

# Review: The causes of epistasis

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## Abstract

Since Bateson's discovery that genes can suppress the phenotypic effects of other genes, gene interactions – called epistasis – have been the topic of a vast research effort. Systems and developmental biologists study epistasis to understand the genotype-phenotype map, while evolutionary biologists recognize the fundamental importance of epistasis for evolution. Depending on its form, epistasis may lead to divergence and speciation, provide evolutionary benefits to sex, and affect the evolvability of organisms. That epistasis can itself be shaped by evolution has only recently been realized. Here, we review the empirical pattern of epistasis and some of the factors that may affect the form and extent of epistasis. Based on their divergent consequences, we distinguish between interactions with or without mean

28 effect, and those affecting the magnitude of fitness effects or their sign. Empirical  
29 work has begun to quantify epistasis in multiple dimensions in the context of  
30 metabolic and fitness landscape models. We discuss possible proximate causes,  
31 such as protein function and metabolic networks, and ultimate factors, including  
32 mutation, recombination, and the importance of natural selection and genetic drift.  
33 We conclude that in general pleiotropy is an important prerequisite for epistasis, and  
34 that epistasis may evolve as an adaptive or intrinsic consequence of changes in  
35 genetic robustness and evolvability.

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39 **Key words:** epistasis, pleiotropy, robustness, evolvability

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41 **1. INTRODUCTION**

42

43 How an organism's genotype determines its phenotype is the focus of vast research  
44 efforts in developmental and systems biology (Costanzo et al. 2010; Moore &  
45 Williams 2005). It is now clear that the mapping between genotype and phenotype is  
46 complex and most phenotypes result from intricate gene interactions. These  
47 interactions, recognized as deviations from additive genetic effects on the phenotype  
48 and collectively called epistasis, are central to evolutionary theories, including those  
49 seeking explanations for divergence and speciation, recombination, genetic  
50 robustness, and evolvability (Phillips 2008; Wolf et al. 2000). These theories make  
51 detailed predictions regarding the consequences of epistasis. By contrast, we know  
52 very little about the causes of epistasis, in particular, how gene interactions are  
53 shaped by natural selection and genetic drift.

54 The notion that epistasis not only influences evolution, but can itself be  
55 altered as a consequence of changes of an organism's genetic architecture, is  
56 relatively recent. In a seminal study, Malmberg (1977) observed that recombination  
57 alleviated epistasis between beneficial mutations in bacteriophage T4. However, it  
58 took almost three decades before theoretical studies addressed how epistasis  
59 evolves (Azevedo et al. 2006; Desai et al. 2007; Gros et al. 2009; Liberman &  
60 Feldman 2005, 2008; Liberman et al. 2007; Martin & Wagner 2009; Misevic et al.  
61 2006). The purpose of this review is to survey existing ideas about the proximate  
62 (mechanistic) and ultimate (evolutionary) causes of epistasis. We will review  
63 definitions and various forms of epistasis, survey the empirical evidence of epistasis,  
64 and discuss theoretical and empirical studies that address its causes.

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67 **2. TERMINOLOGY**

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69 Over a century ago, William Bateson et al. (1905) introduced the term epistasis to  
70 describe the suppression of an allelic phenotype by an allele at another locus. Later,  
71 Ronald Fisher (1918) 'rediscovered' epistasis by finding deviations from expected  
72 additive effects on quantitative traits of alleles occurring at the same (dominance) or  
73 different loci. In the evolutionary literature, in reference to Fisher's definition, the term  
74 epistasis includes all deviations from independent effects of alleles at different loci on  
75 a phenotype (Phillips 1998; Phillips 2008; Wolf et al. 2000). On which scale effects  
76 are called independent depends on the consequences of epistasis one is interested  
77 in. As our focus is on the evolutionary role of epistasis, we focus on epistasis at the  
78 level of fitness, where deviations from multiplicative effects are relevant. We make  
79 two distinctions.

