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# Correlated stabilizing selection shapes the topology of gene regulatory networks

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## Abstract

The evolution of gene expression is constrained by the topology of gene regulatory networks, as co-expressed genes are likely to have their expressions affected together by mutations. Conversely, co-expression can also be an advantage when genes are under joint selection. Here, we assessed theoretically whether correlated selection (selection for a combination of traits) was able to affect the pattern of correlated gene expressions and the underlying gene regulatory networks. We ran individual-based simulations, applying a stabilizing correlated fitness function to three genetic architectures: a quantitative genetics (multilinear) model featuring epistasis and pleiotropy, a quantitative genetics model where each gene has an independent mutational structure, and a gene regulatory model, mimicking the mechanisms of gene expression regulation. Simulations showed that correlated mutational effects evolved in the three genetic architectures as a response to correlated selection, but the response in gene networks was specific. The intensity of gene co-expression was mostly explained by the regulatory distance between genes (largest correlations being associated to genes directly interacting with each other), and the sign of co-expression was associated with the nature of the regulation (transcription activation or inhibition). These results concur to the idea that gene network topologies could partly reflect past correlated selection patterns on gene expression.

**Key words** Evolvability ; Mutation Covariance Matrix ; Evolution of Pleiotropy ; Evolutionary Systems Biology ; Quantitative Genetics.

## Introduction

The development and physiology of living organisms are controlled by large and complex Gene Regulatory Networks (GRNs). The central role of GRNs is documented in all kinds of organisms, e.g. for the control of cell physiology in yeasts (Guelzim *et al.*, 2002), heart development in humans (Olson, 2006), skeleton development in sea urchins (Shashikant *et al.*, 2018), or flower development in angiosperms (Espinosa-Soto *et al.*, 2004). The organization of these networks have long been of central interest for systems biologists, and it is now widely acknowledged that GRNs tend to follow general structural rules: for instance, they tend to be sparse, modular (Wagner *et al.*, 2007; Espinosa-Soto, 2018), and scale-free (i.e., the number of connections per node follows a power law) (Babu *et al.*, 2004; Ouma *et al.*, 2018).

The reasons why real-life GRNs are organized in such a way are not completely clear (Espinosa-Soto, 2018; Taylor *et al.*, 2022). Because the expression of genes affect phenotypic traits, and thus condition the individual fitness, gene expression levels are believed to be driven by natural selection, at least for a subset of genes. For instance, specific sets of genes have been shown to evolve in a direction consistent with prior knowledge in the wild (Philippe *et al.*, 2007; Verta and Jones, 2019; Huang *et al.*, 2021), or during experimental evolution (Philippe *et al.*, 2007; Ghalambor *et al.*, 2015; Jallet *et al.*, 2020). In contrast, the structure of the network itself is less directly subject to natural selection.

46 As multiple gene network topologies are capable of producing the same gene expression patterns, at  
47 least in theory (Wagner and Wright, 2007), the main evolutionary mode of network structure should  
48 follow non-adaptive processes, such as systems drift (Lynch, 2007), or mutation bias (Van Noort *et al.*,  
49 2004). Yet, a direct or indirect effect of selection on the evolution of network topology should not  
50 be excluded. For instance, it has been empirically established that the gene network structure may  
51 be deeply rewired during rapid evolutionary events, including domestication (Swanson-Wagner *et al.*,  
52 2012; Bellucci *et al.*, 2014). Furthermore, the effect of indirect selection favoring evolvability (the  
53 propensity to produce mutant phenotypes with a good fitness) or robustness (the ability to buffer  
54 the effect of mutations) in gene networks remains a theoretical possibility (Wagner, 2008; Mayer and  
55 Hansen, 2017). Overall, there are only few theoretical predictions about how selection may affect the  
56 network topology, and about the possible role of adaptation in shaping GRN structure.

57 May the evolution of GRN topology be predicted from quantitative genetics theory? After all,  
58 gene expressions can be assimilated to quantitative traits, and the complex result of regulations can  
59 be described as epistasis (i.e., non-additive between genes) and pleiotropy (i.e., genes affect several  
60 traits) (Phillips, 2008; Fagny and Austerlitz, 2021). Evolutionary quantitative genetics provide a wide  
61 corpus of evolutionary models (e.g. Walsh and Lynch, 2018), including models designed to focus on the  
62 evolution of pleiotropy and modularity of quantitative characters (Sgrò and Hoffmann, 2004; Pavličev  
63 and Cheverud, 2015). With such theoretical tools, it has been showed that, if the genetic architec-  
64 ture is epistatic, pleiotropy could evolve in response to correlated stabilizing selection (Jones *et al.*,  
65 2014). Correlated selection, which corresponds to the selection of trait combinations (illustrated in  
66 Suppl. Fig. 1A), has been documented for various combinations of phenotypic characters (Sinervo and  
67 Svensson, 2002), but its consequences on the structure of genetic architectures is not well understood  
68 (Uller *et al.*, 2018; Svensson and Berger, 2019; Svensson *et al.*, 2021). The evolutionary mechanism  
69 involved in the evolution of pleiotropy relies on the fact that the genetic load of new mutations is min-  
70 imized when the mutational correlation matches the direction of the fitness function (Suppl. Fig. 1B).  
71 In other terms, the effect of mutations are expected to evolve to promote trait combinations favored  
72 by selection (Jones *et al.*, 2007). As the mutational effects are a direct consequence of the genetic  
73 structure, the simulations by Jones *et al.*, 2014 thus formalises the hypothesis that correlated selection  
74 could favor gene network topologies promoting the co-expression of co-selected genes. Yet, this impor-  
75 tant result from evolutionary quantitative genetics may not be straightforward to translate towards  
76 systems biology, as the genetic architecture in Jones *et al.*, 2014 was based on a bivariate multilinear  
77 model (Hansen and Wagner, 2001), featuring unconstrained and isotropic pleiotropic epistasis (i.e.,  
78 any gene have the potential to modify the pleiotropy of any other gene). In contrast, the epistatic  
79 and pleiotropic effects in GRNs are largely constrained and biased by the topology of gene networks  
80 (Sorrells *et al.*, 2015; Nghe *et al.*, 2018).

81 Here, we intend to understand the propensity of correlated stabilizing selection to shape the struc-  
82 ture of gene networks. We will use the theoretical framework proposed by Wagner, 1994, 1996 to  
83 implement a simple gene regulatory network model as a genotype-phenotype map. We will monitor  
84 the evolution of pleiotropy among gene expressions in individual-based simulations. This framework is  
85 well-suited for being coupled with simulations, as the genotype (the set of regulations between genes)  
86 and the phenotype (gene expressions) are explicit and clearly separated. We will address the evolu-  
87 tion of gene co-expression at two levels: (i) at the gene expression level, can gene networks evolve to  
88 optimize mutational correlation in regard to correlated selection ? (ii) at the network level, what is  
89 the effect of correlated selection on network structure and topology? The evolution of co-expression in  
90 the GRN model will be compared to the evolution of pleiotropy in two quantitative genetics models:  
91 the bivariate multilinear model (Hansen and Wagner, 2001; Jones *et al.*, 2014) and the gene pleiotropy  
92 model (Lande, 1980).

## 93 Material and Methods

94 Our purpose is to measure the evolutionary changes in the properties of the genetic architectures when  
95 submitted to correlated selection, with a particular focus on the propensity of mutations to induce  
96 pleiotropic (correlated) effects on co-selected phenotypic traits. The influence of the nature of the  
97 genotype-phenotype relationship will be addressed by considering three genotype-phenotype models,  
98 explored by individual-based simulations.

## 99 Measurement of pleiotropy *via* the mutational covariance matrix

100 In multivariate quantitative genetics models, the response to directional selection in a complex pheno-  
101 typic space can be predicted from the structure of the (additive) genetic covariances (the  $\mathbf{G}$ -matrix)  
102 in the population (Lande and Arnold, 1983; Blows, 2007). Genetic covariances result from both link-  
103 age disequilibrium (LD), the statistical association of alleles at different loci, and pleiotropy. LD is  
104 reversible, it can be affected by genetic drift, recombination rate, recent directional or stabilizing se-  
105 lection, and gene flow. In contrast, pleiotropy reflects the properties of the genetic architecture of  
106 the traits, and is generally considered as a non-evolvable constraint when studying the adaptation of  
107 quantitative traits (e.g. Jones *et al.*, 2003; Chantepie and Chevin, 2020).

108 Here, our objective is to study the long-term evolution of pleiotropy as a consequence of selection for  
109 trait combinations. Pleiotropy can be formally measured as the propensity of mutations to affect two  
110 or more traits together. The distribution of the multivariate effects of mutations can be summarized by  
111 the matrix  $\mathbf{M}$ , which diagonal and off-diagonal elements stand for mutational variances and covariances  
112 respectively. Most of the following results will focus on two traits, named  $a$  and  $b$ ; the corresponding  
113  $\mathbf{M}$  being:

$$114 \quad \mathbf{M} = \begin{Bmatrix} M_a & M_{a,b} \\ M_{a,b} & M_b \end{Bmatrix} \quad (1)$$

115 where  $M_a$  and  $M_b$  are the mutational variances of traits  $a$  and  $b$ , respectively, and  $M_{b,a} = M_{a,b}$  is the  
116 mutational covariance between traits  $a$  and  $b$ .

117 Two-dimensional covariance matrices can be conveniently represented graphically as ellipses (Cheverud,  
118 1984; Jones *et al.*, 2014), sometimes assimilated to the corresponding 95% confidence interval of a mul-  
119 tivariate Gaussian distribution. We will extract two geometrical properties from these matrices, the  
120 direction (angle) between its main eigenvector and the first trait, measuring the main mutational di-  
121 rection  $\alpha(\mathbf{M})$ , and the ellipse eccentricity  $e(\mathbf{M})$ , measuring the strength of pleiotropy from 0 to 1.  
122 The calculation of the mutational direction is detailed in the Supplementary Methods section; the  
123 eccentricity of the  $\mathbf{M}$  matrix was computed as  $e(\mathbf{M}) = \sqrt{1 - \lambda_2/\lambda_1}$ , where  $\lambda_i$  stands for the  $i^{\text{th}}$  eigen-  
124 value of the matrix  $\mathbf{M}$ . Mutational correlation  $r(\mathbf{M})$  between genes  $a$  and  $b$  were calculated from  $\mathbf{M}$   
125 matrices using the standard formula  $r(\mathbf{M}) = M_{a,b}/(\sqrt{M_a}\sqrt{M_b})$ . The relationship between direction,  
126 eccentricity, and correlation is illustrated in Figure 1A.

127 While the genetic covariance matrix  $\mathbf{G}$  is a population property, the mutational matrix  $\mathbf{M}$  is a  
128 property of a genotype.  $\mathbf{M}_i$  was thus estimated for every individual  $i$  of the population, and variances  
129 and covariances were averaged out to get the population  $\mathbf{M}$ . Thirty independent simulation replicates  
130 were run, and some figures report average values. Average correlations  $\bar{r}$  and eccentricities  $\bar{e}$  were  
131 computed as arithmetic means, while the mean direction  $\bar{\alpha}$  over  $R$  replicates was obtained as a circular  
132 mean restricted to the interval  $(-\pi/2, \pi/2)$  (detailed in the Supplementary Methods).

