

1 Network Architecture and Mutational Sensitivity of the *C. elegans* Metabolome

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40

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63 **Abstract**

64 Background: A fundamental issue in evolutionary systems biology is understanding the
65 relationship between the topological architecture of a biological network, such as a metabolic
66 network, and the evolution of the network. The rate at which an element in a metabolic network
67 accumulates genetic variation via new mutations depends on both the size of the mutational
68 target it presents and its robustness to mutational perturbation. Quantifying the relationship
69 between topological properties of network elements and the mutability of those elements will
70 facilitate understanding the variation in and evolution of networks at the level of populations and
71 higher taxa.

72
73 Results: We report an investigation into the relationship between two topological properties of
74 29 metabolites in the *C. elegans* metabolic network and the sensitivity of those metabolites to
75 the cumulative effects of spontaneous mutation. The relationship between several measures of
76 network centrality and sensitivity to mutation is weak, but point estimates of the correlation
77 between network centrality and mutational variance are positive, with only one exception. There
78 is a marginally significant correlation between core number and mutational heritability. There is
79 a small but significant negative correlation between the shortest path length between a pair of
80 metabolites and the mutational correlation between those metabolites.

81
82 Conclusions: Positive association between the centrality of a metabolite and its mutational
83 heritability is consistent with centrally-positioned metabolites presenting a larger mutational
84 target than peripheral ones, and is inconsistent with centrality conferring mutational robustness,
85 at least *in toto*. The weakness of the correlation between shortest path length and the
86 mutational correlation between pairs of metabolites suggests that network locality is an
87 important but not overwhelming factor governing mutational pleiotropy. These findings provide

88 necessary background against which the effects of other evolutionary forces, most importantly
89 natural selection, can be interpreted.
90

91 **Introduction:**

92 The set of chemical reactions that constitute organismal metabolism is often represented
93 as a network of interacting components, in which individual metabolites are the nodes in the
94 network and the chemical reactions of metabolism are the edges linking the nodes [1].
95 Representation of a complex biological process such as metabolism as a network is
96 conceptually powerful because it offers a convenient and familiar way of visualizing the system,
97 as well as a well-developed mathematical framework for analysis.

98 If the representation of a biological system as a network is to be useful as more than a
99 metaphor, it must have predictive power [2]. Metabolic networks have been investigated in the
100 context of evolution, toward a variety of ends. Many studies have compared empirical metabolic
101 networks to various random networks, with the goal of inferring adaptive features of network
102 architecture (e.g., [1, 3-7]. Other studies have addressed the relationship between network-
103 level properties of individual elements of the network (e.g., node degree, centrality) and
104 properties such as rates of protein evolution [8, 9] and within-species polymorphism [10].

105 One fundamental evolutionary process that remains essentially unexplored with respect
106 to metabolic networks is mutation. Mutation is the ultimate source of genetic variation, and as
107 such provides the raw material for evolution: the greater the input of genetic variation by
108 mutation, the greater the capacity for evolution. However, in a well-adapted population, most
109 mutations are at least slightly deleterious. At equilibrium, the standing genetic variation in a
110 population represents a balance between the input of new mutations that increase genetic
111 variation and reduce fitness, and natural selection, which removes deleterious variants and
112 thereby increases fitness. Because genetic variation is jointly governed by mutation and
113 selection, understanding the evolution of any biological entity, such as a metabolic network,
114 requires an independent accounting of the effects of mutation and selection.

115 The cumulative effects of spontaneous mutations can be assessed in the near absence
116 of natural selection by means of a mutation accumulation (MA) experiment (Figure 1). Selection

117 becomes ineffective relative to random genetic drift in small populations, and mutations with
118 effects on fitness smaller than about the reciprocal of the population size (technically, the
119 genetic effective population size, N_e) will be essentially invisible to natural selection [11]. An MA
120 experiment minimizes the efficacy of selection by minimizing N_e , thereby allowing all but the
121 most strongly deleterious mutations to evolve as if they are invisible to selection [12].

122 Our primary interest is in the relationship between the centrality of a metabolite in the
123 network and the sensitivity of that metabolite to mutation. Roughly speaking, the centrality of a
124 node in a network quantifies some measure of the importance of the node in the network [13].
125 A generic property of empirical networks, including metabolic networks, is that they are
126 (approximately) scale-free; scale-free networks are characterized by a topology with a few "hub"
127 nodes (high centrality) and many peripheral nodes (low centrality; [1]). Scale-free networks are
128 more robust to random perturbation than are randomly-connected networks [14].

129 Mutation is an important source of perturbation to biological systems, and much effort
130 has gone into theoretical and empirical characterization of the conditions under which
131 mutational robustness will evolve [15-17]. Mutational robustness can be assessed in two basic
132 ways: top-down, in which a known element of the system is mutated and the downstream
133 effects of the mutation quantified, or bottom-up, in which mutations are introduced at random,
134 either spontaneously or by mutagenesis, and the downstream effects quantified. Top-down
135 experiments are straightforward to interpret: the greater the effects of the mutation (e.g., on a
136 phenotype of interest), the less robust the system. However, the scope of inference is limited to
137 the types of mutations introduced by the investigator (which in practice are almost always gene
138 knockouts), and provide limited insight into natural variation in mutational robustness.

139 Bottom-up approaches, in which mutations are allowed to accumulate at random,
140 provide insight into the evolution of a system as it actually exists in nature: all else equal, a
141 system, or element of a system ("trait"), that is robust to the effects of mutation will accumulate
142 less genetic variance under MA conditions than one that is not robust (Figure 1b; [18]).

143 However, the inference is not straightforward, because all else may not be equal: different
144 systems or traits may present different mutational targets (roughly speaking, the number of sites
145 in the genome that potentially affect a trait; [19]).

146 Ultimately, disentangling the evolutionary relationship between network architecture,
147 mutational robustness, and mutational target is an empirical enterprise, specific to the system of
148 interest. As a first step, it is necessary to establish the relationship between network
149 architecture (e.g., topology) and the rate of accumulation of genetic variance under MA
150 conditions. If a general relationship emerges, targeted top-down experiments can then be
151 employed to dissect the relationship in more mechanistic detail.

152 In addition to the relationship between metabolite centrality and mutational variance, we
153 are also interested in the relationship between network topology and the mutational correlation
154 (r_M) between pairs of metabolites (Figure 1c). In principle, mutational correlations reflect
155 pleiotropic relationships between genes underlying pairs of traits (but see below for caveats;
156 [20]). Genetic networks are often modular [21], consisting of groups of genes (modules) within
157 which pleiotropy is strong and between which pleiotropy is weak [22]. Genetic modularity
158 implies that mutational correlations will be negatively correlated with the length of the shortest
159 path between network elements. However, it is possible that the network of gene interactions
160 underlying metabolic regulation is not tightly correlated with the metabolic network itself, e.g., if
161 *trans* acting regulation predominates.

162 Here we report results from a long-term MA experiment in the nematode *Caenorhabditis*
163 *elegans*, in which replicate MA lines derived from a genetically homogeneous common ancestor
164 (G0) were allowed to evolve under minimally effective selection ($N_e \approx 1$) for approximately 250
165 generations (Figure 1a). We previously reported estimates from these MA lines of two key
166 quantitative genetic parameters by which the cumulative effects of mutation can be quantified:
167 the per-generation change in the trait mean (the mutational bias, ΔM) and the per-generation
168 increase in genetic variation (the mutational variance, V_M) for the standing pools of 29

169 metabolites [23]; Supplementary Table S1. In this report, we interpret those results, and new
170 estimates of mutational correlations (r_M), in the context of the topology of the *C. elegans*
171 metabolic network.

172

173 **Results and Discussion**

174 Representation of the Metabolic Network – The metabolic network of *C. elegans* was estimated
175 in two ways: (i) by the method of Ma and Zeng [24; MZ], and (ii) by the method of Yilmaz and
176 Walhout [25; YW]. Details of the network construction are given in section I of the Methods;
177 data are presented in Supplementary Appendix A1. For the set of metabolites included (see
178 Methods), MZ and YW give nearly identical results. In the few cases in which there is a
179 discrepancy (~1%; Supplementary Table S2), we use the MZ network, for reasons we explain in
180 the Methods. The resulting network is a directed graph including 646 metabolites, with 1203
181 reactions connecting nearly all metabolites (Figure 2).

182 Properties of networks can be quantified in many ways, and different measures of
183 centrality capture different features of network importance (Table 1). We did not have a strong
184 prior hypothesis about which specific measure(s) of centrality would prove most informative in
185 terms of a relationship with ΔM and/or V_M . Therefore, we assessed the relationship between
186 mutational properties and several measures of network centrality: betweenness, closeness, and
187 degree centrality, in- and out-degree, and core number (depicted in Figure 3). These
188 parameters are all positively correlated. Definitions of the parameters are given in Table 1;
189 correlations between the parameters are included in Table 2. For each of the six parameters,
190 we calculated Spearman's correlation ρ between mutational statistics and the network
191 parameter associated with the metabolite. The strict experiment-wide 5% significance level for
192 these correlations is approximately $P < 0.002$ ($\alpha = 0.05 / [6 \text{ network parameters} \times 4 \text{ mutational}$
193 $\text{parameters}]$).

194 Mutational Parameters – Details of the MA experiment are reported in [26] and outlined in
195 section II of the Methods. The experimental protocol by which metabolite concentrations were
196 measured is reported in [23] and outlined in section III of the Methods; data are archived in
197 Dryad at <http://dx.doi.org/10.5061/dryad.2dn09/1>. For each of the 29 metabolites, the
198 cumulative effects of mutation are summarized by the mutational bias (ΔM), and the mutational
199 variance (V_M). For a trait z , $\Delta M_z = \mu_G \alpha_z$, where μ_G is the genomic mutation rate and α_z is the
200 average effect of a mutation on the trait; $V_M = \mu_G \alpha_z^2$ [27, p. 329]. Details of the estimation of
201 mutational parameters are given in section IV of the Methods.

