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

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

Key words: epistasis, pleiotropy, robustness, evolvability

1. INTRODUCTION

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

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

2. TERMINOLOGY

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

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

Second, within pairs of interacting alleles, one can distinguish between 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).
101
102
103 **3. EMPIRICAL EVIDENCE OF EPISTASIS**
104
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

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

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

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

4. CAUSES OF EPISTASIS

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

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

(a) Metabolic models

Metabolic models have been developed to predict epistasis between mutations that affect either the same or different enzymes. Within a single enzyme, epistasis may result from the quantitative relationship between enzyme activity and fitness. This 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 Szathmáry 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 Szathmáry (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 Segrè 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; Segrè 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 ϕX174. 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 (Szathmáry 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; Jasnos 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

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

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

(d) Robustness

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

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

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

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

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

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

6. CONCLUSION

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

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FIGURE LEGENDS

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

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

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

REFERENCES

1. Agrawal, A. F. & Whitlock, M. C. 2010 Environmental duress and epistasis: how does stress affect the strength of selection on new mutations? *Trends in Ecology and Evolution* 25, 450-458.
2. Azevedo, R. B. R., Lohaus, R., Srinivasan, S., Dang, K. K. & Burch, C. L. 2006 Sexual reproduction selects for robustness and negative epistasis in artificial gene networks. *Nature* 440, 87-90.
3. Barton, N. H. 1995 A general model for the evolution of recombination. *Genetical Research* 65, 123-144.
4. Bateson, W., Saunders, E. R., Punnett, R. C. & Hurst, C. C. 1905 Reports to the Evolution Committee of the Royal Society, Report II (ed. H. a. Sons). London.
5. Bjorkman, J., Nagaev, I., Berg, O. G., Hughes, D. & Andersson, D. I. 2000 Effects of environment on compensatory mutations to ameliorate costs of antibiotic resistance. *Science* 287, 1479-1482.
6. Bonhoeffer, S., Chappey, C., Parkin, N. T., Whitcomb, J. M. & Petropoulos, C. J. 2004 Evidence for positive epistasis in HIV-1. *Science* 306, 1547-1550.
7. Burch, C. L. & Chao, L. 2004 Epistasis and its relationship to canalization in the RNA virus phi6. *Genetics* 157, 559-567.
8. Carneiro, M. & Hartl, D. L. 2009 Adaptive landscapes and protein evolution. *Proceedings of the National Academy of Sciences USA* 107, 1747-1751.
9. Chou, H.-H., Chiu, H.-C., Delaney, N. F., Segrè, D. & Marx, C. J. 2011 Diminishing Returns Epistasis Among Beneficial Mutations Decelerates Adaptation. *Science* 332, 1190-1192.
10. Cooper, T. F. & Lenski, R. E. 2010 Experimental evolution with *E. coli* in diverse resource environments. I. Fluctuating environments promote divergence of replicate populations. *BMC Evolutionary Biology* 10, 11.
11. Cooper, T. F., Ostrowski, E. A. & Travisano, M. 2007 A negative relationship between mutation pleiotropy and fitness effect in yeast. *Evolution* 61, 1495-1499.