## 133 Selection

134 Relative fitness was determined by a multivariate stabilizing bell-shaped fitness function (Lande, 1980):

$$135 \quad w(\mathbf{Z}_i) = \exp\left(-\frac{1}{2}(\mathbf{Z}_i - \boldsymbol{\Theta}_i)^T \mathbf{S}^{-1}(\mathbf{Z}_i - \boldsymbol{\Theta}_i)\right), \quad (2)$$

137 where  $\mathbf{Z}_i$  is the vector of phenotypes for individual  $i$ ,  $\boldsymbol{\Theta}_i$  is the optimal phenotype for trait  $i$  (by  
138 default,  $\boldsymbol{\Theta}_i = 0$  unless specified otherwise), and  $\mathbf{S}$  is the covariance matrix of the fitness function. The  
139 trace of the matrix  $\mathbf{S}$  (the sum of the diagonal elements) represents the width of the fitness function  
140 (the larger the coefficients of  $\mathbf{S}$ , the weaker the selection). The fitness function was parameterized so  
141 that the maximum relative fitness was  $w(\boldsymbol{\Theta}) = 1$ .

142 For simplicity, the number of phenotypic traits on which correlated selection was applied was  
143 reduced to two traits in most simulations. As a consequence, the fitness function was specified by five  
144 parameters: two parameters for the phenotypic optima, and three (co)variance parameters for the  $2 \times 2$   
145 matrix  $\mathbf{S}$  (the strength of selection on traits 1 and 2, and the selection correlation  $r(\mathbf{S})$ ). The main  
146 direction of selection  $\alpha(\mathbf{S})$ , the eccentricity  $e(\mathbf{S})$  and the correlation  $r(\mathbf{S})$  of the fitness function have  
147 the same meaning as for the mutation covariance matrix.

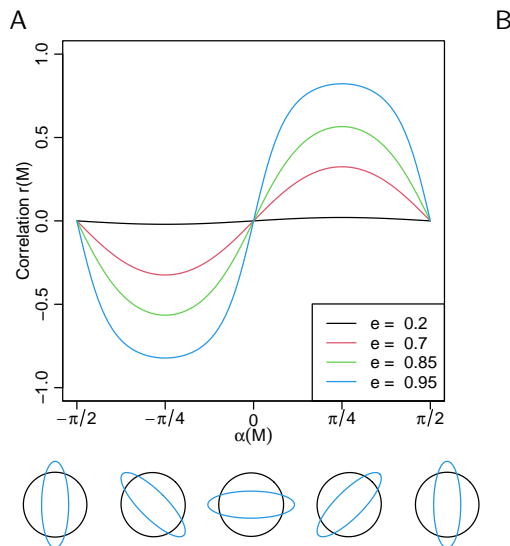


Figure 1: A: The relationship between the eccentricity ( $e$ ), direction ( $\alpha$ ) and correlation ( $r$ ) of a variance-covariance matrix  $\mathbf{M}$ . The geometry of matrices with different directions ( $\mathbf{M} = -\pi/2, -\pi/4, 0, \pi/4, \pi/2$ ) and eccentricities (black:  $e = 0.2$ , blue:  $e = 0.95$ ) is represented below the x-axis. B: Diagram representing our gene regulatory network design.  $a$  and  $b$  are the focal genes (the genes which expression is under correlated selection).  $c$  and  $d$  are genes selected to be activated (optimal phenotype at  $\Theta_i = 0.5$ , corresponding to an optimal expression  $\simeq 0.62$ , slightly above the basal expression  $\kappa = 0.5$ ), independently from each other.  $e$  and  $f$  are free to evolve without affecting the fitness directly, and can thus act as transcription factors.

## 148 Genotype-Phenotype models

149 To simulate the evolution of populations and their  $\mathbf{M}$  matrices, we used three models implementing  
 150 different genotype-phenotype mapping. The first model is a gene regulatory network (GRN) model,  
 151 in which the genotype represents regulations between transcription factors, and the phenotype is  
 152 the expression of the network genes at equilibrium. The second model is a bivariate version of the  
 153 multilinear model (Hansen and Wagner, 2001; Jones *et al.*, 2014), which extends the classical additive  
 154 model with epistatic and pleiotropic interactions. The third model (that we called the Gene Pleiotropy  
 155 model, GP) is based on an implementation of Fisher, 1930's geometric model in which every additive  
 156 locus has its own pleiotropic pattern (Lande, 1980).

157 **Gene regulatory network model** We used a regulatory gene network model inspired from Wagner  
 158 (Wagner 1994, 1996), which is a common abstraction of the transcription regulation process as it is a  
 159 dynamic model with discrete time steps (Bergman and Siegal 2003; Azevedo *et al.* 2006; Leclerc 2008;  
 160 Rhoné and Austerlitz 2011; Rünneburger and Le Rouzic 2016; Espinosa-Soto 2016). The structure of  
 161 the regulation network among  $n$  genes is stored in a  $n \times n$  matrix  $\mathbf{W}$ , corresponding to cis-regulations  
 162 among transcription factors. Element  $W_{ij}$  corresponds to the effect of the product of gene  $j$  on the  
 163 expression of gene  $i$ . Inhibiting regulations are negative values and activating regulations are positive  
 164 values. Zero indicates the absence of direct regulation. Each gene of the network is susceptible to  
 165 act as a transcription factor and to modify the expression of other genes; there was no self-regulation  
 166 ( $W_{ii} = 0$ ).

167 Gene expression was computed dynamically for 24 time steps, which happens to be enough to reach  
 168 equilibrium in our simulations (Suppl. Fig. 5). Initial gene expressions were set to their basal level  
 169 (expression in absence of regulation)  $\kappa = 0.5$ , intermediate between full inhibition and full activation.  
 170 Gene expressions were dynamically updated as a function of the concentration of the other genes of  
 171 the network:

$$172 \quad \mathbf{P}_{t+1} = F(\mathbf{W}\mathbf{P}_t), \quad (3)$$

173 where  $\mathbf{P}$  is a vector of  $n$  quantitative gene expressions, scaled between 0 (no expression) to 1 (maximal  
 174 expression), by a sigmoid function  $F(x_1, \dots, x_n) = (f(x_1), \dots, f(x_n))$ . This scaling function was  
 175  $f(x) = 1/(1 + e^{-4x})$  for the default basal expression  $\kappa = 0.5$  (see supplementary methods for  $\kappa \neq 0.5$ ).  
 176 The phenotype  $\mathbf{Z}$  corresponding to a genotype  $\mathbf{W}$  was computed from the average expression of the  
 177 two first genes of the network (hereafter called "a" and "b") for the 4 last time steps  $\bar{P} = (1/4) \sum_{t=21}^{24} P_t$ ,  
 178 as  $Z_i = \log[\bar{P}_i / (1 - \bar{P}_i)]$ , rescaled over  $(-\infty, +\infty)$  to be directly comparable with the multilinear model;  
 179  $Z_i < 0$  corresponds to underexpression,  $Z_i > 0$  to overexpression, and the phenotypic value  $Z_i = 0$  to  
 180 an expression intermediate between the minimum and maximum.

181 Network topology and the corresponding gene expression evolved because the strength of regulation  
 182  $W_{ij}$  can change by mutation (except for self-regulation  $W_{ii}$ , which was set to a constant 0). The  
 183 mutation rate per individual was  $\mu$ , and each gene had the same probability  $\mu/n$  to be affected by a  
 184 mutation. Mutations changed a single random element of the mutated gene by a Gaussian deviation  
 185 of variance  $\sigma^2 m$  (see Table 2 for parameter values).

186 In order to facilitate the evolution of diverse regulatory motifs in the network (involving more than  
 187 the two target genes), two genes ( $c$  and  $d$ ) were considered as "transcription factors", and selected  
 188 to be up-regulated by including them in the fitness function (equation 2), with an optimum  $\theta_c =$   
 189  $\theta_d = 0.5$  (corresponding to an optimal expression of  $P_c = P_d = 0.62$ ) and a selection strength of  
 190  $S_{c,c} = S_{d,d} = 10$ , selection being uncorrelated ( $S_{c,i \neq c} = 0$ ) (see Figure 1B). In addition to the selection  
 191 on the phenotype, unstable networks were penalized (considering unstable networks as unviable is  
 192 common in the literature, see e.g. Siegal and Bergman 2002). In practice, the individual fitness  $w$  was  
 193 multiplied by a factor  $w_{\text{stab}} = \exp(-s' \sum_i^n V_i)$  where  $s'$  quantifies the selection against unstable gene  
 194 expression, and  $V_i = (1/4) \sum_{t=21}^{24} (P_{it} - \bar{P}_i)^2$  is the variance in the expression of gene  $i$  during the last  
 195 4 steps of the network dynamics (more details in Supplementary methods). We set  $s' = 46,000$ , as  
 196 in Rünneburger and Le Rouzic 2016, which was a large penalty; in practice, unstable networks were  
 197 thus strongly selected against and these genotypes were absent from the simulations except for rare  
 198 spontaneous mutants.

199 **Multilinear model** The multilinear model was originally developed by Hansen and Wagner, 2001.  
 200 Although provided as a multivariate model in its original description, it has been extensively used in  
 201 its univariate form in the quantitative genetics literature (Hermisson *et al.*, 2003; Carter *et al.*, 2005;  
 202 Jones *et al.*, 2007; Le Rouzic *et al.*, 2013), but more rarely in its multivariate implementation (Jones  
 203 *et al.*, 2014).

204 The multilinear model is built as an extension of the additive model, by adding epistatic terms  
 205 proportional to the product of the additive effects across genes. Restricting the model to second-order  
 206 epistasis (interactions between pairs of genes), the phenotypic value  $Z_m$  of a trait  $m$  (among  $K$  traits)  
 207 is:

$$208 \quad Z_m = Z_{0m} + \sum_{i=1}^n y_m^i + \sum_{k=1}^K \sum_{l=1}^K \sum_{i=1}^n \sum_{j>i}^n \varepsilon_{mkl}^{ij} y_k^i y_l^j, \quad (4)$$

209 in which  $y_m^i$  is the effect of the genotype at gene  $i$  on the phenotypic trait  $m$  measured in an arbitrary  
 210 reference genotype where all  $y_m^{j \neq i} = 0$ .  $Z_{0m}$  is the phenotypic reference, i.e., the phenotypic value  
 211 corresponding to an arbitrary reference genotype for which all  $y_m^i = 0$ . For every combination of  
 212 traits, the epistatic coefficient  $\varepsilon^{ij}$  quantifies the directional epistasis between genes  $i$  and  $j$ . The  
 213 coefficients  $\varepsilon_{mmm}$  describe "classical" epistasis, i.e., the interaction of allelic effects  $y_m^i$  and  $y_m^j$  on trait  
 214  $m$ . In contrast,  $\varepsilon_{mkl}$  with  $k$  and/or  $l$  different from  $m$ , correspond to interactions involving pleiotropy,  
 215 i.e., how trait  $m$  is influenced by the interaction between the effects of alleles on traits  $k$  and  $l$ . When  
 216 all  $\varepsilon^{ij} = 0$ , this model collapses towards an additive model. When  $\varepsilon_{mkl} \neq 0$ , pleiotropy can evolve  
 217 (traits can become more or less dependent). In total, there are  $K^3$  combinations of  $K$  traits, and for  
 218  $n$  genes,  $n(n-1)/2$  independent epistatic coefficients (because  $j > i$ ) for each combination of traits.

219 In the multilinear model, evolution occurs because  $y_m^i$  can change. Mutations affect genes indepen-  
 220 dently, and a mutation at gene  $i$  affects all traits at once (the effect of mutations being independently  
 221 drawn in Gaussian distributions of variance  $\sigma_m^2$ ). In contrast, the  $\varepsilon$  coefficients ( $2^3 \times 6 \times 5/2 = 120$  in  
 222 the default setting) could not evolve. They were drawn in a Gaussian distribution  $\varepsilon \sim \mathcal{N}(0, 1)$  at the  
 223 beginning of each simulation run and kept constant throughout generations, as in Jones *et al.*, 2014.



224 **Gene Pleiotropy model** We also considered a model in which gene contributions were additive  
225 (i.e., pleiotropy was not modeled as epistasis), but each locus had its own pleiotropic structure (termed  
226 "orientation heterogeneity" in Chevin *et al.*, 2010). This setting is inspired from Lande, 1980 and is  
227 regularly used to study the evolution of modularity (Chevin *et al.*, 2010).

