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The red death meets the abdominal bristle: Polygenic mutation for susceptibility to a bacterial pathogen in *Caenorhabditis elegans*

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Understanding the genetic basis of susceptibility to pathogens is an important goal of medicine and of evolutionary biology. A key first step toward understanding the genetics and evolution of any phenotypic trait is characterizing the role of mutation. However, the rate at which mutation introduces genetic variance for pathogen susceptibility in any organism is essentially unknown. Here, we quantify the per-generation input of genetic variance by mutation (VM) for susceptibility of *Caenorhabditis elegans* to the pathogenic bacterium *Pseudomonas aeruginosa* (defined as the median time of death, LT50). VM for LT50 is slightly less than VM for a variety of life-history and morphological traits in this strain of *C. elegans*, but is well within the range of reported values in a variety of organisms. Mean LT50 did not change significantly over 250 generations of mutation accumulation. Comparison of VM to the standing genetic variance (VG) implies a strength of selection against new mutations of a few tenths of a percent. These results suggest that the substantial standing genetic variation for susceptibility of *C. elegans* to *P. aeruginosa* can be explained by polygenic mutation coupled with purifying selection.

KEY WORDS: **LT50, mutation accumulation, mutational bias, mutational variance, *Pseudomonas aeruginosa*, spontaneous mutation.**

Different individuals exposed to the same pathogen often vary in their response, and in many cases the variation in these responses has a genetic component. Susceptibility to a pathogen is often determined by one or a few genes of large effect (Wilfert and Schmid-Hempel 2008; Chappell and Rausher 2011), but in other cases the genetic basis of disease susceptibility is polygenic, wherein alleles with small to moderate effects at multiple loci underlie variation in susceptibility (Lefebvre and Palloix 1996; Daub et al. 2013). Susceptibility to pathogens is a particularly important quantitative trait, for at least two reasons. First, pathogens are among the leading causes of human morbidity and mortality, inflicting enormous economic costs. Second, the host–pathogen relationship figures importantly in evolutionary biology, with respect to the evolution of sex and recombination

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(Bell 1982; Kondrashov 1993) and to the maintenance of genetic variation (Haldane 1949; Clarke 1976; Tellier and Brown 2007).

Given the importance of the host–pathogen relationship, it is surprising that, to the best of our knowledge, essentially nothing is known about the mutational properties of quantitative genetic variation for pathogen susceptibility. For example, the per-generation input of genetic variation by mutation (the mutational heritability, h_m^2) for a variety of traits ranges over about three orders of magnitude, from approximately 10^{-2}/generation (some morphological traits in mouse, maize, and fruit flies; see Table 1 of Houle et al. 1996) to approximately 10^{-5}/generation (transcript abundance in *Caenorhabditis elegans* [Denver et al. 2005] and *Drosophila melanogaster* [Rifkin et al. 2005]). Whether the mutational properties of susceptibility to a *specific* pathogen are more like those of a classical polygenic trait (e.g., Drosophila bristle number) or of a trait closely associated with a single gene (e.g., transcript abundance) is not known.

Here, we report the results of an experiment designed to (1) elucidate the quantitative genetic mutational properties of susceptibility of the nematode *C. elegans* to the pathogenic effects of the PA14 strain of the bacterium *Pseudomonas aeruginosa*, and (2) compare the mutational variance (VM) to the standing genetic variance (VG) for the same trait to infer the strength and mode of selection acting on mutations that affect susceptibility to a specific pathogen. The trait "susceptibility to the pathogen" is defined as the median time to death in the presence of the pathogen (*LT50Pa*) and as such conflates "resistance" and "tolerance" as defined in the host–pathogen literature (Restif and Koella 2004) and behavioral avoidance of the pathogen (Reddy et al. 2009; Chang et al. 2011). However, defining "susceptibility" as we do here is relevant from the perspective of evolution: when the plague hits the village, villagers may survive or perish for many different reasons that are equivalent from the perspective of relative fitness.

Methods

MUTATION ACCUMULATION LINES

One hundred mutation accumulation (MA) lines were initiated in March 2001 from a single highly inbred hermaphrodite of the PB306 strain and propagated by single-hermaphrodite descent ($N_e \approx 1$) for 250 generations under standard conditions (20°C, NGM agar plates, fed on the OP50 strain of *Escherichia coli*; see Baer et al. (2005) for details). Under these conditions, mutations that reduce fitness by less than approximately 25% ($4N_e s < 1$) will accumulate at the neutral rate (Kimura 1962; Keightley and Caballero 1997). The common ancestor of the MA lines (G0) was cryopreserved at the time of initiation of the MA experiment; all surviving MA lines were cryopreserved at generation 250.

WILD ISOLATES

A worldwide collection of 114 wild isolates of *C. elegans* (Table S1) was assayed for pathogen susceptibility. Each strain was propagated for five generations without starvation, crowding, or burrowing on modified Nematode Growth Medium plates (Andersen et al. 2014) before pathogen susceptibility was measured.

PATHOGEN

Pseudomonas aeruginosa is an opportunistic pathogen of a wide spectrum of eukaryotes (Rahme et al. 2000; Pukatzki et al. 2002). The PA14 strain is known to be pathogenic to *C. elegans* under the conditions of this experiment (Tan et al. 1999). Whether *C. elegans* commonly encounters *P. aeruginosa* in its natural environment is not known, although *P. aeruginosa* has been identified from collections of microbes associated with *C. elegans* taken from nature (B. Samuel, pers. comm.). Different strains of *P. aeruginosa* have been shown to kill nematodes via different mechanisms (Gallagher and Manoil 2001); the PA14 strain typically kills nematodes by means of oxidative stress mediated by phenazines (Cezairliyan et al. 2013).

SLOW KILLING ASSAY

A detailed description of the SKA assay is given in Supporting Information Methods S3; the experimental design is described in detail in Supporting Information Methods S5 and is depicted in Figure S2. Briefly, pathogen challenge was performed on 35 mm SKA agar plates (Tan et al. 1999) inoculated with 5 μl of a saturated culture of the PA14 strain of *P. aeruginosa*. Thirty to forty immature (L3/L4 stage) hermaphrodites from a synchronized population were introduced onto an assay plate at time $t = 0$. Beginning at time $t = 32$ h, all worms on a plate were scored as live/dead at 12-h intervals (MA lines) or 8-h intervals (wild isolates) by the criterion of responsiveness to the touch of a worm pick. The assay was terminated after 128 h.

Seventy MA lines were assayed over eight blocks; 40 MA lines were included in two blocks and 30 lines in a single block. Each assay block included 14 G250 MA lines and three ancestral G0 control pseudolines; each (pseudo)line was replicated threefold. The assay of wild isolates was identical except all isolates were assayed in a single block.

OTHER TRAITS

To put the results of these experiments in a comparative context, we reanalyzed several existing datasets from the same set of PB306 MA lines, resulting in estimates of mutational parameters for five additional traits: (1) Lifetime reproductive output under standard conditions at 25° (*W25*; Baer et al. 2006), (2) fecundity over three days of reproduction in liquid culture at 20° measured by large-particle flow cytometry (W_{SORT}; see Supporting Information Methods S1 for details), (3) egg-to-adult viability under

MA conditions (*Surv*; see Supporting Information Methods S2 for details), (4) median life span under MA conditions (*LT50MA*; Joyner-Matos et al. 2009; see Supporting Information Methods S4 for details), and (5) body volume at 72 h posthatching (*Size*; Ostrow et al. 2007).