202 Comparisons of variation among traits or groups require that the variance be measured
203 on a common scale. V_M is commonly scaled either relative to the trait mean, in which case V_M is
204 the squared coefficient of variation and is often designated I_M , or relative to the residual
205 variance, V_E ; V_M/V_E is the mutational heritability, h_M^2 . I_M and h_M^2 have different statistical
206 properties and evolutionary interpretations [28], so we report both. I_M and I_E are standardized
207 relative to the mean of the MA lines.

208 Network centrality and sensitivity to mutation –

209 (i) *Mutational bias (ΔM)*. It is reasonable to expect that metabolite concentrations are under
210 some degree of stabilizing selection, in which case sufficiently large changes in either direction
211 are deleterious. Neither ΔM nor $|\Delta M|$ showed a clear association with any measure of network
212 centrality (Table 1). Four metabolites (adenosine, nicotinamide, succinic acid, and xanthine)
213 have atypically large, positive ΔM (Supplementary Table S1), among the largest values of ΔM
214 ever reported for any trait [23], and those four metabolites all have core number $k = 2$ ($P = 0.03$,
215 exact probability). However, the large ΔM for those traits is probably an artifact of scaling,
216 because those four metabolites had very low concentrations (near zero) in the G0 ancestor.

217 (ii) *Mutational variance (V_M)*. We report V_M scaled in two ways: relative to the trait mean (I_M) and
218 relative to the residual ("environmental") variance, h_M^2 . Of the twelve correlations (two measures
219 of mutational variance \times six measures of centrality), the correlation with betweenness centrality

220 is very slightly negative; the rest are positive (Table 2), although only the correlation between
221 core number and h_M^2 approaches statistical significance at the experiment-wide 5% level
222 ($\rho=0.48$, $P<0.008$). The 29 metabolites in our data set have core number of either one or two
223 (the maximum core number of any metabolite in the network is two). Mean h_M^2 of metabolites of
224 core number = 2 is approximately 2.5X greater than that of metabolites of core number = 1
225 (0.002 vs. 0.0008). To put that result in context, the average h_M^2 for a wide variety of traits in a
226 wide variety of organisms is on the order of 0.001 [28].

227 Core number is a discrete interval variable, whereas the other measures of network
228 centrality are continuous variables. As an alternative analysis, we performed ordinary linear
229 regression (equivalent to analysis of variance in the case of a binary categorical variable) of
230 $\log(h_M^2)$ on core number; the results are similar to the rank correlation ($F_{1,27} = 10.53$, $P<0.0032$;
231 Pearson's $r = 0.53$).

232 The conservative interpretation of these results is that there is no relationship between
233 network centrality and any measure of mutational sensitivity. If so, there are various possible
234 explanations. For example, it may be that mutational target and mutational robustness
235 effectively cancel each other out. More worryingly, it may be that the representation of the *C.*
236 *elegans* metabolic network used here misrepresents the network as it actually exists *in vivo*.
237 The topology of the dynamic metabolic network of the bacterium *E. coli* varies depending on the
238 environmental context [29], and it seems intuitive that the greater spatiotemporal complexity
239 inherent to a multicellular organism would exacerbate that problem. More mundanely, it may be
240 that the sampling variance associated with the relatively small number of mutations and MA
241 lines drowns out any signal of an association. Or it may be that there simply is no functional
242 relationship between the centrality of a metabolite in a network and its sensitivity to mutation.

243 The liberal interpretation is that the near-significant correlation of mutational heritability
244 with core number represents a weak signal emerging from a small sample from a noisy system.

245 Quantifying centrality in terms of core number is analogous to categorizing a set of size
246 measurements into "small" and "large": power is increased, at the cost of losing the ability to
247 discriminate between more subtle differences.

248 The raw mutational variance, V_M , appears in the numerator of both h_M^2 and I_M ; the
249 difference lies in the denominator, which is the residual variance V_E for h_M^2 and the square of the
250 trait mean for I_M . For some replicates of some metabolites, estimated metabolite concentrations
251 were atypically low and near zero; I_M is more sensitive to low outliers than is h_M^2 . However, the
252 correlation between I_M and the trait mean is small ($r = -0.11$) and not significantly different from
253 zero. Alternatively, it is possible that V_M does not vary consistently with metabolite centrality,
254 but that metabolites with low centrality (core number = 1) are more susceptible to random
255 microenvironmental variation ("noise") than are metabolites with high centrality (core number =
256 2), in which case V_E would be greater for metabolites with low centrality and h_M^2 would be lower.
257 Unfortunately, the variance is correlated with the trait mean, so the least biased way to address
258 that question is by comparing the residual squared coefficients of variation, I_E . There is no hint
259 of correlation between core number and I_E ($\rho=0.025$, $P>0.89$; Table 2), and I_E is uncorrelated
260 with the trait mean ($r = -0.12$, $P>0.54$), so the association between h_M^2 and core number cannot
261 obviously be attributed to differential sensitivity to random noise.

262 The relationship between mutational correlation (r_M) and shortest path length – In an MA
263 experiment, the cumulative effects of mutations on a pair of traits i and j may covary for two,
264 nonexclusive reasons [20]. More interestingly, individual mutations may have consistently
265 pleiotropic effects, such that mutations that affect trait i also affect trait j in a consistent way.
266 Less interestingly, but unavoidably, individual MA lines will have accumulated different numbers
267 of mutations, and if mutations have consistently directional effects, as would be expected for
268 traits correlated with fitness, lines with more mutations will have more extreme trait values than
269 lines with fewer mutations, even in the absence of consistent pleiotropy. Estes et al. [20]

270 simulated the sampling process in *C. elegans* MA lines with mutational properties derived from
271 empirical estimates from a variety of traits and concluded that sampling is not likely to lead to
272 large absolute mutational correlations in the absence of consistent pleiotropy ($|r_M| \leq 0.25$).

273 Ideally, we would like to estimate the full mutational (co)variance matrix, \mathbf{M} , from the joint
274 estimate of the among-line (co)variance matrix. However, with 25 traits, there are $(25 \times 26)/2 =$
275 325 covariances, and with only 43 MA lines, there is insufficient information to jointly estimate
276 the restricted maximum likelihood of the full \mathbf{M} matrix. To proceed, we calculated mutational
277 correlations from pairwise REML estimates of the among-line (co)variances, i.e., $r_M =$
278 $\frac{COV_L(X,Y)}{\sqrt{VAR_L(X)VAR_L(Y)}}$ [30, 31]. Pairwise estimates of r_M are shown in Supplementary Table S3. To
279 assess the extent to which the pairwise correlations are sensitive to the underlying covariance
280 structure, we devised a heuristic bootstrap analysis. For a random subset of 12 of the 300 pairs
281 of traits, we randomly sampled six of the remaining 23 traits without replacement and estimated
282 r_M between the two focal traits from the joint REML among-line (co)variance matrix. For each of
283 the 12 pairs of focal traits, we repeated the analysis 100 times.

284 There is a technical caveat to the preceding bootstrap analysis. Resampling statistics
285 are predicated on the assumption that the variables are exchangeable [32], which metabolites
286 are not. For that reason, we do not present confidence intervals on the resampled correlations,
287 only the distributions. However, we believe that the analysis provides a meaningful heuristic by
288 which the sensitivity of the pairwise correlations to the underlying covariance structure can be
289 assessed.

290 Distributions of resampled correlations are shown in Supplementary Figure S2. In every
291 case the point estimate of r_M falls on the mode of the distribution of resampled correlations, and
292 in 11 of the 12 cases, the median of the resampled distribution is very close to the point
293 estimate of r_M . However, in six of the 12 cases, some fraction of the resampled distribution falls
294 outside two standard errors of the point estimate. The most important point that the resampling

295 analysis reveals is this: given that 29 metabolites encompass only a small fraction of the total
296 metabolome of *C. elegans* (<5%), even had we been able to estimate the joint likelihood of the
297 full $29 \times 30/2$ M -matrix, the true covariance relationships among those 29 metabolites could
298 conceivably be quite different from those estimated from the data.

299 Correlations are properties of pairs of variables, so we expect *a priori* that network
300 parameters that apply to pairs of elements are more likely to contain information about the
301 mutational correlation between a pair of metabolites than will the pairwise average of a
302 parameter that applies to individual elements of a network. The shortest path length is the
303 simplest network property that describes the relationship between two nodes, although since the
304 metabolic network is directed, the shortest path from element i to element j is not necessarily
305 the same as the shortest path from j to i . For each pair of metabolites i and j , we calculated the
306 shortest path length from i to j and from j to i , without repeated walks (Supplementary Table S4).
307 We then calculated Spearman's correlation ρ between the mutational correlation r_M and the
308 shortest path length.

309 Statistical assessment of the correlation between mutational correlations (r_M) and
310 shortest path length presents a problem of nonindependence, for two reasons. First, all
311 correlations including the same variable are non-independent. Second, even though the
312 mutational correlation between metabolites i and j is the same as the correlation between j and
313 i , the shortest path lengths need not be the same, and moreover, the path from i to j may exist
314 whereas the path from j to i may not. To account for non-independence of the data, we devised
315 a parametric bootstrap procedure; details are given in section V of the Methods. Three
316 metabolites (L-tryptophan, L-lysine, and Pantothenate) lie outside of the great strong component
317 of the network [33] and are omitted from the analysis.

