
15	N2	0.29 (0.17)	0.80 (0.23)	5.85 (0.37)	6.12 (0.27)	1.69 (0.96)	3.12 (0.94)
	PB	0.45 (0.25)	2.21 (0.56)	4.95 (0.36)	10.56 (0.46)	4.54 (1.61)	
16	N2	0.28 (0.17)	0.49 (0.16)	6.27 (0.40)	5.38 (0.24)	0.74 (0.79)	1.04 (0.61)
	PB	0.29 (0.21)	0.74 (0.21)	7.04 (0.52)	6.02 (0.26)	1.35 (0.92)	
17	N2	0	0.16 (0.05)	2.14 (0.13)	2.21 (0.10)	1.45 (0.51)	1.97 (0.58)

Trait	***Strain***	***VL,G0***	***VL,MA***	***VE,G0***	***VE,MA***	h_m^2 **(x 10^4)**	**ave** h_m^2
	PB	0.08 (0.06)	0.34 (0.10)	1.70 (0.13)	2.61 (0.11)	2.48 (1.05)	
18	N2	0.04 (0.02)	0.06 (0.01)	0.21 (0.01)	0.35 (0.02)	1.09 (1.59)	1.91 (1.05)
	PB	0.02 (0.01)	0.06 (0.02)	0.28 (0.02)	0.30 (0.01)	2.73 (1.38)	
19	N2	0.64 (0.58)	3.44 (0.99)	29.11 (1.84)	26.30 (1.16)	2.02 (0.84)	1.64 (0.62)
	PB	1.44 (1.03)	3.51 (1.07)	32.80 (2.36)	33.08 (1.42)	1.26 (0.91)	
Mean	N2					1.41 (0.23)	1.71 (0.05)
	PB					2.00 (0.07)	
Median	N2					1.12	1.52
	PB					1.92	

Table S4. Raw variances of unstandardized traits and mutational heritabilities. Standard errors in parentheses. Column headings are: *VL,G0*, among-line variance of G0 pseudolines; *VL,MA*, among-line variance of MA lines; *VE,G0*, within-line variance of G0 pseudolines; *VE,MA*, within-line variance of MA lines; h_m^2, mutational heritability (x 10^4); *ave* h_m^2, average mutational heritability of the two strains. Standard errors of h_m^2 for individual traits are calculated from the square-root of the sum of the sampling variances of the G0 pseudolines and MA lines. Standard errors of the mean h_m^2 are calculated as the among-trait variance divided by the square-root of the number of traits.

Trait	VL_{MA} ($\times 10^3$)	VL_{G0} ($\times 10^3$)	VE_{MA} ($\times 10^2$)	VE_{G0} ($\times 10^2$)	VM ($\times 10^5$)	h^2_m ($\times 10^3$)	VG ($\times 10^3$)	t_P
W20 (# offspr)*					*15.49*	*1.57*	*13.7*	*88.4*
W25 (# offspr)	*99.11 (39.03)*	*9.71 (33.96)*	*34.63 (3.81)*	*48.01 (6.22)*	*20.32 (11.76)*	*0.49*		
W_{SORT} (# offspr)	*55.57 (12.74)*	*3.05 (1.96)*	*19.78 (2.12)*	*14.90 (2.29)*	*10.50 (2.58)*	*4.64*	*17.5*	*166.7*
Surv (Pct)	*16.72 (10.32)*	*0*	*16.58 (1.50)*	*12.62 (1.38)*	*3.39 (2.64)*	*0.23*		
LT50MA (hrs)	*33.37 (9.43)*	*3.91 (4.49)*	*2.52 (0.40)*	*1.42 (4.62)*	*5.72 (2.10)*	*2.91*		
LT50Pa (hrs)	*8.97 (3.26)*	*0*	*1.62 (1.56)*	*1.45 (2.22)*	*1.79 (0.65)*	*1.17*	*6.0*	*335.2*
Size (mm^3)*	*54.15 (15.53)*	*1.08 (3.66)*	*36.93 (7.32)*	*37.63 (7.22)*	*13.35 (3.19)*	*3.58*	*2.3*	*17.2*
Mean / Median	*44.6 / 43.8*	*3.0 / 2.1*	*18.7 / 18.2*	*19.3 / 13.8*	*9.2 / 8.1*	*2.2 / 2.0*	*9.9 / 9.9*	