DATA ANALYSIS

Median time until death (LT50) was estimated by nonlinear least squares from the equation $FA = 1 - \frac{1}{1+e^{B-[G\times\log(t)]}}$, where *FA* is the fraction alive at time *t*; LT50 is equal to $e^{(B/G)}$ (Tan et al. 1999; Reddy et al. 2009). Mean LT50 of the G0 controls in each block was estimated by least squares from the linear model $LT50 = Line + Replicate(Line)$, where the among-replicate variance is the residual variance. LT50 was mean-standardized (*stdLT50*) by dividing each datapoint (MA and G0 control) by the G0 control mean for a given block.

Mutational variance (VM)

The VM for a trait *Z* is the product of the genome-wide mutation rate (*U*), the fraction of the genome with the potential to affect the trait (the mutational target, Q_Z), and the square of the average effect of a mutant allele on the trait, α^2, that is, $VM = UQ_Z\alpha^2$ (Barton 1990; Kondrashov and Turelli 1992). VM is typically standardized in one of two ways that have meaningful differences (Houle 1992; Houle et al. 1996; Hereford et al. 2004). The first, more traditional quantification is the mutational heritability, $h_m^2 = VM/VE$, where VE is the environmental (residual) component of variance; the second is the mean-standardized variance, that is, the squared coefficient of variation. Both measures are dimensionless and permit comparison of traits measured on different scales. Heritability has the critical property of dependence on the environmental variance, whereas the squared CV depends only on the genetic variance (Hereford et al. 2004). However, some traits cannot be meaningfully mean-standardized (e.g., traits that can take negative values), and h_m^2 has been more widely reported. Following Crow (1958), we use the term *I* to refer to the squared CV; we report both I_M and h_m^2 and discuss each in context.

In this experiment, the relevant mean by which the variance is standardized is the mean of the experimental block. Downstream analyses of data standardized by the block mean (*stdLT50*) should in principle account for sampling variance of the block mean (this is typically done by resampling lines within a block), but in this experiment the number of ancestral control (G0) pseudolines in a block is too small to meaningfully resample. Accordingly, we analyze both the raw (unscaled) LT50 and LT50 scaled by the G0 block mean (*stdLT50*) to assess the statistical significance of the change in the trait mean and the change in the among-line component of variance. We then analyze the mean-standardized data (*stdLT50*) to parameterize the per-generation% change in the trait mean (the "mutational bias", ΔM) and the mutational variance (I_M). We report significance tests of both unstandardized and standardized variances; the results are qualitatively similar, although the *P* values of tests on the mean-standardized data are somewhat smaller.

Variance components were estimated by restricted maximum likelihood (REML) as implemented in the MIXED procedure in SAS version 9.3 from the linear model:

$$\begin{aligned} LT50(\text{or } stdLT50) = {} & Block + Generation + Block \\ & \times Treatment + Line + Line \times Block \\ & + Line \times Block \times Treatment \\ & + Replicate\{\text{Line[Block(Treatment)]}\} \end{aligned} \quad (1)$$

Generation is treated as a continuous variable representing generations of MA and equals 0 for the G0 controls and 250 for the MA lines; *Treatment* is a categorical variable denoting G0 control or MA (and is equivalent to *Generation*). *Line* represents MA lines and G0 pseudolines. *Replicate* represents the experimental unit (a plate of worms); the among-replicate variance is the residual variance. *Generation* is considered a fixed effect, the other variables as random effects. Variance components of the random effects were estimated for each treatment group individually using the "/GROUP =" option in the RANDOM or REPEATED statement in PROC MIXED (Fry 2004). Degrees of freedom (df) were assigned by the Kenward–Roger method (Kenward and Roger 1997).

The mutational variance VM is calculated from the equation $\Delta VL = (VL_{MA} - VL_{G0})/2t$, where VL_{MA} is the among-line component of variance of the MA lines, VL_{G0} is the among-line component of variance of the G0 pseudoline, and *t* is the number of generations of MA (= 250; Lynch and Walsh 1998). We tested the hypothesis that $\Delta VL > 0$ by likelihood-ratio test (LRT) of the model with among-line variance estimated separately for the MA and G0 control groups versus a model with a single among-line variance component. Because the models are nested, twice the difference between the log-likelihoods of the two models is asymptotically chi-square distributed with df equal to the difference in the number of parameters estimated in the two models.

Analyses of the other traits are analogous to that of mean-standardized *LT50Pa*, with one exception. The experiments involving the variables *W25*, *Surv*, *LT50MA*, and *Size* were done in two blocks and any individual line was present in only one of the two blocks; W_{SORT} was assayed in one block. We initially estimated the mean of the G0 control for each block by least squares from the linear model, $Value = Line + Replicate(Line)$, as before. Each datapoint was then divided by the G0 block mean, resulting in a mean-standardized variable. Variance components

Table 1. Variances of mean-standardized traits (= squared coefficients of variation); SEs are in parentheses.

Trait	VL_{MA} ($\times 10^3$)	VL_{G0} ($\times 10^3$)	VE_{MA} ($\times 10^2$)	VE_{G0} ($\times 10^2$)	I_M ($\times 10^5$)	h_m^2 ($\times 10^3$)	$P(I_M > 0)$
LT50Pa (h)	8.97 (3.26)	0	1.62 (1.56)	1.45 (2.22)	1.79 (0.65)	1.17	0.006
LT50Pa (QN)	5.37 (2.93)	0	1.66 (1.63)	1.45 (2.22)	1.07 (0.59)	0.69	>0.07
W25 (# offspring)	99.11 (39.03)	9.71 (33.96)	34.63 (3.81)	48.01 (6.22)	20.32 (11.76)	0.49	> 0.09
W25 (QN)	67.60 (31.48)	9.71 (33.96)	33.95 (3.70)	48.01 (6.22)	13.16 (11.58)	0.32	>0.22
W_{SORT} (no. of offspring)	55.57 (12.74)	3.05 (1.96)	19.78 (2.12)	14.90 (2.29)	10.50 (2.58)	4.64	≪0.0001
W_{SORT} (QN)	43.27 (10.24)	3.05 (1.96)	19.72 (2.14)	14.90 (2.29)	8.05 (2.09)	4.65	≪0.0001
Surv (proportion)	16.72 (10.32)	0	16.58 (1.50)	12.62 (1.38)	3.39 (2.64)	0.23	> 0.09
Surv (QN)	8.77 (8.88)	0	16.51 (1.50)	12.62 (1.38)	1.76 (1.78)	0.12	> 0.31
LT50MA (h)	33.37 (9.43)	3.91 (4.49)	2.52 (0.40)	1.42 (4.62)	5.72 (2.10)	2.91	0.019
LT50MA (*QN*)	27.23 (8.19)	3.91 (4.49)	2.47 (0.40)	1.42 (4.62)	4.66 (1.87)	2.40	0.036
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	<0.0001
Size (*QN*)	41.87 (12.31)	1.08 (3.66)	35.92 (6.86)	37.63 (7.22)	10.20 (2.57)	2.77	<0.0002

SEs of *VL* and VE were determined by REML (see Methods); SEs for I_M are calculated as the square-root of the sum of the squared SEs of the MA and G0 lines. Column headings are *Trait* (units in parentheses, see text for definitions; QN designates "quasinormal" lines); 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; I_M, mutational variance; h_m^2, mutational heritability; *P*(VM > 0), *P*-value of LRT of different mean-standardized among-line variances of MA lines and G0 control pseudolines (see Methods for details).

were then estimated from the linear model, $stdValue = Generation + Line(Treatment) + Replicate[Line(Treatment)]$ where the variables are defined as before. VM is then calculated and significance assessed as described above for *LT50Pa*, except that block is implicitly treated as a fixed effect.