12. Costanzo, M., Baryshnikova, A., Bellay, J., Kim, Y., Spear, E. D. & al. 2010 The genetic landscape of a cell. *Science* 327, 425-431.
13. Crow, J. F. & Kimura, M. 1979 Efficiency of truncation selection. *Proceedings of the National Academy of Sciences USA* 76, 396-399.
14. da Silva, J., Coetzer, M., Nedellec, R., Pastore, C. & Mosier, D. E. 2010 Fitness Epistasis and Constraints on Adaptation in a Human Immunodeficiency Virus Type 1 Protein Region. *Genetics* 185, 293–303.
15. de la Peña, M., Elena, S. F. & Moya, A. 2000 Effect of deleterious mutation-accumulation on the fitness of RNA bacteriophage MS2. *Evolution* 54, 686-691.
16. de Visser, J. A. G. M. & Elena, S. F. 2007 The evolution of sex: empirical insights into the roles of epistasis and drift. *Nature Reviews Genetics* 8, 139-149.
17. de Visser, J. A. G. M., Hermisson, J., Wagner, G. P., Ancel Meyers, L., Bagheri-Chaichian, H., Blanchard, J. L., Chao, L., Cheverud, J. M., Elena, S. F., Fontana, W., Gibson, G., Hansen, T. F., Krakauer, D., Lewontin, R. C., Ofria, C., Rice, S. H., von Dassow, G., Wagner, A. & Whitlock, M. C. 2003 Perspective: Evolution and detection of genetic robustness. *Evolution* 57, 1959-1972.
18. de Visser, J. A. G. M., Hoekstra, R. F. & van den Ende, H. 1996 The effect of sex and deleterious mutations on fitness in Chlamydomonas. *Proceedings of the Royal Society of London, series B* 263, 193-200.
19. de Visser, J. A. G. M., Hoekstra, R. F. & van den Ende, H. 1997a An experimental test for synergistic epistasis and its application in Chlamydomonas. *Genetics* 145, 815-819.
20. de Visser, J. A. G. M., Hoekstra, R. F. & van den Ende, H. 1997b Test of interaction between genetic markers that affect fitness in *Aspergillus niger*. *Evolution* 51, 1499-1505.
21. de Visser, J. A. G. M. & Lenski, R. E. 2002 Long-term experimental evolution in *Escherichia coli*. XI. Rejection of non-transitive interactions as cause of declining rate of adaptation. *BMC Evolutionary Biology* 2, 19.
22. de Visser, J. A. G. M., Park, S.-C. & Krug, J. 2009 Exploring the effect of sex on empirical fitness landscapes. *American Naturalist* 174, S15-S30.
23. Dean, A. M., Dykhuizen, D. E. & Hartl, D. L. 1986 Fitness as a function of beta-galactosidase function in *Escherichia coli*. *Genetical Research* 48, 1-8.

24. DePristo, M. A., Weinreich, D. M. & Hartl, D. L. 2005 Missense meanderings in sequence space: a biophysical view of protein evolution. *Nature Reviews Genetics* 6, 678-687.
25. Desai, M. M., Weissman, D. & Feldman, M. W. 2007 Evolution can favor antagonistic epistasis. *Genetics* 177, 1001-1010.
26. Elena, S. F. 1999 Little evidence for synergism among deleterious mutations in a nonsegmented RNA virus. *Journal of Molecular Evolution* 49, 703-707.
27. Elena, S. F. & Lenski, R. E. 1997 Test of interaction between deleterious mutations in *E. coli*. *Nature* 390, 395-398.
28. Feist, A. M., Henry, C. S., Reed, J. L., Krummenacker, M., Joyce, A. R., Karp, P. D., Broadbelt, L. J., Hatzimanikatis, V. & Palsson, B. Ø. 2007 A genome-scale metabolic reconstruction for *Escherichia coli* K-12 MG1655 that accounts for 1260 ORFs and thermodynamic information. *Molecular Systems Biology* 3, 121.
29. Fisher, R. A. 1918 The correlations between relatives on the supposition of Mendelian inheritance. *Transactions of the Royal Society of Edinburgh* 52, 399–433.
30. Fisher, R. A. (ed.) 1958 *The genetical theory of natural selection*. New York: Dover.
31. Franke, J., Klözer, A., de Visser, J. A. G. M. & Krug, J. 2011 Evolutionary accessibility of mutational pathways. *PLOS Computational Biology* in press.
32. Gros, P.-A., Le Nagard, H. & Tenaillon, O. 2009 The Evolution of Epistasis and Its Links With Genetic Robustness, Complexity and Drift in a Phenotypic Model of Adaptation. *Genetics* 182, 277–293.
33. Hall, D. W., Agan, M. & Pope, S. C. 2010 Fitness Epistasis among 6 Biosynthetic Loci in the Budding Yeast *Saccharomyces cerevisiae*. *Journal of Heredity* 101, S75-S84.
34. Hamilton, W. D., Axelrod, R. & Tanese, R. 1990 Sexual reproduction as an adaptation to resist parasites (a review). *Proceedings of the National Academy of Sciences USA* 87, 3566-3573.
35. Hayashi, Y., Aita, T., Toyota, H., Husimi, Y., Urabe, I. & Yomo, T. 2006 Experimental Rugged Fitness Landscape in Protein Sequence Space. *PLoS One* 1, e96.
36. Hayden, E. J., Ferrada, E. & Wagner, A. 2011 Cryptic genetic variation promotes rapid evolutionary adaptation in an RNA enzyme. *Nature* 474, 92-95.