Table S5. Variances of mean-standardized life history traits and body volume at maturity; standard errors in parentheses. All traits are from worms grown under MA conditions (on NGM agar plates at 20° C, fed on *E. coli* OP50) unless noted otherwise. Column headings are: *Trait* (units in parentheses, definitions below); VL_{MA}, among-line variance of MA lines; VL_{G0}, among-line variance of G0 controls; VE_{MA}, within-line variance of MA lines; VE_{G0}, within-line variance of G0 controls; VM, mutational variance; h^2_m, mutational heritability; VG, genetic variance. Trait abbreviations are: *W20*, lifetime reproduction weighted by survival; *W25,* lifetime reproduction weighted by survival at 25° C; W_{SORT}, lifetime reproduction of worms grown individually in liquid media in microplates; *Surv*, proportion of embryos surviving to 72 hrs; *LT50MA*, median lifespan under MA conditions; *LT50Pa*, median lifespan of worms

exposed to the pathogenic bacteria *Pseudomonas aeruginosa*; *Size*, body volume at maturity. VG for traits marked with an asterisk is not estimated from the same set of wild isolates included in this study. Experimental details are reported in ETIENNE ET AL. 2015.

Figure Legends

Figure 1 - Tracking and measurement of cell-division traits in the first mitotic division of *C. elegans*: (A) Automatic tracking of the spindle (green), centrosomes (blue), cellular boundary (orange, last panel), and position of the division plane (orange, last panel). Measurements for Traits 15-17 are shown in the last panel. Scale bar 10 microns. (B) Pole-to-pole distance as a function of time (red dots). The blue curve is the sigmoid function fitted to the data (see Methods and Materials). Measurements for Traits 1-4 and Trait 18 are shown. Trait 4 (Elongation time) is multiplied by a factor of 4 in the image for ease of visualization (C) Spindle oscillation as a function of time (red dots). The distance of the posterior centrosome from the long axis of the embryo is plotted as a function of time. Measurements for Traits 5 and 6 are shown.

Figure 2. Raw VM (PB306) plotted against raw VM (N2). The dashed line represents the line of equality.

Figure 3. Mean-standardized VG* plotted against mean-standardized VM. The solid black line shows the best-fit of the spindle trait data; the dashed black line represents the extension of the best-fit line. The orange dashed line shows $4N_eVM$ for $N_e = 10^4$. See text for description of labeled traits and experimental details. Traits labeled in orange were measured on the same set of wild isolates included in this study.

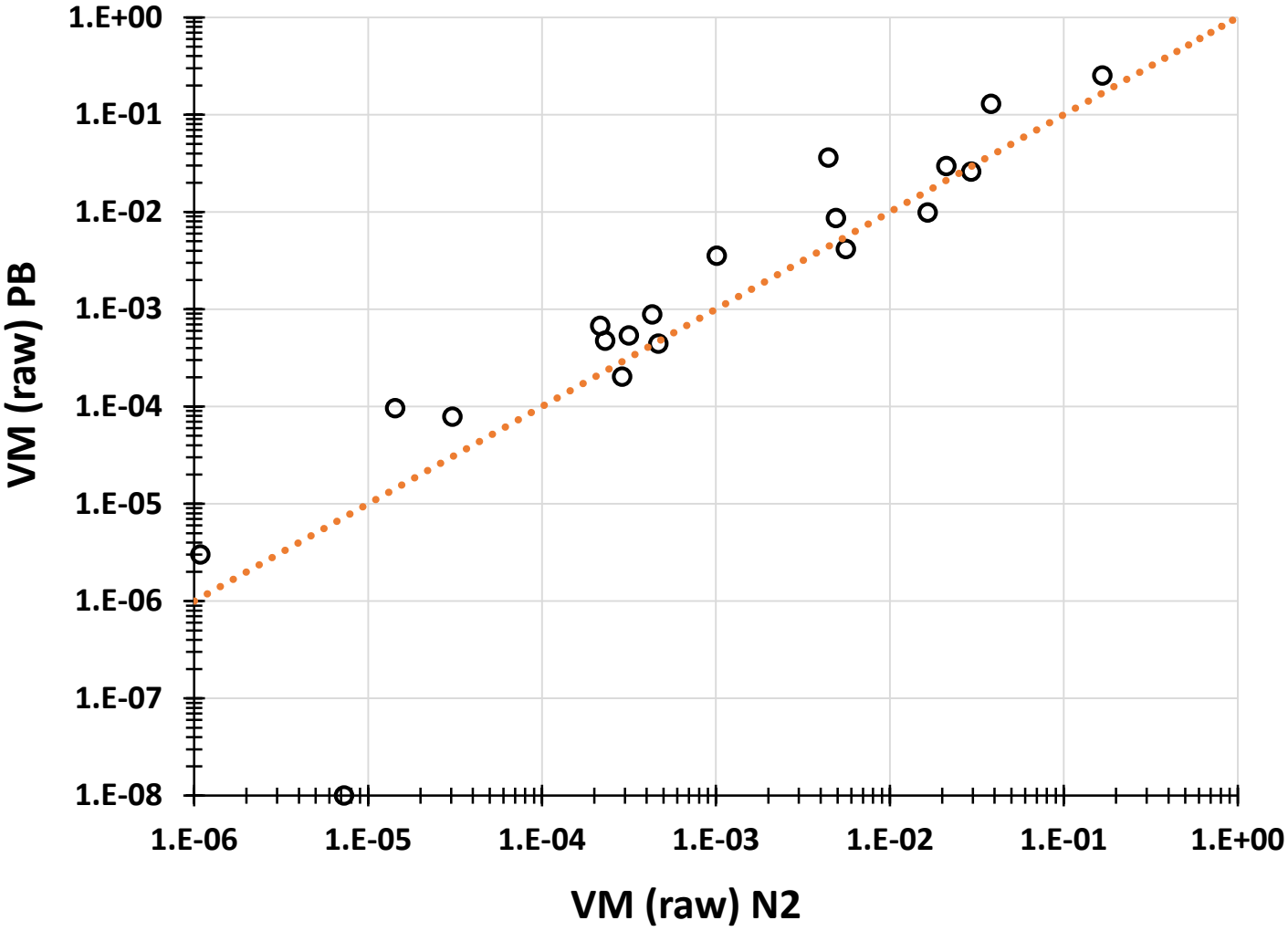
Supplementary Figure S1. (a) Principal Component Analysis of N2. Left panel, Scree plot of eigenvalues of the phenotypic correlation matrix. Right panel, cumulative phenotypic variance explained by each PC. See Text for details of the PCA. (b) Same figure for PB306.