228 In practice, every gene  $i$  was featured by its own mutational matrix  $\mathbf{M}_i = \mu_i \mathbf{C}_i$ , where the covari-  
229 ance matrix  $\mathbf{C}_i$  quantifies the pleiotropy at gene  $i$ . The covariance matrix  $\mathbf{C}_i$  was constant, but the  
230 mutation rate  $\mu_i$  was evolvable, opening the possibility for the gene to increase or decrease its overall  
231 contribution to the mutational properties of the genotype. Gene-specific covariance matrices  $\mathbf{C}_i$  were  
232 computed in order to cover equally-spread angles between  $-\pi/2$  and  $\pi/2$ , with a strong eccentricity  
233 ( $e(\mathbf{C}_i) = 0.9$ ). The genotype was encoded in the same way as in the multilinear model,  $y_m^i$  being  
234 the additive effect of gene  $i$  on trait  $m$ , and the genotype-phenotype map was additive  $Z_m = \sum_i y_m^i$ .  
235 There are two kinds of mutations: "regular" mutations affecting the traits ("trait mutations"), and  
236 mutations affecting the gene mutation rate ("rate mutations"). Trait mutations occurred with a rate  
237  $\mu\mu_i / \sum_j \mu_j$  at gene  $i$ ; they affect all traits at once, and mutational effects were correlated according  
238 to the covariance matrix  $\mathbf{C}_i$ . Mutation rates were normalized so that the mutation rate per individual  
239 and per generation is  $\mu$ , as in the other models (the mutation rate of genes evolved relative to each  
240 other, but the total mutation rate remained constant). Rate mutations occurred with a rate  $\mu^*$  per  
241 genotype and per generation (for convenience,  $\mu^* = \mu$ ), and may affect all loci with the same prob-  
242 ability. Their effect was Gaussian on the multiplicative scale (the mutation rate after mutation was  
243  $\mu'_i \sim \exp[\mathcal{N}(\log \mu_i, \sigma_m^*)]$ , and the effect of rate mutation was fixed to  $\sigma_m^* = 0.1$  (in average, a rate  
244 mutation changed the mutation rate by  $\simeq 8.3\%$ ).

245 The similarities and differences among the three models are summarized in Table 1.

## 246 Simulation model

247 All data presented in this article have been generated by computer simulation of evolving populations  
248 with the C++ program Simevolv (Rünneburger and Le Rouzic 2016: <https://github.com/lerouzic/simevolv.git>). The analysis scripts have been written in R and are available at [https://github.com/apetit8/Mmatrix\\_paper.git](https://github.com/apetit8/Mmatrix_paper.git).

251 **Reproduction** Simulations followed a traditional Wright-Fisher framework. Populations consisted  
252 in  $N$  haploid, sexually-reproducing hermaphrodite individuals. The genotype was encoded as  $n$  freely  
253 recombining genes, the (multivariate) phenotype being computed from the genotype according to one of  
254 the genotype-phenotype models described above. Generations were non-overlapping; for each offspring,  
255 two parents were picked with a probability proportional to their relative fitness, and the two  $n$ -gene  
256 haploid gametes were recombined to form a new haploid genotype. Populations evolved during 10,000  
257 generations and were submitted to genetic drift, selection, and mutations.

258 **Mutations** Mutations affect the genotype immediately after recombination, before the computation  
259 of the phenotype of individuals. Mutations occurred with a rate  $\mu$  per gamete, and affect random genes  
260 as described above. Mutational effects were cumulative, the new allelic value was drawn in Gaussian  
261 distributions centered on the former values.

262 **Model output** The simulation software reports the means, variances and co-variances of the popu-  
263 lation phenotypes and genotypes at regular time points. In addition, the population average mutation  
264 co-variance matrix  $\mathbf{M}$  was estimated in the following way: 6 mutations were simulated for each of the  
265  $N = 5,000$  individuals  $i$ , leading to 5,000 covariance matrices that were averaged out and multiplied  
266 by the mutation rate  $\mu$ .

## 267 Simulation parameters

268 Default simulation parameters were set as displayed in Table 2. For the multilinear model, the epistasis  
269 parameters were inspired from Jones et al., 2014 (Jones *et al.*, 2014). Parameters for the three models  
270 were adjusted to produce  $\mathbf{M}$  matrices of similar sizes.

271 All simulations start with genotypic values ( $y_i$  in the multilinear and GP models,  $W_{ij}$  in the gene  
272 network model) set at 0, unless specified otherwise. In some simulations, the initial gene network

	Regulatory network	Multilinear	Gene pleiotropy
Genotype	$n \times n$ regulation matrix ( $\mathbf{W}$ ) between $n$ genes; $W_{ij}$ : how much gene $j$ regulates gene $i$ .	$n \times K$ matrix ( $n$ genes, $K$ traits). $y_m^i$ is the reference effect of gene $i$ to trait $m$ .	$n \times K$ matrix, $y_m^i$ is the effect of gene $i$ to trait $m$ .
Phenotype	A vector $\mathbf{P} \in [0, 1]$ of $n$ equilibrium gene expressions, transformed to $\mathbf{Z} = \log[\mathbf{P}/(1 - \mathbf{P})]$	A vector $\mathbf{Z}$ of $K$ quantitative traits	A vector $\mathbf{Z}$ of $K$ quantitative traits
Genotype-Phenotype map	Emergent from the dynamic regulation model: $\mathbf{P}_{t+1} = f(\mathbf{P}_t \mathbf{W})$	Multilinear: $Z_m = \sum_i y_m^i + \sum_{i,j,k,l} \varepsilon_{mkl}^{ij} y_k^i y_l^j$	Additive: $Z_m = \sum_i y_m^i$ .
Mutation	Independent for each regulation: $W'_{ij} \sim \mathcal{N}(W_{ij}, \sigma_m^2)$ .	Uncorrelated for the $K$ traits: $\mathbf{y}^{it} \sim \mathcal{M}(\mathbf{y}^i, \sigma_m^2 \mathbf{I})$	Correlated for the $K$ traits: $\mathbf{y}^{it} \sim \mathcal{M}(\mathbf{y}^i, \sigma_m^2 \mathbf{C}_i)$
Selection	Gene expressions under correlated stabilizing selection ( $\mathbf{S}$ ). Some additional selection constraints (see text)	Traits under correlated stabilizing selection ( $\mathbf{S}$ )	Traits under correlated stabilizing selection ( $\mathbf{S}$ )
Epistasis	Emerging from network gene regulation	Explicit, proportional to the product of additive effects (multilinear)	None
Pleiotropy	Emerging from network gene regulation	Explicit, mathematically equivalent to epistasis between traits	Explicit, mutational correlations at each gene
$\mathbf{M}$ matrix evolution	Consequence of the network topology	Consequence of the non-linear interactions between allelic effects	Consequence of the differential mutation rates among genes

Table 1: Comparative table for the three models.  $\mathcal{N}(\mu, \sigma^2)$ : Normal distribution of mean  $\mu$  and variance  $\sigma^2$ ;  $\mathcal{M}(\boldsymbol{\mu}, \boldsymbol{\Sigma})$ : Multivariate normal distribution of means  $\boldsymbol{\mu}$  and covariances  $\boldsymbol{\Sigma}$ .  $\mathbf{I}$  stands for the identity matrix of the adequate dimension. Other symbols are described in the text.



Parameter	GRN	Multilinear	Gene pleiotropy
Generations	10000	10000	10000
Population size $N$	5000	5000	5000
Genes $n$	6	6	6
Correlationally selected traits	2	2	2
Haplotype mutation rate $\mu$	0.1	0.1	0.1
Mutation effect $\sigma_m$	0.1	0.0369	0.0369
Optimum phenotype	0	0	0
$\mathbf{S}$ matrix size (trace)	10	10	10
$\mathbf{S}$ matrix eccentricity	0.94	0.94	0.94

Table 2: Default parameters for the three models.

273 topology was manipulated (positive or negative initial correlation) by setting some initial regulations  
 274 ( $W_{ab}$  and  $W_{ba}$ ) with positive (+0.5), negative (-0.5), or null (0) values. The corresponding slots of  
 275 the  $\mathbf{W}$  matrix ( $W_{ab}$  and  $W_{ba}$ ) were not evolvable and remained to their initial values, while the rest  
 276 of the network was free to evolve.

277 The bivariate stabilizing selection ( $\mathbf{S}$  variance matrix) was parameterized in each simulation run  
 278 by setting the angle of the major axis (between  $-\pi/2$  and  $\pi/2$ ); the matrix size  $\text{tr}(\mathbf{S})$  and eccentricity  
 279 remained constant (see Table 2 and orange ellipses in Figure 2).

## 280 Results

### 281 Mutational correlations can evolve in all models

282 We compared the evolution of simulated populations based on three genetic architectures: a gene  
 283 regulatory network architecture (GRN model), considering gene expression levels as phenotypic traits,  
 284 quantitative traits controlled by a multilinear genetic architecture (as in Jones *et al.*, 2014), additive  
 285 traits controlled by several genes displaying different pleiotropic patterns (Lande, 1980) (GP model).  
 286 Two traits (two gene expressions for the GRN model) were submitted to correlated stabilizing selec-  
 287 tion, the fitness function being defined by the direction  $\alpha(\mathbf{S})$  of the optimal trait combination. Our  
 288 expectation was that pleiotropy (measured as the shape and direction of the mutational covariance  
 289 matrix  $\mathbf{M}$ ) should evolve in order to match the direction of the fitness function.

290 In the multilinear and GP models simulations, the alignment between the main axis of the mu-  
 291 tational matrix  $\mathbf{M}$  and the direction of the correlated fitness function was convincing after less than  
 292 500 generations (Figure 2A, B). This result confirmed the conclusions from Jones *et al.*, 2014, based  
 293 on the multilinear model. In contrast, our gene network model did not always evolved towards the  
 294 best alignment, even after 10,000 generations (e.g. in Figure 2B): the sign of mutational correlations  
 295 matched the sign of fitness correlations, but there was a discrepancy at equilibrium.

296 The different nature of the response to correlated selection in the three models is illustrated in  
 297 Figure 2C. For both the multilinear and GP models, the response to the direction of the fitness  
 298 function  $\alpha(\mathbf{S})$  was homogeneous in all directions, and the shape (eccentricity) of the  $\mathbf{M}$  matrix did  
 299 not depend on  $\alpha(\mathbf{S})$ . In contrast, with the GRN model, although both the direction and eccentricity  
 300 of  $\mathbf{M}$  evolved, pleiotropy evolved along preferential directions:  $\alpha(\mathbf{M})$  did match the sign of  $\alpha(\mathbf{S})$ ,  
 301 but not the precise direction of the fitness function. Intermediate angles ( $\pi/4$ : both gene expressions  
 302 affected equally by mutations, and  $-\pi/4$ : opposite effects on both genes) were frequently observed, and  
 303 mutational independence ( $\alpha(\mathbf{M}) = \pm\pi/2$  or 0) was difficult to achieve. In the GRN model, evolving  
 304 different mutational effects for both selected traits was more difficult, leading to frequent round (weak  
 305 eccentricity)  $\mathbf{M}$  matrices. This appears to reflect a property of gene network architectures, as GRNs  
 306 tend to evolve towards this pattern even when starting from a better alignment (Suppl. Fig. 3).

