THE MUTATIONAL STRUCTURE OF METABOLISM IN CAENORHABDITIS ELEGANS

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Abstract

A properly functioning organism must maintain metabolic homeostasis. Deleterious mutations degrade organismal function, presumably at least in part via effects on metabolic function. Here we present an initial investigation into the mutational structure of the Caenorhabditis elegans metabolome by means of a mutation accumulation experiment. We find that pool sizes of 29 metabolites vary greatly in their vulnerability to mutation, both in terms of the rate of accumulation of genetic variance (the mutational variance, VM) and the rate of change of the trait mean (the mutational bias, ΔM). Strikingly, some metabolites are much more vulnerable to mutation than any other trait previously studied in the same way. Although we cannot statistically assess the strength of mutational correlations between individual metabolites, principal component analysis provides strong evidence that some metabolite pools are genetically correlated, but also that there is substantial scope for independent evolution of different groups of metabolites. Averaged over MA lines, PC3 is positively correlated with relative fitness, but a model in which metabolites are uncorrelated with fitness is nearly as good by Akaike’s Information Criterion (AIC).

Keywords

Fitness; Metabolome; Mutation accumulation; Mutational Bias; Mutational variance

INTRODUCTION

In the long run, the course of phenotypic evolution depends on the quantity and qualities of new mutations. The quantity and quality of available mutations must, in turn, depend on the functional architectures – the networks of matter, energy and signals that regulate the development of living things and their workings as adults. These thoughts, put together, lead to the notion that functional architectures constrain the evolution of living things, making some paths more likely than others, quite independently of the adaptive demands of the environment (Maynard Smith et al. 1985; Houle 1991; Arnold 1992; Brakefield 2006;
Stoltzfus 2006; Hendrikse et al. 2007; Psujek and Beer 2008; Braendle et al. 2010). They also lead to another thought: that if we can learn enough about the structure of functional architectures we may be able to predict the quality and quantity of mutations available for natural selection and hence the ability of living things to respond to new adaptive challenges (Ibarra et al. 2002; Dean and Thornton 2007; Lewis et al. 2010; Papp et al. 2011).

This, in brief, is the structuralist programme in evolutionary biology (Gould 2002) – or, at least, one version of it. Its execution faces two difficulties. The first is that it is difficult to estimate the quantity and quality of variation that mutation produces each generation. The second difficulty is that, for all that we have learned about how organisms build themselves and work, our causal models remain fragmentary.

The first of these difficulties - assessing the input of genetic variation by mutation - can be addressed by means of evolution experiments in which the effects of natural selection are minimized, leaving only those of mutation. This is the principle upon which mutation accumulation (MA) experiments depend. In a MA experiment, many replicate lines derived from a genetically homogeneous common ancestor are allowed to evolve in the near absence of natural selection by minimizing effective population size. Over the course of many generations, the lines accumulate mutations and so diverge from each other. The among-line variance reflects the input of new genetic variance by mutation - the mutational variance, \( VM \), or more generally, the mutational variance-covariance matrix, \( M \) (Lynch and Walsh 1998). The change in the mean of a trait over the course of the experiment represents the phenotypic bias of new mutations, \( \Delta M \).

Mutation accumulation experiments have been carried out in a number of species – all of them small and possessed of rapid generation times (reviewed in Drake et al. 1998; Baer et al. 2007; Halligan and Keightley 2009). The nematode *Caenorhabditis elegans* is a particular favorite since it is a selfing hermaphrodite, thus immune to the malign effects of inbreeding, and has a 3–4 day generation cycle (e.g., Keightley and Caballero 1997; Vassilieva and Lynch 1999; Azevedo et al. 2002; Baer et al. 2005; Begin and Schoen 2006; Katju et al. 2015). Most MA studies have focused on gross phenotypes, in particular those related to fitness such as fecundity and longevity (but see Denver et al. 2005). For a variety of reasons, it is interesting to know the mutational properties of fitness-related traits, but, since the networks that regulate such traits are complex and poorly known, we have no way of predicting their mutational structure. To give a concrete instance: the molecular regulation of, and physiological connections between, *C. elegans'* lifespan and reproduction have been much studied (Hansen et al. 2013), yet we still have no causal model that offers anything resembling a prediction as to how spontaneous mutations will affect either trait individually or jointly. This is so even though decades of life-history models have assumed they are bound to each other by an energetic trade-off (Leroi 2001).