80 First, we distinguish between *unidimensional* and *multidimensional* epistasis  
81 (Kondrashov & Kondrashov 2001). Unidimensional epistasis refers to deviations from  
82 a linear relationship between *mean* log fitness and the number of alleles affecting  
83 fitness (figure 1(a)). This form of epistasis has also been called directional or mean  
84 epistasis, and can be positive or negative depending on whether the fitness of  
85 genotypes carrying multiple mutations is higher or lower than expected from  
86 independent effects, respectively. Antagonistic epistasis among deleterious  
87 mutations and synergistic epistasis among beneficial mutations represent positive  
88 epistasis, while the opposite situations represent negative epistasis. Multidimensional  
89 epistasis refers to the individual interactions among a given set of alleles and  
90 provides a more complete description of the interactions within a fitness landscape  
91 involving these alleles (figure 1(b)). This description includes features such as the  
92 variation of epistasis among pairs of alleles, the number of fitness maxima, and  
93 measures of the accessibility of particular genotypes and pathways. Importantly, this  
94 type of epistasis can be common even if unidimensional epistasis is absent.

95 Second, within pairs of interacting alleles, one can distinguish between  
96 magnitude and sign epistasis. Magnitude epistasis refers to interactions where the

97 combined effect of two alleles deviates from multiplicative effects, but in a way that  
98 does not change the sign of either allele's fitness effect. Sign epistasis refers to  
99 'stronger' interactions where the sign of an allele's contribution to fitness changes  
100 with genetic background (Weinreich et al. 2005).

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### 103 **3. EMPIRICAL EVIDENCE OF EPISTASIS**

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#### 105 ***(a) Unidimensional epistasis***

106 Motivated by its relevance for explaining the evolution of sex (Kondrashov 1988;  
107 Barton 1995) and because its detection involves less effort, most empirical work on  
108 epistasis has focused on finding unidimensional epistasis among random mutations.  
109 Studies have examined epistasis in a variety of organisms, from viruses to plants and  
110 fruitflies (reviewed in de Visser & Elena 2007; Kouyos et al. 2007). Some studies  
111 reported negative epistasis (de Visser et al. 1996; de Visser et al. 1997a; Mukai  
112 1969; Salathé & Ebert 2003; Whitlock & Bourguet 2000), but others found positive  
113 epistasis (Jasnos & Korona 2007; Lenski et al. 1999; Maisnier-Patin et al. 2005;  
114 Sanjuán et al. 2004; Zeyl 2005) or no prevailing epistasis (de la Peña et al. 2000; de  
115 Visser et al. 1997b; Elena 1999; Elena & Lenski 1997; Hall et al. 2010; Kelly 2005).

116

#### 117 ***(b) Multidimensional epistasis***

118 Two recent research themes seek to provide a more complete empirical picture of  
119 epistasis. The first seeks to understand the metabolic basis and general organization  
120 of epistasis by studying pairwise interactions among deleterious mutations at a  
121 genome-wide scale. These analyses show (i) no (Costanzo et al. 2010; Segrè et al.  
122 2005) or prevailing positive epistasis (He et al. 2010; Jasnos & Korona 2007), (ii)  
123 extensive variation in the sign of epistasis, (iii) a modular pattern of epistasis, with  
124 similar interaction profiles for genes involved in the same functional module

125 (Costanzo et al. 2010; He et al. 2010; Segrè et al. 2005), and (iv) a hierarchical  
126 network structure, with most genes having few, but some ('hubs') many interactions  
127 (Costanzo et al. 2010).