318 There is a weak, but significant, negative correlation between r_M and the shortest path
319 length between the two metabolites ($\rho = -0.128$, two-tailed $P < 0.03$; Supplementary Figure S1a),
320 whereas $|r_M|$ is not significantly correlated with shortest path length ($\rho = -0.0058$, two-tailed

321 $P > 0.45$; Supplementary Figure S1b). The correlation between r_M and the shortest path in the
322 undirected network is similar to the correlation between r_M and the shortest path in the directed
323 network ($\rho = -0.105$, two-tailed $P > 0.10$; Supplementary Figure S1c).

324 An intuitive possible cause of the weak negative association between shortest path
325 length and mutational correlation would be if a mutation that perturbs a metabolic pathway
326 toward the beginning of the pathway has effects that propagate downstream in the same
327 pathway, but the effect of the perturbation attenuates. The attenuation could be due either to
328 random noise or to the effects of other inputs into the pathway downstream from the
329 perturbation (or both). The net effect would be a characteristic pathway length past which the
330 mutational effects on two metabolites are uncorrelated, leading to an overall negative correlation
331 between r_M and path length. The finding that the correlations between r_M and the shortest path
332 length in the directed and undirected network are very similar reinforces that conclusion. The
333 negative correlation between r_M and shortest path length is reminiscent of a finding from
334 Arabidopsis, in which sets of metabolites significantly altered by single random gene knockouts
335 are closer in the global metabolic network than expected by chance [34].

336 Conclusions and Future Directions

337 The proximate goal of this study was to find out if there are topological properties of the *C.*
338 *elegans* metabolic network (node centrality, shortest path length) that are correlated with a set
339 of statistical descriptions of the cumulative effects of spontaneous mutations (ΔM , V_M , r_M).

340 Ultimately, we hope that a deeper understanding of those mathematical relationships will shed
341 light on the mechanistic biology of the organism. Bearing in mind the statistical fragility of the
342 results, we conclude:

343 *(i) Network centrality may be associated with mutational sensitivity (V_M), it is not associated with*
344 *mutational robustness ($1/V_M$).* If the liberal interpretation of the results is true, the most plausible
345 explanation is that metabolites that are central in the network present a larger mutational target
346 than do metabolites that peripherally located. However, although $1/V_M$ is a meaningful measure

347 of mutational robustness [35], it does not necessarily follow that highly-connected metabolites
348 are therefore more robust to the effects of *individual* mutations [19, 36].

349 *(ii) Pleiotropic effects of mutations affecting the metabolome are predominantly local, as*
350 evidenced by the significant negative correlation between shortest path length between a pair of
351 metabolites and the mutational correlation, r_M , between that pair of metabolites. That result is
352 not surprising in hindsight, but the weakness of the correlation suggests that there are other
353 important factors that underlie pleiotropy beyond network proximity.

354 To advance understanding of the mutability of the *C. elegans* metabolic network, three
355 things are needed. First, it will be important to cover a larger fraction of the metabolic network.
356 Untargeted mass spectrometry of cultures of *C. elegans* reveals many thousands of features
357 (Art Edison, personal communication); 29 metabolites are only the tip of a large iceberg. For
358 example, our intuition leads us to believe that the mutability of a metabolite will depend more on
359 its in-degree (mathematically, the number of edges leading into a node in a directed graph;
360 biochemically, the number of reactions in which the metabolite is a product) than its out-degree.
361 The point-estimate of the correlation of h_M^2 with in-degree is twice that of the correlation of h_M^2
362 with out-degree (Table 2), although the difference is not statistically significant.

363 Second, to more precisely partition mutational (co)variance into within- and among-line
364 components, more MA lines are needed. We estimate that each MA line carries about 80
365 unique mutations (see Methods), thus the mutational (co)variance is the result of about 3500
366 total mutations, distributed among 43 MA lines. The MA lines were a preexisting resource, and
367 the sample size was predetermined. It is encouraging that we were able to detect significant
368 mutational variance for 25/29 metabolites (Supplementary Table S1b), but only 14% (42/300) of
369 pairwise mutational correlations are significantly different from zero at the experiment-wide 5%
370 significance level, roughly corresponding to $|r_M| > 0.5$ (Supplementary Table S3); 18 of the 42
371 significant mutational correlations are not significantly different from $|r_M| = 1$. It remains
372 uncertain how sensitive estimates of mutational correlations are to the underlying covariance

373 structure of the metabolome. It also remains to be seen if the mutability of specific features of
374 metabolic networks are genotype or species-specific, and the extent to which mutability
375 depends on environmental context.

376 Third, it will be important to quantify metabolites (static concentrations and fluxes) with
377 more precision. The metabolite data analyzed in this study were collected from large cultures
378 ($n > 10,000$ individuals) of approximately age-synchronized worms, and were normalized relative
379 to an external quantitation standard [23]. Ideally, one would like to characterize the
380 metabolomes of single individuals, assayed at the identical stage of development. That is not
381 yet practical with *C. elegans*, although it is possible to quantify hundreds of metabolites from a
382 sample of 1000 individuals [37], and preliminary studies suggest it will soon be possible to
383 reduce the number of individuals to 100 or even ten (M. Witting, personal communication).
384 Minimizing the number of individuals in a sample is important for two reasons; (1) the smaller
385 the sample, the easier it is to be certain the individuals are closely synchronized with respect to
386 developmental stage, and (2) knowing the exact number of individuals in a sample makes
387 normalization relative to an external standard more interpretable. Ideally, data would be
388 normalized relative to both an external standard and an internal standard (e.g., total protein;
389 [30]).

390 This study provides an initial assessment of the relationship between mutation and
391 metabolic network architecture. To begin to uncover the relationship between metabolic
392 architecture and natural selection, the next step is to repeat these analyses with respect to the
393 standing genetic variation (VG). There is some reason to think that more centrally-positioned
394 metabolites will be more evolutionarily constrained (i.e., under stronger purifying selection) than
395 peripheral metabolites [8], in which case the ratio of the mutational variance to the standing
396 genetic variance (VM/VG) will increase with increasing centrality.

397

398 **Methods and Materials:**

399 I. Metabolic Network:

400 The metabolic network of *C. elegans* was estimated in two ways: (i) by the static, purely
401 graphical method of Ma and Zeng ([24]; updated at <http://www.ibiodesign.net/kneva/>; we refer to
402 this method as MZ), and (ii) by the dynamical, flux-balance analysis (FBA) method of Yilmaz
403 and Walhout ([25]; <http://wormflux.umassmed.edu/>; we refer to this method as YW).

404 Subnetworks that do not contain at least one of the 29 metabolites were excluded from
405 downstream analyses. The MZ method includes several *ad hoc* criteria for retaining or omitting
406 specific metabolites from the analysis (criteria are listed on p. 272 of [24]). The set of reactions
407 in the MZ and YW networks are approximately 99% congruent; in the few cases in which there
408 is a discrepancy (listed in Supplementary Table S2), we chose to use the MZ network because
409 we used the MZ criteria for categorizing currency metabolites (defined below).

410 To begin, the 29 metabolites of interest were identified and used as starting sites for the
411 network. Next, all forward and reverse reactions stemming from the 29 metabolites were
412 incorporated into the subnetwork until all reactions either looped back to the starting point or
413 reached an endpoint. Currency metabolites were removed following the MZ criteria (a currency
414 metabolite is roughly defined as a molecule such as water, proton, ATP, NADH, etc., that
415 appears in a large fraction of metabolic reactions but is not itself an intermediate in an
416 enzymatic pathway). Metabolic networks in which currency metabolites are included have much
417 shorter paths than networks in which they are excluded. When currency metabolites are
418 included in the network reported here, all shortest paths are reduced to no more than three
419 steps, and most shortest paths consist of one or two steps. The biological relevance of path
420 length when currency metabolites are included in the network is unclear [24].

421 A graphical representation of the network was constructed with the Pajek software
422 package (<http://mrvar.fdv.uni-lj.si/pajek/>) and imported into the networkX Python package [38],
423 which was used to generate network statistics. Proper importation from Pajek to networkX was
424 verified by visual inspection.

425 II. Mutation Accumulation Lines

426 A full description of the construction and propagation of the mutation accumulation (MA) lines is
427 given in [26]. Briefly, 100 replicate MA lines were initiated from a nearly-isogenic population of
428 N2-strain *C. elegans* and propagated by single-hermaphrodite descent at four-day (one
429 generation) intervals for approximately 250 generations. The long-term N_e of the MA lines is
430 very close to one, which means that mutations with a selective effect less than about 25% are
431 effectively neutral [39]. The common ancestor of the MA lines ("G0") was cryopreserved at the
432 outset of the experiment; MA lines were cryopreserved upon completion of the MA phase of the
433 experiment. Based on extensive whole-genome sequencing [40; A. Saxena and CFB, in prep],
434 we estimate that each MA line carries about 80 mutant alleles in the homozygous state.

435 At the time the metabolomics experiments reported in [23] were initiated, approximately
436 70 of the 100 MA lines remained extant, of which 43 ultimately provided sufficient material for
437 Gas Chromatography/Mass Spectrometry (GC-MS). Each MA line was initially replicated five-
438 fold, although not all replicates provided data of sufficient quality to include in subsequent
439 analyses; the mean number of replicates included per MA line is 3.9 (range = 2 to 5). The G0
440 ancestor was replicated nine times. However, the G0 ancestor was not subdivided into
441 "pseudolines" [41], which means that inferences about mutational variances and covariances
442 are necessarily predicated on the assumption that the among-line (co)variance of the ancestor
443 is zero. Each replicate consisted of age-synchronized young-adult stage worms taken from a
444 single 10 cm agar plate.