Supplementary Figure S2. Mean-standardized VM (PB306) plotted against mean-standardized VM (N2). The dashed line represents the line of equality.

Supplementary Figure S3. Raw VG* plotted against raw VM. The solid black line shows the best-fit of the spindle trait data. The orange dashed line shows $4N_eVM$ for $N_e = 10^4$.

Supplementary Figure S4. The distribution of eigenvalues of PCA of randomized data from the N2 lines (G0 and control). Data were randomized by randomly sorting data for each of the 19 traits independently and carrying out PCA as described in the Methods; this procedure was repeated 1000 times to generate a distribution of random eigenvalues. The randomized data are uncorrelated between traits. The distribution of mean random eigenvalues is shown in blue, the maximum value (out of 1000) of each random eigenvalue is shown in orange and the observed distribution of eigenvalues is shown in gray.

Figure 2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1.E-07
1.E-08
VM (raw) PB
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1.E-04
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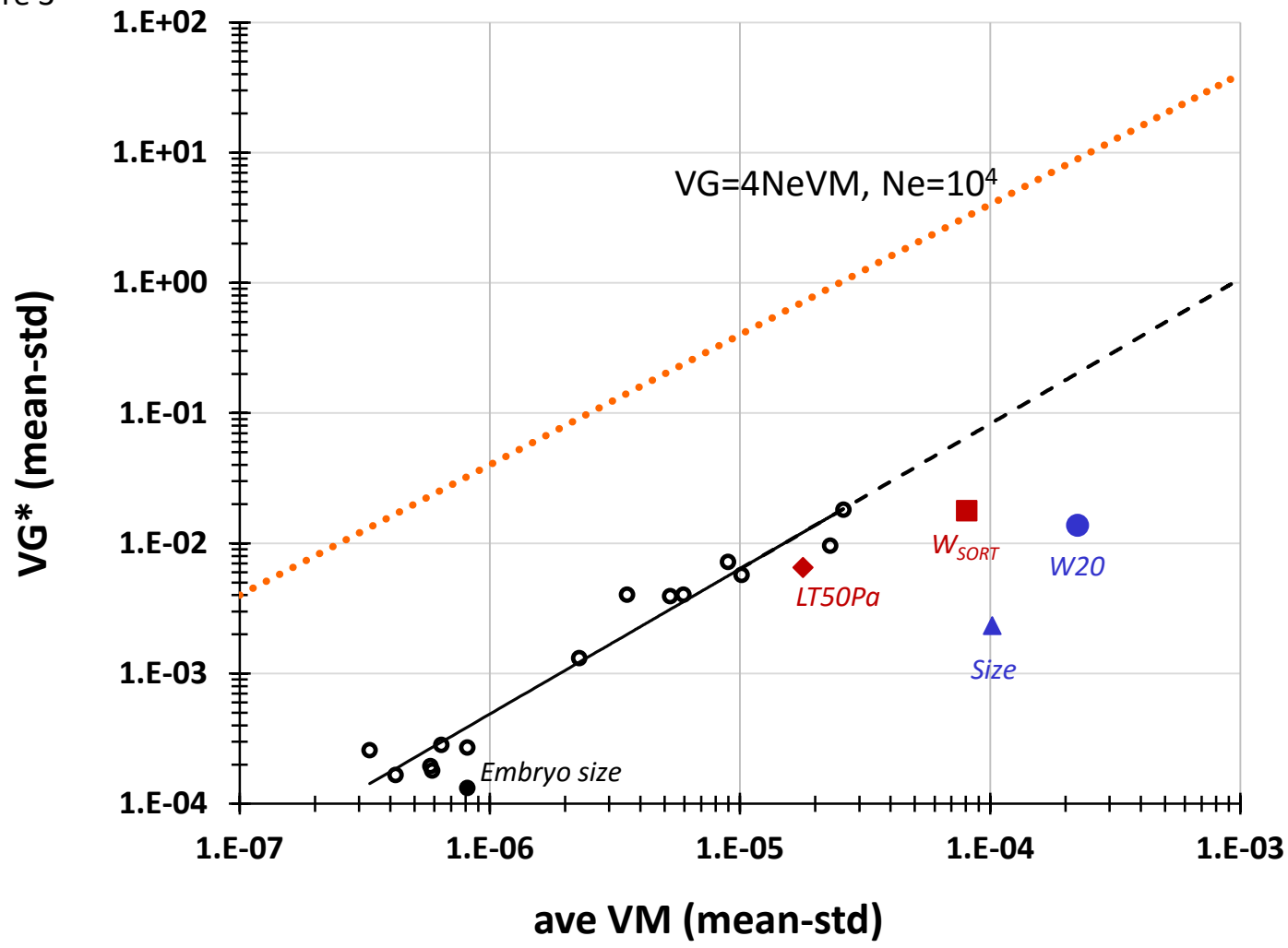
1.E-02
1.E-01
1.E+00
VM (raw) N2

Figure 3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1.E-03
1.E-04
VG* (mean-std)
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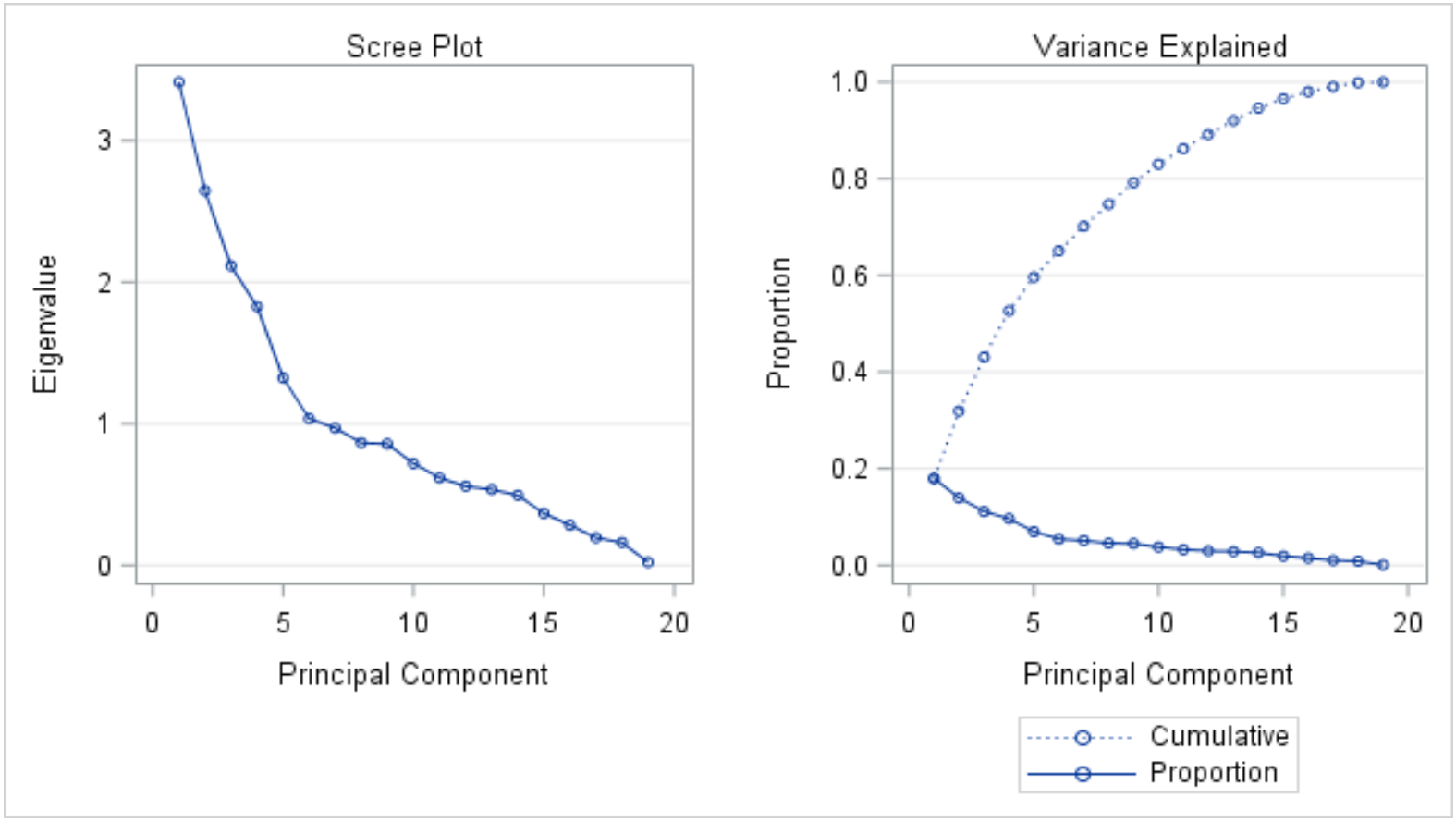
1.E-06
1.E-05
1.E-04
1.E-03
ave VM (mean-std)
VG=4NeVM, Ne=10^4
W_SORT
W20
LT50Pa
Size
Embryo size