If, then, we wish to link mutational properties (\( VM \), \( \Delta M \), or more ambitiously, \( M \)) to functional architecture, it seems that we have to start by looking at simpler, lower-level, intermediary phenotypes. Braendle et al. (2010), for example, estimated \( VM \) and \( \Delta M \) for cell fates in the exceptionally well-studied nematode vulva; Farhadifar et al. (2015) estimated the full covariance matrix for a set of six traits associated with the first mitotic cell division. In
the same spirit, here we use an MA experiment to estimate the mutational properties of part of the worm’s metabolic network. Metabolomic analysis of *C. elegans* has already been widely used to study a range of different biological questions; changes in elements of the metabolome have been associated with organismal development (Swire et al. 2009), aging (Fuchs et al. 2010), responses to physiological stresses (Stupp et al. 2013), and behavior (Srinivasan et al. 2012). Here we use GC-MS (Gas chromatography–mass spectrometry) for metabolic profiling (Swire et al. 2009; Fuchs et al. 2010; Geier et al. 2011; Geier et al. 2013; Stupp et al. 2013) to characterize the evolution of 29 metabolites in an existing set of mutation accumulation lines. Further, since we also have fitness data on the same set of MA lines, we ask whether the mutational structure of fitness can be predicted from features of the metabolome.

**MATERIALS AND METHODS**

**(i) Mutation accumulation lines: assay**

The origin and husbandry of the MA lines included in this study are outlined in Baer et al. (2005). By the time the lines were assayed for metabolomic analysis they had accumulated mutations under minimally efficient selection \((N_e \approx 1)\) for 250 generations. Of the 100 original MA lines, ~70 remained extant at the time this study was initiated. All remaining MA lines, and their ancestral control (G0), were sent to Imperial College for metabolomic analysis where they were washed and instantly frozen at ~80°C. Of those lines, 43 were ultimately included in the assay. Upon thawing, lines were cleaned by bleaching with hypochlorite solution to kill bacterial and fungal contaminants and surviving L1 larvae were chunk-transferred onto NGM plates seeded with *E. coli* OP50 food (Stiernagle 2006). We then grew five replicate plates for each MA line; some replicates were subsequently discarded because they had low biomass and hence poor metabolomic signals, leaving 2–5 replicate samples per line (mean = 3.9). For the ancestral control line we grew 9 replicate plates. All lines were allowed to reproduce until plates had reached a point suitable for synchronization by treatment with hypochlorite (Stiernagle 2006). Following synchronization, worms were incubated at 20°C until young adulthood, defined here as the point at which some eggs were seen on plates but no second generation worms had hatched. At this point, worms were washed from plates with M9 buffer and allowed to settle naturally at the bottom of a 15 ml centrifuge tube for three minutes. The M9 supernatant was then removed and the worm pellet transferred to a microcentrifuge tube, snap-frozen with liquid nitrogen and stored at ~80°C.

Fitness assays were carried out in the Baer laboratory at generations 200 (20° C) and 220 (25° C) by counting the number of live progeny produced over a three day period (Baer et al. 2006).