307 While pleiotropy (and absence of pleiotropy) could not evolve in the GRNs as freely as in the other  
 308 models, GRN models displayed the best response of mutational effect correlation  $r(\mathbf{M})$  to the fitness  
 309 correlation  $r(\mathbf{S})$ :  $\beta_{\text{GRN}}$  (linear regression coefficient) of 0.63, against  $\beta_{\text{GP}} = 0.48$  and  $\beta_{\text{multilin}} = 0.50$   
 310 (Figure 2D). The constraints on the evolution of pleiotropy did not translate into the genetic covariance  
 311 matrix  $\mathbf{G}$ , which was aligned on selection for all models (Suppl. Fig. 4) due to the contribution of linkage  
 312 disequilibrium. Overall, all three models evolve under correlated selection through different strategies

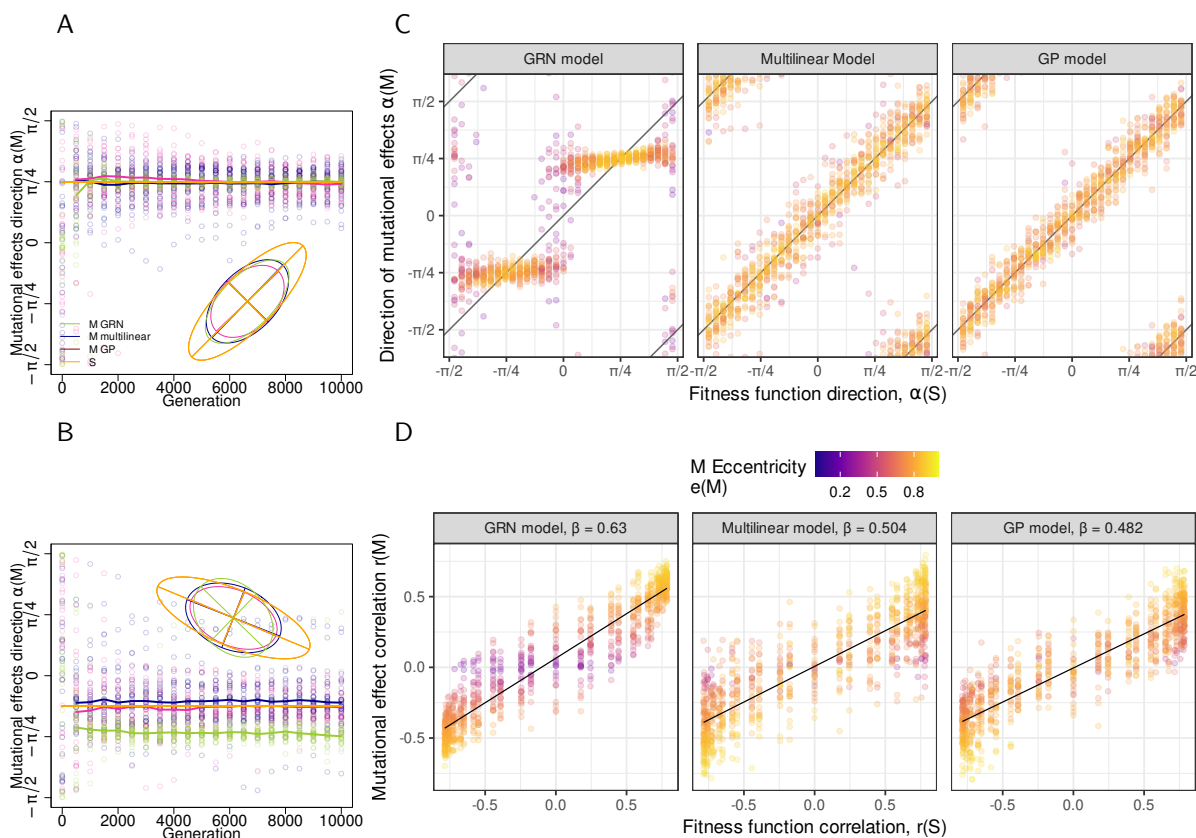


Figure 2: A, B: Evolution of the angle of the main axis of the mutation matrix ( $\alpha(\mathbf{M})$ ) along generations. Orange ellipses illustrate the fitness function (scaled  $\times 0.025$ ), which direction was  $\alpha(\mathbf{S}) = +\pi/4$  (panel A), and  $\alpha(\mathbf{S}) = -\pi/8$  (panel B). Dots represent 30 simulation replicates, plain lines stand for circular means  $\bar{\alpha}(\mathbf{M})$ . Ellipses are the geometric representation of  $\mathbf{M}$  matrices at the last generation (10,000), averaged over the 30 replicates. C:  $\alpha(\mathbf{M})$  as a function of  $\alpha(\mathbf{S})$  for the three models. Dots represents the direction of the main axis of the  $\mathbf{M}$  matrix. D:  $r(\mathbf{M})$  as a function of  $r(\mathbf{S})$  for the three models. For C and D: Data obtained after 10,000 generations in 30 simulation replicates for 31 values of  $\alpha(\mathbf{S})$  regularly spaced between  $-\pi/2$  and  $\pi/2$ ; the color scale encodes the eccentricity of  $\mathbf{M}$ .  $\beta$  is the linear regression coefficient between  $r(\mathbf{M})$  and  $r(\mathbf{S})$ .

313 : the direction of the  $\mathbf{M}$  matrix tends to evolve quantitatively in the GP and multilinear models, while  
 314 the response of GRNs is rather discrete (positive, negative, or no pleiotropy).

### 315 Mutational correlation is determined by local regulatory motifs

316 In the previous section, we used quantitative genetics tools to describe the structure of mutational  
 317 correlations among traits and its evolution. Here we aim at deciphering the changes in the regulatory  
 318 motifs that underlie the evolution of co-expression in gene networks.

319 We measured the correlation between each of the 30 network regulations  $\mathbf{W}_{ij}$  and the quantitative  
 320 descriptors of  $\mathbf{M}$  ( $\alpha(\mathbf{M})$ ,  $e(\mathbf{M})$  and  $r(\mathbf{M})$ ) (Figure 3). The regulations affecting  $\mathbf{M}$  the most were the  
 321 direct regulations between target genes  $a$  and  $b$ . In contrast, regulations between the rest of the network  
 322 (especially the overexpressed transcription factors  $c$  and  $d$ ) towards the focal genes  $a$  and  $b$  decreased  
 323 pleiotropy. The other regulations did not affect the direction or eccentricity of the mutational matrix,  
 324 strongly suggesting that the co-expression between two genes is determined by the local regulatory  
 325 motif.

326 The influence of direct regulations was further assessed by forcing their value to positive (activation),  
 327 negative (repression), or zero (no regulation allowed). Fixing regulations between the focal genes  
 328 prevented the evolution of the direction of the mutational matrix (Figure 4A and B), and  $\alpha(\mathbf{M})$  was  
 329 constrained by the sign of the direct regulation (mutually activating genes were always positively co-

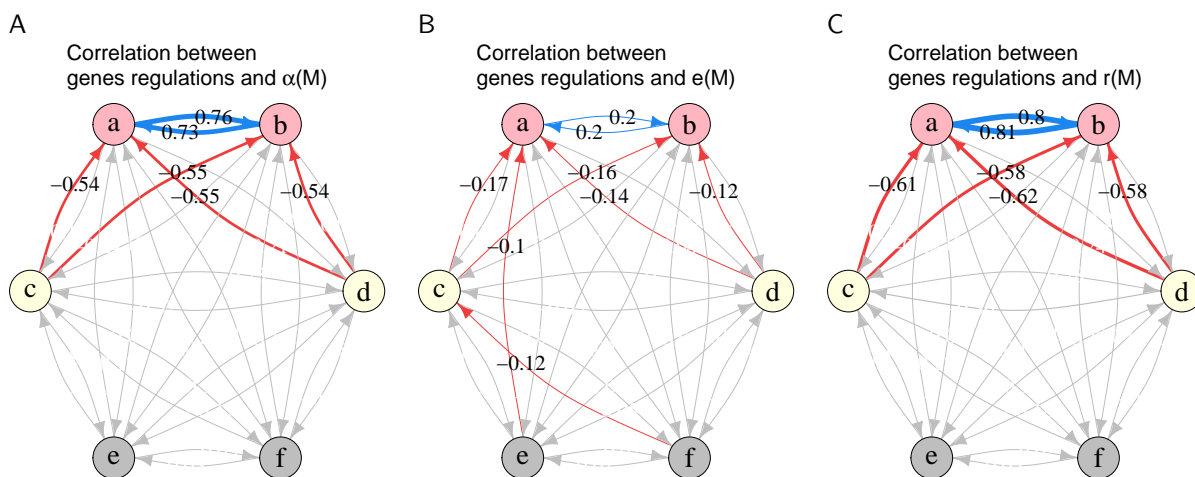


Figure 3: Network graph (off-diagonal elements of the regulatory matrix  $\mathbf{W}$ ) with edges width proportional to the correlation between genes regulations  $\mathbf{W}_{ij}$  and A: the mutational matrix direction  $\alpha(\mathbf{M})$ , B: the mutational matrix eccentricity  $e(\mathbf{M})$ , and C: the mutational correlation  $r(\mathbf{M})$ . Positive correlation are blue, negative correlations are red. Correlations inferior at 0.4 for A and C and inferior at 0.1 for B are represented in grey. Data used to calculate correlations are the one represented in Figure 2D (930 networks in total, corresponding to 31 regularly distributed angles for the correlated fitness function, with 30 simulation replicates for each angle).

330 expressed, mutually inhibiting genes were always negatively co-expressed). When direct regulations  
 331 were prevented, co-expression could evolve, but to a lesser extent (Figure 4C). Direct regulations are  
 332 thus the main contributor to the evolution of the pleiotropy in gene networks.

333 We manipulated the network topology to increase further the network distance between focal genes  
 334 and assessed the effect of network distance on mutational correlations (Figure 5). While direct regu-  
 335 lations between two genes allowed for the evolution of mutational correlations ranging from  $-0.6$  to  
 336  $+0.7$ , correlation intensity decreased with the network distance, as it barely spanned  $\pm 0.2$  with one  
 337 intermediate gene, and  $\pm 0.05$  with two intermediate genes. No mutational correlation was detected  
 338 with more than two intermediate genes. Similar simulations with larger networks displayed the same  
 339 trend (Suppl. Fig. 5).

### 340 Correlated selection can shape gene networks at a large scale

341 So far, we assessed whether the fitness correlation between two genes only could shape the local  
 342 topology of the gene network, which was arguably a favorable scenario that maximizes the chances for  
 343 the genetic architecture to respond. Realistic selection pressures are probably more complex, involving  
 344 many genes and a complex pattern of fitness correlations among them. To check whether the observed  
 345 pattern was maintained at a larger scale, we simulated the evolution large GRNs in which all genes  
 346 were under stabilizing selection (diagonal elements of the matrix  $\mathbf{S}$  were set to 10), while all pairwise  
 347 correlations were drawn randomly. Although the association between the fitness correlation and the  
 348 mutational correlation weakened with the number of genes, the response was still observable with up  
 349 to 30 genes (Figure 6). This confirms that network topology can be shaped by selection at a large  
 350 scale, and that our results are not an artifact of focusing on small network motifs.

351 The effect of network size ( $n$ ), as well as five other parameters (population size  $N$ , mutation size  
 352  $\sigma_m$ , strength of selection, and basal expression  $\kappa$ ) have been explored in Suppl. Fig. 6. Our main  
 353 result (the mutational structure responds to correlated selection) appeared to be robust to parameter  
 354 changes, and may only be affected by extreme parameter values. The default parameter set was not  
 355 necessarily optimal, as larger co-expression could evolve in large populations ( $N = 10,000$ ).