Mutational bias (ΔM)

The slope of the regression of *stdLT50* on Generation is the mutational bias, ΔM. We tested the hypothesis that $\Delta M \neq 0$ by *F*-test with type III sums of squares and df derived from the linear models described previously.

Standing genetic variance (VG)

Data were mean-standardized and variance components were calculated as for the MA data from the linear model $LT50Pa = Line + Replicate(Line)$. The hypothesis that VG > 0 was tested by LRT against a model with the *Line* term omitted.

Mutational correlations

We define relative fitness, w_i, as the mean *W25* (defined above) of MA line *i*, divided by the mean *W25* of the set of MA lines. The mutational correlations between *LT50Pa* and *W25* and between *LT50Pa* and *LT50MA* were calculated from the among-line components of (co)variance derived from multivariate analysis of variance (MANOVA) as implemented in the MIXED procedure of SAS version 9.3 following (Fry 2004). Because *W25* was measured after 220 generations of MA and *LT50Pa* was measured after 250 generations, the last 30 generations of MA do not contribute to the covariance of *LT50Pa* with *W25*. We multiplied the variance in *LT50Pa* by (220/250) in the calculation of the correlation coefficient, assuming that mutations accumulate linearly with time.

Mutational target size (Q_Z) and average-squared effect (α^2)

Changes in trait means (ΔM) and variances (VM) can be directly estimated from data, whereas the underlying parameters U, Q_Z, and α^2 can only be inferred indirectly. To assess the goodness-of-fit of various combinations of the underlying parameters to the data, for each combination of parameter values U, Q_Z, and α^2 shown in Table S3, we simulated 1000 datasets, maintaining the block structure and distribution of sample sizes of the actual experiment. The parameters were constrained such that the product $UQ_Z\alpha^2 = \text{VM}$, where VM is the estimated mutational variance (I_M, Table 1). We assume that mutational effects (α) are normally distributed with mean 0 and variance α^2; we justify the assumption of normality on the grounds that the observed distribution of block mean standardized MA line means is very close to normal with mean 0 (Fig. S3) and that the residual variance (V_E) is not very much greater than the among-line variance (Table 1). Each simulated MA line was assigned its unique set of mutations; mutations were Poisson distributed among lines with parameter Ut, where U is the haploid genomic mutation rate and t is the number of generations of MA (= 250 generations). The haploid mutation rate is used even though the worms are diploid to account for the 50% probability than any mutation is lost prior to fixation. Individual mutations are assumed to affect trait Z with probability Q_Z. Each MA line i has a unique genotypic value g_i equal to the sum of its mutational effects. Each replicate of an MA line is assigned a unique environmental effect drawn from a Normal distribution with mean 0 and variance equal to the observed V_E (Table 1). Finally, for each MA line, the arithmetic mean is taken as the estimate of the average phenotypic value. For each of the 18 combinations of U, Q_Z, and α^2, we simulated 1000 samples

of line means of the same size as the observed line means (n = 70), and compared the distribution of the simulated sample with the observed distribution of means, using as a metric the empirical estimate of the Kullback–Liebler (KL) divergence (Hausser and Strimmer 2009). The smaller the KL distance, the better the fit. The higher the proportion of simulated datasets with a small KL divergence for a given parameter combination, the better that particular parameter combination explained the observed data. As a result, for each of the 18 combinations of the parameters, we accumulated 1000 samples of a measure of discrepancy between the observed and the simulated distribution of means: the KL divergence. If a large proportion of these 1000 samples of the KL divergence accumulated close to 0, it means that a large proportion of the simulated distributions of means were similar to the observed distribution of means. Thus, by plotting the empirical cumulative distribution of the KL divergence measures for each one of the 18 parameter combinations, we could visually pick, for each of the three mutation rates (U = 0.5, 1, and 2), the combination of Q_Z and α^2 that led to the smallest difference from the observed sample. We repeated this process with two VM sizes, 1.074×10^{-5} (the quasinormal value) and 1.79×10^{-5} (the observed value including all lines). In Table S4, we include for each parameter combination the proportion of the 1000 simulated distributions that resulted in a KL divergence below 0.10. For instance, if that proportion is equal to 0.90, it means that 90% of the simulated distributions had a KL divergence less than or equal to 0.10. The parameter combination with the highest proportion corresponds to the parameter combination that resulted in the largest number of simulations with a distribution closest to the observed distribution of line means.

Results and Discussion

MUTATIONAL VARIANCE FOR SUSCEPTIBILITY TO PATHOGENESIS BY *P. AERUGINOSA* IN *C. ELEGANS*

We employed the "slow-killing assay" of Tan et al. (1999) to determine the median time to death (*LT50Pa*) of worms exposed to the pathogenic PA14 strain of *P. aeruginosa*. Although we did not run a parallel control to determine LT50 in the absence of the pathogen (which would have doubled the size of the experiment), three lines of evidence lead to the strong conclusion that almost all mortality over the course of the assay resulted from the pathogenic effects of infection by *P. aerugnosa*. First, and most convincingly, almost all dead and dying worms exhibited the characteristic vesicles symptomatic of infection by *P. aeruginosa* (Irazoqui et al. 2010), and some worms also exhibited the "red death" syndrome, resulting from Pseudomonas quinolone signal + Fe^{3+} (PQS + Fe^{3+}) upon infection by *P. aeruginosa* (Zaborin et al. 2009). Second, *LT50Pa* of the ancestral control lines (approximately 80 h) was only about 20% of the previously reported LT50 under nonpathogenic conditions at 25° (approximately 18–19 days [Leiser et al. 2011]). Third, the among-line (= mutational) correlation between *LT50Pa* and LT50 previously estimated for these same lines under MA conditions (*LT50MA*, Joyner-Matos et al. 2009; described in Supporting Information Methods S4) is small ($r_M \approx 0.1$) and not significantly different from zero. Thus, we confidently conclude that variation in *LT50Pa* reflects underlying variation in susceptibility to the pathogenic effects of *P. aeruginosa* rather than more general variation in life span unrelated to infection by *P. aeruginosa*.

I_M for *LT50Pa* estimated from the full set of MA lines is 1.8×10^{-5}/generation ($P < 0.006$, LRT of unstandardized LT50; $P < 0.006$; LRT of mean-standardized LT50); h_m^2 is approximately 1.2×10^{-3}/generation. To put these results in a broader context, we compared I_M for *LT50Pa* to I_M for five other traits from the same MA lines; the traits are described in the Methods and the results are presented in Table 1. We expect the two measures of reproductive success to present the largest mutational target, which, all else equal, would result in the largest I_M, and *LT50Pa* to present the smallest target, and that result is borne out (Table 1). I_M for *W25*, a measure of fitness that incorporates both survival and fecundity, is an order of magnitude greater (2.0×10^{-4}/generation, $P > 0.1$, LRT) than I_M for *LT50Pa*, albeit with large sampling variance. However, I_M for *LT50Pa* is not very much less than for egg-to-adult survivorship (*Surv*; 3.4×10^{-5}/generation, $P < 0.09$,) or for median life span under MA conditions (*LT50MA*; 5.2×10^{-5}, $P < 0.04$). An additional consideration is that the analysis of *LT50Pa* separates the block-by-line component of variance from the among-line variance (see Methods); ignoring the interaction with block approximately doubles the among-line variance for *LT50Pa*.