Figure S1.a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3
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5
10
15
20
Principal Component
Variance Explained
Proportion
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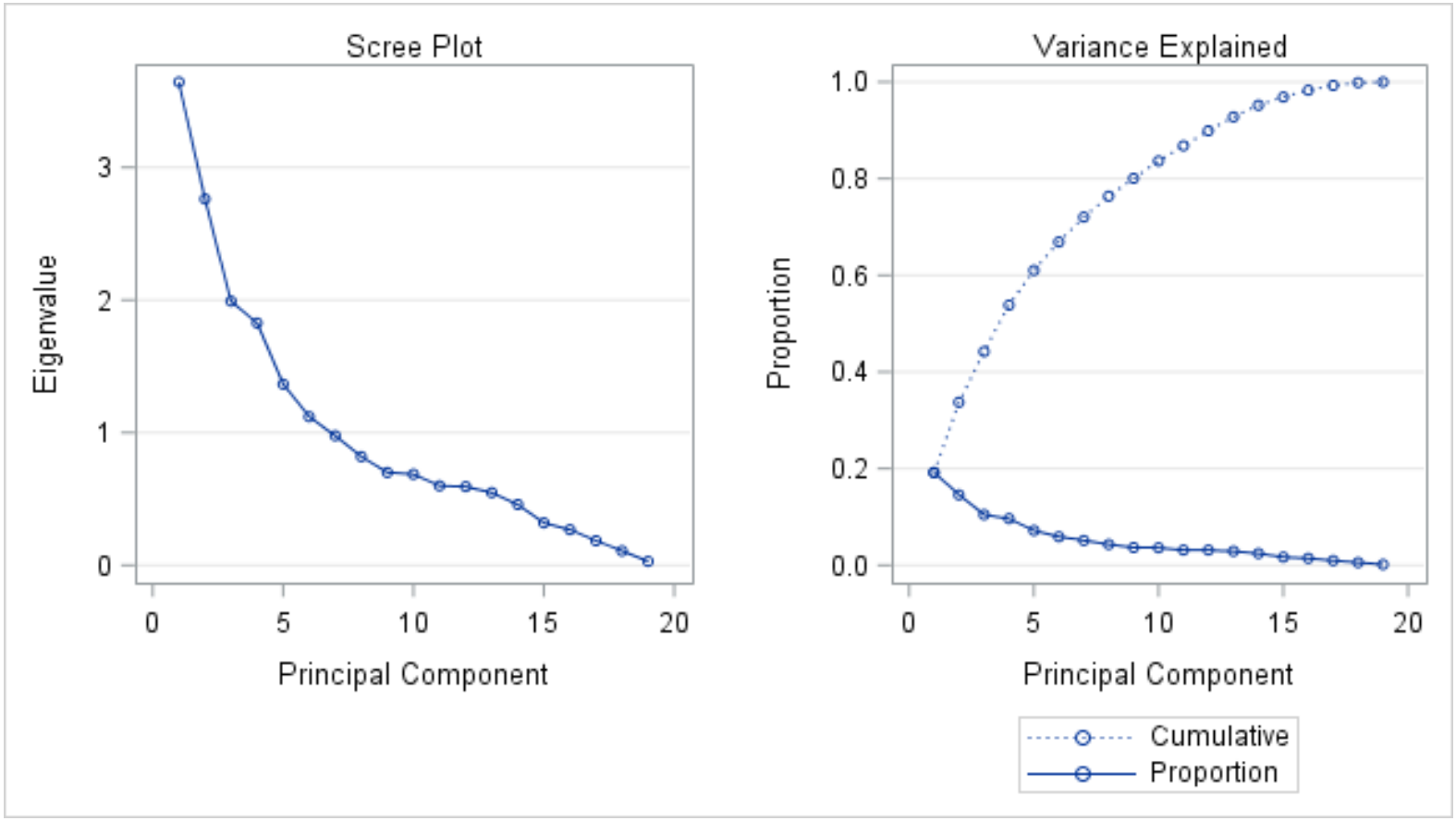
1.0
0
5
10
15
20
Principal Component
Cumulative
Proportion