128         The second approach has been to study all possible (i.e.  $2^n$ ) interactions  
129 among a given set of  $n$  — often beneficial — mutations. Such complete sets provide  
130 a detailed view of part of the fitness landscape for a given environment (Fig. 1(b)),  
131 including the extent of sign epistasis and the accessibility of the global peak under  
132 defined evolutionary scenarios (Carneiro & Hartl 2009; Franke et al. 2011; Weinreich  
133 et al. 2006). At present, fitness landscape data exist for sets of four to eight  
134 mutations for the enzymes isopropylmalate dehydrogenase (Lunzer et al. 2005),  
135 TEM-1  $\beta$ -lactamase (Weinreich et al. 2006) and sesquiterpene synthetase (O'Maille  
136 et al. 2008), the malaria parasite *Plasmodium falciparum* (Lozovsky et al. 2009), the  
137 fungus *Aspergillus niger* (de Visser et al. 2009; Franke et al. 2011), and the bacteria  
138 *Escherichia coli* (Khan et al. 2011) and *Methylobacterium extorquens* (Chou et al.  
139 2011).

140         These studies, as well as studies examining incomplete subsets of mutants  
141 (Costanzo et al. 2010; da Silva et al. 2010; Elena & Lenski 1997; Hall et al. 2010;  
142 Hinkley et al. 2011; Jasnos & Korona 2007; Khan et al. 2011; Kvitek & Sherlock  
143 2011; MacLean et al. 2010; Rokyta et al. 2011; Salverda et al. 2011; Whitlock &  
144 Bourguet 2000), show that: (i) multidimensional epistasis can be strong even when  
145 no significant unidimensional epistasis is detected, and (ii) sign epistasis, although  
146 not ubiquitous, is quite common and sometimes leads to fitness landscapes with  
147 multiple maxima (de Visser et al. 2009; Franke et al. 2011; Hayashi et al. 2006). In  
148 addition, some recent studies have found prevailing negative epistasis among  
149 beneficial mutations (Chou et al. 2011; Khan et al. 2011; Kvitek & Sherlock 2011;  
150 MacLean et al. 2010; Rokyta et al. 2011), which may explain the declining rate of  
151 adaptation often observed during long-term evolution in a constant environment (de  
152 Visser & Lenski 2002; Kryazhimskiy et al. 2009).

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#### 155 **4. CAUSES OF EPISTASIS**

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157 Given the abundant evidence for epistasis, understanding its causes is required to  
158 understand its evolutionary role. Epistasis results from the way in which genetic  
159 elements interact with each other in their ‘causation’ of a phenotype and ultimately  
160 fitness. For instance, intra-gene epistasis may result from non-independent effects of  
161 mutations on RNA stability or enzyme activity or stability, while inter-gene epistasis  
162 may result from protein interactions and the structure of metabolic networks (see  
163 Lehner [2011] for a recent extensive review of molecular mechanisms of epistasis).  
164 Predicting these interactions and their effects on fitness requires the full  
165 consideration of an organism’s development and physiology, and remains a major  
166 long-term goal of systems biology. Some progress has been made. For example, a  
167 model of bacteriophage T7 predicts aspects of growth dynamics (You & Yin 2002),  
168 and metabolic models can predict the effect of gene deletions on growth efficiency  
169 (Feist et al. 2007; Szappanos et al. 2011).

170 Besides lacking insight into the direct causation of epistasis, we do not yet  
171 understand how evolution shapes the various genetic architectures associated with  
172 different patterns of epistasis. Here, we will discuss how epistasis arises from the  
173 workings and pleiotropic constraints of enzymes and their metabolic networks, from  
174 environmental conditions, and from its effect on robustness and evolvability.

175

##### 176 **(a) Metabolic models**

177 Metabolic models have been developed to predict epistasis between mutations that  
178 affect either the same or different enzymes. Within a single enzyme, epistasis may  
179 result from the quantitative relationship between enzyme activity and fitness. This  
180 relationship is typically linear only at low enzyme activity levels, rapidly leveling off at

181 higher levels such that further increases in activity will cause only small fitness gains  
182 (Dean et al. 1986; Kacser & Burns 1973). For this reason, mutations with additive  
183 effect on enzyme activity will typically show negative epistasis for fitness (figure 2;  
184 Szathmary 1993).