Closer inspection of the data reveals one line with anomalously small *LT50Pa* (Fig. 1A). When that line is omitted, $I_M = 1.07 \times 10^{-5}$/generation ($P > 0.11$, LRT of unstandardized LT50; $P > 0.07$, LRT of mean-standardized LT50), there is a 40% reduction. However, visual inspection of the data shows that all the other traits also exhibit outlying lines (not always the same lines; Fig. 1B–F). Re-analysis of the data with obviously anomalous lines removed reduces I_M by as little as 19% (*LT50MA*) and by as much as approximately 50% (*Surv*); the average reduction in I_M when anomalous lines are omitted is approximately 30% (we follow Mukai et al. (1972) in referring to the reduced set of lines as "quasinormal" lines).

MUTATIONAL BIAS (ΔM)

A second important collective property of new mutations is the cumulative change in the trait mean (ΔM). A directional change in the trait mean under MA conditions implies the existence of a mutational bias, which in turn implies the trajectory of evolution upon relaxation of purifying selection. *LT50Pa* declines

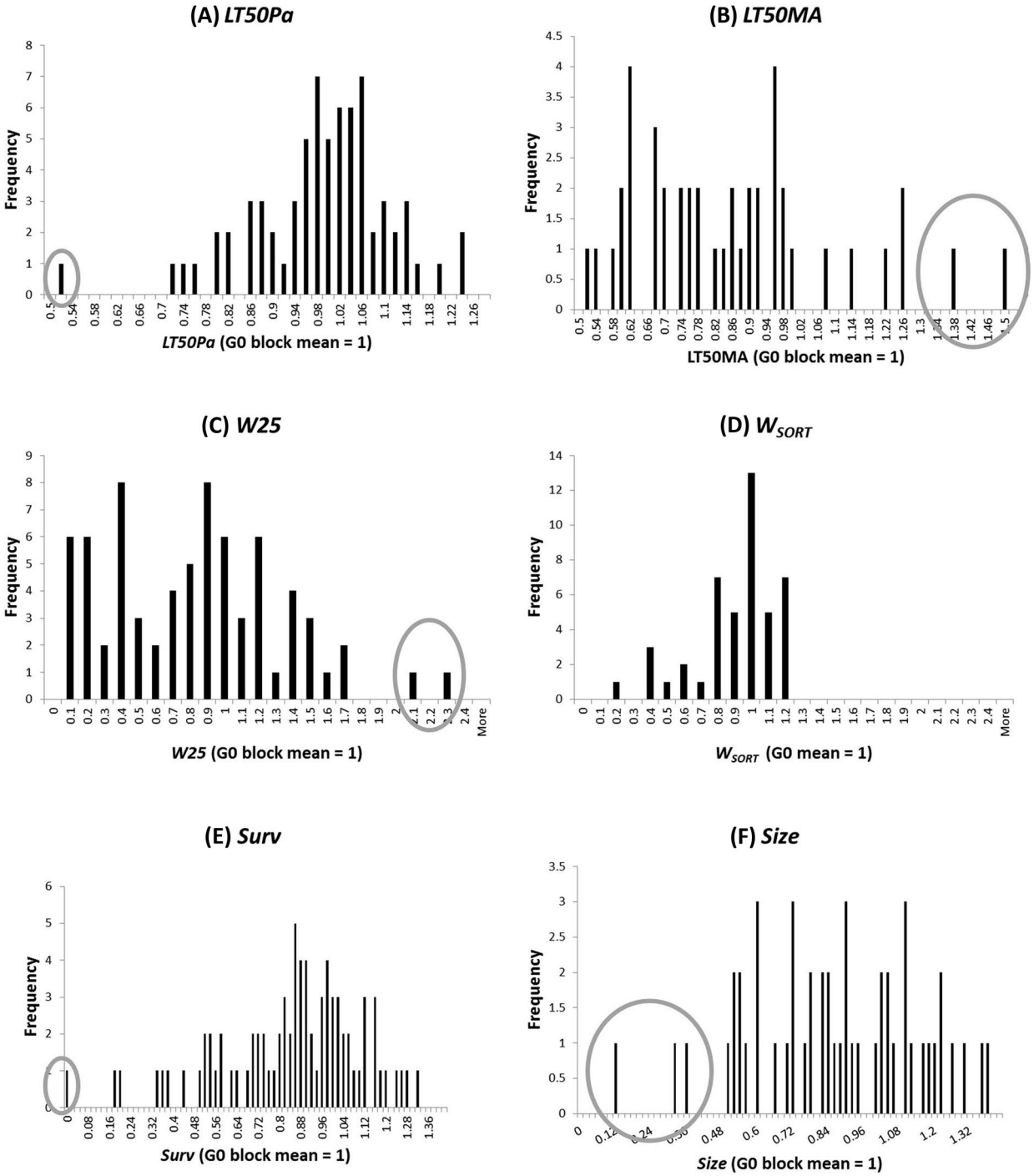

Figure 1. **Frequency distributions of MA line means of mean-standardized traits. In all cases the mean of the G0 ancestral pseudolines in an experimental block is set to a value of 1. Lines that are circled are outliers that were omitted from the "quasinormal" estimates. Trait abbreviations are defined in the text. (A) *LT50Pa*, (B) *LT50MA*, (C) *W25*, (D) W_{SORT}, (E) *Surv*, (F) *Size*.**

only very slowly and not significantly different from zero ($\Delta M = -1.1 \times 10^{-4}$/generation, $P > 0.28$, F-test); if the one atypically short-lived line is removed, the decline is further reduced by ~30% ($\Delta M = -0.8 \times 10^{-4}$/generation, $P > 0.45$). Moreover, MA line means are almost symmetrically distributed around the ancestral value (Fig. 1A; Fig. S3). The very slow change in mean *LT50Pa* stands in stark contrast to every other fitness-related trait we have investigated in these lines, all of which exhibit significant changes in the direction of lower fitness (Table 2) and for which the distributions of line means are strongly biased in the

Table 2. Trait means, SEs in parentheses.