37. He, X., Qian, W., Wang, Z., Li, Y. & Zhang, J. 2010 Prevalent positive epistasis in *Escherichia coli* and *Saccharomyces cerevisiae* metabolic networks. *Nature Genetics* 42, 272-276.
38. Hinkley, T., Martins, J., Chappey, C., Haddad, M., Stawiski, E., Whitcomb, J. M., Petropoulos, C. J. & Bonhoeffer, S. 2011 A systems analysis of mutational effects in HIV-1 protease and reverse transcriptase. *Nature Genetics* 43, 487-490.
39. Jasnos, L. & Korona, R. 2007 Epistatic buffering of fitness loss in yeast double deletion strains. *Nature Genetics* 39, 550-554.
40. Jasnos, L., Tomala, K., Paczesniak, D. & Korona, R. 2008 Interactions between stressful environment and gene deletions alleviate the expected average loss of fitness in yeast. *Genetics* 178, 2105-2111.
41. Kacser, H. & Burns, J. A. 1973 The control of flux. *Symposium of the Society of Experimental Biology* 32, 65-104.
42. Kashtan, N. & Alon, U. 2005 Spontaneous evolution of modularity and network motifs. *Proceedings of the National Academy of Sciences USA* 102, 13773-13778.
43. Kelly, J. 2005 Epistasis in monkeyflowers. *Genetics* 171, 1917-1931.
44. Khan, A. I., Dinh, D. M., Schneider, D., Lenski, R. E. & Cooper, T. F. 2011 Negative Epistasis Between Beneficial Mutations in an Evolving Bacterial Population. *Science* 332, 1193-1196.
45. Kishony, R. & Leibler, S. 2003 Environmental stresses can alleviate the average deleterious effect of mutations. *Journal of Biology* 2, 14.
46. Kondrashov, A. S. 1988 Deleterious mutations and the evolution of sexual reproduction. *Nature* 336, 435-440.
47. Kondrashov, F. A. & Kondrashov, A. S. 2001 Multidimensional epistasis and the disadvantage of sex. *Proceedings of the National Academy of Sciences USA* 98, 12089-12092.
48. Korona, R., Nakatsu, C. H., Forney, L. J. & Lenski, R. E. 1994 Evidence for multiple adaptive peaks from populations of bacteria evolving in a structured habitat. *Proceedings of the National Academy of Sciences USA* 91, 9037-9041.

49. Kouyos, R. D., Silander, O. K. & Bonhoeffer, S. 2007 Epistasis between deleterious mutations and the evolution of recombination. *Trends in Ecology and Evolution* 22, 308-315.
50. Kryazhimskiy, S., Tkacik, G. & Plotkin, J. B. 2009 The dynamics of adaptation on correlated fitness landscapes. *Proceedings of the National Academy of Sciences USA* 276, 3035-3035.
51. Kvitek, D. J. & Sherlock, G. 2011 Reciprocal Sign Epistasis between Frequently Experimentally Evolved Adaptive Mutations Causes a Rugged Fitness Landscape. *PLoS Genetics* 7, e1002056.
52. Lehner, B. 2011 Molecular mechanisms of epistasis within and between genes. *Trends in Genetics*, in press.
53. Lenski, R. E. 1988 Experimental studies of pleiotropy and epistasis in *Escherichia coli*. II. Compensation for maladaptive effects associated with resistance to virus T4. *Evolution* 42, 433-440.
54. Lenski, R. E., Ofria, C., Collier, T. C. & Adami, C. 1999 Genome complexity, robustness and genetic interactions in digital organisms. *Nature* 400, 661-664.
55. Levin, B. R., Perrot, V. & Walker, N. 2000 Compensatory mutations, antibiotic resistance and the population genetics of adaptive evolution in bacteria. *Genetics* 154, 985-997.
56. Liberman, U. & Feldman, M. 2008 On the evolution of epistasis III: The haploid case with mutation. *Theoretical Population Biology* 73, 307-316.
57. Liberman, U. & Feldman, M. W. 2005 On the evolution of epistasis I: diploids under selection. *Theoretical Population Biology* 67, 141-160.
58. Liberman, U., Puniyani, A. & Feldman, M. W. 2007 On the evolution of epistasis II: a generalized Wright-Kimura framework. *Theoretical Population Biology* 71, 230-238.
59. Lozovsky, E. R., Chookajorn, T., Browna, K. M., Imwong, M., Shaw, P. J., Kamchonwongpaisan, S., Neafsey, D. E., Weinreich, D. M. & Hartl, D. L. 2009 Stepwise acquisition of pyrimethamine resistance in the malaria parasite. *Proceedings of the National Academy of Sciences USA* 106, 12015 – 12030.
60. Lunzer, M., Miller, S. P., Felsheim, R. & Dean, A. M. 2005 The biochemical architecture of an ancient adaptive landscape. *Science* 310, 499-501.