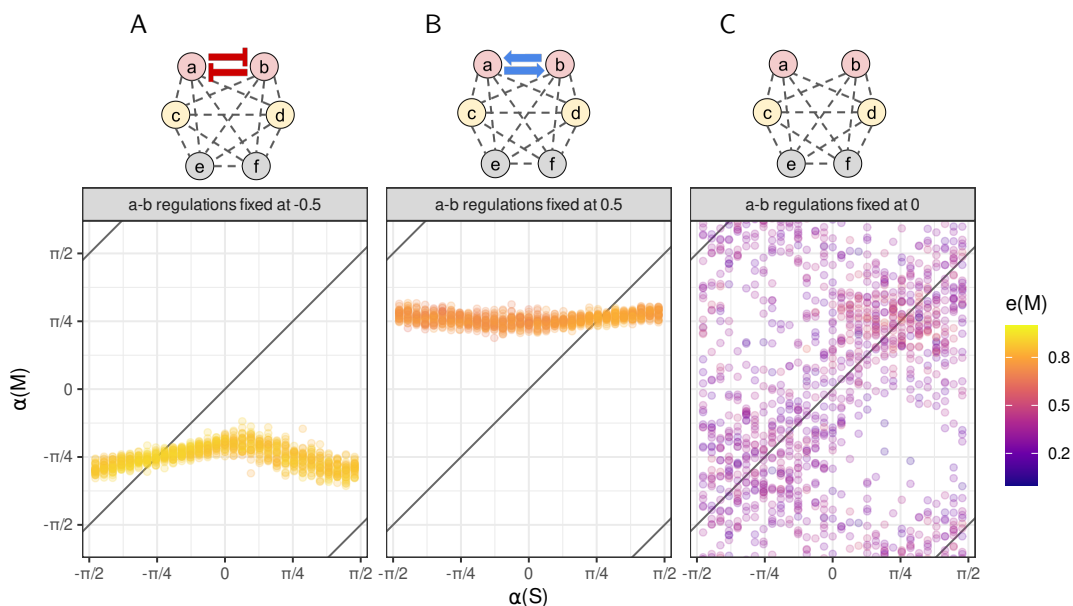


Figure 4: Direction of mutational matrices ( $\alpha(M)$ ) after 10,000 generations of evolution as a function of the direction of the correlated fitness function ( $\alpha(S)$ ). The representation is the same as in Figure 2D and E. A: fixed negative (inhibition) regulation between genes  $a$  and  $b$ , B: fixed positive (activation) regulation, C: no regulation. The corresponding networks are represented above the scatterplots (red arrows: constant inhibition, blue arrows: constant activation, grey hyphenated connections: evolvable regulations; whether or not such regulations evolved in the simulations differed among replicates). Other conditions were the same as in Figure 2E.

## 356 Discussion

357 Constant stabilizing selection is often thought to promote stasis, and thus prevent the evolution of  
 358 traits, but it is also suspected to modify the structure of genetic architectures, in particular through  
 359 the minimization of the fitness load due to environmental disturbances and mutations (Wagner *et al.*,  
 360 1997). When considering phenotypic traits independently, stabilizing selection promotes genetic back-  
 361 grounds that reduce mutational effects (Rice, 2002; Hermisson *et al.*, 2003). Simulations based in gene  
 362 network models have reproduced this predictions, and have associated the decrease in the effects of  
 363 mutations with systematic changes at the network level, including feedback loops, global network size  
 364 and properties, and redundancy (Masel and Siegal, 2009; Payne and Wagner, 2015; Rünneburger and  
 365 Le Rouzic, 2016).

366 Quantitative genetics theory predicts that mutational correlation between traits (pleiotropy) can  
 367 evolve as the result of correlated stabilizing selection (Cheverud, 1984; Jones *et al.*, 2014). Yet, this  
 368 theoretical evolutionary force is weak and indirect, and could easily be overwhelmed by genetic drift,  
 369 correlations with phenotypic trait values, and mutation bias. We simulated the evolution of mutational  
 370 correlations in three genetic architectures to assess whether the evolution of pleiotropy depends on  
 371 the mechanisms underlying trait expression. In both models derived from traditional multivariate  
 372 quantitative genetics settings (the multilinear model and the gene pleiotropy model), the evolution  
 373 of mutational correlations was similar: the mutation covariance matrix aligned with the direction  
 374 of the fitness function, and correlations remained modest. In contrast, in gene regulatory networks,  
 375 correlations could also evolve qualitatively, but the response was not isotropic in the phenotypic space.  
 376 Correlation patterns were mostly driven by the regulatory distance and nature (activation or inhibition)  
 377 of regulations between genes. Simulations confirmed that the correlation between gene expressions  
 378 decreased with the network distance among genes, and that strongest correlations were associated  
 379 with direct regulations. As correlated stabilizing selection promotes mutational correlation, it thus  
 380 promotes network topologies where selected genes are closely connected. Taken together, our results  
 381 exemplify how the shape of a constant, stabilizing fitness function can theoretically drive the structure  
 382 of the underlying genetic architecture.

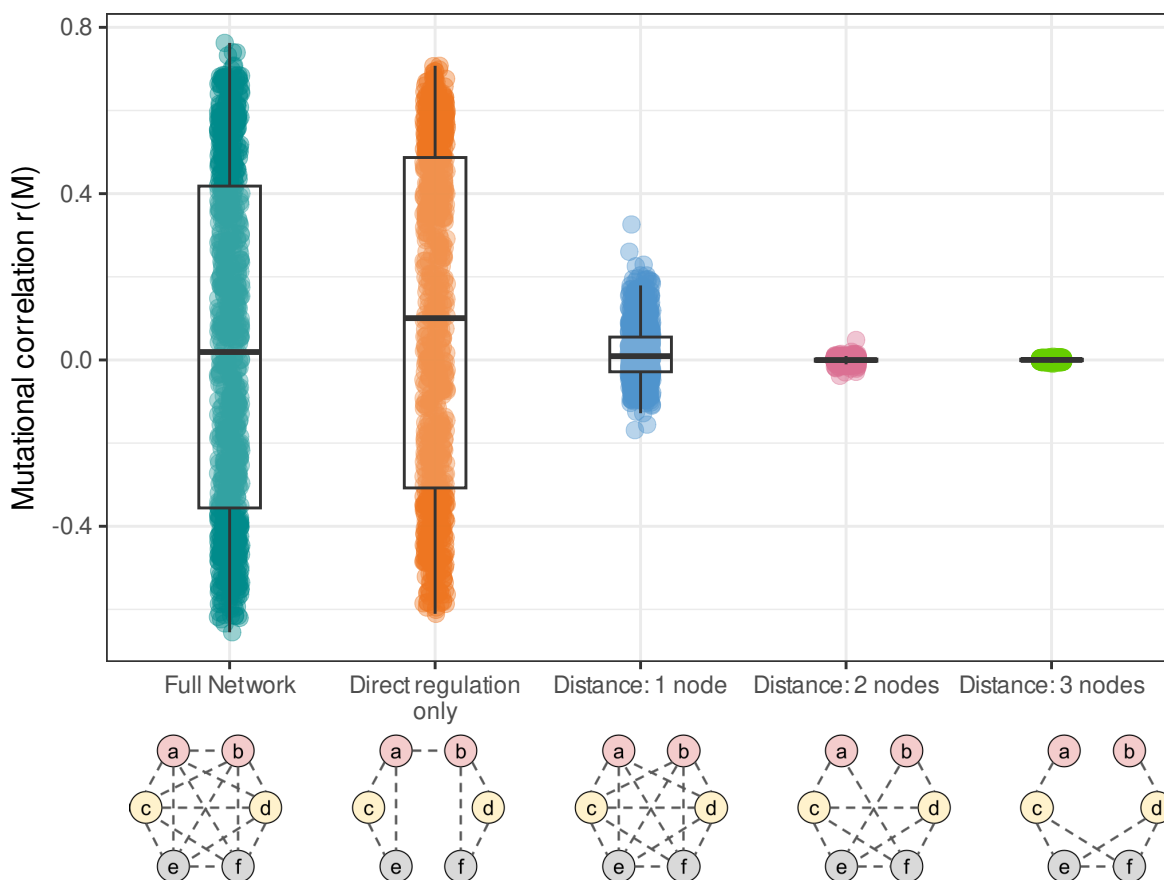


Figure 5: Distribution of mutational correlations  $r(\mathbf{M})$  in networks of different topologies, illustrated under the x-axis. All networks have evolved in the same set of conditions as in Figure 4, i.e., 31 correlated fitness functions oriented in various directions. Genes colored in pink ( $a$  and  $b$ ) are the genes under correlated selection, yellow genes (transcription factors  $c$  and  $d$ ) are under non-correlated selection, and grey genes are not selected (sans color code as in Figures 1 and 4). Regulatory connections indicate which regulations were possible; whether or not such regulations evolved in the simulations differed among replicates.

383 From a multivariate quantitative genetics point of view, organizing the phenotypic space along  
 384 measurable traits is a necessary consequence of how phenotypes are estimated empirically, but most  
 385 models can easily be redefined for any linear combination of traits. For instance, when the bivariate  
 386 multilinear model is parameterized in such a way that epistasis and pleiotropy are identical in all direc-  
 387 tions of the phenotypic space, mutational effects evolve indifferently towards robustness or pleiotropy  
 388 depending on the direction of the fitness function (Jones *et al.*, 2014 and our simulations). This is where  
 389 the quantitative genetics predictions break when applied to gene networks: in network models, genes  
 390 both define observable phenotypes and structure the regulatory patterns. As a consequence, correlated  
 391 selection can also induce the evolution of pleiotropic gene expression, as predicted by theory, but the  
 392 mechanisms involved into this response are different from those involved into the evolution of robust-  
 393 ness. Our simulations pointed out the major effect of the network distance on co-expression, showing  
 394 that evolving pleiotropic gene expression requires to rewire the network and reduce the distance (and  
 395 possibly the sign of regulations) between co-selected genes.

## 396 Model properties

397 All three models implemented in our simulation software are based on fundamentally different princi-  
 398 ples, although they all allow for the evolution of pleiotropy.

399 In the multilinear model, pleiotropy arises as a consequence of the gene-gene interactions (epista-  
 400 sis). Under stabilizing selection, the genetic contributions ( $y^i$  in the mathematical model description)



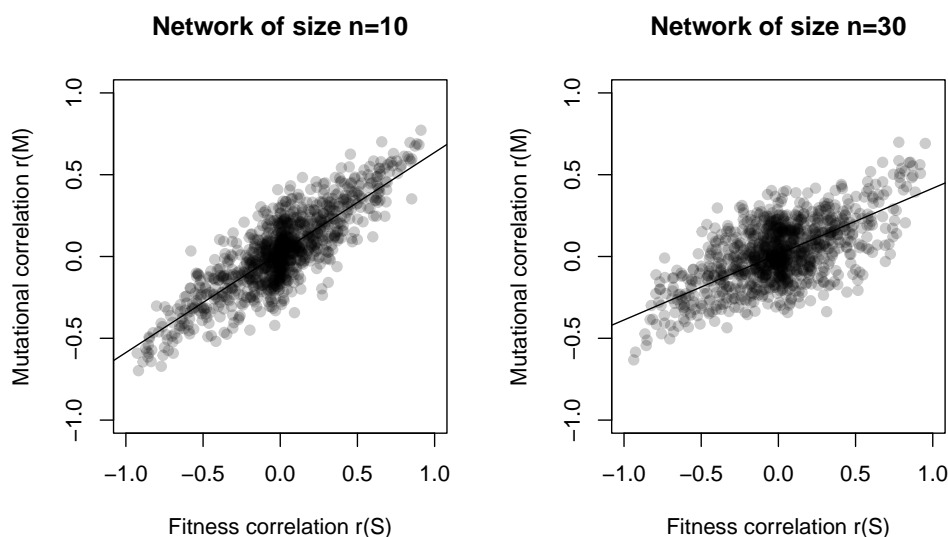


Figure 6: Mutational correlation after 20000 generations of evolution, as a function of the fitness correlation in networks of various sizes ( $n = 10$  and  $n = 30$ ). Each pair of genes was under correlated selection, correlations ranging from  $-0.9$  to  $0.9$ . To ensure that the correlation matrix was positive definite, selection correlations were the non-diagonal elements of the  $n \times n$  matrix  $uu^T$ , where  $u$  was drawn in a uniform  $(-1; 1)$  distribution. The mutation rate was scaled across simulations to ensure a constant mutation rate by gene.