**(ii) Metabolomics: extraction and data acquisition**

We based our metabolite extraction protocol on a method previously validated by Geier et al. (2011): briefly, we added 1.2 ml methanol to each tube containing an approximately 0.3 ml worm pellet, and used a Precellys Dual bead beater (Bertin Technologies) to homogenize the samples. We repeated this process with an 80% methanol wash; all extraction solvents used
were ice cold. After centrifugation for 5 minutes at 13000 g, we combined supernatants from
the two steps, transferred them to new tubes and vacuum–dried them overnight. We then
added 20 μl quantitation standard mixture (1.5mM 2,2,3,2H3-leucine, 1.3mM 13C- [U]-
glucose) to the samples before vacuum–drying them in an Agilent 2 ml autosampler vial. We
derivatized samples by adding 40 μl methoxyamine hydrochloride (20 mg ml⁻¹ in pyridine)
to each tube and incubating at 37 °C for 2 hours with shaking (Lisec et al. 2006). Following
incubation, 70 μl N-Methyl-N-(trimethylsilyl)-trifluoroacetamide (MSTFA) and 10 μl
injection standard (2-fluorobiphenyl) was added to each tube. This was followed by a further
incubation period of 30 minutes at 37°C with shaking.

We analyzed our samples (1 μl injected volumes) on an Agilent 5975c quadrupole mass
spectrometer with a 7890 gas chromatograph, equipped with a 30m × 0.25mm HP–5ms
column, and splitless injector. The injector port was heated to 230°C and the source to
200°C; the carrier gas was helium (flow rate 1 ml min⁻¹). The temperature program was
isothermal at 70°C for 5 minutes, followed by a 5 °C min⁻¹ ramp to 230 °C, followed by a
10 °C /min ramp to 330 °C, and then isothermal for 5 minutes. The mass spectrometer was
set to scan from 50–600 Da at 5,000 Da/s.

To identify metabolites we used the Fiehn Library (Kind et al. 2009) and AMDIS, a
deconvolution program (Halket et al. 1999). We then manually inspected all peaks, set
individual integration boundaries, and reintegrated the peaks using the GAVIN Matlab script
(Behrends et al. 2011). This gave 34 identified metabolites, of which 29 were included in
this study.

Normalized metabolite data have been archived in Dryad (http://dx.doi.org/10.5061/dryad.
dn09)

(iii) Estimation of quantitative genetic parameters

To examine the directional mutational bias that each metabolite is subject to we first
calculated the mean ratio log₂(M_MAJ/M_0) where M_MAJ is the mean pool size of the jth MA
line and M_0 is the mean of the replicate ancestral (G0) samples. The global mean and
confidence intervals were then calculated from these log₂ ratios (Figure 1A). The relative per
generation change in the mean was calculated as ΔM = ((M_MAJ − M_0)/t)/M_0 * 100 where
M_MAJ is the global mean of the MA lines (Azevedo et al. 2002).

To examine the rate at which mutational variance accumulated in the MA lines we estimated
the between-line variance, V_L, and the environmental variance, V_E, using ANOVA with line
as a random variate. Since the residuals were not normally distributed we assessed the
significance of V_L by bootstrapping. The among-line variance can be used to estimate the
per-generation input of genetic variation from new mutation (the mutational variance, V_M),
which is V_L/2t where t is the number of generations of mutation accumulation (Lynch and
Walsh 1998, p. 330). Comparisons of mutational variances are complicated by scaling
effects (Houle et al. 1996; Fry and Heinsohn 2002) that are not necessarily obviated by log-
transformation. We therefore estimated the mutational coefficient of variation CV_M =
(V_M)^{1/2}/M_0 *100 as a percentage. However, if M_MAJ changes drastically during the course
of the experiment, which it does here in some cases, comparisons of CV_M_0 will underestimate
the extent of relative variation, so we also scale by the mean of the MA lines, $M_{MA}$, to give two measures, $CV_{M_0}$ and $CV_{M_MA}$. Both $\Delta M$ and the CVs were calculated using the median rather than the mean as the measure of central tendency because the median is less sensitive to outliers.