445 III. Metabolomics:

446 Details of the extraction and quantification of metabolites are given in [23]. Briefly, samples were
447 analyzed using an Agilent 5975c quadrupole mass spectrometer with a 7890 gas
448 chromatograph. Metabolites were identified by comparison of GC-MS features to the Fiehn
449 Library [42] using the AMDIS deconvolution software [43], followed by reintegration of peaks
450 using the GAVIN Matlab script [44]. Metabolites were quantified and normalized relative to an

451 external quantitation standard. 34 metabolites were identified, of which 29 were ultimately
452 included in the analyses. Normalized metabolite data are archived in Dryad
453 (<http://dx.doi.org/10.5061/dryad.2dn09>).

454 IV. Quantitative Genetic Analyses: There are three quantitative genetic parameters of interest:
455 (i) the per-generation proportional change in the trait mean, referred to as the mutational bias,
456 ΔM ; (ii) the per-generation increase in the genetic variance, referred to as the mutational
457 variance, V_M ; and (iii) the genetic correlation between the cumulative effects of mutations
458 affecting pairs of traits, the mutational correlation, r_M . Details of the calculations of ΔM and V_M
459 are reported in [23]; we reprise the basic calculations here.

460 (i) *Mutational bias (ΔM)* – The mutational bias is the change in the trait mean due to the
461 cumulative effects of all mutations accrued over one generation. $\Delta M_z = \mu_G \alpha_z$, where μ_G is the per-
462 genome mutation rate and α_z is the average effect of a mutation on trait z , and is calculated as
463 $\Delta M_z = \frac{\bar{z}_{MA} - \bar{z}_0}{t \bar{z}_0}$, where \bar{z}_{MA} and \bar{z}_0 represent the MA and ancestral (G0) trait means and t is the
464 number of generations of MA.

465 (ii) *Mutational variance (V_M)* - The mutational variance is the increase in the genetic variance
466 due to the cumulative effects of all mutations accrued over one generation. $V_M = \mu_G \alpha_z^2$ and is
467 calculated as $V_M = \Delta V_L = \frac{V_{L,MA} - V_{L,G0}}{2t}$, where $V_{L,MA}$ is the variance among MA lines, $V_{L,G0}$ is the
468 among-line variance in the G0 ancestor, and t is the number of generations of MA [27, p. 330].
469 In this study, we must assume that $V_{L,G0} = 0$.

470 (iii) *Mutational correlation, r_M* – Pairwise mutational correlations were calculated from the
471 among-line components of (co)variance, which were estimated by REML as implemented in the
472 in the MIXED procedure of SAS v. 9.4, following JD Fry [45]. Statistical significance of
473 individual correlations was assessed by Z-test, with a global 5% significance criterion of
474 approximately $P < 0.000167$.

475 V. Analysis of the correlation between mutational correlation (r_M) and shortest path length -

476 Each off-diagonal element of the 24x24 mutational correlation matrix ($r_{ij}=r_{ji}$) was associated with
477 a random shortest path length sampled with probability equal to its frequency in the empirical
478 distribution of shortest path lengths between all metabolites included in the analysis. Next, we
479 calculated the Spearman's correlation ρ between r_M and the shortest path length. The
480 procedure was repeated 10,000 times to generate an empirical distribution of ρ , to which the
481 observed ρ can be compared. This comparison was done for the raw mutational correlation, r_M ,
482 the absolute value, $|r_M|$, and between r_M and the shortest path length in the undirected network
483 (i.e., the shorter of the two paths between metabolites i and j).

484

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490

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600

601

Figure Legends

Figure 1. (a) Schematic diagram of the mutation accumulation (MA) experiment. An MA experiment is simply a pedigree. The genetically homogeneous ancestral line (G0) was subdivided into 100 MA lines, of which 43 are included in this study. Lines were allowed to accumulate mutations for $t=250$ generations. At each generation, lines were propagated by a single randomly chosen hermaphrodite ($N=1$). Mutations, represented as colored blocks within a homologous pair of chromosomes, arise initially as heterozygotes and are either lost or fixed over the course of the experiment. At the culmination of the experiment, each line has accumulated its own unique set of mutations. MA lines were compared to the cryopreserved G0 ancestor, which is wild-type at all loci. After [12]. (b) Expected outcome of an MA experiment. As mutations accumulate over time, relative fitness (solid dark blue line) declines from its initial value of 1 at rate ΔM per generation and the genetic component of variance (solid orange line) increases from its initial value of 0 at rate V_M per generation. Trait X (light blue dashed line) is positively correlated with fitness and declines with MA; trait Y (green dashed line) is negatively correlated with fitness and increases with MA. Trajectories are depicted as linear, but they need not be. (c) Accumulation of mutational covariance in an MA experiment. Coordinate axes represent two traits, X and Y. Concentric ellipses show the increase in genetic covariance with MA, beginning from the initial value of zero; the orientation of the ellipses represents the linear relationship between pleiotropic mutational effects on the two traits.

Figure 2. Graphical depiction of the metabolic network including all 29 metabolites. Pink nodes represent included metabolites with core number = 1, red nodes represent included metabolites with core number = 2. Gray nodes represent metabolites with which the included 29 metabolites directly interact. Metabolite identification numbers are: 1, L-Serine; 2, Glycine; 3, Nicotinate; 4, Succinate; 5, Uracil; 6, Fumarate; 7, L-Methionine; 8, L-Alanine. 9, L-Aspartate;

10, L-3-Amino-isobutanoate; 11, trans-4-Hydroxy-L-proline; 12, (S) – Malate; 13, 5-Oxoproline; 14, L-Glutamate; 15, L-Phenylalanine; 16, L-Asparagine; 17, D-Ribose; 18, Putrescine; 19, Citrate; 20, Adenine; 21, L-Lysine; 22, L-Tyrosine; 23, Pantothenate; 24, Xanthine; 25, Hexadecanoic acid; 26, Urate; 27, L-Tryptophan; 28, Adenosine; 29, Alpha;alpha-Trehalose.

Figure 3. Schematic depiction of the k -cores of a graph. The k -core of a graph is the largest subgraph that contains nodes of degree at least k . The colored balls represent nodes in a network and the black lines represent connecting edges. Each red ball in the darkest gray area has core number $k=3$; note that each node with $k=3$ is connected to at least three other nodes. After Batagelj and Zaveršnik [46].