570 61. Lynch, M. & Conery, J. S. 2003 The origins of genome complexity. *Science* 302, 1401-
571 1404.
572 62. MacLean, R. C., Bell, G. & Rainey, P. B. 2004 The evolution of a pleiotropic fitness
573 tradeoff in *Pseudomonas fluorescens*. *Proceedings of the National Academy of Sciences*
574 *USA* 101, 8072-8077.
575 63. MacLean, R. C., Perron, G. G. & Gardner, A. 2010 Diminishing Returns From Beneficial
576 Mutations and Pervasive Epistasis Shape the Fitness Landscape for Rifampicin
577 Resistance in *Pseudomonas aeruginosa*. *Genetics* 186, 1345–1354.
578 64. Maisnier-Patin, S., Roth, J. R., Fredriksson, A., Nyström, T., Berg, O. G. & Andersson, D.
579 I. 2005 Genomic buffering mitigates the effects of deleterious mutations in bacteria.
580 *Nature Genetics* 37, 1376-1379.
581 65. Malmberg, R. L. 1977 The evolution of epistasis and the advantage of recombination in
582 populations of bacteriophage T4. *Genetics* 86, 607-621.
583 66. Martin, G., Elena, S. F. & Lenormand, T. 2007 Distributions of epistasis in microbes fit
584 predictions from a fitness landscape model. *Nature Genetics* aop.
585 67. Martin, O. C. & Wagner, A. 2009 Effects of recombination on complex regulatory circuits.
586 *Genetics* 183, 673-684.
587 68. Misevic, D., Ofria, C. & Lenski, R. E. 2006 Sexual reproduction reshapes the genetic
588 architecture of digital organisms. *Proceedings of the Royal Society London B* 273, 457-
589 464.
590 69. Moore, J. H. & Williams, S. M. 2005 Traversing the conceptual divide between biological
591 and statistical epistasis: systems biology and a more modern synthesis. *BioEssays* 27,
592 637-646.
593 70. Mukai, T. 1969 The genetic structure of natural populations of Drosophila melanogaster.
594 VII Synergistic interaction of spontaneous mutant polygenes controlling viability. *Genetics*
595 61, 749-761.
596 71. O'Maille, P. E., Malone, A., Dellas, N., Hess Jr., B. A., Smentek, L., Sheehan, I.,
597 Greenhagen, B. T., Chappell, J., Manning, G. & Noel, J. P. 2008 Quantitative exploration
598 of the catalytic landscape separating divergent plant sesquiterpene synthaes. *Nature*
599 *Chemical Biology* 4, 617-623.

600 72. Phillips, P. C. 1998 The Language of Gene Interaction (Perspective). *Genetics* 149,
601 1167–1171.
602 73. Phillips, P. C. 2008 Epistasis -- the essential role of gene interactions in the structure and
603 evolution of genetic systems. *Nature Reviews Genetics* 9, 855-867.
604 74. Poon, A. & Chao, L. 2005 The rate of compensatory mutation in the DNA bacteriophage
605 phiX174. *Genetics* 170, 989-999.
606 75. Poon, A. F. Y. & Chao, L. 2006 Functional origins of fitness effect-sizes of compensatory
607 mutations in the DNA bacteriophage phiX174. *Evolution* 60, 2032-2043.
608 76. Remold, S. K. & Lenski, R. E. 2004 Pervasive joint influence of epistasis and plasticity on
609 mutational effects in *Escherichia coli*. *Nature Genetics* 36, 423-426.
610 77. Rokyta, D. R., Joyce, P., Caudle, S. B., Miller, C., Beisel, C. J. & Wichman, H. A. 2011
611 Epistasis between Beneficial Mutations and the Phenotype-to-Fitness Map for a ssDNA
612 Virus. *PLoS Genetics* 7, e1002075.
613 78. Rozen, D. E., Habets, M. G. J. L., Handel, A. & De Visser, J. A. G. M. 2008
614 Heterogeneous adaptive trajectories of small populations on complex fitness landscapes.
615 *PLoS One* 3, e1715.
616 79. Salathé, P. & Ebert, D. 2003 The effects of parasitism and inbreeding on the competitive
617 ability in *Daphnia magna*: evidence for synergistic epistasis. *Journal of Evolutionary*
618 *Biology* 16, 976-985.
619 80. Salverda, M. L. M., Dellus, E., Gorter, F. A., Debets, A. J. M., Van der Oost, J., Hoekstra,
620 R. F., Tawfik, D. S. & de Visser, J. A. G. M. 2011 Initial mutations direct alternative
621 pathways of protein evolution. *PLoS Genetics* 7, e1001321.
622 81. Sanjuán, R. & Elena, S. F. 2006 Epistasis correlates to genomic complexity. *Proceedings*
623 *of the National Academy of Sciences USA* 103, 14402-14405.
624 82. Sanjuán, R., Moya, A. & Elena, S. F. 2004 The contribution of epistasis to the architecture
625 of fitness in an RNA virus. *Proceedings of the National Academy of Sciences USA* 101,
626 15376-15379.
627 83. Sanjuán, R. & Nebot, M. R. 2008 A network model for the correlation between epistasis
628 and genomic complexity. *PLoS One* 3, e2663.

