

Review

The causes of epistasis

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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, whereas 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 robustness and 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.

Keywords: 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 [1,2]. 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 [3,4]. These theories make detailed predictions regarding the consequences of epistasis. In 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 also itself be altered as a consequence of changes of an organism's genetic architecture, is relatively recent. In a seminal study, Malmberg [5] observed that recombination alleviated epistasis between beneficial mutations in bacteriophage T4. However, it took almost three decades before theoretical studies addressed how epistasis evolves [6–13]. 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, Bateson *et al.* [14] introduced the term epistasis to describe the suppression of an allelic phenotype by an allele at another locus. Later, Fisher [15] '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, with reference to Fisher's definition, the term epistasis includes all deviations from independent effects of alleles at different loci on a phenotype [3,4,16]. On which scale effects are called independent depends on the consequences of epistasis in which one is interested. As our focus is on the evolutionary role of epistasis, we consider epistasis at the level of fitness, where deviations from multiplicative effects are relevant.

We make two distinctions. First, we distinguish between *unidimensional* and *multidimensional* epistasis [17]. Unidimensional epistasis refers to deviations from a linear relationship between *mean* log fitness and the number of alleles affecting fitness (figure 1a). 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

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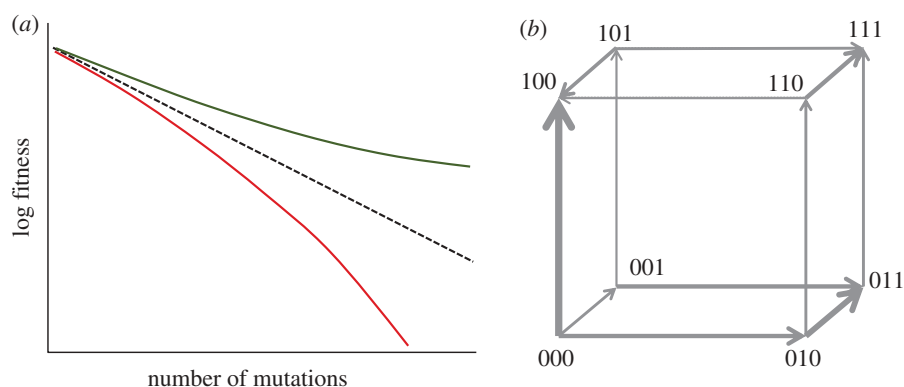


Figure 1. (a) Unidimensional epistasis. The dashed line indicates the multiplicative null model (no epistasis) for the average fitness of mutants carrying the same number of mutations, here with negative effect; the green and red curved lines are examples of positive and negative epistasis, respectively. (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. 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 $100 \Rightarrow 101$ versus $110 \Rightarrow 111$) and two fitness maxima (100 and 111).

deleterious mutations and synergistic epistasis among beneficial mutations represent positive epistasis, whereas 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 1b). 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 combined effect of two alleles deviates from multiplicative effects, but in a way that does not change the sign of either allele's fitness effect. Sign epistasis refers to 'stronger' interactions, where the sign of an allele's contribution to fitness changes with genetic background [18].

3. EMPIRICAL EVIDENCE OF EPISTASIS

(a) *Unidimensional epistasis*

Motivated by its relevance for explaining the evolution of sex [19,20], and because its detection involves less effort, most empirical work on epistasis has focused on finding unidimensional epistasis among random mutations. Studies have examined epistasis in a variety of organisms, from viruses to plants and fruitflies (reviewed in earlier studies [21,22]). Some studies reported negative epistasis [23–27], but others found positive epistasis [28–32] or no prevailing epistasis [33–38].

(b) *Multidimensional epistasis*

Two recent research themes seek to provide a more complete empirical picture of epistasis. The first theme seeks to understand the metabolic basis and general organization of epistasis by studying pairwise interactions among deleterious mutations at a genome-wide scale. These analyses show (i) no epistasis [1,39] or prevailing positive epistasis [28,40]; (ii) extensive variation in the sign of

epistasis; (iii) a modular pattern of epistasis, with similar interaction profiles for genes involved in the same functional module [1,39,40]; and (iv) a hierarchical network structure, with most genes having few, but some ('hubs') having many interactions [1].