With 43 MA lines, we can jointly estimate the full $M$-matrix for at most eight variables: the number of observations in the data (i.e., MA lines) must be at least $[n(n+1)/2]$, where $n = $ the dimension of the covariance matrix. To gain insight into the multivariate structure of the metabolome, we employed principal components analysis (PCA). We first calculated PCs based on the individual observations, normalized to zero mean and unit variance, using the PRINCOMP procedure in SAS v. 9.4 with eigenvectors scaled to unit length. We then calculated within and among-line components of covariance with the first eight PCs as response variables, using restricted maximum likelihood (REML) as implemented in the MIXED procedure in SAS v. 9.4, following Fry (2004). We compared the full model with unstructured covariances, using the TYPE=UNR option in the RANDOM and REPEATED statements, respectively, to a model in which the off-diagonal elements of the among-line covariance matrix were constrained equal to zero using the PARMS option in the RANDOM statement. The two models were compared using the corrected Akaike Information Criterion (AICc).

To assess the relationship between the metabolome and fitness while taking into account the correlation structure of the metabolome, we performed PCA on the line means as outlined in the previous paragraph. Lifetime reproduction weighted by survival (“Total fitness”, $W$) of some of these MA lines has been previously assayed at generation 200 (31/43 lines) at 20°C and 220 (35/43 lines) at 25°C (Baer et al. 2006). Scaling $W$ by the mean of the ancestral control provides a measure of relative fitness, $w$. Averaging the two assays, mean $W$ of the 36 MA lines for which we have measurements of both metabolites and fitness was 71% that of the ancestral control (range 0.3%–123%). With 36 MA lines, we can jointly estimate the among-line covariances of at most eight variables, so we calculated the correlation between line mean relative fitness and PC1-PC7, using the same linear model strategy outlined in the previous paragraph but with only a single hierarchical level (= line means). Because fitness was measured after 200 or 220 generations of MA and metabolite traits were measured after 250 generations, the among-line correlation calculated from the data will underestimate the mutational correlation, because some fraction (50/250 or 30/250 respectively) of the among-line variance in the metabolite traits will be due to mutations that occurred subsequent to measurement of fitness and thus cannot contribute to the correlation. The correlation between metabolic traits and $w$ was calculated from the equation

$$r_{xw} = \frac{Cov(x,w)}{\sqrt{\frac{t_x}{t_w}}} \frac{\text{Var}(x)}{\text{Var}(w)}$$

where the index $x$ designates the metabolite, $t_x$ is 250 generations of MA and $t_w$ is 210 generations, the unweighted average of the two fitness assays (Braendle et al. 2010). Statistical significance of correlations of relative fitness with PC1-PC7 was assessed pairwise by Likelihood Ratio Test of a model with unstructured covariance compared to a model with the off-diagonal elements constrained to zero. The models are nested and differ by a single parameter, so twice the difference in the log-
likelihoods is asymptotically chi-square distributed with a single degree of freedom. We accounted for multiple tests by applying the sequential Bonferroni correction.

RESULTS

Some metabolites are vulnerable to mutations of very large effect

To initially examine the directional effects of mutation we calculated the mean log$_2$(MA/G0) for each metabolite. We found that most metabolites showed a significant directional bias (Fig. 1A, Supplementary Table S1): 7 increased, 18 decreased; we failed to detect a directional change in 4 (one-sample t-tests on log$_2$ ratios, $mu=0$, df = 42). Succinic acid showed the greatest increase (12-fold); asparagine showed the greatest decrease (66%) relative to the ancestral strain. We calculated the per-generation relative change, $\Delta M$, for each metabolite and found that, on average, the metabolites changed by 0.73% ($\pm$ 0.59, 95%CI) per generation, independent of the sign of the change (Supplementary Table S1, abs($\Delta M$) (%)). By way of comparison, lifetime reproduction ("Total Fitness") in the same set of lines decreases by about 0.1%/generation (Baer et al. 2006). Some metabolites, such as succinic acid, xanthine, nicotinic acid and adenosine, increased by more than 2% per generation, but others showed far smaller changes, or no detectable change at all. Thus it appears that metabolite pool sizes vary greatly in their susceptibility to mutation.

The per-generation increase in the among-line component of variance reflects the mutational variance, $V_M$. Of the 29 metabolites, seven (glycine, glutamate, putrescine, 3-aminoisobutyric acid, lysine, alanine, asparagine) showed no significant $V_M$ insofar that their 95% confidence intervals overlapped zero (Supplementary Table S1).