401 can evolve at all genes, provided that the changes are compensated by other genes (so that the phe-  
402 notype remains constant). Due to the non-linear genotype-phenotype mapping, the average effect of a  
403 mutation can thus evolve when the genetic background changes, which modified mutational variances  
404 and covariances.

405 The interaction coefficients  $\varepsilon$  have an empirical meaning, as they measure the curvature of the  
406 genotype-phenotype map in some specific directions (Hansen and Wagner, 2001; Le Rouzic, 2014). Yet,  
407 the number of coefficients grow very fast with the complexity of the model (proportionally to  $n^o K^3$   
408 for a  $n$ -gene  $K$ -trait model when considering epistatic interactions of order  $o$ ), and even our simple  
409 setting ( $n = 6$ ,  $o = 2$ ,  $K = 2$ ) made it complicated to control the properties of the genetic architecture  
410 by finely setting these coefficients. In particular, isotropy (the fact that traits were interchangeable) is  
411 not a general property of the multilinear model, but rather a consequence of distributing  $\varepsilon$  coefficients  
412 independently.

413 In contrast, the gene pleiotropy model is perfectly additive, and the effects of mutations are constant  
414 throughout the simulations. Each gene has a specific, non-evolvable pleiotropic pattern, and the  
415 mutational covariances change at the genotype level because the differential mutation rates of all genes  
416 can evolve. It is thus the relative gene contribution to the  $\mathbf{M}$  matrix that drives the evolution of  
417 mutational correlations. Contrary to the multilinear model, changing the size of the  $\mathbf{M}$  matrix was  
418 not possible (i.e., robustness to mutations could not evolve for both traits at once), and only the shape  
419 and the direction of the mutational covariance matrix was evolvable.

420 The gene network model used for the simulation was based on the popular 'Wagner' model (Wagner,  
421 1994, 1996). This model has already been explored in evolutionary biology to study the evolvability,  
422 the modularity, and the canalization of gene regulatory networks (Siegal and Bergman, 2002; Ciliberti  
423 *et al.*, 2007; Rünneburger and Le Rouzic, 2016, see Fierst and Phillips, 2015 for review). This model  
424 is computationally fast and requires few parameters in addition to the structure of the regulation net-  
425 work itself. Contrary to both previous models, which were based on quantitative genetics (statistical)  
426 principles, the gene network model implements mechanistic interactions among genes, so that com-  
427 plexity emerges from the model structure. More specifically, epistasis emerges from the non-linearity  
428 of the sigmoid regulation scaling function, and pleiotropy is due to the causal relationship between  
429 the expression level of regulatory genes and the consequences on the expression of regulated genes.  
430 Its lack of realism at the biochemical and cellular level (discrete time steps, no degradation kinetics,  
431 no compartments, arbitrary dose-response function) makes it less popular for physiological models of



432 known regulation pathways (alternative models could be found in e.g. [Karlebach and Shamir, 2008](#)),  
433 but it is a convenient framework for theoretical studies of network evolution on evolutionary time  
434 scales.

435 Theoretical approaches to the evolution of genetic architectures are often limited by the oversim-  
436 plification of the selective constraints. In a multicellular organism, phenotype is many-dimensional  
437 and encompass morphological, behavioral, and physiological traits in a complex temporal and spatial  
438 context, accounting to different developmental stages, different cell types, and different environmental  
439 conditions. In contrast, the Genotype-to-Fitness map in our simulations was simple (multivariate bell-  
440 shaped) and phenotypic optima were constant and close to their initial value (no adaptive evolution  
441 in the simulations). Realistic patterns and strengths of selection in high phenotypic dimensions are  
442 not really known, but recent statistical and experimental progress makes it possible to expect reliable  
443 empirically-based estimates in the near future, e.g., for gene expressions in a transcriptome ([Whitehead  
444 and Crawford, 2006](#); [Koch and Guillaume, 2020](#); [Price \*et al.\*, 2022](#)).

## 445 Co-expression in gene regulatory network

446 Whether or not stabilizing selection could affect gene network topology is not a trivial question. It  
447 is not clear whether structural features of biological networks result from an adaptive process. For  
448 instance, modularity can emerge from different mechanisms, including direct selection for efficiency  
449 ([Clune \*et al.\*, 2013](#)), adaptation to modular environments ([Kashtan and Alon, 2005](#)), indirect selection  
450 for evolvability, or mutation bias ([Wagner \*et al.\*, 2007](#)). Large-scale mathematical properties, such  
451 as scale-freeness, may not have any impact on fitness, and the evolution of gene networks may be  
452 dominated by non-adaptive mechanisms, including genetic drift and mutation bias ([Lynch, 2007](#)). The  
453 mechanisms of gene regulation generate a lot of epistasis and pleiotropy at the gene expression level,  
454 but these are not expected to be uniformly distributed in the phenotypic space. In particular, co-  
455 expression among the genes belonging to the same regulatory module is unavoidable, suggesting that  
456 evolving expression independence might be more difficult than evolving correlated expression, as it  
457 requires to rewire the network and change its modularity.

458 We observed repeatedly that gene networks were evolving to match the fitness function qualitatively,  
459 but often failed to align to the correct direction. We could discard the possibility that some networks  
460 could be trapped at a local optimum, since starting close to the direction of the fitness function evolved  
461 to imperfect alignment. Several hypotheses can be proposed to explain this gene-network specific  
462 observation : (i) mutations affecting gene co-expression have direct negative side effects (change in gene  
463 expression, decrease of the network stability), so that the fitness peak corresponds to a sub-optimal  
464 pleiotropic pattern; (ii) mutations affecting gene co-expression have indirect negative side effects (e.g.,  
465 increase the size of  $\mathbf{M}$ ) and actually do not decrease the genetic load; (iii) some  $\mathbf{M}$  matrices cannot  
466 be obtained with this gene network model. It was difficult to investigate this question further based  
467 on our simulation setting.

468 Here, we showed theoretically how correlated stabilizing selection on gene expression could deter-  
469 ministically alter the topology of gene networks, by shortening the network distance between genes  
470 which expression levels interact at the fitness level. Simulations show that the evolution of regulatory  
471 connections as a result of correlated selection can realistically happen in non-restrictive conditions, even  
472 if it is difficult to estimate the extent by which real gene networks are affected by this phenomenon.  
473 Nevertheless, correlated selection is not the only form of selection that may promote specific network  
474 topologies. Directional selection on a multivariate phenotype may for instance indirectly favor genetic  
475 backgrounds generating mutational variation towards the optimum. Fluctuating selection could sim-  
476 ilarly promote pleiotropy when the optimal phenotypes are correlated among traits ([Crombach and  
477 Hogeweg, 2008](#)).

478 Independently, fluctuating selection also opens the possibility for the organisms to gather cues  
479 about the environment and evolve an adaptive plastic response ([Via and Lande, 1985](#)). If, as intuited  
480 by [Waddington \(1942\)](#), complex genetic architectures respond to mutational and environmental distur-  
481 bances through shared molecular mechanisms, it is likely that mutational and environmentally-induced  
482 co-expressions will be similar – a property of complex genetic system that could fasten genetic adap-  
483 tation ([Brun-Usan \*et al.\*, 2021](#); [Chevin \*et al.\*, 2021](#)). Direct selection for correlated gene expression  
484 plasticity among sets of genes thus appears as a potentially powerful force that could drive the evolu-  
485 tion of the topology of gene networks, perhaps strong enough to overcome the influence of correlated  
486 selection illustrated in our simulations.

487 Even if our results explored the theoretical possibility for selection to affect the modularity of  
488 genetic architectures, interpreting gene network topologies as systematic consequences of an adaptive  
489 process would be largely premature. The influence of correlated selection on the topology and on  
490 co-expression in real gene networks remains virtually unknown. Adaptive (e.g., plasticity) and non-  
491 adaptive (mutation bias or genetic drift) forces are also prone to alter network topology, and condition  
492 the long-term evolvability of these complex genetic architectures. Since gene networks can be shaped  
493 by selection, but can also constrain the mutational availability of evolutionary path, the long-term  
494 influence of past environment on phenotypic variability and evolvability remains a challenging question  
495 in both quantitative genetics and evolutionary systems biology.

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## 502 Conflict of interest

503 The authors declare no conflict of interest.

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## 663 Supplementary material

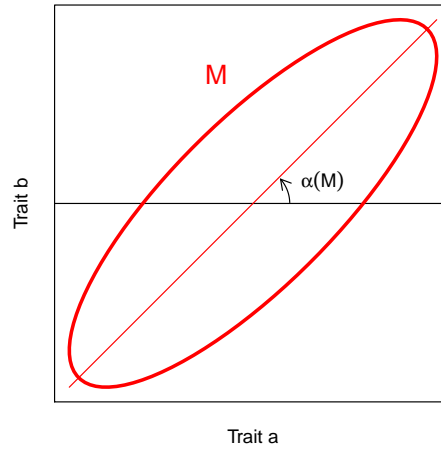
### 664 Supplementary Methods

665 **Mutational direction** The mutational direction  $\alpha(\mathbf{M})$  is the angle between trait  $a$  and the main  
 666 axis of the  $2 \times 2$  mutational matrix  $\mathbf{M}$  between the two focal traits  $a$  and  $b$ . It was expressed in the  
 667 interval  $(-\pi/2, \pi/2)$ , and calculated as:

$$\alpha'(\mathbf{M}) = \text{acos}(m_{1,1}) \pmod{\pi},$$

$$\alpha(\mathbf{M}) = \begin{cases} \alpha'(\mathbf{M}) & \text{if } \alpha'(\mathbf{M}) < \pi/2 \\ \alpha'(\mathbf{M}) - \pi & \text{otherwise.} \end{cases}$$

668 where  $m_{1,1}$  is the first element of the first eigenvector of  $\mathbf{M}$ .



669

670 Whenever necessary, the mean direction  $\bar{\alpha}$  over  $R$  replicates was obtained by a circular mean  
 671 restricted to the  $(-\pi/2, \pi/2)$  interval:

$$\bar{\alpha} = \frac{1}{2} \left[ \text{atan2} \left( \frac{1}{R} \sum_{i=1}^R \sin 2\alpha_i, \frac{1}{R} \sum_{i=1}^R \cos 2\alpha_i \right) \right] \pmod{\pi},$$

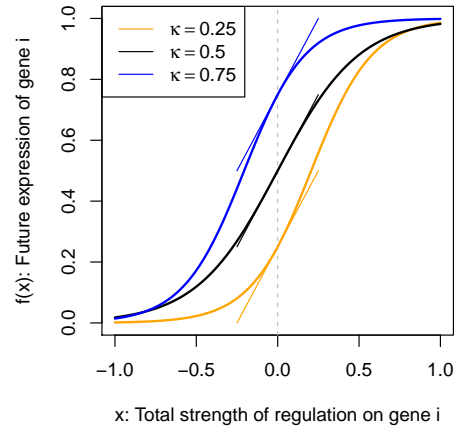
672 where  $\text{atan2}(x, y) = 2 \text{atan} [y/(x + \sqrt{x^2 + y^2})]$  is the angle between the X-axis and the vector  $(x, y)$ .

673 **Regulation scaling function** Quantitative gene network models require a scaling function that  
674 maps the strength of regulation on a gene (the total effects of all transcription factors acting on the  
675 gene) and gene expression. Here, we used the same scaling function as in [Rünneburger and Le Rouzic](#)  
676 [2016](#):

$$f(x) = \frac{1}{1 + \left(\frac{1}{\kappa} - 1\right) \exp\left(-\frac{x}{\kappa(1-\kappa)}\right)}$$

677 where  $\kappa \in (0, 1)$  stands for the basal expression level. By definition,  $f(0) = \kappa$  (in absence of regulation,  
678 the gene is expressed at its basal level), and the function is scaled so that  $df/dx|_{x=0} = 1$ , in order to  
679 ensure that effects of genotype changes are comparable across simulations with different basal levels.