The second approach involves the study of 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 (figure 1b), including the extent of sign epistasis and the accessibility of the global peak under defined evolutionary scenarios [41–43]. At present, fitness landscape data exist for sets of four to eight mutations for the enzymes isopropylmalate dehydrogenase [44], TEM-1 β -lactamase [43] and sesquiterpene synthetase [45], the malaria parasite *Plasmodium falciparum* [46], the fungus *Aspergillus niger* [42,47], and the bacteria *Escherichia coli* [48] and *Methylobacterium extorquens* [49].

These studies, as well as studies examining incomplete subsets of mutants [1,27,28,36,37,48,50–55], 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 [42,47,56]. In addition, some recent studies have found prevailing negative epistasis among beneficial mutations [48,49,52–54], which may explain the declining rate of adaptation often observed during long-term evolution in a constant environment [57,58].

4. CAUSES OF EPISTASIS

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 and enzyme activity or stability, whereas inter-gene epistasis may result from protein interactions and the structure of metabolic networks (see [59] for a detailed 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 [60], and metabolic models can predict the effect of gene deletions on growth efficiency [61,62].

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 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 levelling off at higher levels such that further increases in activity will cause only small fitness gains [63,64]. For this reason, mutations with additive effect on enzyme activity will typically show negative epistasis for fitness [65].

Enzymes typically function together in metabolic networks, and the interactions inherent in these relationships play a key role in determining epistasis. Szathmáry [65] modelled a linear pathway to study this relationship, assuming that mutations had additive effects on enzyme activity and that activity was near the optimum. Four regimes were considered, fitness being proportional to either maximum or optimum flux, or maximum or optimum metabolite concentration. When mutations affected different enzymes, the direction of epistasis depended on the selection regime: mutations interacted positively when selection was for maximum flux, but negatively when selection was for optimum flux or metabolite concentration. Similar to enzymes in a linear pathway under selection for maximum flux, mutations affecting transcription and translation showed positive epistasis in *Pseudomonas aeruginosa* [66].

Segrè *et al.* [39] used a large-scale model of the yeast metabolic network to predict epistasis between pairs of gene-knockout mutations. If mutations affected serial steps of a rate-limiting pathway, then they tended to have redundant effects leading to positive epistasis (figure 2, green line). However, if mutations affected steps in different pathways, then the sign of epistasis depended on the redundancy and relatedness of the affected pathways. If they are unrelated, then mutations tend to show no epistasis (figure 2, black line). If they are related pathways producing the same product, then mutations tend to interact negatively (figure 2, red line), provided that no other pathways exist. As two random mutations will probably affect different pathways, the variation in observed patterns of epistasis seen in different yeast studies [1,28,39,40] may be explained by variation in the metabolic function and average fitness effect of affected genes within each dataset [28], or, alternatively, by differences in the statistical power to detect epistasis [67].

The observation of prevailing negative epistasis among beneficial mutations (see above) and the frequent reports of positive epistasis among deleterious mutations

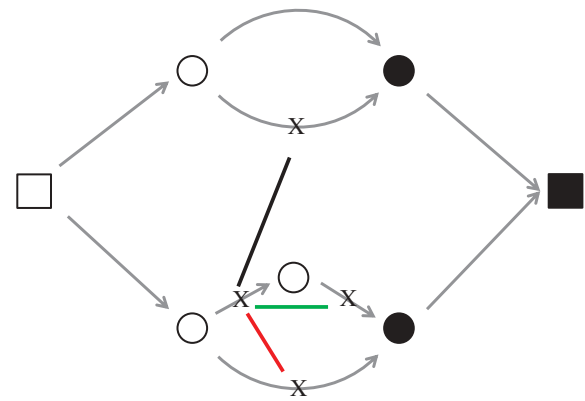


Figure 2. A simple metabolic network showing examples of positive (green line), negative (red line) and no (black line) epistasis between loss-of-function gene mutations (X). The synthesis of biomass (black square) from biomass components (such as amino acids or nucleotides, black circles) requires an optimal allocation of a common nutrient (white square) through intermediate metabolites (white circles). 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, then multiplicative effects between the two mutations are to be expected (black line). Adapted from Segrè *et al.* [39].