Figure 1

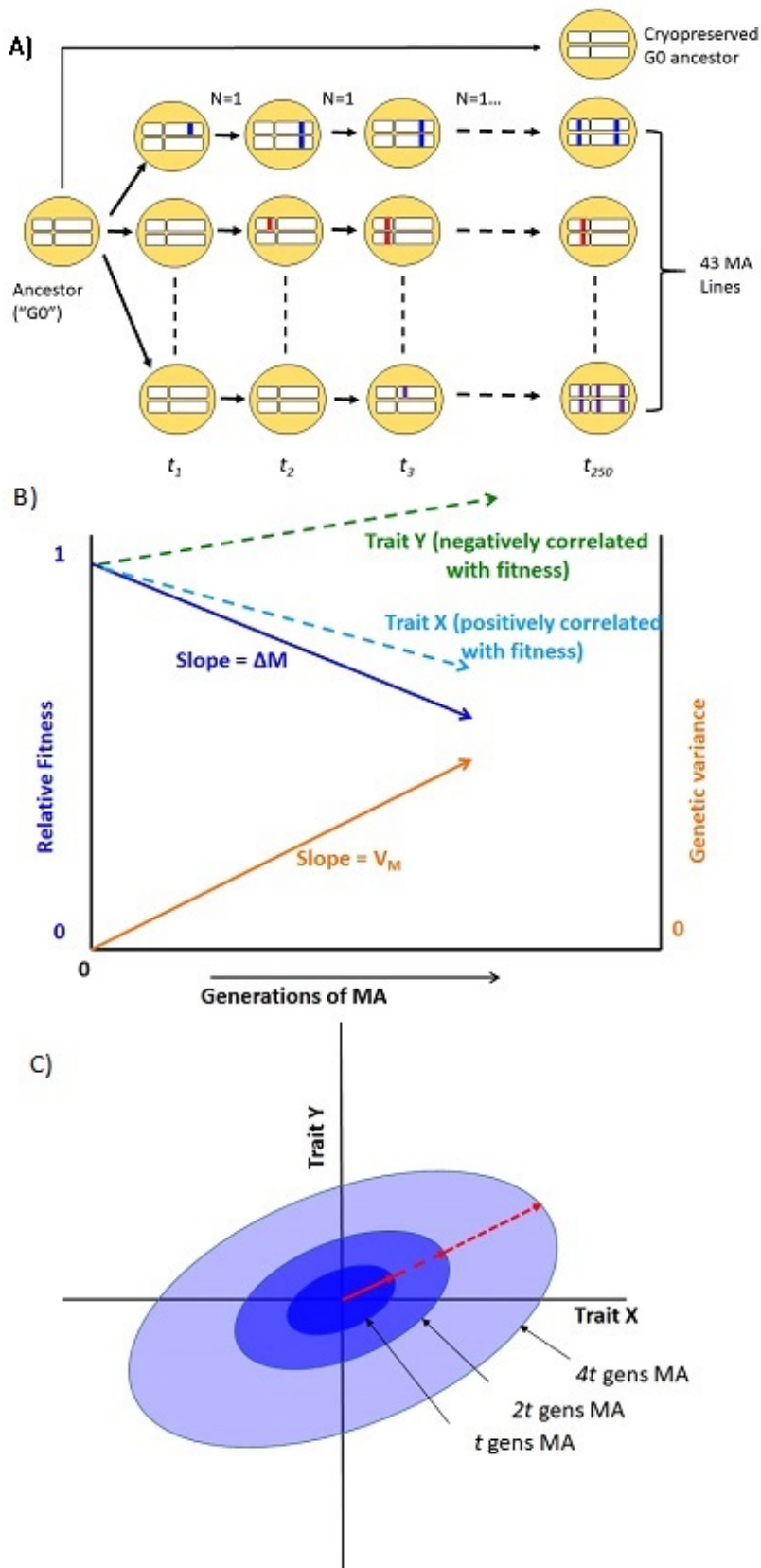


Figure 2

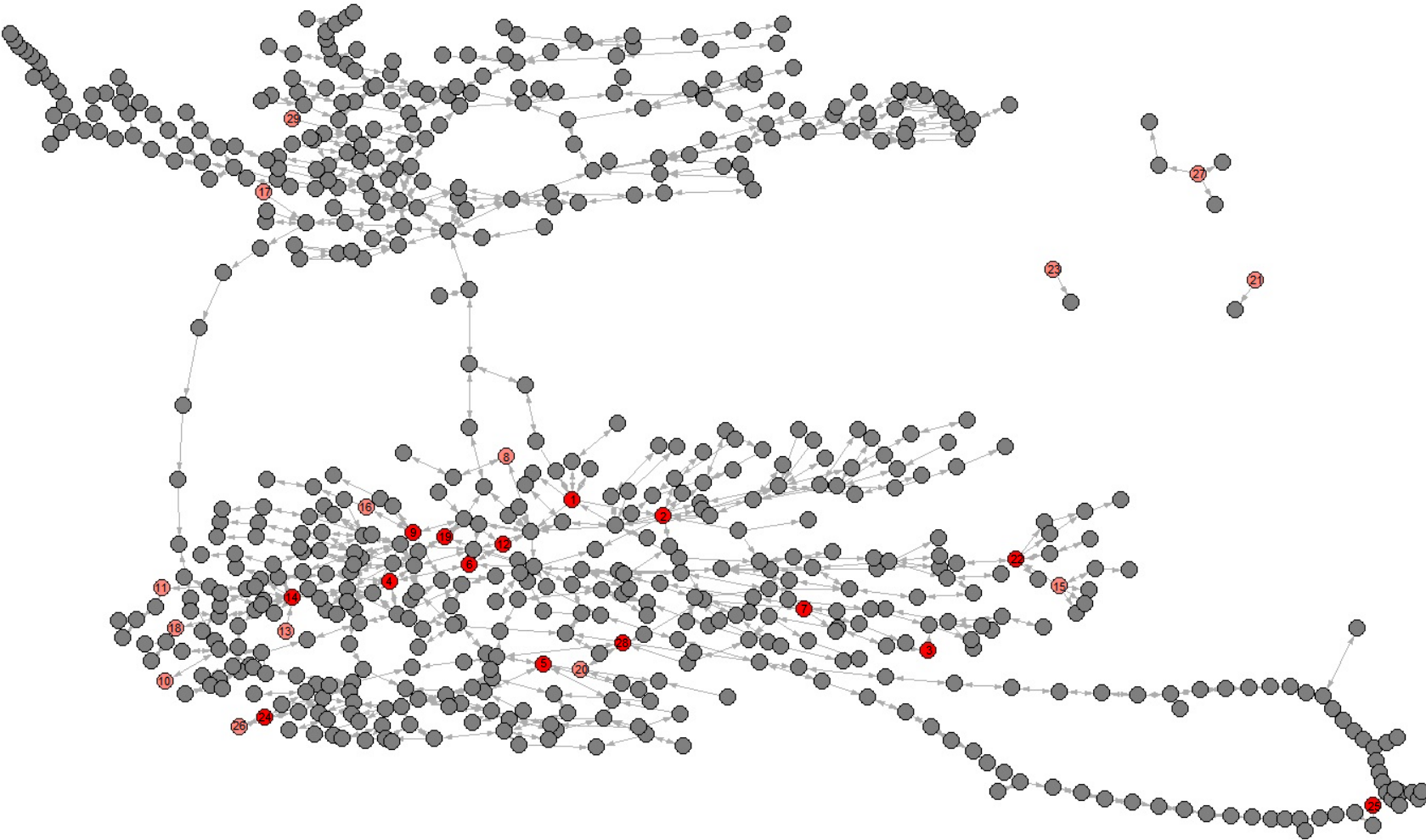
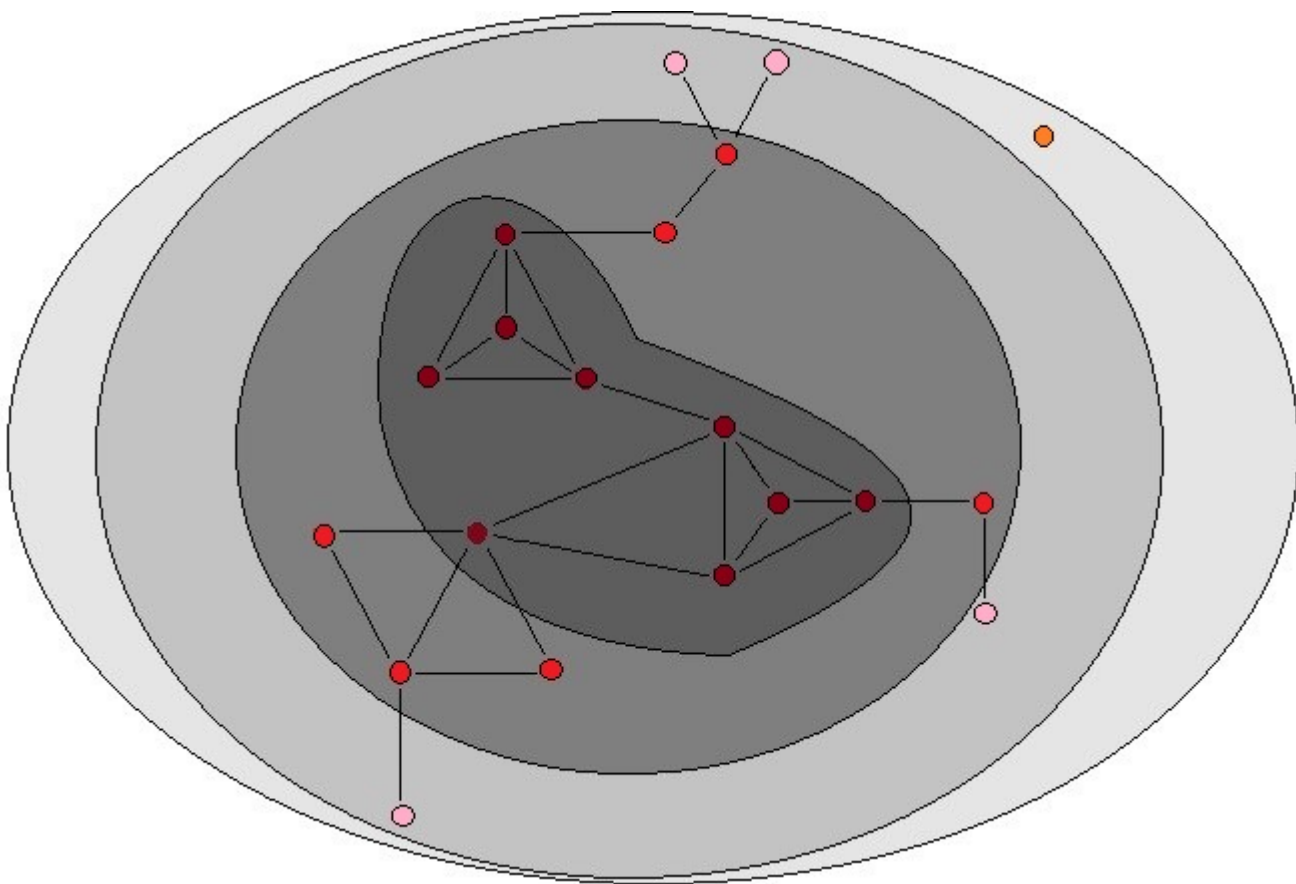


Figure 3



| Parameter | Heuristic Definition | Formal Definition |
|--|--|---|
| In Degree (IN ^o), $deg^+(v)$ | The number of incoming edges to node v in a directed graph. | self-explanatory |
| Out Degree (OUT ^o), $deg^-(v)$ | The number of outgoing edges from node v in a directed graph. | self-explanatory |
| Shortest Path Length, $d(v, u)$ | Shortest distance from node v to another node u with no repeated walks | self-explanatory |
| Betweenness Centrality (BET), $c_B(v)$ | Betweenness centrality of node v is the sum of the fraction of all-pairs shortest paths that pass through v . The greater $c_B(v)$, the greater the fraction of shortest paths that pass through node v . | $\frac{c_B(v)}{(n-1)(n-2)}$, where $c_B(v) = \sum_{s,t \in V} \frac{\sigma(s,t v)}{\sigma(s,t)}$, V is the set of nodes, $\sigma(s, t)$ is the number of shortest paths from node s to node t , $\sigma(s, t v)$ is the number of paths from s to t that pass through node v , and n is the number of nodes in the graph. The denominator $(n-1)(n-2)$ is the normalization factor for a directed graph that scales $c_B(v)$ between 0 and 1. |
| Closeness Centrality (CLO), | Closeness centrality of node v is the reciprocal of | $C(v) = \frac{n-1}{\sum_{u=1}^{n-1} d(u,v)}$, where n is the number of |