Comparisons of variances are only meaningful when the different groups are measured on comparable scales. Metabolite pools differ greatly in size so in order to compare the rates at which they accumulate mutational variance we need to scale $V_M$ in some fashion (see Materials and Methods). $V_M$ is typically scaled either relative to the trait mean (i.e., the mutational coefficient of variation, $CV_M$) or relative to the environmental component of variance (the mutational heritability, $h^2_M$). $CV_M$ can be scaled relative either to the mean of the ancestral control ($CV_{M_C}$) or to the mean of the MA lines ($CV_{M_MA}$; Baer et al. 2006). If it is assumed that the effects of mutations are multiplicative (Barton 1990), it is appropriate to scale by the mean of the MA lines; scaling by the MA mean is approximately equivalent to calculating variances of log-transformed data (Fry and Heinsohn 2002). Since the mean pool sizes of some metabolites changed so much in the course of the experiment, in some cases the two methods give very different estimates of $CV_M$, and so we report both (Supplementary Table S1). Scaling by the ancestral control line means gives a mean $CV_{M_C}$ across metabolites of 7.00% ($\pm$ 95%CI: 5.90; range: 0.25 – 72.38%); scaling by the MA line mean gives a mean $CV_{M_MA}$ across metabolites of 1.60% ($\pm$ 95%CI: $\pm$ 0.33; range: 0.42 – 4.11%). The correlation between these two ways of estimating $CV_M$ is moderate but significant: $r^2 = 0.64$ ($P < 0.01$). Thus, depending on scaling, by this measure, metabolites vary between one and two orders of magnitude in their vulnerability to mutation.

As noted, there are estimates of mutational parameters in C. elegans for a variety of phenotypes such as fitness components (productivity, longevity, and survival to maturity),

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body size and various behavioral traits and traits related to mitotic cell division (Supplementary Table S2). For gross phenotypes $\Delta M$ is typically a fraction of a percent per generation; the most rapid relative decline recorded is $\sim 0.2\%$ per generation for productivity (Vassilieva et al. 2000; Joyner-Matos et al. 2011) and lifespan (Joyner-Matos et al. 2009).

Although the overall rate of directional change in metabolite pool levels is somewhat larger than that of gross phenotypes (0.73%), some metabolites show rates of directional change on the order 2–6%. These extraordinary changes in pool size are not due to a few extreme lines (Figure 1B). On average, coefficients of mutational variation ($\text{CV}_{M, MA}$) for gross phenotypes and metabolome pool sizes are similar to those previously observed for life-history traits (around 1–2% generation), but a few metabolites show increases of up to 4%, among the largest reported for any trait (Houle et al. 1996). Mutational heritabilities average about $10^{-3}$, a typical value for gross phenotypes (Houle et al. 1996) but approximately an order of magnitude greater than the typical $h^2_{md}$ estimated for transcript abundance (Rifkin et al. 2005; Landry et al. 2007; McGuigan et al. 2014).

These results imply that the metabolome is much more subject to large effects of mutation than are gross phenotypes such as fitness, growth or behavior. Another striking difference can be seen in the direction of the mutational effects. Whereas $\Delta M$ of gross phenotypes is almost always negative, that is, mutations tend to cause the trait to decline in magnitude, for these extremely sensitive metabolites, $\Delta M$ is positive, that is, pool size increases.

**Correlation and modularity are both hallmarks of the metabolic mutational structure**

To assess the multivariate architecture of the metabolome, we first carried out PCA on the normalized metabolite concentrations (Supplementary Figure S1; Supplementary Table S3) and then addressed whether the principal components vary significantly among lines using the general linear model outlined in the Methods. Of the first eight principal components, there is significant among-line variance (i.e., $V_M$) for PC2-PC7 ($Z$-test, $P < 0.003$ in all cases); only PC1 does not vary significantly among MA lines. The non-heritable PC1 explains 25.8% of the total phenotypic variance, and presumably captures the variation due to microenvironmental fluctuations and/or experimental error. PC2 explains 18% of the phenotypic variance, and the first eight PCs collectively explain about 78% of the variance. Mutational heritabilities of PC2-PC7 are all on the order of $10^{-3}$/generation (Supplementary Table S4).