Figure S1.b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Principal Component
Variance Explained
Proportion
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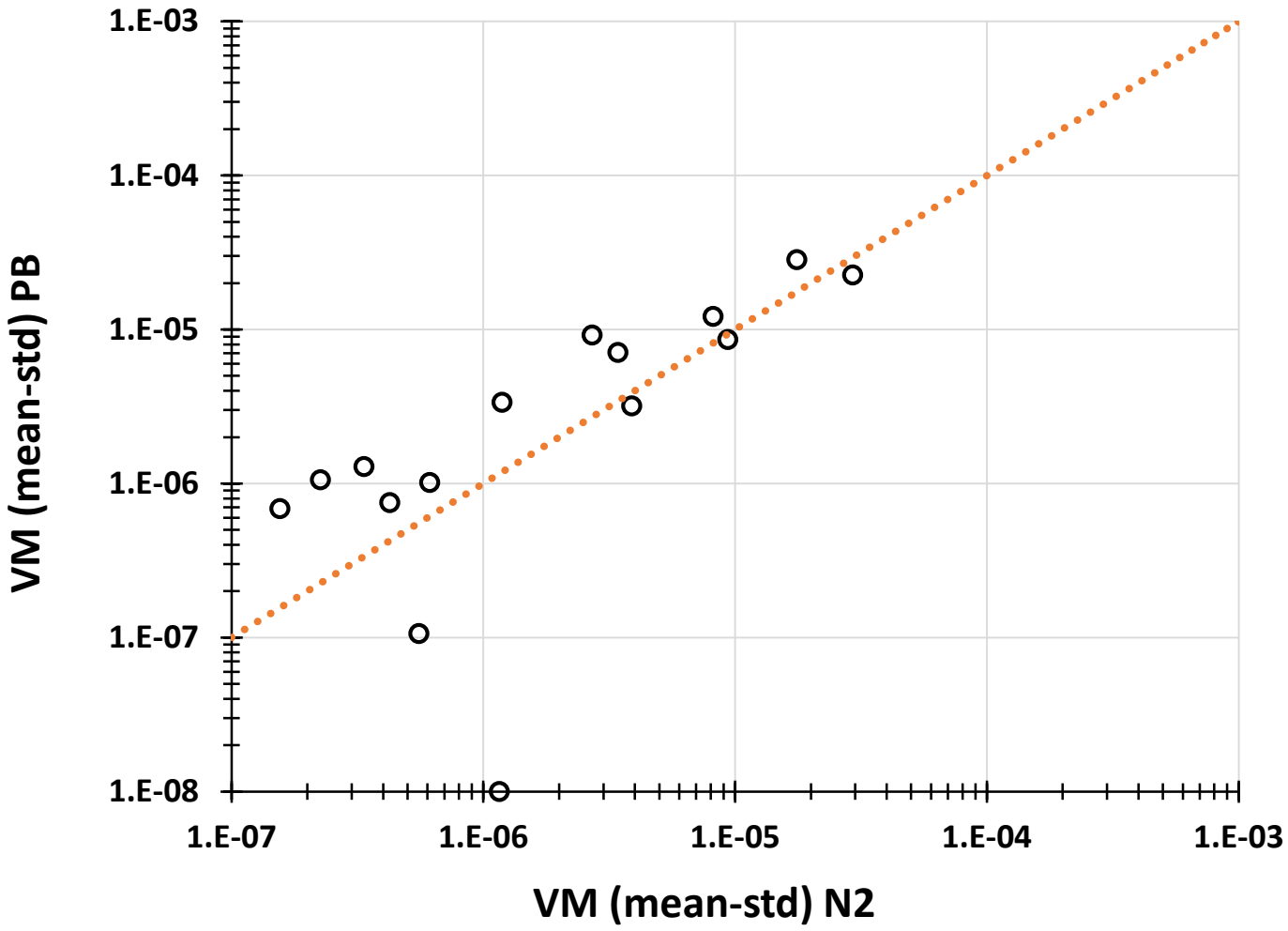
0.8
1.0
0
5
10
15
20
Principal Component
Cumulative
Proportion