Trait	Trait mean (G0)	Trait Mean (MA)	STD mean (G0)	STD mean (MA)	ΔM ($\times 10^3$)	$P(\Delta M = 0)$
LT50Pa (h)	85.27 (2.83)	83.50 (2.57)	1.007 (0.013)	0.976 (0.022)	−0.12 (0.10)	>0.25
LT50Pa (*QN*)	85.27 (2.83)	84.26 (2.48)	1.007 (0.013)	0.986 (0.020)	−0.08 (0.10)	>0.39
W25 (no. of offspring)	90.89 (22.34)	74.78 (14.60)	1.001 (0.057)	0.818 (0.055)	−0.83 (0.36)	0.024
W25 (*QN*)	90.89 (22.34)	72.03 (17.53)	1.001 (0.057)	0.790 (0.051)	−0.96 (0.35)	0.008
W_{SORT} (no. of offspring)	171.93 (2.87)	147.64 (6.26)	1.000 (0.017)	0.859 (0.036)	−0.57 (0.16)	0.0008
W_{SORT} (*QN*)	171.93 (2.87)	150.60 (5.64)	1.000 (0.017)	0.876 (0.033)	−0.50 (0.15)	0.0013
Surv (proportion)	0.71 (0.03)	0.61 (0.02)	0.989 (0.027)	0.854 (0.027)	−0.54 (0.15)	0.0005
Surv (*QN*)	0.71 (0.03)	0.62 (0.02)	0.989 (0.027)	0.867 (0.025)	−0.48 (0.15)	0.0016
LT50MA (h)	407.82 (43.46)	339.36 (14.35)	0.998 (0.030)	0.843 (0.123)	−0.62 (0.51)	0.0017
LT50MA (*QN*)	407.82 (43.46)	334.53 (11.46)	0.998 (0.030)	0.830 (0.110)	−0.67 (0.46)	0.0007
Size (mm^3)	1.60×10^{-3} (0.48×10^{-3})	1.38×10^{-3} (0.91×10^{-3})	0.998 (0.023)	0.857 (0.043)	−0.70 (0.25)	0.0022
Size (*QN*)	1.60×10^{-3} (0.48×10^{-3})	1.43×10^{-3} (0.72×10^{-3})	0.998 (0.023)	0.889 (0.035)	−0.54 (0.21)	0.0098

SE of ΔM is the SE of the slope of the regression of the trait value on generations of MA. Column headings: trait (units in parentheses; see text for definitions); trait mean G0; trait mean (MA); STD mean (G0), trait value/G0 mean; STD mean (MA), trait value/G0 mean; ΔM, per-generation change in the trait mean; $P(\Delta M = 0)$, *P*-value of *F*-test of the slope of the regression of trait mean on generation MA.

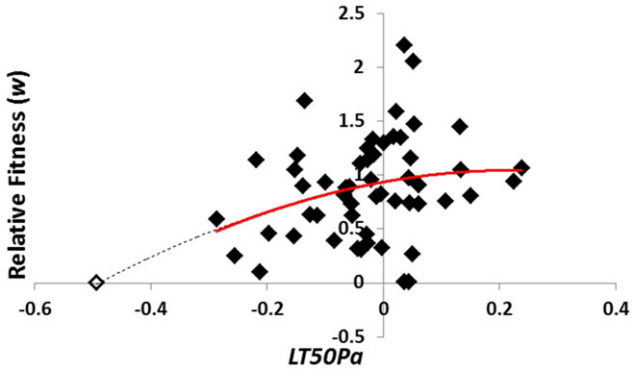

Figure 2. Polynomial regression of relative fitness (*w*) on *LT50Pa*; datapoints are MA line means. See text for details of the calculation of relative fitness. The dashed line is the best-fit polynomial regression with all MA lines included; the solid line is the regression with the outlying line (open symbol) excluded.

direction of lower fitness (Fig. 1B–F). The qualitative difference between *LT50Pa* and the other fitness-related traits remains when only quasinormal lines are included in the analyses, in which case average absolute value of ΔM changes by about 10–15%. Most, but not all, outlying lines occur in the direction of extremely low fitness (Fig. 1).

RELATIONSHIP BETWEEN *LT50Pa* AND RELATIVE FITNESS (W25)

We used the estimates of lifetime reproductive output at 25° after 220 generations of MA (reported in Baer et al. 2006) as a proxy for absolute fitness; dividing by the mean of the MA lines provides an estimate of relative fitness (w). This measure incorporates both fecundity and survivorship in the absence of the pathogen. Data on relative fitness exist for 55 of the 70 MA lines included in the present study. The mutational correlation between w and mean-standardized *LT50Pa*, estimated from the among-line components of (co)variance, is $r_M = 0.68$ ($P < 0.02$). The large, significantly positive mutational correlation is consistent with previous estimates of mutational correlations in this strain, which in most cases are large and positive (Baer et al. 2006; Ostrow et al. 2007; Joyner-Matos et al. 2009).

The lack of mutational bias is unusual for a trait under directional selection. Visual inspection of Figure 2 suggests that the fitness function is curvilinear, such that beyond some point, further increases in *LT50Pa* are, if not downright costly, at least are not associated with a further increase in fitness.

STANDING VG FOR *LT50Pa*

We applied a slight modification of the same *P. aeruginosa* SK assay to a set of 114 wild isolates of *C. elegans* (Table S1), the modification being that survival was assessed at 8-h intervals rather than 12-h intervals. The wild isolates are

highly homozygous (Andersen et al. 2012), so the among-isolate component of variance represents (twice) the genotypic variance (Lynch and Walsh 1998); this variance is directly comparable to VM calculated from the MA lines. The squared CV (I_G) for *LT50Pa* is 0.0060 (SE = 0.0012, LRT, df = 2, chi-square = 192.2, $P << 0.0001$); the distribution of isolate means is shown in Figure S1 and tabulated in Table S2. By way of comparison, there exist estimates of I_G among *C. elegans* wild isolates for lifetime reproductive output under MA conditions (*W20* [Salomon et al. 2009]), *Size* (Salomon et al. 2009), and W_{SORT} (ECA, unpublished); these values are given in Table S3. I_G for *LT50Pa* is somewhat less than I_G for the two estimates of fitness (*W20*, $I_G = 0.0137$; W_{SORT}, $I_G = 0.0175$) and somewhat larger than I_G for Size ($I_G = 0.0023$), although it is important to note that the estimates for *W20* and *Size* are from a different set of wild isolates than *LT50Pa*. To extend the comparison, these estimates of I_G are remarkably similar to the additive genetic variances (I_A) reported for a set of life-history and morphological traits in *D. melanogaster*; the point estimate of I_G for *LT50Pa* is almost identical to the average of the *D. melanogaster* I_A values (see Table 1 of Houle 1992).

The fraction of the total phenotypic variance (VP) contributed by VG is the broad-sense heritability, $H^2 = \text{VG/VP}$. H^2 for *LT50Pa* is 29%, similar to that for W_{SORT} (28%) and greater than that for *W20* (7%) and *Size* (5%).

The ratio of VM to VG is expected to approximate the strength of selection acting on deleterious mutations at mutation–selection balance under a fairly general set of conditions, provided that mutations are uniformly deleterious (Barton 1990; Wayne and Mackay 1998; Zhang and Hill 2005); in the limiting case of a neutral trait in a predominantly selfing population, $\text{VG} = 4N_e\text{VM}$ at mutation–drift equilibrium (Lynch and Hill 1986). There is some consensus that N_e in *C. elegans* is in the order of 10^4 or greater, although that number varies somewhat depending on the chromosomal context (Andersen et al. 2012). Taking 10^4 as a reasonable (probably low) estimate of N_e and assuming VM is that estimated from the quasinormal lines, for *LT50Pa*, $4N_e\text{VM} \approx 0.4$, about 60-fold greater than the estimated value of VG ($I_G = 0.0060$, Table S3). For *LT50Pa*, VM/VG = 0.0028 or 0.0016 for quasinormal lines. Thus, we infer that the strength of selection acting on new mutations that affect *LT50Pa* is about 0.1–0.3%, and that selection has both a directional (linear) and stabilizing (quadratic) component. Inspection of the distribution of *LT50Pa* among wild isolates (Fig. S1) reveals a slight positive skew that is consistent with asymmetric curvilinear selection with stronger selection against alleles that reduce *LT50* by a given amount than against alleles that increase *LT50* by the same amount.