[28–32,68,69] evoke the general view that epistasis results from the buffering effects of physiological homeostasis. If correct, then it remains unclear to what extent this pattern of epistasis arises intrinsically from metabolic kinetics and network organization, when compared with as a direct consequence of natural selection, perhaps for increased robustness or evolvability (see below).

(b) *Pleiotropy as a precondition for epistasis*

The simple metabolic models mentioned above assume that mutations affect a single phenotype. However, mutations are often pleiotropic, simultaneously affecting multiple phenotypes. Pleiotropy has been suggested as a source of epistasis on the basis of Fisher's geometric model, which describes the relationship between multiple phenotypes and fitness [70,71]. This is well illustrated by negative pleiotropy, where mutations with a positive effect on one phenotype have a negative effect on another phenotype. In the context of adaptive evolution, negative pleiotropy is a precondition for sign epistasis, because it allows compensatory mutations to specifically 'repair' the negative pleiotropic effects of previously selected mutations (figure 3).

A common form of pleiotropy within proteins is the simultaneous effects of mutations on enzyme activity and stability [72,73]. Mutations that stabilize proteins carrying an activity-increasing mutation have been found to be neutral or deleterious by themselves [73], an example of sign epistasis. At a genomic scale, compensatory mutations that undo the negative pleiotropic effects of antibiotic-resistant [74–77] or other adaptive mutations [78] may have negative effects in the wild-type background. These results yield the view of adaptation initiated by large-benefit mutations with substantial pleiotropic costs [79], followed by compensatory mutations that repair negative pleiotropic effects.

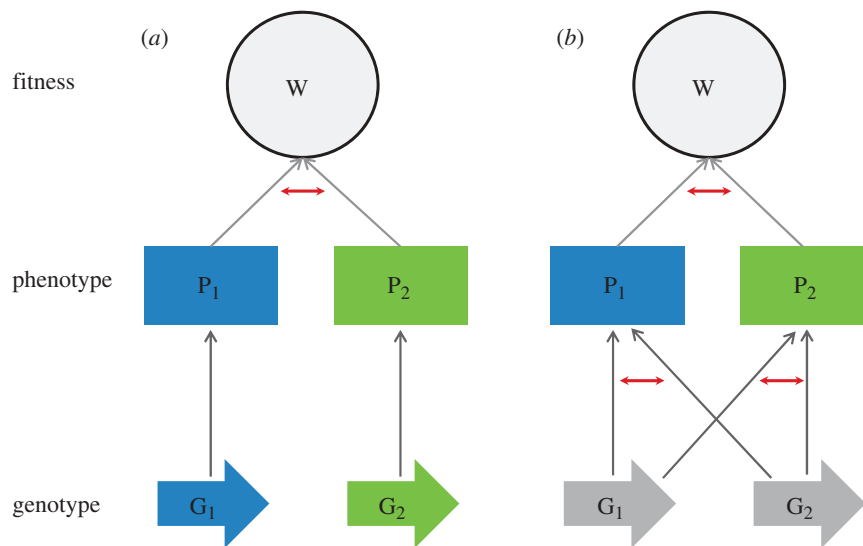


Figure 3. Pleiotropy provides opportunities for epistasis. P_1 and P_2 are two phenotypes with effects on fitness (W) encoded by genes G_1 and G_2 . (a) No pleiotropy: genes encoding P_1 or P_2 have no pleiotropic effects and lack opportunities for mutual epistatic interactions (red double arrows), except at the level of fitness. (b) Pleiotropy: owing to pleiotropic effects of G_1 and G_2 , additional opportunities for epistatic interactions arise at the level of the phenotype. When P_1 and P_2 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 G_1 and G_2 allow compensatory (i.e. sign epistatic) mutations to alleviate negative pleiotropic effects of previous mutations with a net beneficial effect.

Poon & Chao [80,81] studied the frequency and functional origins of compensatory mutations in bacteriophage ϕ X174. They found that compensatory mutations were common and often occurred in the same gene as the deleterious mutation. Compensatory mutations were most effective when both they and the original deleterious mutation had strong effects on the local physical properties, and thus were most likely to have pleiotropic consequences.