The substantial departure from uniformity of the eigenvalues of the PCs indicates the presence of underlying phenotypic correlations between metabolites (Supplementary Figure S2). To investigate the extent to which the PCs are themselves genetically correlated (i.e., coefficients of different PCs covary among MA lines), we compared a model in which the first eight PCs were allowed to covary among lines (unstructured covariance) to a model in which the off-diagonal elements of the among-line covariance matrix were constrained to zero, using AICc as the criterion for goodness-of-fit. The simpler model with the off-diagonals constrained to zero provides a more parsimonious fit (AICc = 4515.1 vs. AICc = 4530.6, relative likelihood $>2300$). This finding suggests that there are many potential avenues available for the evolution of the metabolome that are relatively unentangled by genetic constraints - or in other words, the metabolome has a modular structure. However,
caution is in order; some of the point estimates of the among-line correlations between PC eigenvectors are large (Supplementary Table S5), and the significantly better fit of the constrained model may simply reflect the limitations of power.

The association of metabolite pool size with fitness

Given that the production of progeny requires the synthesis and utilization of many metabolites, we might expect that metabolite pool sizes are correlated with fitness. Because the metabolites are themselves correlated, we initially carried out a PCA based on MA line means (Supplementary Table S6) and then calculated the correlations of relative fitness with the first seven PCs (Supplementary Table S7). PC3 is marginally significantly correlated with relative fitness ($r_{PC3,w} = 0.495$, df=34, $P<0.0069$, sequential Bonferroni-adjusted $P_{α=0.05} = 0.0071$).

These results suggest that it might be possible to predict the relative fitness of a given MA line from knowing its metabolome. We investigated the relationship between metabolites and relative fitness by means of stepwise multiple regression, using the scores of the first seven principal components calculated from line means as explanatory variables. One line (MA577) had a relative fitness of only about 1%, which is probably an anomalous measurement, so we omitted that line from the analysis. Beginning with a model with all variables (PC1-PC7), we removed variables stepwise by their F-value to find the best-fitting model, using AICc as the criterion for goodness-of-fit. The model with only PC3 has the smallest (best) AICc (6.1), but a model with only a random intercept fits almost as well (AICc=8.6, relative probability $\approx 3.5:1$).

DISCUSSION

Here we estimate, for the first time, the sensitivity of a metabolic network, or part of one, to spontaneous mutations (for a different but related perspective see Clark et al. 1995), who reported the effects of spontaneous mutations on the activities of 12 metabolic enzymes in Drosophila melanogaster). Obviously, there are many more than 29 metabolites present in a worm, so our view of the worm's metabolome is restricted. Nevertheless, this study lays the groundwork for future studies with greater coverage of the metabolome. More generally, it is one of the first phenomic studies of M beyond the transcriptome in any species.

By two criteria – the per-generational relative rate of directional change, $ΔM$, and the standardized per-generation rate of increase in genetic variation, $CV_M$ – we find that the pool sizes of metabolites vary greatly in their vulnerability to mutation. We also find that some metabolites are much more vulnerable to the directional effects of mutation than any fitness-related, morphological or behavioral trait studied until now. In other words, some metabolites show relatively little genetic canalization (cf Fraser and Schadt 2010).

Several studies have reported mutational heritabilities for large numbers of gene transcripts (Rifkin et al. 2005; Landry et al. 2007; McGuigan et al. 2014), and the average $h^2_M$ for transcript abundance is typically on the order of $10^{-4}$ or less. The contrast in mutational heritability between the transcriptome and the metabolome (or at least a small fraction of the metabolome) is noteworthy, inasmuch as the relative influence of genes and environment on
the metabolome appear more similar to those of gross phenotypic traits than of gene transcripts.