Figure S2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VM (mean-std) PB
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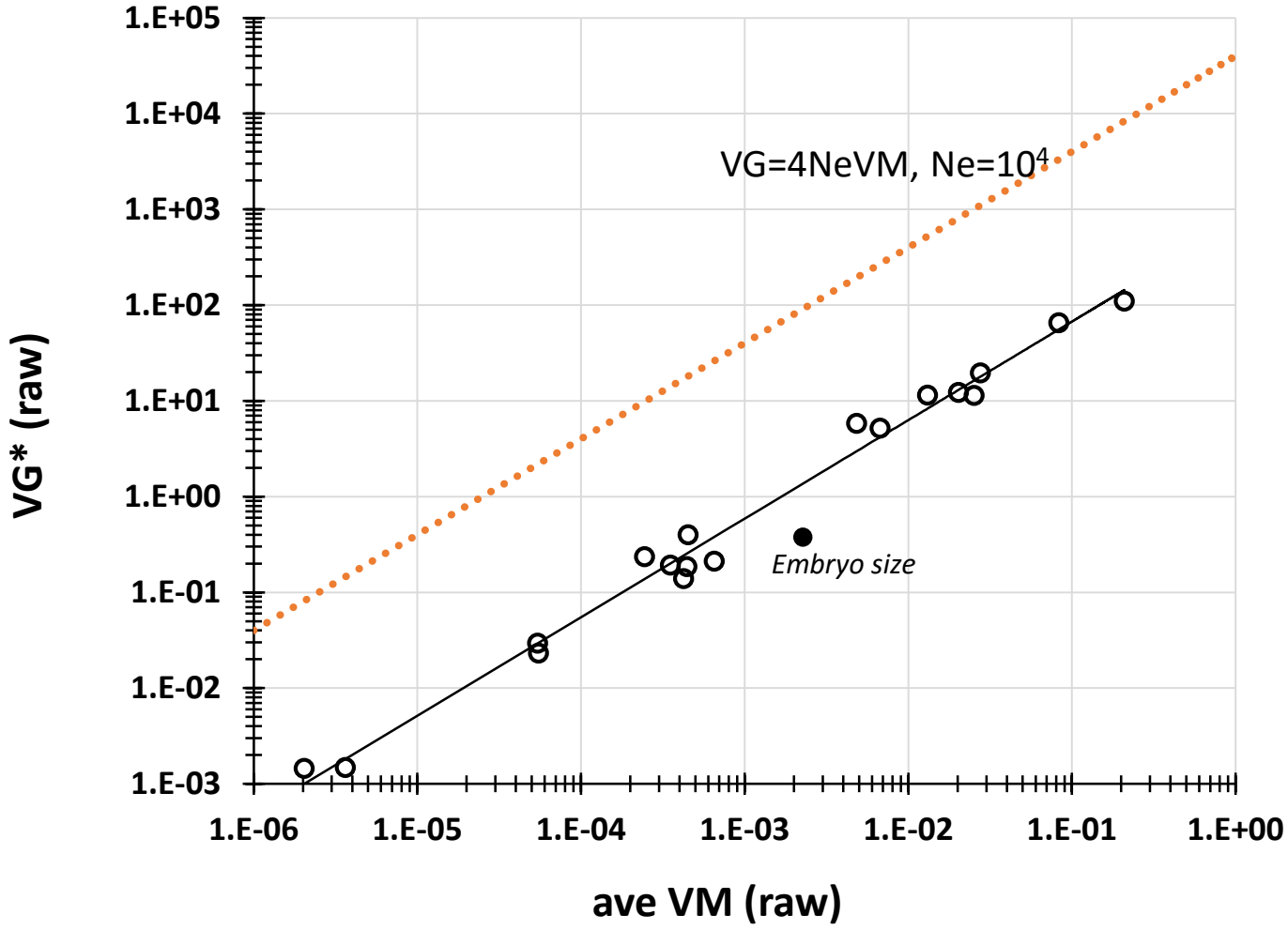
1.E-05
1.E-04
1.E-03
VM (mean-std) N2