185 Enzymes typically function together in metabolic networks, and the  
186 interactions inherent in these relationships play a key role in determining epistasis.  
187 Szathmary (1993) modeled a linear pathway to study this relationship, assuming that  
188 mutations had additive effects on enzyme activity and that activity was near the  
189 optimum. Four regimes were considered, fitness being proportional to either  
190 maximum or optimum flux, or to maximum or optimum metabolite concentration.  
191 When mutations affected different enzymes, the direction of epistasis depended on  
192 the selection regime: mutations interacted positively when selection was for  
193 maximum flux, but negatively when selection was for optimum flux or metabolite  
194 concentration. Similar to enzymes in a linear pathway under selection for maximum  
195 flux, mutations affecting transcription and translation showed positive epistasis in  
196 *Pseudomonas aeruginosa* (Trindade et al. 2009).

197 Segre et al. (2005) used a large-scale model of the yeast metabolic network  
198 to predict epistasis between pairs of gene knockout mutations. If mutations affected  
199 serial steps of a rate-limiting pathway they tended to have redundant effects, leading  
200 to positive epistasis (figure 2, green line). However, if mutations affected steps in  
201 different pathways, the sign of epistasis depended on the redundancy and  
202 relatedness of the affected pathways. If they are unrelated, mutations tend to show  
203 no epistasis (figure 2, black line). If they are related pathways producing the same  
204 product, mutations tend to interact negatively (figure 2, red line), provided that no  
205 other pathways exist. Since two random mutations will probably affect different  
206 pathways, the variation in observed patterns of epistasis seen in different yeast  
207 studies (Costanzo et al. 2010; He et al. 2010; Jasnos & Korona 2007; Segre et al.  
208 2005) may be explained by variation in the metabolic function and average fitness



209 effect of affected genes within each data set (Jasnos & Korona 2007), or,  
210 alternatively, by differences in the statistical power to detect epistasis (Agrawal &  
211 Whitlock 2010).

212         The observation of prevailing negative epistasis among beneficial mutations  
213 (see above) and the frequent reports of positive epistasis among deleterious  
214 mutations (Bonhoeffer et al. 2004; Burch & Chao 2004; Jasnos & Korona 2007;  
215 Lenski et al. 1999; Maisnier-Patin et al. 2005; Sanjuán et al. 2004; Zeyl 2005) evoke  
216 the general view that epistasis results from the buffering effects of physiological  
217 homeostasis. If correct, it remains unclear to what extent this pattern of epistasis  
218 arises intrinsically from metabolic kinetics and network organization, compared to as  
219 a direct consequence of natural selection, perhaps for increased robustness or  
220 evolvability (see below).

221

#### 222         **(b) Pleiotropy as a precondition for epistasis**

223 The simple metabolic models mentioned above assume that mutations affect a single  
224 phenotype. However, mutations are often pleiotropic, simultaneously affecting  
225 multiple phenotypes. Pleiotropy has been suggested as a source of epistasis on the  
226 basis of Fisher's geometric model, which describes the relationship between multiple  
227 phenotypes and fitness (Fisher 1958; Martin et al. 2007). This is well illustrated by  
228 negative pleiotropy, where mutations with a positive effect on one phenotype have a  
229 negative effect on another phenotype. In the context of adaptive evolution, negative  
230 pleiotropy is a precondition for sign epistasis, because it allows compensatory  
231 mutations to specifically 'repair' the negative pleiotropic effects of previous  
232 substitutions (figure 3).