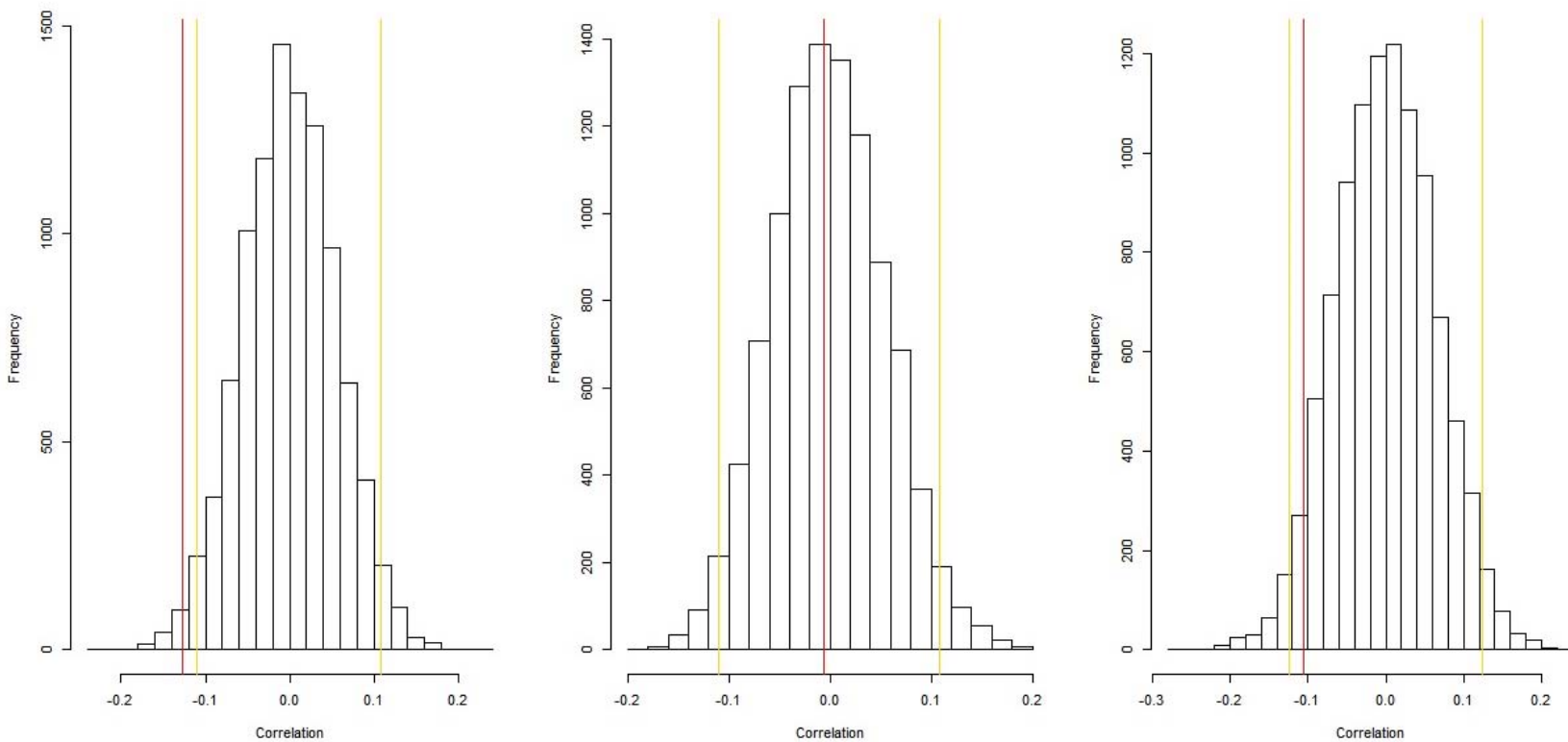
| Parameter | Heuristic Definition | Formal Definition |
|--|--|--|
| $C(v)$ | the sum of the shortest path lengths to all $n-1$ other nodes, normalized by the sum of minimum possible distances $n-1$. The greater $C(v)$, the closer v is to other nodes. | nodes and $d(u, v)$ is the shortest path distance between u and v . |
| Degree Centrality (DEG), $C_D(v)$ | Degree centrality of node v is the fraction of nodes in the network that node v is connected to. | $C_D(v) = \frac{deg^+(v)+deg^-(v)}{n-1}$, where n is the number of nodes in the network. |
| Core Number (CORE) | A k -core is the largest subgraph that contains nodes of at least degree k . The core number of node v is the largest value k of a k -core containing node v . | Calculated using the algorithm of Batagelj and Zaversnik (2011). |
| Mutational Bias (ΔM) | Per-generation rate of change of the trait mean in an MA experiment. Equivalent to the product of the genome-wide mutation rate, μ_G , and the average effect of a mutation on the trait, α . | $\Delta M_z = \frac{\bar{z}_{MA} - \bar{z}_0}{t}$; \bar{z}_{MA} and \bar{z}_0 represent the MA and ancestral (G0) trait means and t is the number of generations of MA. |
| Mutational Variance (V_M) | Per-generation rate of increase in genetic variance for a trait in an MA experiment. | $V_M = \Delta V_L = \frac{V_{L,MA} - V_{L,G0}}{2t}$, where $V_{L,MA}$ is the variance among MA lines, $V_{L,G0}$ is the among- |

| Parameter | Heuristic Definition | Formal Definition |
|---|--|---|
| | Equivalent to the product of the genome-wide mutation rate, μ_G , and the square of the average effect of a mutation on the trait, α^2 . | line variance in the G0 ancestor, and t is the number of generations of MA |
| Squared coefficient of variation (I_M, I_E) | I_M is the mutational variance (V_M) scaled by the square of the trait mean, and provides a measure of the evolvability of a trait. I_E is the residual variance (V_E) scaled in the same way. | |
| Mutational heritability (h_M^2) | Mutational variance (V_M) scaled as a fraction of the residual variance (V_E). Provides a measure of the short-term response to selection on mutational variance. | $h_M^2 = \frac{V_M}{V_E}$ |
| Mutational correlation (r_M) | Genetic correlation between two traits in MA lines. Provides an estimate of pleiotropic effects of new mutations. | $r_M = \frac{COV_M(X,Y)}{\sqrt{V_M(X)V_M(Y)}}$, where COV_M is the mutational covariance and V_M is the mutational variance. |

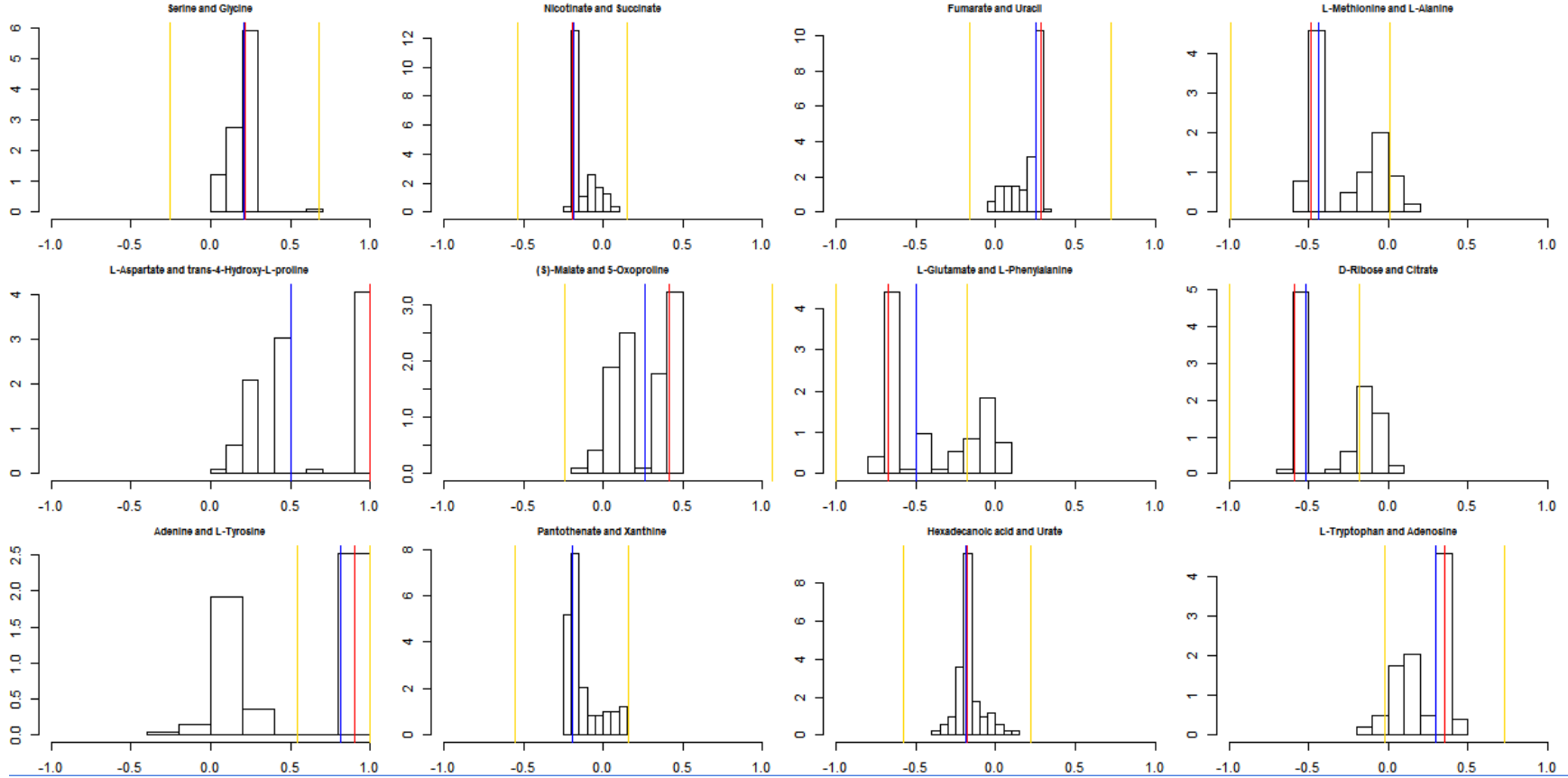
Table 1. Definitions of network parameters, following the documentation of NetworkX, v.1.11 (Hagberg et al. 2008) and quantitative genetic parameters. Abbreviations of the parameters used in Table 2 follow the parameter name in parentheses in bold type.