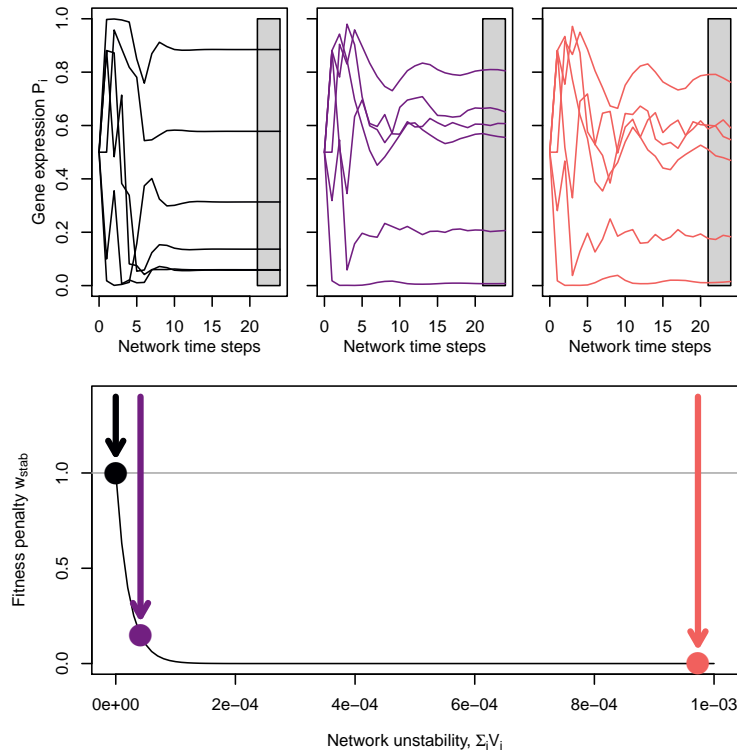
680 With the default basal expression  $\kappa = 0.5$ , the scaling function reduces to  $f(x) = 1/(1 + e^{-4x})$ .



681

682 In the simulations, the regulation on gene  $i$  was obtained by adding up the effect of transcription  
683 factors, proportionally to their concentration:  $x_i = \sum_{j \neq i} P_j W_{ij}$ .

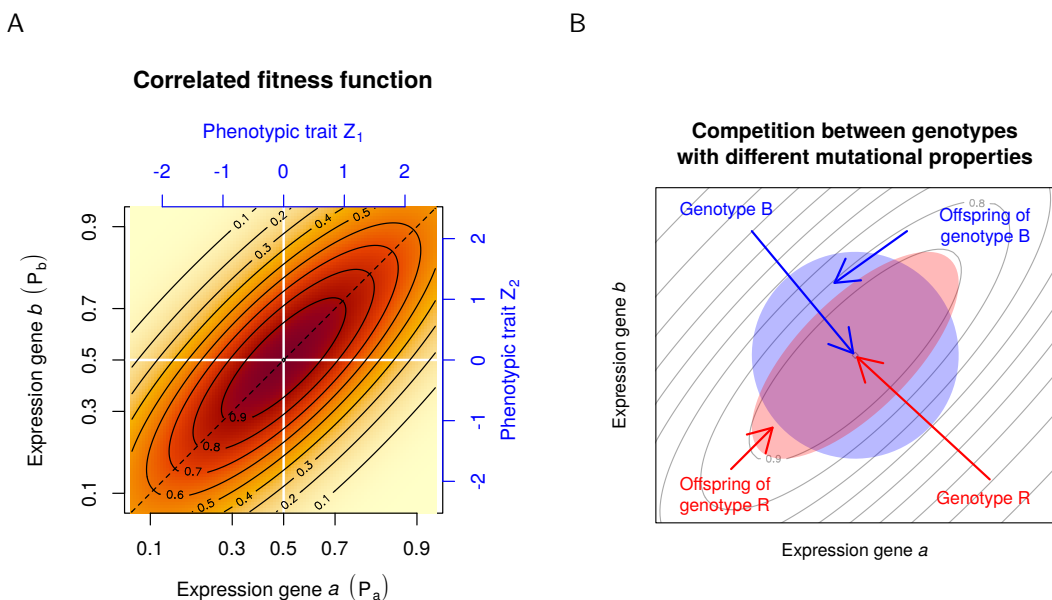
684 **Selection against unstable networks** The fitness component associated with selection against  
 685 unstable (cyclic) networks was a negative exponential function of the variance in gene expression. This  
 686 exponential scaling ensures that the fitness penalty is nil when the network is stable ( $w_{stab} = 1$  when  
 687  $\sum V_i = 0$ ), and that the individual is virtually not viable when the network is unstable ( $w_{stab} \rightarrow 0$   
 688 when  $\sum V_i$  is large).



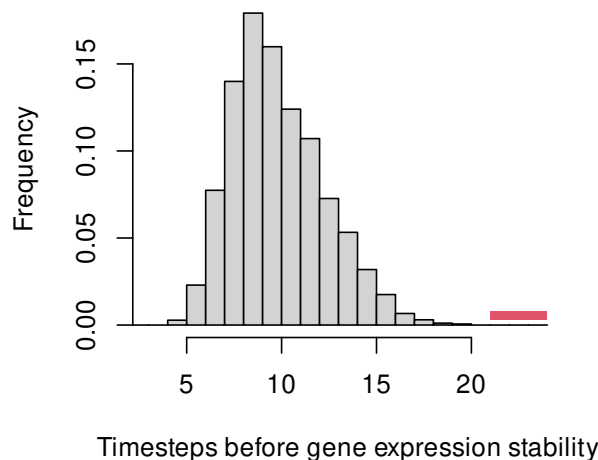
689

690 The figure displays the dynamics of three arbitrary 6-gene networks displaying diverse stability  
 691 behavior. Gene expression stability is measured in the gray areas (steps 21 to 24). The bottom panel  
 692 represents the fitness penalty used in the simulations ( $w_{stab} = e^{-s' \sum V_i}$ , with  $s' = 46,000$ ). The fitness  
 693 effect associated with the stable network (black, left) is  $w_{stab} \simeq 1$ , which does not penalize the fitness  
 694 function. In contrast, fitness is multiplied by  $w_{stab} \simeq 0$  for the unstable network (red, right), making  
 695 the individual unviable regardless of the gene expression level. With the strong selection coefficient  $s'$ ,  
 696 even slightly fluctuating networks (violet) were substantially penalized.

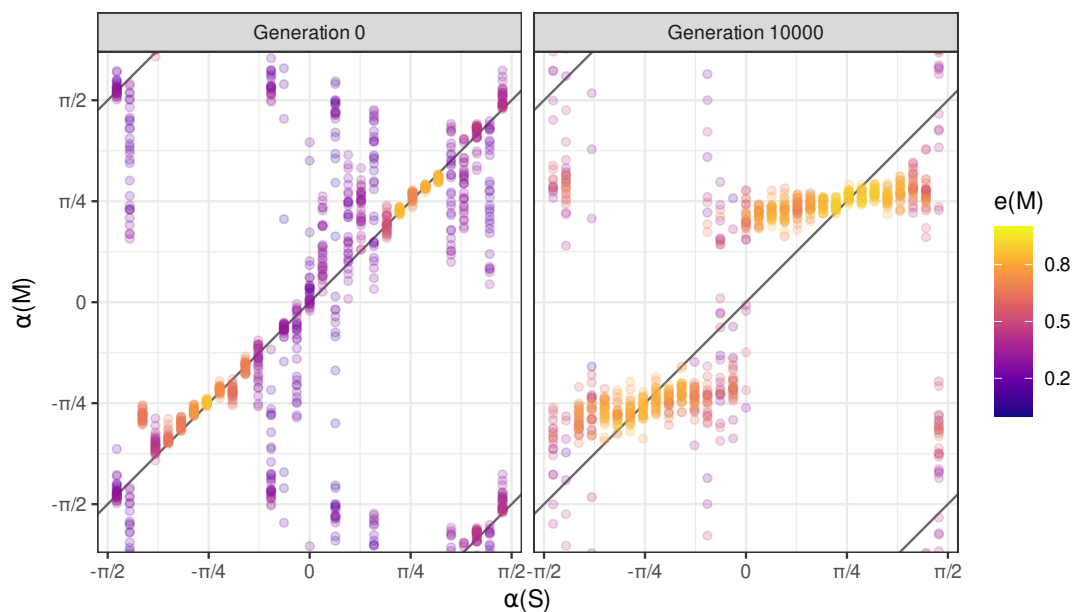
697 **Supplementary Figures**



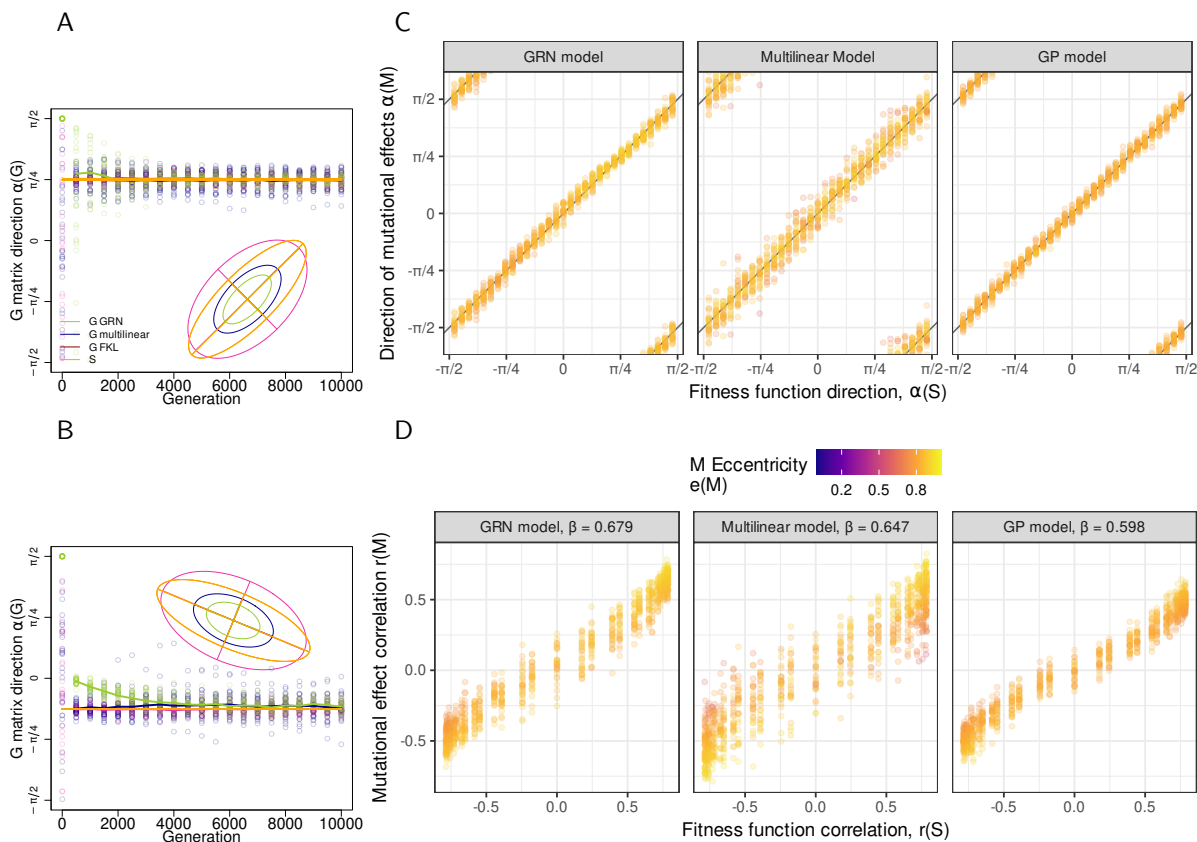
Supplementary Figure 1: A: representation of the bivariate correlated fitness function used in the simulations (here, with an direction  $\alpha(\mathbf{M}) = \pi/4$ ). Axes display both gene expression levels ( $\mathbf{P}$ ) scaled between 0 (no expression) and 1 (full expression) in black, and the corresponding rescaled phenotypes  $\mathbf{Z}$  from which fitness was computed in all three models (in blue). White lines highlight the optimal phenotype (for which the fitness is maximal). Any deviation from the optimal phenotype is penalized, but the penalty is weaker when both traits change together. B: Cartoon representation of the advantage of a genotype in which mutational effects are correlated (red, R) over a genotype in which mutational effects are uncorrelated (blue, B). Both genotypes display the optimal gene expression and thus have the same fitness; mutant offspring from both genotypes deviate from the optimum within the same range, but due to the genetic correlation, the average fitness of the mutant offspring (iso-fitness lines in gray) from genotype R is higher than the offspring from genotype B: the R lineage will progressively replace the B lineage in the population.