Figure S3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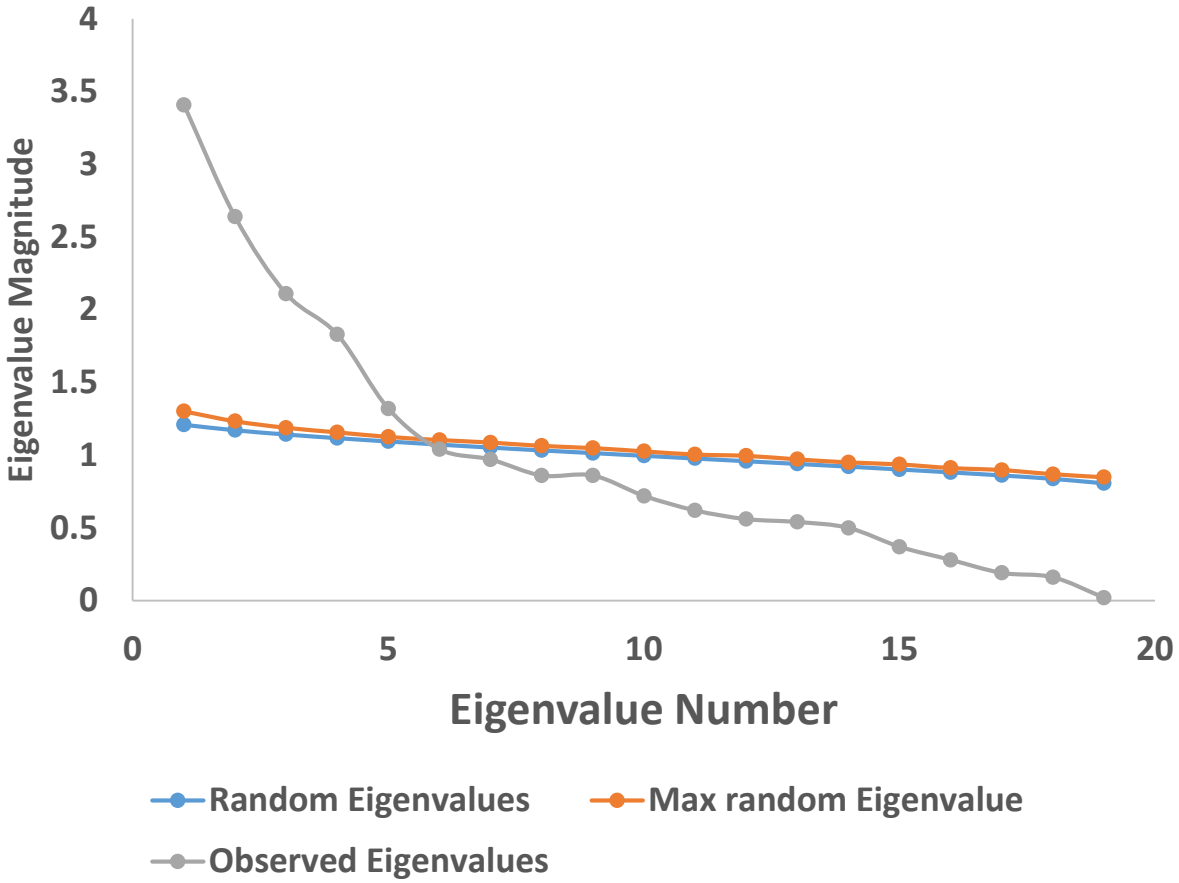
1.E+04
1.E+03
1.E+02
1.E+01
1.E+00
1.E-01
1.E-02
1.E-03
VG* (raw)
VG=4NeVM, Ne=10^4
Embryo size
1.E-06
1.E-05
1.E-04
1.E-03
1.E-02
1.E-01
1.E+00
ave VM (raw)

Figure S4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2.5
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1.5
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0
0
5
10
15
20
Eigenvalue Number
Random Eigenvalues
Max random Eigenvalue
Observed Eigenvalues