| | BTW | CLO | DEG | IN° | OUT° | CORE | ΔM | $ \Delta M $ | I_M | I_E | h_M^2 |
|------|-----|------|------|------|------|------|---------------|---------------|---------------|---------------|---------------|
| BTW | | 0.60 | 0.84 | 0.86 | 0.66 | 0.79 | -0.009 (0.96) | -0.055 (0.77) | -0.007 (0.97) | -0.122 (0.52) | 0.128 (0.51) |
| CLO | | | 0.54 | 0.51 | 0.47 | 0.54 | 0.012 (0.94) | 0.297 (0.11) | 0.119 (0.53) | 0.034 (0.86) | 0.089 (0.64) |
| DEG | | | | 0.88 | 0.92 | 0.83 | 0.038 (0.84) | -0.078 (0.68) | 0.178 (0.35) | -0.062 (0.74) | 0.218 (0.25) |
| IN° | | | | | 0.65 | 0.85 | 0.099 (0.60) | 0.043 (0.82) | 0.188 (0.32) | 0.007 (0.97) | 0.277 (0.14) |
| OUT° | | | | | | 0.68 | 0.031 (0.87) | -0.200 (0.29) | 0.133 (0.49) | -0.096 (0.62) | 0.139 (0.47) |
| CORE | | | | | | | 0.245 (0.20) | 0.104 (0.59) | 0.298 (0.11) | 0.025 (0.89) | 0.481 (0.008) |

Table 2. Spearman's rank correlation ρ between network parameters (rows/first five columns) and between network parameters and mutational parameters (rows/last four columns). Abbreviations of network parameters are: BTW, betweenness centrality; CLO, closeness centrality; DEG, degree centrality; IN°, in-degree, OUT°, out-degree; CORE, core number. Network parameters are defined mathematically and heuristically in Table 1. Abbreviations of mutational parameters are: ΔM , per-generation change in the trait mean; $|\Delta M|$, absolute value of ΔM ; I_M , squared mutational coefficient of variation; I_E , squared residual coefficient of variation; h_M^2 , mutational heritability. See text and Supplementary Table S1 for details of mutational parameters. Uncorrected P-values of mutational parameters are given in parentheses.



Supplementary Figure S1 - Parametric bootstrap distributions of random correlations ρ between (a) r_M and the shortest path length in the directed network, (b) $|r_M|$ and the shortest path length in the directed network, (c) r_M and shortest path length in the undirected network (i.e., the shorter of the two path lengths between metabolites i and j in the directed network). Red lines show the observed values of ρ , yellow lines show the 95% confidence interval of the distribution of the correlation between the mutational correlation and a random shortest path length drawn from the observed distribution of shortest path lengths. See Methods for details.



Supplementary Figure S2. Bootstrap distributions of mutational correlations (r_M) calculated from the joint REML estimate of the among-line components of covariance from a pair of focal metabolites (listed at the top of each panel) and six other metabolites randomly sampled without replacement. Each distribution is based on 100 resamples from the data. Red lines show the observed

r_M , blue lines show the median of the resampled values, yellow lines show \pm two standard errors of the observed r_M . Details of the bootstrap analysis are given in the Methods.

| Metabolite | ID # | BTW | CLO | DEG | IN° | OUT° | CORE |
|---------------------------|-------------|------------|------------|------------|------------|-------------|-------------|
| trans-4-Hydroxy-L-proline | 11 | 0.004482 | 0.002761 | 0.004658 | 2 | 1 | 1 |
| L-3-Amino-isobutanoate | 10 | 0 | 0 | 0.001553 | 1 | 0 | 1 |
| Adenine | 20 | 0.003335 | 0.032205 | 0.009317 | 2 | 4 | 2 |
| Adenosine | 28 | 0.005516 | 0.032198 | 0.009317 | 4 | 2 | 2 |
| L-Alanine | 8 | 0 | 0 | 0.001553 | 1 | 0 | 1 |
| L-Asparagine | 16 | 0.002236 | 0.052579 | 0.004658 | 1 | 2 | 1 |
| L-Aspartate | 9 | 0.055054 | 0.056756 | 0.012422 | 3 | 5 | 2 |
| Citrate | 19 | 0.171567 | 0.058576 | 0.012422 | 4 | 4 | 2 |
| Fumarate | 6 | 0.028317 | 0.053784 | 0.007764 | 3 | 2 | 2 |
| L-Glutamate | 14 | 0.036659 | 0.048283 | 0.020186 | 5 | 8 | 2 |
| Glycine | 2 | 0.02661 | 0.044377 | 0.017081 | 5 | 6 | 2 |
| L-Lysine | 21 | 0 | 0.004658 | 0.004658 | 0 | 3 | 1 |
| (S) – Malate | 12 | 0.029016 | 0.057446 | 0.007764 | 2 | 3 | 2 |
| L-Methionine | 7 | 7.49E-05 | 0.004969 | 0.007764 | 2 | 3 | 2 |
| Nicotinate | 3 | 0.00221 | 0.046122 | 0.006211 | 2 | 2 | 2 |
| Hexadecanoic acid | 25 | 0.066162 | 0.019529 | 0.01087 | 2 | 5 | 2 |

| Metabolite | ID # | BTW | CLO | DEG | In° | Out° | Core |
|-----------------------|-------------|------------|------------|------------|------------|-------------|-------------|
| Pantothenate | 23 | 0 | 0.001553 | 0.001553 | 0 | 1 | 1 |
| L-Phenylalanine | 15 | 0.001137 | 0.040279 | 0.009317 | 1 | 5 | 1 |
| Putrescine | 18 | 0.003362 | 0.002795 | 0.003106 | 1 | 1 | 1 |
| 5-Oxoproline | 13 | 0 | 0.045387 | 0.001553 | 0 | 1 | 1 |
| D-Ribose | 17 | 0 | 0.044346 | 0.001553 | 0 | 1 | 1 |
| L-Serine | 1 | 0.034582 | 0.057313 | 0.017081 | 5 | 6 | 2 |
| Succinate | 4 | 0.02215 | 0.051067 | 0.01087 | 4 | 3 | 2 |
| Alpha,alpha-Trehalose | 29 | 0 | 0.042179 | 0.001553 | 0 | 1 | 1 |
| L-Tryptophan | 27 | 0 | 0.004969 | 0.004658 | 0 | 3 | 1 |
| L-Tyrosine | 22 | 0.004518 | 0.041547 | 0.012422 | 3 | 5 | 2 |
| Uracil | 5 | 0.078303 | 0.044365 | 0.01087 | 4 | 3 | 2 |
| Urate | 26 | 0 | 0.029702 | 0.003106 | 1 | 1 | 1 |
| Xanthine | 24 | 0.004393 | 0.031003 | 0.01087 | 3 | 4 | 2 |

Supplementary Table S1(a), above. Network parameters for the 29 metabolites. Column headings are the abbreviations for the network parameters given in Tables 1 and 2. ID# is the number of the metabolite in the network shown in Figure 1.

| Metabolite | ID # | Mean (G0) | Mean (MA) | ΔM (%) | VL | VM | VE | I_M | $h_M^2 (x 10^3)$ |
|---------------------------|-------------|------------------|------------------|----------------------------------|-------------------|--------------------|--------------------|-------------------------|------------------------------------|
| trans-4-Hydroxy-L-proline | 11 | 228.6 (10.4) | 149.4 (14.5) | 0.14 (0.02) | 1058.0 (349.0) | 2.12 (0.70) | 3577.4 (1052.3) | 9.45E-05 | 0.59 (0.26) |
| L-3-Amino-isobutanoate | 10 | 20.0 (3.1) | 14.5 (0.8) | -0.11 (0.06) | 3.58 (4.32) | 0.007 (0.009) | 95.0 (9.86) | 3.43E-05 | 0.08 (0.09) |
| Adenine | 20 | 11.3 (1.4) | 13.2 (0.8) | 0.07 (0.06) | 15.4 (4.4) | 0.03 (0.009) | 28.0 (4.02) | 0.000178 | 1.10 (0.35) |
| Adenosine | 28 | 2.1 (0.7) | 26.0 (3.4) | 4.39 (0.62) | 407.7 (78.2) | 0.82 (0.16) | 186.5 (75.9) | 0.001261 | 4.37 (1.97) |
| L-Alanine | 8 | 5.5 (1.5) | 6.1 (0.6) | 0.04 (0.12) | 5.48 (3.43) | 0.011 (0.007) | 30.3 (5.0) | 0.000278 | 0.36 (0.23) |
| L-Asparagine | 16 | 3.7 (1.3) | 1.2 (0.1) | -0.27 (0.14) | 0.11 (0.15) | 0.0002 (0.0003) | 2.4 (0.4) | 0.000135 | 0.09 (0.13) |
| L-Aspartate | 9 | 37.7 (2.7) | 19.0 (1.2) | -0.20 (0.03) | 48.2 (19.2) | 0.096 (0.038) | 60.2 (18.2) | 0.00026 | 1.60 (0.80) |
| Citrate | 19 | 11.4 (2.2) | 5.7.1 (0.5) | -0.16 (0.08) | 4.56 (1.54) | 0.009 (0.003) | 19.8 (6.8) | 0.000182 | 0.46 (0.22) |