233         A common form of pleiotropy within proteins is the simultaneous effects of  
234 mutations on enzyme activity and stability (DePristo et al. 2005; Wang et al. 2002).  
235 Mutations that stabilize proteins carrying an activity-increasing mutation have been  
236 found to be neutral or deleterious by themselves (Wang et al. 2002), an example of

237 sign epistasis. At a genomic scale, compensatory mutations that undo the negative  
238 pleiotropic effects of antibiotic-resistant (Bjorkman et al. 2000; Lenski 1988; Levin et  
239 al. 2000; Schoustra et al. 2007) or other adaptive mutations (MacLean et al. 2004)  
240 may have negative effects in the wild-type background. These results yield the view  
241 of adaptation initiated by large-benefit mutations with substantial pleiotropic costs  
242 (Cooper et al. 2007), followed by compensatory mutations that repair negative  
243 pleiotropic effects.

244 Poon and Chao (2005; 2006) studied the frequency and functional origins of  
245 compensatory mutations in bacteriophage  $\phi$ X174. They found that compensatory  
246 mutations were common and often occurred in the same gene as the deleterious  
247 mutation. Compensatory mutations were most effective when both they and the  
248 original deleterious mutation had strong effects on the local physical properties and  
249 thus were most likely to have pleiotropic consequences.

250

### 251 (c) Environment

252 As fitness is the product of a genotype in an environment, environmental conditions  
253 may have direct effects on epistasis (Remold & Lenski 2004). An intuitive source of  
254 negative epistasis among deleterious mutations is truncation selection (Crow &  
255 Kimura 1979). When resources are scarce, the effect of combinations of deleterious  
256 mutations might cause a much larger fitness cost, perhaps even death, than in a  
257 benign environment. Several authors have suggested this connection based on  
258 ecological (Crow & Kimura 1979; Hamilton et al. 1990; Kondrashov 1988) or  
259 metabolic arguments (Szathmary 1993; You & Yin 2002). Some studies have looked  
260 at the effect of environmental stress on the form of epistasis, but without consistent  
261 effects (Kishony & Leibler 2003; Yeh et al. 2009; Jasnos et al. 2008; de Visser &  
262 Elena 2007).

263 The degree of environmental complexity might also influence the evolution of  
264 epistasis. If in multiple-niche environments beneficial mutations have negative

265 pleiotropic effects on adaptation to alternative niches, there would be scope for sign  
266 epistasis and rugged fitness landscapes. Consistently, evolved bacterial populations  
267 showed greater divergence in complex than in simple environments (Cooper &  
268 Lenski 2010; Korona et al. 1994; Rozen et al. 2008). Moreover, if environmental  
269 conditions fluctuate, a modular organization of epistatic interactions may evolve, as  
270 was found during artificial selection of electronic circuits in environments with  
271 modularly varying goals, but not with fixed or randomly varying goals (Kashtan &  
272 Alon 2005).

273 Finally, environmental conditions can have long-term effects on epistasis by  
274 influencing the strength of selection relative to drift, e.g. through changes in  
275 population size, with possible consequences for the evolution of genetic robustness  
276 and genome complexity, which are both associated with particular patterns of  
277 epistasis.

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279

#### 280 **(d) Robustness**

281 Based on the predicted correlation between the effect-size of individual deleterious  
282 mutations and the strength of unidimensional epistasis, epistasis has been  
283 associated with genetic robustness — the insensitivity of organisms to the impact of  
284 mutations (de Visser et al. 2003; Wagner 2005). The relationship between genetic  
285 robustness and epistasis is, however, complex, and it is unclear whether it is an  
286 intrinsic or an adaptive feature of genomes. Recently, models have been used to  
287 study the evolution of alleles that modify epistasis among deleterious mutations when  
288 populations are close to a fitness optimum (Desai et al. 2007; Gros et al. 2009;  
289 Liberman & Feldman 2005, 2008; Liberman et al. 2007). These models suggest that  
290 both positive and negative epistasis can evolve as a consequence of purifying  
291 selection against deleterious mutations, depending on whether selection for  
292 robustness is driven by the negative impact of single or multiple mutations. They

293 assume that drift and recombination challenge organisms with more mutations than  
294 strong selection and clonal reproduction; hence, robustness is determined by the  
295 reduced fitness effect of multiple and single mutations, respectively. If the mean cost  
296 of single mutations is reduced by selection, interactions may become more negative,  
297 as the combined cost is likely to increase if one assumes that total fitness variation  
298 remains constant (Wilke & Adami 2001); the reciprocal argument predicts positive  
299 epistasis whenever robustness is selected to decrease the cost of multiple mutations.