84. Schoustra, S. E., Debets, A. J. M., Slakhorst, M. & Hoekstra, R. F. 2007 Mitotic recombination accelerates adaptation in the fungus *Aspergillus nidulans*. *PLoS Genetics* 3, e68.
85. Segrè, D., A., D., Church, G. M. & Kishony, R. 2005 Modular epistasis in yeast metabolism. *Nature Genetics* 37, 77-83.
86. Szappanos, B., Kovács, K., Szamecz, B., Honti, F., Costanzo, M., Baryshnikova, A., Gelius-Dietrich, G., Lercher, M. J., Jelasity, M., Myers, C. L., Andrews, B. J., Boone, C., Oliver, S. G., Pál, C. & Papp, B. 2011 An integrated approach to characterize genetic interaction networks in yeast metabolism. *Nature Genetics* 43, 656-662.
87. Szathmáry, E. 1993 Do deleterious mutations interact synergistically? Metabolic control theory provides a partial answer. *Genetics* 133, 127-132.
88. Trindade, S., Sousa, A., Bivar Xavier, K., Dionisio, F., Godinho Ferreira, M. & Gordo, I. 2009 Positive Epistasis Drives the Acquisition of Multidrug Resistance. *PLoS Genetics* 5, e1000578.
89. Wagner, A. 2005 *Robustness and evolvability in living systems (Princeton Studies in Complexity)*. Princeton: Princeton University Press.
90. Wagner, G. P., Pavlicev, M. & Cheverud, J. M. 2007 The road to modularity. *Nature Reviews Genetics* 8, 921-931.
91. Wang, X., Minasov, G. & Shoichet, B. K. 2002 Evolution of an antibiotic resistance enzyme constrained by stability and activity trade-offs. *Journal of Molecular Biology* 320, 85-95.
92. Watson, R. A., Weinreich, D. M. & Wakeley, J. 2011 Genome structrue and the benefits of sex. *Evolution* 65, 523–536.
93. Weinreich, D. M. & Chao, L. 2005 Rapid evolutinoary escape by large populations from local fitness peaks is likely in nature. *Evolution* 59, 1175-1182.
94. Weinreich, D. M., Delaney, N. F., DePristo, M. A. & Hartl, D. L. 2006 Darwinian evolution can follow only very few mutational paths to fitter proteins. *Science* 312, 111-114.
95. Weinreich, D. M., Watson, R. A. & Chao, L. 2005 Perspective: Sign epistasis and genetic constraint on evolutionary trajectories. *Evolution* 59, 1165-1174.

96. Whitlock, M. C. & Bourguet, D. 2000 Factors affecting the genetic load in Drosophila: synergistic epistasis and correlations among fitness components. *Evolution* 54, 1654-1660.

97. Wilke, C. O. & Adami, C. 2001 Interaction between directional epistasis and average mutational effects. *Proceedings of the Royal Society London B* 268, 1469-1474.

98. Wilke, C. O., Wang, J. L., Ofria, C., Lenski, R. E. & Adami, C. 2001 Evolution of digital organisms at high mutation rates leads to survival of the flattest. *Nature* 412, 331-333.

99. Wolf, J. B., Brodie, E. D. & Wade, M. J. (ed.) 2000 *Epistasis and the evolutionary process*. Oxford: Oxford University press.

100. Yeh, P. J., Hegreness, M. J., Aiden, A. P. & Kishony, R. 2009 Drug interactions and the evolution of antibiotic resistance. *Nature Reviews Microbiology* 7, 460-466.

101. You, L. & Yin, J. 2002 Dependence of epistasis on environment and mutation severity as revealed by *in silico* mutagenesis of phage T7. *Genetics* 160, 1273-1281.

102. Zeyl, C. 2005 The number of mutations selected during adaptation in a laboratory population of *Saccharomyces cerevisiae*. *Genetics* 169, 1825-1831.