(c) Environment

As fitness is the product of a genotype in an environment, environmental conditions may have direct effects on epistasis [82]. An intuitive source of negative epistasis among deleterious mutations is truncation selection [83]. When resources are scarce, the effect of combinations of deleterious mutations might cause a much larger fitness cost, perhaps even death, than in a benign environment. Several authors have suggested this connection based on ecological [20,83,84] or metabolic [60,65] arguments. Some studies have looked at the effect of environmental stress on the form of epistasis, but without consistent effects [21,85–87].

The degree of environmental complexity might also influence the evolution of epistasis. If in multiple-niche environments beneficial mutations have negative pleiotropic effects on adaptation to alternative niches, then there would be scope for sign epistasis and rugged fitness landscapes. Consistently, evolved bacterial populations showed greater divergence in complex than in simple environments [88–90]. Moreover, if environmental conditions fluctuate, then 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 [91].

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, both of which are 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 [92,93]. 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 [7–11]. 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 assume that drift and recombination challenge organisms with more mutations than strong selection and clonal reproduction; hence, robustness is determined by the reduced fitness effect of multiple and single mutations, respectively. If the mean cost of single mutations is reduced by selection, then interactions may become more negative, as the combined cost is likely to increase if one assumes that total fitness variation remains constant [94]; the reciprocal argument predicts positive epistasis whenever robustness is selected to decrease the cost of multiple mutations.

Another link between robustness and epistasis is via the buffering effect of specialized chaperones. These modifiers of robustness can cause positive epistasis if they are induced by the accumulation of deleterious mutations [30]. Yet another suggested robustness mechanism is genetic redundancy, thought to be common in

complex genomes. This form of robustness has been associated with negative epistasis [95]. Mutations at one copy of a duplicated element are silent as long as the other copy remains unmutated; the more copies of the element exist, the more negative epistasis should be [96]. However, this mechanism seems inconsistent with the predicted importance of drift owing to small effective population size in organisms with complex genomes [97], where robustness should be associated with positive epistasis [8]. This discrepancy may be explained by the fact that the model predicting positive epistasis under drift does not allow genome size to evolve, thereby preventing negative epistasis from evolving as a result of increased genetic redundancy.

(e) *Evolvability*

Organism evolvability has been associated with particular patterns of epistasis. For instance, high mutation rates have two potential consequences for the evolution of epistasis. First, high mutation rates can weakly select for genetic robustness [92,98]. 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 [99].

The realization that recombination may change epistatic interactions involving newly arising mutations originated from the work of Malmberg [5], 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 [6]. Interestingly, this response might promote the maintenance of recombination through the more efficient elimination of deleterious mutations [20]. It was also found that circuits evolved with recombination were enriched for *cis*-regulatory complexes [12], and hence had an increased modular structure. Evolution experiments with digital organisms similarly found that recombination increased robustness and modularity, and reduced unidimensional epistasis [13].

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 [100,101]. 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 [21,102].

5. 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 endeavour 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, depending on the selective and genetic environment. Understanding these dynamics 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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REFERENCES

- 1 Costanzo, M. *et al.* 2010 The genetic landscape of a cell. *Science* **327**, 425–431. (doi:10.1126/science.1180823)
- 2 Moore, J. H. & Williams, S. M. 2005 Traversing the conceptual divide between biological and statistical epistasis: systems biology and a more modern synthesis. *BioEssays* **27**, 637–646. (doi:10.1002/bies.20236)
- 3 Phillips, P. C. 2008 Epistasis: the essential role of gene interactions in the structure and evolution of genetic systems. *Nat. Rev. Genet.* **9**, 855–867. (doi:10.1038/nrg2452)
- 4 Wolf, J. B., Brodie, E. D. & Wade, M. J. 2000 *Epistasis and the evolutionary process*. Oxford, UK: Oxford University Press.
- 5 Malmberg, R. L. 1977 The evolution of epistasis and the advantage of recombination in populations of bacteriophage T4. *Genetics* **86**, 607–621.
- 6 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. (doi:10.1038/nature04488)
- 7 Desai, M. M., Weissman, D. & Feldman, M. W. 2007 Evolution can favor antagonistic epistasis. *Genetics* **177**, 1001–1010. (doi:10.1534/genetics.107.075812)
- 8 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. (doi:10.1534/genetics.108.099127)
- 9 Liberman, U. & Feldman, M. W. 2008 On the evolution of epistasis III: the haploid case with mutation. *Theor.*