Unfortunately, we have no estimate of VG for *W25*, our proxy for fitness. Estimates of VM/VG for *W20* (a proxy for fitness under the MA conditions), *Size*, and W_{SORT} are 1–2%, 6–7%, and 0.6–0.7%, respectively.

MUTATIONAL TARGET

As manifested in the composite statistic *LT50*, susceptibility of *C. elegans* to pathogenesis-induced mortality resulting from infection by the PA14 strain of *P. aeruginosa* appears to be a relatively typical quantitative trait, with mutational properties (I_M, h_m^2) and standing genetic variance (I_G, H^2) that are similar to average estimates from life-history and morphological characters from a wide range of taxa (Houle et al. 1996). For example, the quasinormal value of I_M for *LT50Pa* is approximately 1.1×10^{-5} (Table 1), remarkably close to the median of 15 estimates of I_M for bristle number in *D. melanogaster* (approximately 1.3×10^{-5}; see Table 1 in Houle et al. 1996). In many cases, however, susceptibility of a host to a specific pathogen is based on one or a few loci of large effect (Wilfert and Schmid-Hempel 2008), and in such cases we would expect the mutational target to be much smaller than for polygenic traits, with a concomitantly larger average effect size.

To home in on the plausible size of the mutational target and the average effect size, we proceeded as follows. The mutational variance for a trait Z is the product of the total genomic mutation rate (U_{Total}, henceforth U without the subscript), the fraction of mutable loci that potentially affect the trait (the mutational target, Q_Z), and the squared average mutational effect (α^2). Note that the genomic mutation rate as it appears in the classical MA literature ("Big U") is almost always conceptually equivalent to the product $U \times Q_Z$ in our terminology. Although there is not yet a reliable estimate of U in *C. elegans* that includes all classes of mutations (single nucleotide polymorphism, indels, short tandem repeats, copy-number variants, other structural variants, transposable elements), a reasonable guess is about one new mutation per haploid genome per generation, most of which occur at short tandem repeat loci and especially at mononucleotide repeats (Denver et al. 2004, 2012; Phillips et al. 2009). Next, we asked, for a given U, what combination of Q_Z and α^2 provides the best fit to the data? I_M for *LT50Pa* is $\approx 10^{-5}$/generation; a range of values of U, Q_Z, and α^2 for which $UQ_Z\alpha^2 = 10^{-5}$ is shown in Table S4, along with the expected number of mutations per MA line and in the entire experiment. For each combination of parameter values U, Q_Z, and α^2, we simulated 1000 datasets. Goodness-of-fit of the simulated data to the observed was assessed by estimating the KL divergence between the observed distribution and each of the simulated distributions. Details of the simulations and analysis are presented in the Methods; results are presented in Figure 3 and in the last column in Table S4.

Given the many assumptions and sources of uncertainty, the assessment of mutational target size and average (squared) effect is heuristic at best. However, visual inspection of Figure 3 clearly shows that, for any of the mutation rates considered ($U = 0.5$, 1, and 2), the model fit is qualitatively better for the three large target/small effect categories ($Q = 0.5$, 0.05, 0.005) than for the three small target/large effect categories, but is essentially indistinguishable within those two broad categories. The intuitive

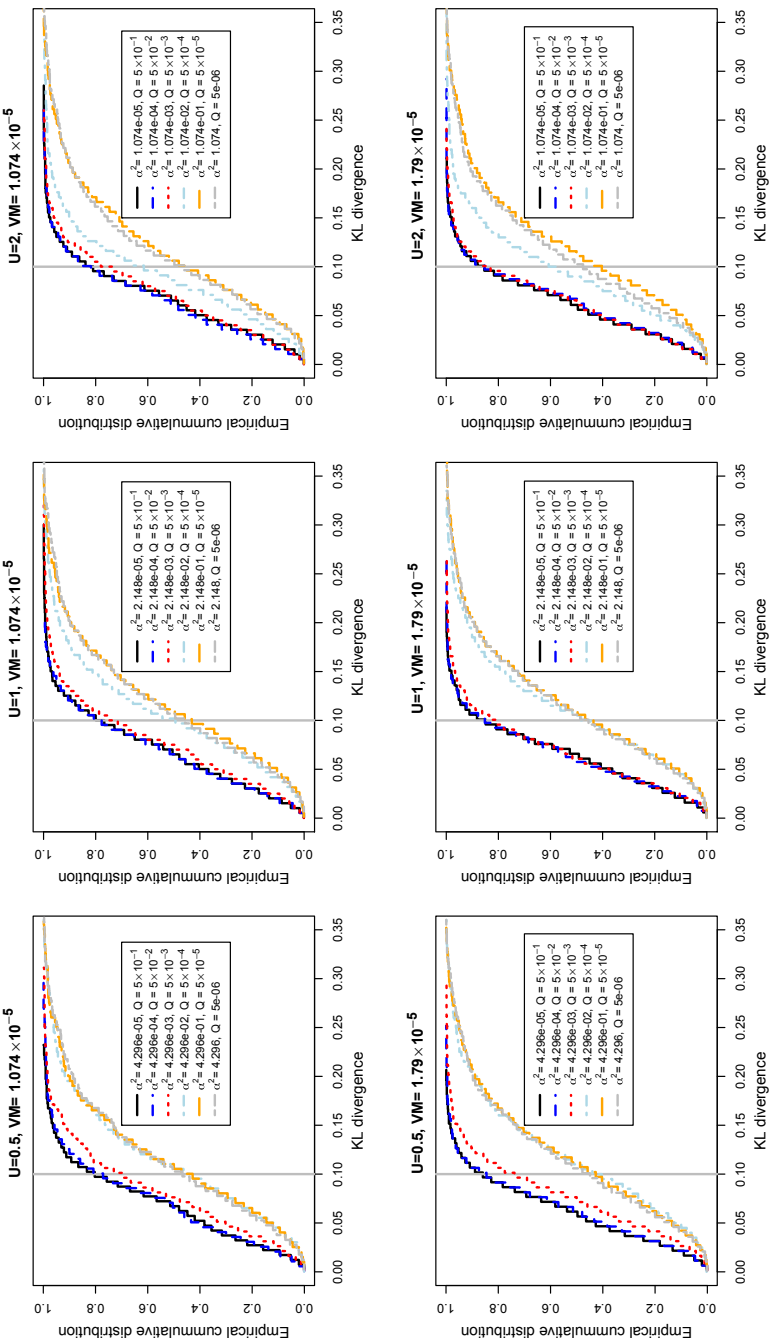

Figure 3. Empirical cumulative distributions of the estimated KL divergence between the observed distribution of means and each of the 1000 simulated distributions of means, for 36 parameter combinations. The gray vertical lines mark the proportion of these simulated distributions that had a KL divergence less than or equal to 0.10. If that proportion was equal to 0.90, it means that 90% of the simulated distributions had a KL divergence less than or equal to 0.10. The parameter combination with the highest proportion corresponds to the parameter combination that resulted in the largest number of simulations closest in distribution to the observed distribution of line means.

explanation for the difference between the two categories is that once mutational effects become too large (greater than about 15–20%), one mutation is likely to take the phenotype outside of the observed range and it would require a second mutation with an effect in the opposite direction to return the distribution to within the observed range.