300 Another link between robustness and epistasis is via the buffering effect of  
301 specialized chaperones. These modifiers of robustness can cause positive epistasis  
302 if they are induced by the accumulation of deleterious mutations (Maisnier-Patin et al.  
303 2005). Yet another suggested robustness mechanism is genetic redundancy, thought  
304 to be common in complex genomes. This form of robustness has been associated  
305 with negative epistasis (Sanjuán & Elena 2006). Mutations at one copy of a  
306 duplicated element are silent as long as the other copy remains unmutated; the more  
307 copies of the element exist, the more negative epistasis should be (Sanjuán & Nebot  
308 2008). However, this mechanism seems inconsistent with the predicted importance  
309 of drift due to small effective population size in organisms with complex genomes  
310 (Lynch & Conery 2003), where robustness should be associated with positive  
311 epistasis (Gros et al. 2009). This discrepancy may be explained, because the model  
312 predicting positive epistasis under drift does not allow genome size to evolve,  
313 thereby preventing negative epistasis to evolve as a result of increased genetic  
314 redundancy.

315

### 316 **(e) Evolvability**

317 Organism evolvability has been associated with particular patterns of epistasis. For  
318 instance, high mutation rates have two potential consequences for the evolution of  
319 epistasis. First, high mutation rates can weakly select for genetic robustness (de  
320 Visser et al. 2003; Wilke et al. 2001). Depending on the relative importance of drift

321 and selection and the time scale considered, this may lead to positive or, more likely,  
322 negative epistasis. Second, high mutation rates and large population sizes may  
323 facilitate selection of combinations of individually deleterious mutations that would be  
324 unlikely to arise in conditions where mutations fix sequentially (Weinreich & Chao  
325 2005).

326         The realization that recombination may change epistatic interactions involving  
327 newly arising mutations originated from the work of Malmberg (1977), who studied  
328 adaptation of bacteriophage T4 to resistance against the drug proflavin in  
329 populations with varying recombination. He found significant positive epistasis in low-  
330 recombination lines and effectively no epistasis in high-recombination lines. In other  
331 words, recombination selected for ‘generalist’ adaptive mutations that conferred a  
332 benefit on many genetic backgrounds, whereas the mutations accumulating in the  
333 absence of recombination made up positively interacting co-adapted complexes.

334         More recently, the effect of recombination on epistasis has been studied  
335 using models of gene regulatory circuits. Recombination caused increased genetic  
336 robustness and negative unidimensional epistasis (Azevedo et al. 2006).  
337 Interestingly, this response might promote the maintenance of recombination through  
338 the more efficient elimination of deleterious mutations (Kondrashov 1988). It was also  
339 found that circuits evolved with recombination were enriched for *cis*-regulatory  
340 complexes (Martin & Wagner 2009), hence had an increased modular structure.  
341 Evolution experiments with digital organisms similarly found that recombination  
342 increased robustness and modularity and reduced unidimensional epistasis (Misevic  
343 et al. 2006).

344         A modular organization of gene interactions enhances evolvability by  
345 reducing constraints from epistasis and pleiotropy. Reduced pleiotropy allows the  
346 relatively independent evolution of functions encoded by the modules, thereby  
347 increasing evolvability in sexual populations (Wagner et al. 2007; Watson et al.  
348 2011). Modular epistasis may thus have evolved as a consequence of its association

349 with evolvability. Similarly, recombination may have found ways to bolster its own  
350 evolution: by generating robust genomes showing negative and modular epistasis it  
351 may have enhanced selection against deleterious mutations and increased its long-  
352 term evolvability (de Visser & Elena 2007; Hayden et al. 2011).