- Popul. Biol.* **73**, 307–316. (doi:10.1016/j.tpb.2007.11.010)
- 10 Liberman, U. & Feldman, M. W. 2005 On the evolution of epistasis. I: diploids under selection. *Theor. Popul. Biol.* **67**, 141–160. (doi:10.1016/j.tpb.2004.11.001)
 - 11 Liberman, U., Puniyani, A. & Feldman, M. W. 2007 On the evolution of epistasis. II: a generalized Wright–Kimura framework. *Theor. Popul. Biol.* **71**, 230–238. (doi:10.1016/j.tpb.2006.10.002)
 - 12 Martin, O. C. & Wagner, A. 2009 Effects of recombination on complex regulatory circuits. *Genetics* **183**, 673–684. (doi:10.1534/genetics.109.104174)
 - 13 Misevic, D., Ofria, C. & Lenski, R. E. 2006 Sexual reproduction reshapes the genetic architecture of digital organisms. *Proc. R. Soc. B* **273**, 457–464. (doi:10.1098/rspb.2005.3338)
 - 14 Bateson, W., Saunders, E. R., Punnett, R. C. & Hurst, C. C. 1905 Reports to the Evolution Committee of the Royal Society, Report II. London, UK: Harrison and Sons.
 - 15 Fisher, R. A. 1918 The correlations between relatives on the supposition of Mendelian inheritance. *Trans. R. Soc. Edinburgh* **52**, 399–433.
 - 16 Phillips, P. C. 1998 The language of gene interaction (perspective). *Genetics* **149**, 1167–1171.
 - 17 Kondrashov, F. A. & Kondrashov, A. S. 2001 Multi-dimensional epistasis and the disadvantage of sex. *Proc. Natl Acad. Sci. USA* **98**, 12 089–12 092. (doi:10.1073/pnas.211214298)
 - 18 Weinreich, D. M., Watson, R. A. & Chao, L. 2005 Perspective: sign epistasis and genetic constraint on evolutionary trajectories. *Evolution* **59**, 1165–1174.
 - 19 Barton, N. H. 1995 A general model for the evolution of recombination. *Genet. Res.* **65**, 123–144. (doi:10.1017/S0016672300033140)
 - 20 Kondrashov, A. S. 1988 Deleterious mutations and the evolution of sexual reproduction. *Nature* **336**, 435–440. (doi:10.1038/336435a0)
 - 21 de Visser, J. A. G. M. & Elena, S. F. 2007 The evolution of sex: empirical insights into the roles of epistasis and drift. *Nat. Rev. Genet.* **8**, 139–149. (doi:10.1038/nrg1985)
 - 22 Kouyos, R. D., Silander, O. K. & Bonhoeffer, S. 2007 Epistasis between deleterious mutations and the evolution of recombination. *Trends Ecol. Evol.* **22**, 308–315. (doi:10.1016/j.tree.2007.02.014)
 - 23 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*. *Proc. R. Soc. Lond. B* **263**, 193–200. (doi:10.1098/rspb.1996.0031)
 - 24 de Visser, J. A. G. M., Hoekstra, R. F. & van den Ende, H. 1997 An experimental test for synergistic epistasis and its application in *Chlamydomonas*. *Genetics* **145**, 815–819.
 - 25 Mukai, T. 1969 The genetic structure of natural populations of *Drosophila melanogaster*. VII: synergistic interaction of spontaneous mutant polygenes controlling viability. *Genetics* **61**, 749–761.
 - 26 Salathé, P. & Ebert, D. 2003 The effects of parasitism and inbreeding on the competitive ability in *Daphnia magna*: evidence for synergistic epistasis. *J. Evol. Biol.* **16**, 976–985. (doi:10.1046/j.1420-9101.2003.00582.x)
 - 27 Whitlock, M. C. & Bourguet, D. 2000 Factors affecting the genetic load in *Drosophila*: synergistic epistasis and correlations among fitness components. *Evolution* **54**, 1654–1660. (doi:10.1111/j.0014-3820.2000.tb.00709.x)
 - 28 Jasnos, L. & Korona, R. 2007 Epistatic buffering of fitness loss in yeast double deletion strains. *Nat. Genet.* **39**, 550–554. (doi:10.1038/ng1986)