Supplementary Figure 2: Number of dynamic steps during the "development phase" necessary for genes to reach a stable expression, i.e a variance between time-steps  $< 0.0001$ . The equilibrium expression is the mean expression of time-steps 21 to 24, indicated in red. The last generation of all of our 8281 GRN simulations presented in the main text are represented in this figure.

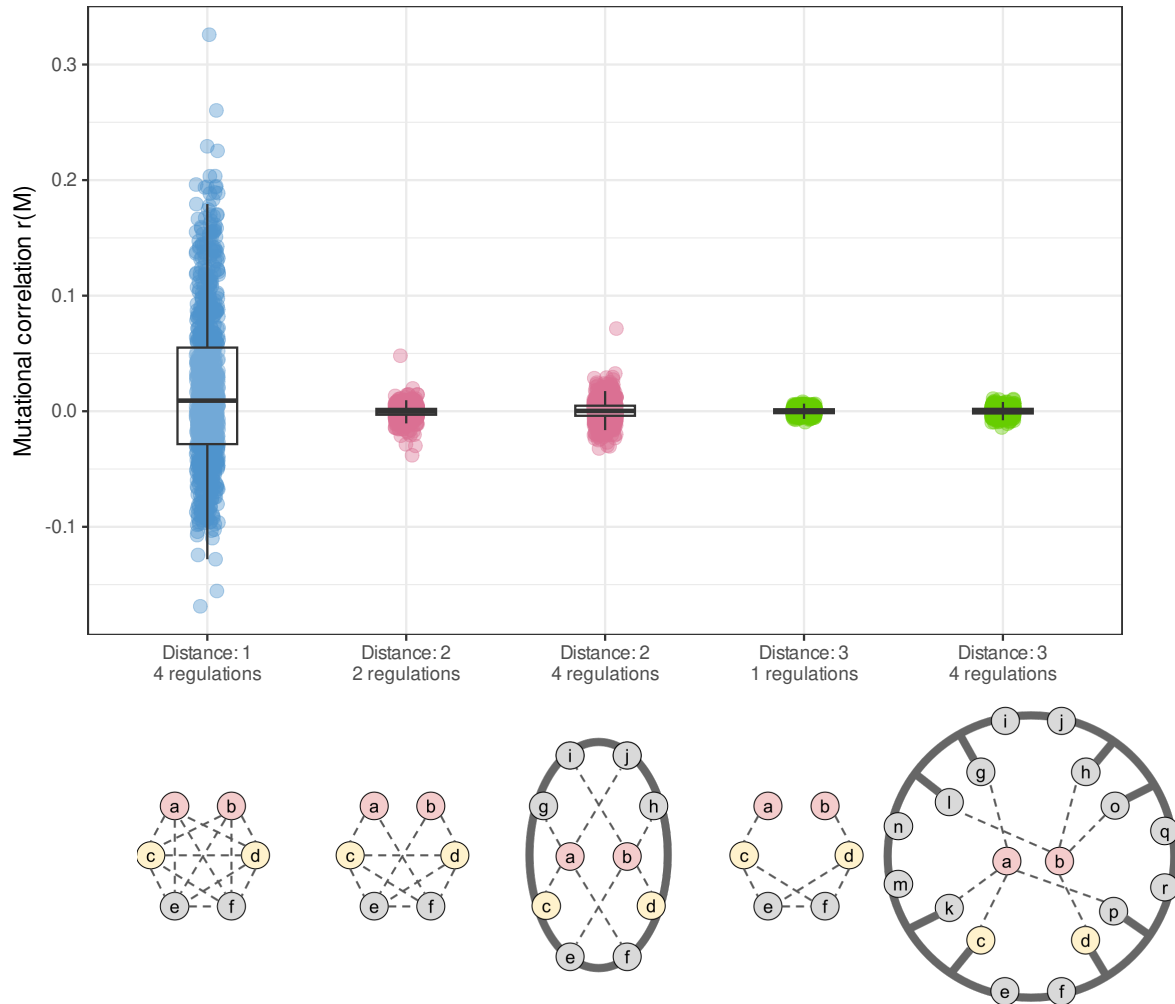


Supplementary Figure 3: Evolution of  $\alpha(\mathbf{M})$  in response to  $\alpha(\mathbf{S})$ , starting simulations with networks giving the closest alignment in previous simulations (Figure 2C, GRN model). For the same  $\alpha(\mathbf{S})$ , replicates started with the same GRN. The variance obtained in the  $\alpha(\mathbf{M})$  at generation 0 is due to sampling effects when computing  $\mathbf{M}$ , which was substantial when  $\mathbf{M}$  were close to round (no well-defined direction).

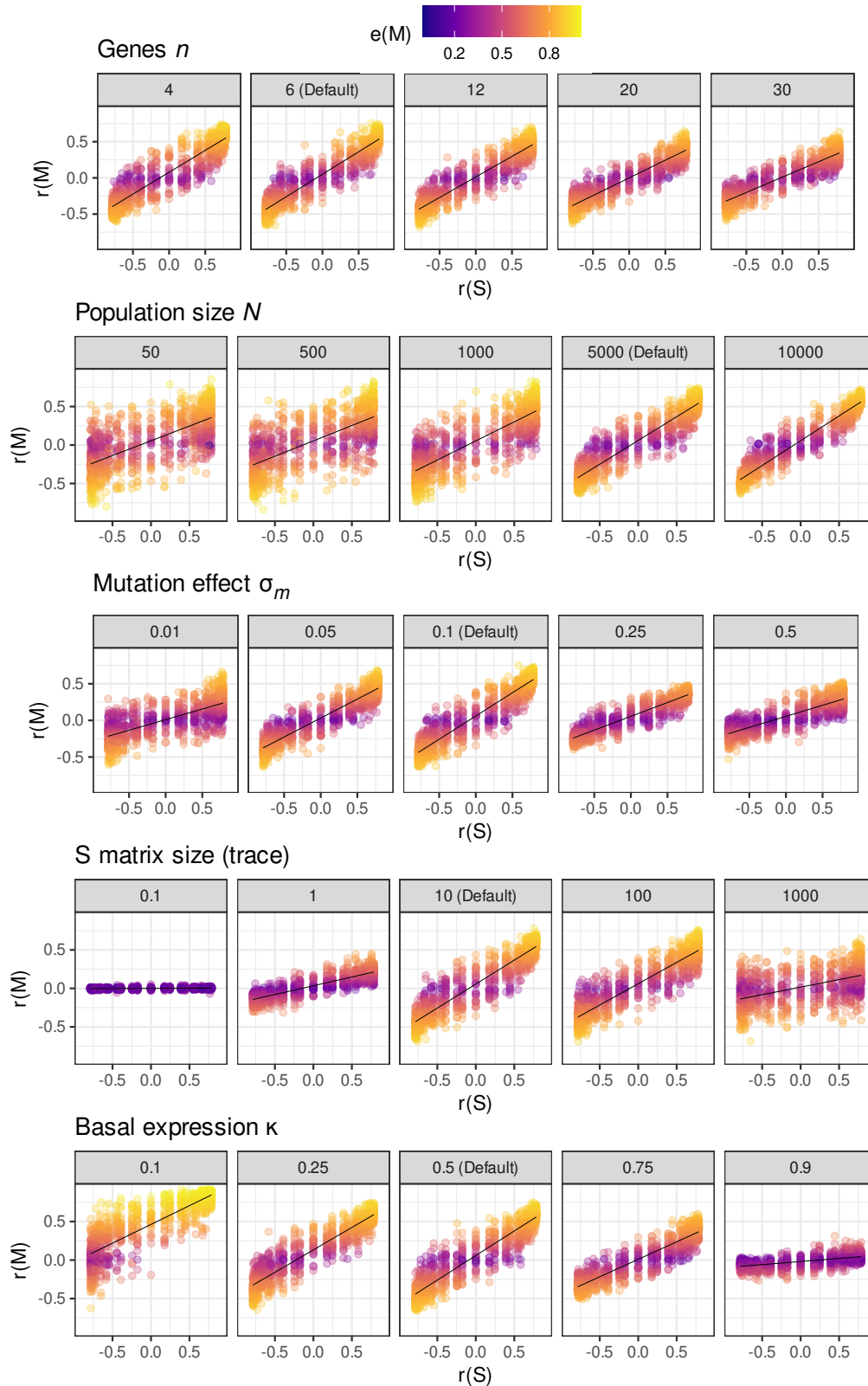


Supplementary Figure 4: The same as Figures 2, but for the  $\mathbf{G}$  matrix. A, B: Evolution of the angle of the main axis of the mutation matrix ( $\alpha(\mathbf{G})$ ) along generations. Orange ellipses represent the fitness function (scaled  $\times 0.025$ ), which direction was  $\alpha(\mathbf{S}) = +\pi/4$  (panel A), and  $\alpha(\mathbf{S}) = -\pi/8$  (panel B). Dots illustrate 30 simulation replicates, plain lines stand for circular means  $\bar{\alpha}(\mathbf{G})$ . Differences in the shape of  $\mathbf{M}$  and  $\mathbf{G}$  matrices can be explained by linkage disequilibrium (the more LD, the more  $\mathbf{G}$  can be similar to  $\mathbf{S}$ ). In A and B, the sizes of the  $\mathbf{G}$  matrix differ between the models more than their  $\mathbf{M}$  matrix, due to different responses of the three models to LD. The  $\mathbf{G}$  matrix in the GP model is the least affected by linkage disequilibrium, as evolution tends to decrease the effective number of loci contributing to the traits. In contrast, regulatory sites in the promoter of genes are completely linked in the GRN model, allowing for the evolution of strong and persistent LD.





Supplementary Figure 5: Distribution of mutational correlations with different networks (same color code as in Figure 5). Two topologies were compared for the same network distance : one with the number of genes conserved (6 genes), and one with the number of edges connected to *a* and *b* conserved (4 possible regulations). Genes located on grey circle are all connected to each other; genes connected to the grey circle can interact with every gene on the circle but not with each other.



Supplementary Figure 6: Parameter exploration on the response from the mutational correlation  $r(\mathbf{M})$  to the selection correlation  $r(\mathbf{S})$ . When changing the network size  $n$ , the mutation rate per individual  $\mu$  was adjusted to keep the same mutation rate per gene. For the smallest values of  $\sigma_m$  0.01 and 0.05), the simulation duration was changed to 100000 and 20000 generations, respectively, to ensure that the population has reached a similar equilibrium. Gene expression optima  $\theta$  were adjusted to follow the basal expression  $\kappa$ .