For the sake of comparison, MA estimates of U (our UQ_Z) and α for fitness related traits in *C. elegans* (Keightley and Caballero 1997; Vassilieva et al. 2000; Azevedo et al. 2002) can be reframed using our assumed values of U. For example, if $U = 1$, then the average estimate of $Q_Z \approx 0.3$–3% and the average estimate of $\alpha \approx 10$–20%, very consistent with the results of this study. For another example, Caballero and Keightley estimated $|\alpha|$ for bristle number in *D. melanogaster* to be 7% (Table 1 in Caballero and Keightley 1994). Thus, the inference that the susceptibility to pathogens is a typical quantitative trait is reinforced by considering the mutational target and the average effect size estimated in this way.

Scaling the mutational target in terms of the fraction of the *C. elegans* genome in base pairs seems artificial; a more intuitive measure is the expected number of mutations distributed across the "population" of 70 MA lines. Based on these results (and these assumptions), the number of mutations is unlikely to be much less than a few dozen unless the mutation rate is low, and a scenario in which hundreds or even thousands of mutations of small effect contribute to the mutational variance is consistent with the data.

Conclusions

The standing VG within *C. elegans* for susceptibility to *one genotype of one pathogen* is of the same order as that for body size and lifetime reproduction. There are at least three possible causes for the high level of genetic variance. First, *C. elegans* may never encounter *P. aeruginosa* in its natural environment, in which case susceptibility to *P. aeruginosa* is a neutral trait. Second, balancing selection may maintain high levels of genetic variation. Third, there may be a high mutational input of genetic variation in the face of purifying selection. We cannot rule out the possibility that susceptibility to *P. aeruginosa* is a neutral trait, but unless the drift dynamics (including hitchhiking) of the underlying loci are very different from the genome-wide average, N_e predicted from the equation $\mathrm{VG} = 4N_e\mathrm{VM}$ would have to be on the order of 10^3, an order of magnitude less than the value of N_e inferred from the standing polymorphism (Andersen et al. 2012). The finding of mutational variance for *LT50Pa* similar to other life-history and morphological traits means that mutation, coupled with reasonably strong purifying selection, is sufficient to explain the high level of genetic variation without the need to invoke any sort of balancing selection.

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DATA ARCHIVING

The doi for our data is 10.5061/dryad.33fv8.

LITERATURE CITED

Andersen, E. C., J. P. Gerke, J. A. Shapiro, J. R. Crissman, R. Ghosh, J. S. Bloom, M.-A. Felix, and L. Kruglyak. 2012. Chromosome-scale selective sweeps shape *Caenorhabditis elegans* genomic diversity. Nat. Genet. 44:285–290.

Andersen, E. C., J. S. Bloom, J. P. Gerke, and L. Kruglyak. 2014. A variant in the neuropeptide receptor NPR-1 is a major determinant of *Caenorhabditis elegans* growth and physiology. Plos Genet. 10:e1004156.

Azevedo, R. B. R., P. D. Keightley, C. Lauren-Maatta, L. L. Vassilieva, M. Lynch, and A. M. Leroi. 2002. Spontaneous mutational variation for body size in *Caenorhabditis elegans*. Genetics 162:755–765.

Baer, C. F., F. Shaw, C. Steding, M. Baumgartner, A. Hawkins, A. Houppert, N. Mason, M. Reed, K. Simonelic, W. Woodard, et al. 2005. Comparative evolutionary genetics of spontaneous mutations affecting fitness in rhabditid nematodes. Proc. Natl. Acad. Sci. USA 102:5785–5790.

Baer, C. F., N. Phillips, D. Ostrow, A. Avalos, D. Blanton, A. Boggs, T. Keller, L. Levy, and E. Mezerhane. 2006. Cumulative effects of spontaneous mutations for fitness in Caenorhabditis: role of genotype, environment and stress. Genetics 174:1387–1395.

Barton, N. H. 1990. Pleiotropic models of quantitative variation. Genetics 124:773–782.

Bell, G. 1982. The masterpiece of nature: the evolution and genetics of sexuality. University of California Press, Berkeley, CA.

Caballero, A., and P. D. Keightley. 1994. A pleiotropic nonadditive model of variation in quantitative traits. Genetics 138:883–900.

Cezairliyan, B., N. Vinayavekhin, D. Grenfell-Lee, G. J. Yuen, A. Saghatelian, and F. M. Ausubel. 2013. Identification of *Pseudomonas aeruginosa* Phenazines that Kill *Caenorhabditis elegans*. Plos Pathog. 9:e1003101.

Chang, H. C., J. Paek, and D. H. Kim. 2011. Natural polymorphisms in *C. elegans* HECW-1 E3 ligase affect pathogen avoidance behaviour. Nature 480:525–529.

Chappell, T. M., and M. D. Rausher. 2011. Genetics of resistance to the rust fungus coleosporium ipomoeae in three species of morning glory (Ipomoea). PLoS One 6:e28875.

Clarke, B. 1976. The ecological genetics of host-parasite relationships. Pp. 87–103 *in* A. E. R. Taylor and R. Muller, eds. Genetic aspects of host-parasite relationships. Blackwell, Oxford, U.K.

Crow, J. F. 1958. Some possibilities for measuring selection intensities in man. Hum. Biol. 30:1–13.

Daub, J. T., T. Hofer, E. Cutivet, I. Dupanloup, L. Quintana-Murci, M. Robinson-Rechavi, and L. Excoffier. 2013. Evidence for polygenic adaptation to pathogens in the human genome. Mol. Biol. Evol. 30:1544–1558.

Denver, D. R., K. Morris, A. Kewalramani, K. E. Harris, A. Chow, S. Estes, M. Lynch, and W. K. Thomas. 2004. Abundance, distribution, and mutation rates of homopolymeric nucleotide runs in the genome of *Caenorhabditis elegans*. J. Mol. Evol. 58:584–595.

Denver, D. R., K. Morris, J. T. Streelman, S. K. Kim, M. Lynch, and W. K. Thomas. 2005. The transcriptional consequences of mutation and natural selection in *Caenorhabditis elegans*. Nat. Genet. 37:544–548.

Denver, D. R., L. J. Wilhelm, D. K. Howe, K. Gafner, P. C. Dolan, and C. F. Baer. 2012. Variation in base-substitution mutation in experimental and natural lineages of Caenorhabditis nematodes. Genome Biol. Evol. 4:513–522.

Fry, J. D. 2004. Estimation of genetic variances and covariances by restricted maximum likelihood using PROC MIXED. *In* A. M. Saxton, ed. Genetic analysis of complex traits using SAS. SAS Institute, Inc., Cary, NC.

Gallagher, L. A., and C. Manoil. 2001. *Pseudomonas aeruginosa* PAO1 kills *Caenorhabditis elegans* by cyanide poisoning. J. Bacteriol. 183:6207–6214.

Haldane, J. B. S. 1949. Disease and evolution. La Ricerca Scientifica 19:68–76.