353

354

## 355 **6. CONCLUSION**

356

357 Epistasis plays a prominent role in many evolutionary processes and has been the  
358 subject of substantial theoretical attention. Experiments have measured mean and  
359 individual epistatic effects over deleterious, random and beneficial mutations. These  
360 studies generally seek to link observed patterns of epistasis to metabolic functions  
361 and models, or quantify the complete pattern of epistasis in all dimensions among  
362 limited sets of mutations to explore the structure of fitness landscapes. This  
363 endeavor has just begun and, from both theoretical and experimental perspectives,  
364 key questions remain largely unexplored. We have argued that the potential for  
365 feedback in the relationship between selection and epistasis is one such question.  
366 Both the mean effect of epistasis and the type of individual interactions between  
367 selected alleles can change, dependent on the selective and genetic environment.  
368 Understanding this dynamic is necessary to determine the role of epistasis in  
369 evolution. In the future, the challenge will be to develop technical and statistical  
370 approaches to determine these changes and to further develop theory that, by  
371 considering epistasis as a dynamic property of organisms, considers how the  
372 feedback between selection and epistasis can influence evolutionary outcomes.

373

374

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382

383 **FIGURE LEGENDS**

384

385 **Figure 1.** (a) Unidimensional epistasis. The dashed line indicates the linear null  
386 model (no epistasis) averaged over mutants carrying the same number of mutations,  
387 here with negative effect; the green and red curved lines are examples of positive  
388 and negative epistasis, respectively. (b) Multidimensional epistasis. The cube shows  
389 an example of a fitness landscape of three loci, where the nodes are genotypes with  
390 mutant ("1") or wild-type ("0") alleles at each of three loci. The arrows point towards  
391 genotypes with higher fitness and their thickness indicates the size of the fitness  
392 increment. In this example, a description of multidimensional epistasis includes the  
393 presence of sign epistasis (the same allele having opposite fitness effects in different  
394 backgrounds, e.g. apparent from the addition of allele "1" at the third locus in 100 ⇒  
395 101 versus 110 ⇒ 111) and two fitness maxima (100 and 111).

396

397

398 **Figure 2.** A simple metabolic network showing examples of positive (green line),  
399 negative (red line and half circle) and no (black line) epistasis between loss-of-  
400 function gene mutations (X). The synthesis of biomass (full square) from biomass  
401 components (such as amino acids or nucleotides, full dots) requires an optimal  
402 allocation of a common nutrient (empty square) through intermediate metabolites  
403 (empty dots). Mutations affecting the same gene always show negative epistasis (red  
404 half circle). Negative epistasis requires that the two pathways affected are the only  
405 two involved in the production of an essential biomass component (leading to  
406 'synthetic lethality' if the mutations are knockouts); if alternative pathways exist or  
407 when affected pathways are involved in distant parts of the metabolism, multiplicative  
408 effects between the two mutations are to be expected (black line). Adapted from  
409 Segrè *et al.* (2005).



410

411

412 **Figure 3.** Pleiotropy provides opportunities for epistasis. P1 and P2 are two  
413 phenotypes with effects on fitness ( $W$ ) encoded by genes G1 and G2. **(a)** No  
414 pleiotropy: genes encoding P1 or P2 have no pleiotropic effects and lack  
415 opportunities for mutual epistatic interactions (red double arrows), except at the level  
416 of fitness. **(b)** Pleiotropy: due to pleiotropic effects of G1 and G2, additional  
417 opportunities for epistatic interactions arise at the level of the phenotype. When P1  
418 and P2 are phenotypes that show a fitness trade-off (e.g. survival and reproduction  
419 for organisms, or enzyme activity and stability for proteins), pleiotropic effects of G1  
420 and G2 allow compensatory (i.e. sign epistatic) mutations to alleviate negative  
421 pleiotropic effects of previous mutations with a net beneficial effect.

422

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