Although the mutational vulnerability of the metabolome per se has not been previously studied, the relative sensitivity of the metabolome to mutational perturbation is not unexpected (Clark et al. 1995). A variety of circumstantial evidence suggests that the metabolome is more sensitive to perturbation than are gross phenotypes. Indeed, metabolomics has been advocated as a way to study the functions of otherwise phenotypically “silent” mutations (Raamsdonk et al. 2001; Blaise et al. 2007). Metabolic networks function to maintain the flux of metabolites critical to fitness in the face of environmental and mutational perturbation, and they do so by redirecting flux through different parts of the network, which results in altered metabolite pool sizes.

The discovery of strong directional mutational biases in some metabolites prompts the question of how the integrity of the metabolome is preserved in nature. We found that several metabolites showed modest correlations with relative fitness across MA lines, collectively manifested in PC3, which implies that the metabolome is under selection. If metabolite pool sizes are at evolutionary equilibrium, however, then selection must act in a particular way: it must oppose the mutational bias for any given metabolite. For example, if mutational bias tends to increase pool size, then at equilibrium selection must oppose this increase, that is, there must be a negative genetic correlation between pool size and relative fitness. Is this in fact so? Of the 29 metabolites, 20 showed a significant directional bias and a significant mutational variance. For each of these 20, we asked whether $\Delta M$ and $r_{w, metabolite}$ were in the same or opposite directions. We found that in 10 cases, they were the same direction and 10 they were not; so there is no tendency for natural selection to oppose the mutational bias. Looking at it another way, of the four metabolites – succinic acid, xanthine, nicotinic acid and adenosine – that were most vulnerable to directional mutation, each having $\Delta M > 2\%$, none showed significant pairwise correlations with fitness, much less in a way implying selection against the mutations that altered their pool sizes so spectacularly. This suggests one of two possibilities: firstly, that some metabolite pool sizes are evolving rapidly under mutation pressure; or, secondly, and more plausibly, that our laboratory studies have not captured all of the fitness consequences of these mutations, and hence all of the selective forces that maintain the integrity of the metabolome. It should of course also be borne in mind that it is not generally meaningful to assign a single biological interpretation to the pool size of any one metabolite. Furthermore, our current analysis contains no information on cellular or tissue localization specific changes in metabolism, and while this a natural consequence of the methods we used, it does mean that future research could still pick up effects based on changes in metabolite concentrations within an individual that are not detected when looking at whole organism extracts.

Since evolution ultimately depends on new mutations, the presence of strong directional mutational biases and large mutational variances suggests that the evolution of the metabolome may be far less constrained than gross phenotypes. The presence of significant mutational heritability of seven of the first eight phenotypic principal components reinforces the conclusion that there is substantial scope for evolution in metabolic space, as does the lack of significant among-line covariance among those PCs. On the other hand, the skewed
distribution of the eigenvalues indicates the presence of significant phenotypic correlations, which may reflect underlying genetic constraint.

Recently, several studies have compared the vulnerability to mutation of various traits such as body size, fitness and vulval morphology among different strains and species of nematodes (Baer et al. 2005; Ostrow et al. 2007; Braendle et al. 2010). They have shown that species potentially vary substantially in the quality and quantity of new mutations affecting these traits, and that the cumulative mutational effects may be context-dependent (Matsuba et al. 2013). Given the size of the mutational effects detected here, it would be of great interest to know whether this is also true for the metabolome.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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LITERATURE CITED


Figure 1.
Mutational bias in metabolite pool size. A. Most metabolites show directional changes, of those that do, most decrease in pool size (25 show significant change at $p < 0.05$; 22 at $p < 0.005$, one-group t-test, $h_0 = 0$). Error bars show one SEM. See also Table S1 for estimates of $\Delta M$. B. Frequency distribution of pool sizes for individual lines for the four most rapidly changing metabolites: orange arrowheads indicate means of ancestral control line; green arrowheads indicate means of MA lines.