Hausser, J., and K. Strimmer. 2009. Entropy inference and the James-Stein estimator, with application to nonlinear gene association networks. J. Mach. Learn. Res. 10:1469–1484.

Hereford, J., T. F. Hansen, and D. Houle. 2004. Comparing strengths of directional selection: how strong is strong? Evolution 58:2133–2143.

Houle, D. 1992. Comparing evolvability and variability of quantitative traits. Genetics 130:195–204.

Houle, D., B. Morikawa, and M. Lynch. 1996. Comparing mutational variabilities. Genetics 143:1467–1483.

Irazoqui, J. E., E. R. Troemel, R. L. Feinbaum, L. G. Luhachack, B. O. Cezairliyan, and F. M. Ausubel. 2010. Distinct pathogenesis and host responses during infection of *C. elegans* by *P. aeruginosa* and *S. aureus*. PLoS Pathog. 6:e1000982.

Joyner-Matos, J., A. Upadhyay, M. P. Salomon, V. Grigaltchik, and C. F. Baer. 2009. Genetic (co)variation for life span in rhabditid nematodes: role of mutation, selection, and history. J. Gerontol. A Biol. Sci. Med. Sci. 64:1134–1145.

Keightley, P. D., and A. Caballero. 1997. Genomic mutation rates for lifetime reproductive output and lifespan in *Caenorhabditis elegans*. Proc. Natl. Acad. Sci. USA 94:3823–3827.

Kenward, M. G., and J. H. Roger. 1997. Small sample inference for fixed effects from restricted maximum likelihood. Biometrics 53:983–997.

Kimura, M. 1962. On probability of fixation of mutant genes in a population. Genetics 47:713–719.

Kondrashov, A. S. 1993. Classification of hypotheses on the advantage of amphimixis. J. Hered. 84:372–387.

Kondrashov, A. S., and M. Turelli. 1992. Deleterious mutations, apparent stabilizing selection and the maintenance of quantitative variation. Genetics 132:603–618.

Lefebvre, A., and A. Palloix. 1996. Both epistatic and additive effects of QTLs are involved in polygenic induced resistance to disease: a case study, the interaction pepper-Phytophthora capsici Leonian. Theor. Appl. Genet. 93:503–511.

Leiser, S. F., A. Begun, and M. Kaeberlein. 2011. HIF-1 modulates longevity and healthspan in a temperature-dependent manner. Aging Cell 10:318–326.

Lynch, M., and W. G. Hill. 1986. Phenotypic evolution by neutral mutation. Evolution 40:915–935.

Lynch, M., and B. Walsh. 1998. Genetics and analysis of quantitative traits. Sinauer, Sunderland, MA.

Mukai, T., S. I. Chigusa, J. F. Crow, and L. E. Mettler. 1972. Mutation rate and dominance of genes affecting viability in *Drosophila melanogaster*. Genetics 72:335–355.

Ostrow, D., N. Phillips, A. Avalos, D. Blanton, A. Boggs, T. Keller, L. Levy, J. Rosenbloom, and C. F. Baer. 2007. Mutational bias for body size in rhabditid nematodes. Genetics 176:1653–1661.

Phillips, N., M. Salomon, A. Custer, D. Ostrow, and C. F. Baer. 2009. Spontaneous mutational and standing genetic (co)variation at dinucleotide microsatellites in *Caenorhabditis briggsae* and *Caenorhabditis elegans*. Mol. Biol. Evol. 26:659–669.

Pukatzki, S., R. H. Kessin, and J. J. Mekalanos. 2002. The human pathogen *Pseudomonas aeruginosa* utilizes conserved virulence pathways to infect the social amoeba *Dictyostelium discoideum*. Proc. Natl. Acad. Sci. USA 99:3159–3164.

Rahme, L. G., F. M. Ausubel, H. Cao, E. Drenkard, B. C. Goumnerov, G. W. Lau, S. Mahajan-Miklos, J. Plotnikova, M. W. Tan, J. Tsongalis, et al. 2000. Plants and animals share functionally common bacterial virulence factors. Proc. Natl. Acad. Sci. USA 97:8815–8821.

Reddy, K. C., E. C. Andersen, L. Kruglyak, and D. H. Kim. 2009. A polymorphism in npr-1 Is a behavioral determinant of pathogen susceptibility in *C. elegans*. Science 323:382–384.

Restif, O., and J. C. Koella. 2004. Concurrent evolution of resistance and tolerance to pathogens. Am. Nat. 164:E90–E102.

Rifkin, S. A., D. Houle, J. Kim, and K. P. White. 2005. A mutation accumulation assay reveals a broad capacity for rapid evolution of gene expression. Nature 438:220–223.

Salomon, M. P., D. Ostrow, N. Phillips, D. Blanton, W. Bour, T. E. Keller, L. Levy, T. Sylvestre, A. Upadhyay, and C. F. Baer. 2009. Comparing mutational and standing genetic variability for fitness and size in *Caenorhabditis briggsae* and *C. elegans*. Genetics 183:685–692.

Tan, M. W., S. Mahajan-Miklos, and F. M. Ausubel. 1999. Killing of *Caenorhabditis elegans* by *Pseudomonas aeruginosa* used to model mammalian bacterial pathogenesis. Proc. Natl. Acad. Sci. USA 96:715–720.

Tellier, A., and J. K. M. Brown. 2007. Polymorphism in multilocus host-parasite coevolutionary interactions. Genetics 177:1777–1790.

Vassilieva, L. L., A. M. Hook, and M. Lynch. 2000. The fitness effects of spontaneous mutations in *Caenorhabditis elegans*. Evolution 54:1234–1246.

Wayne, M. L., and T. F. C. Mackay. 1998. Quantitative genetics of ovariole number in *Drosophila melanogaster*. II. Mutational variation and genotype-environment interaction. Genetics 148:201–210.

Wilfert, L., and P. Schmid-Hempel. 2008. The genetic architecture of susceptibility to parasites. BMC Evol. Biol. 8:187.

Zaborin, A., K. Romanowski, S. Gerdes, C. Holbrook, F. Lepine, J. Long, V. Poroyko, S. P. Diggle, A. Wilke, K. Righetti, et al. 2009. Red death in *Caenorhabditis elegans* caused by *Pseudomonas aeruginosa* PAO1. Proc. Natl. Acad. Sci. USA 106:6327–6332.

Zhang, X. S., and W. G. Hill. 2005. Genetic variability under mutation selection balance. Trends Ecol. Evol 20:468–470.

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Supporting Information

Additional Supporting Information may be found in the online version of this article at the publisher's website:

Figure S1. Frequency distribution of wild isolate line means of mean-standardized *LT50Pa*.
Figure S2. Schematic diagram of one block of the experimental assay of the MA lines. "SKA" is the slow-killing assay (see Supplementary Methods S4 for details). Subscripts on lines represent generations subsequent to thawing.
Figure S3. Q-Q plot of MA line mean *LT50Pa*.
Table S1. Collection information for the wild isolates included in this study.
Table S2. Estimates of *LT50Pa* and associated standard errors for the wild isolates included in this study.
Table S3. Variance among wild isolates of mean-standardized traits (= squared coefficient of variation), SEMs in parentheses.
Table S4. Combinations of genome-wide mutation rate (U), fraction of the genome that potentially affects the trait (Q), and average mutational effect on the trait ($|\alpha|$) that result in the observed value of VM for *LT50Pa*.