| Metabolite | ID # | Mean (G0) | Mean (MA) | ΔM (%) | VL | VM | VE | I_M | h_M^2 ($\times 10^3$) |
|-------------------|-------------|------------------|------------------|----------------------------------|--------------------|------------------|--------------------|-------------------------|--|
| Fumarate | 6 | 33.0 (2.1) | 25.5 (1.2) | -0.09 (0.03) | 35.1 (9.2) | 0.070 (0.018) | 84.1 (26.1) | 0.000109 | 0.83 (0.34) |
| L-Glutamate | 14 | 115.2 (24.5) | 93.2 (6.3) | -0.08 (0.09) | 973.0 (556.8) | 1.94 (1.11) | 2703.7 (556.9) | 0.000214 | 0.72 (0.44) |
| Glycine | 2 | 47.1 (5.3) | 52.8 (4.5) | 0.05 (0.06) | 606.7 (411.6) | 1.21 (0.82) | 939.3 (323.7) | 0.000393 | 1.29 (0.98) |
| L-Lysine | 21 | 6.7 (2.6) | 5.0 (0.6) | -0.10 (0.16) | 4.43 (3.99) | 0.009 (0.008) | 38.4 (7.01) | 0.00032 | 0.23 (0.21) |
| (S) – Malate | 12 | 44.7 (4.0) | 71.9 (4.2) | 0.24 (0.05) | 397.8 (135.4) | 0.80 (0.27) | 1179.7 (276.2) | 0.000157 | 0.67 (0.28) |
| L-Methionine | 7 | 47.9 (4.1) | 32.2 (1.7) | -0.13 (0.04) | 101.2 (20.5) | 0.202 (0.041) | 72.4 (13.4) | 0.000197 | 2.79 (0.77) |
| Nicotinate | 3 | 4.9 (0.6) | 33.0 (3.5) | 2.29 (0.29) | 473.9 (88.1) | 0.95 (0.18) | 120.3 (21.4) | 0.000863 | 7.88 (2.03) |
| Hexadecanoic acid | 25 | 238.4 (22.3) | 265.1 (15.0) | -0.03 (0.04) | 6712.9 (1579.6) | 13.43 (3.16) | 9579.1 (2985.7) | 0.000194 | 1.40 (0.55) |

| Metabolite | ID # | Mean (G0) | Mean (MA) | ΔM (%) | VL | VM | VE | I_M | $h_M^2 (x 10^3)$ |
|-----------------------|-------------|-------------------|-------------------|----------------------------------|---------------------|--------------------|----------------------|-------------------------|------------------------------------|
| Pantothenate | 23 | 22.9 (0.8) | 14.4 (0.6) | -0.15 (0.02) | 12.8 3.2 | 0.026 (0.006) | 16.0 (3.17) | 0.000123 | 1.60 (0.51) |
| L-Phenylalanine | 15 | 87.4 (7.9) | 94.4 (6.3) | 0.03 (0.05) | 678.9 (263.6) | 1.36 (0.53) | 3835.0 (810.3) | 0.000155 | 0.35 (0.16) |
| Putrescine | 18 | 73.7 (15.3) | 57.4 (3.4) | -0.09 (0.08) | 70.9 (70.6) | 0.14 (0.14) | 1672.3 (193.2) | 4.15E-05 | 0.08 (0.08) |
| 5-Oxoproline | 13 | 701.6 (40.0) | 528.2 (22.2) | -0.10 (0.03) | 7275.6 (3649.0) | 14.56 (7.30) | 52146.6 (13671.9) | 5.24E-05 | 0.28 (0.16) |
| D-Ribose | 17 | 5.6 (0.8) | 13.3 (1.5) | 0.51 (0.11) | 76.3 (22.8) | 0.15 (0.05) | 66.3 (27.2) | 0.000841 | 2.30 (1.17) |
| L-Serine | 1 | 130.0 (49.8) | 85.8 (3.7) | -0.14 (0.15) | 373.7 (117.7) | 0.75 (0.24) | 1221.7 (376.9) | 9.87E-05 | 0.61 (0.27) |
| Succinate | 4 | 7.3 (0.8) | 91.1 (9.7) | 4.52 (0.51) | 3216.6 (688.3) | 6.43 (1.38) | 2797.7 (932.6) | 0.000778 | 2.30 (0.91) |
| Alpha,alpha-Trehalose | 29 | 1772.2 (147.2) | 2525.4 (277.6) | 0.19 (0.07) | 2118803 (105069) | 4237.6 (2101.4) | 4355039 (1656108) | 0.000584 | 0.97 (0.61) |

| Metabolite | ID # | Mean (G0) | Mean (MA) | ΔM (%) | VL | VM | VE | I_M | h_M^2 ($\times 10^3$) |
|-------------------|-------------|------------------|------------------|----------------------------------|-----------------|------------------|-----------------|-------------------------|--|
| L-Tryptophan | 27 | 107.7 (14.3) | 92.2 (2.8) | -0.06 (0.05) | 205.7 (87.3) | 0.411 (0.174) | 496.9 (64.0) | 4.93E-05 | 0.83 (0.37) |
| L-Tyrosine | 22 | 74.7 (9.3) | 47.9 (2.9) | -0.14 (0.05) | 197.2 (70.9) | 0.394 (0.142) | 643.3 (99.7) | 0.00017 | 0.61 (0.24) |
| Uracil | 5 | 9.5 (1.0) | 8.8 (0.4) | -0.03 (0.05) | 4.67 (1.59) | 0.009 (0.003) | 8.3 (1.4) | 0.000123 | 1.13 (0.43) |
| Urate | 26 | 20.7 (3.4) | 11.5 (1.2) | -0.18 (0.07) | 45.3 (20.1) | 0.091 (0.040) | 45.3 (4.75) | 0.000654 | 2.00 (0.91) |
| Xanthine | 24 | 0.4 (0.3) | 6.7 (1.0) | 6.64 (1.09) | 36.5 (8.04) | 0.073 (0.016) | 15.5 (2.8) | 0.001711 | 4.70 (1.34) |
| Mean | | | 0.73* (0.30) | | | | 1.46 (0.31) | | |
| Median | | | 0.14 | | | | 0.83 | | |

Supplementary Table S1(b). Mutational statistics of the 29 metabolites, reprised from Supplementary Table S1 of Davies et al. (2016). Column Headings are: *ID#*, the number of the metabolite in the network shown in Figure 1; *Mean (G0)*, mean metabolite pool of the G0 ancestor; *Mean (MA)*, mean metabolite pool of the MA lines; *ΔM (%)*, percent change per-generation in mean trait

value; VL , among-line component of variance, VM , mutational variance; $CV_{M,G0}$, mutational coefficient of variation standardized by the G0 mean; $CV_{M,MA}$, mutational coefficient of variation standardized by the MA mean; VE , environmental (= within-line) component of variance; h_M^2 , mutational heritability.

* - Mean ΔM (%) is the mean absolute value.

| Reaction Number | Reaction |
|-----------------|---|
| R01579 | D-Glutamine + H ₂ O = D-Glutamate + NH ₃ |
| R01887 | gamma-Amino-gamma-cyanobutanoate + 2 H ₂ O = DL-Glutamate + NH ₃ |
| R04936 | Se-Adenosylselenohomocysteine + H ₂ O = Adenosine +Selenohomocysteine |
| R00891 | L-Serine + Hydrogen sulfide = L-Cysteine + H ₂ O |
| R09099 | L-Serine + 5,6,7,8-Tetrahydromethanopterin = 5,10-Methylenetetrahydromethanopterin + Glycine + H ₂ O |
| R02853 | D-O-Phosphoserine + H ₂ O = D-Serine + Orthophosphate |
| R00904 | 3-Aminopropanal + NAD ⁺ + H ₂ O = beta-Alanine + NADH + H ⁺ |
| R03542 | alpha-Aminopropionitrile + 2 H ₂ O = Alanine + NH ₃ |
| R01324 | Citrate = Isocitrate |
| R00483 | ATP + L-Aspartate + NH ₃ = AMP + Diphosphate + L-Asparagine |
| R01221 | Glycine + Tetrahydrofolate + NAD ⁺ = 5,10-Methylenetetrahydrofolate+ NH ₃ + CO ₂ + NADH + H ⁺ |
| R02078 | 3,4-Dihydroxy-L-phenylalanine + L-Tyrosine + Oxygen = Dopaquinone+ 3,4-Dihydroxy-L-phenylalanine + H ₂ O |
| R01706 | Hexadecanoyl-[acp] + H ₂ O = Acyl-carrier protein + Hexadecanoicacid |
| R04666 | 3-Ureidoisobutyrate + H ₂ O = 3-Aminoisobutyric acid + CO ₂ + NH ₃ |

Supplementary Table S2. Discrepancies between the metabolic networks constructed using the MZ and YW methods. All reactions listed here are in the Ma and Zeng database (<http://www.ibiodesign.net/kneva/>) but not in the Wormflux database (<http://wormflux.umassmed.edu/>) and were used in the generation of the metabolic network. There is a total of 1203 reactions in the network, these represent about 1% of all reactions.