 - 29 Lenski, R. E., Ofria, C., Collier, T. C. & Adami, C. 1999 Genome complexity, robustness and genetic interactions in digital organisms. *Nature* **400**, 661–664. (doi:10.1038/23245)
 - 30 Maisnier-Patin, S., Roth, J. R., Fredriksson, A., Nyström, T., Berg, O. G. & Andersson, D. I. 2005 Genomic buffering mitigates the effects of deleterious mutations in bacteria. *Nat. Genet.* **37**, 1376–1379. (doi:10.1038/ng1676)
 - 31 Sanjuán, R., Moya, A. & Elena, S. F. 2004 The contribution of epistasis to the architecture of fitness in an RNA virus. *Proc. Natl Acad. Sci. USA* **101**, 15 376–15 379. (doi:10.1073/pnas.0404125101)
 - 32 Zeyl, C. 2005 The number of mutations selected during adaptation in a laboratory population of *Saccharomyces cerevisiae*. *Genetics* **169**, 1825–1831. (doi:10.1534/genetics.104.027102)
 - 33 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. (doi:10.1554/0014-3820(2000)054[0686:EODMAO]2.0.CO;2)
 - 34 de Visser, J. A. G. M., Hoekstra, R. F. & van den Ende, H. 1997 Test of interaction between genetic markers that affect fitness in *Aspergillus niger*. *Evolution* **51**, 1499–1505. (doi:10.2307/2411202)
 - 35 Elena, S. F. 1999 Little evidence for synergism among deleterious mutations in a nonsegmented RNA virus. *J. Mol. Evol.* **49**, 703–707. (doi:10.1007/PL00000082)
 - 36 Elena, S. F. & Lenski, R. E. 1997 Test of interaction between deleterious mutations in *E. coli*. *Nature* **390**, 395–398. (doi:10.1038/37108)
 - 37 Hall, D. W., Agan, M. & Pope, S. C. 2010 Fitness epistasis among 6 biosynthetic loci in the budding yeast *Saccharomyces cerevisiae*. *J. Hered.* **101**, S75–S84. (doi:10.1093/jhered/esq007)
 - 38 Kelly, J. 2005 Epistasis in monkeyflowers. *Genetics* **171**, 1917–1931. (doi:10.1534/genetics.105.041525)
 - 39 Segrè, D. A. D., Church, G. M. & Kishony, R. 2005 Modular epistasis in yeast metabolism. *Nat. Genet.* **37**, 77–83. (doi:10.1038/ng1489)
 - 40 He, X., Qian, W., Wang, Z., Li, Y. & Zhang, J. 2010 Prevalent positive epistasis in *Escherichia coli* and *Saccharomyces cerevisiae* metabolic networks. *Nat. Genet.* **42**, 272–276. (doi:10.1038/ng.524)
 - 41 Carneiro, M. & Hartl, D. L. 2009 Adaptive landscapes and protein evolution. *Proc. Natl Acad. Sci. USA* **107**, 1747–1751. (doi:10.1073/pnas.0906192106)
 - 42 Franke, J., Klözer, A., de Visser, J. A. G. M. & Krug, J. 2011 Evolutionary accessibility of mutational pathways. *PLoS Comput. Biol.* **7**, e1002134. (doi:10.1371/journal.pcbi.1002134)
 - 43 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. (doi:10.1126/science.1123539)
 - 44 Lunzer, M., Miller, S. P., Felsheim, R. & Dean, A. M. 2005 The biochemical architecture of an ancient adaptive landscape. *Science* **310**, 499–501. (doi:10.1126/science.1115649)
 - 45 O'Maille, P. E. *et al.* 2008 Quantitative exploration of the catalytic landscape separating divergent plant sesquiterpene synthases. *Nat. Chem. Biol.* **4**, 617–623. (doi:10.1038/nchembio.113)
 - 46 Lozovsky, E. R., Chookajorn, T., Browne, 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. *Proc. Natl Acad. Sci. USA* **106**, 12 015–12 030. (doi:10.1073/pnas.0905922106)

- 47 de Visser, J. A. G. M., Park, S.-C. & Krug, J. 2009 Exploring the effect of sex on empirical fitness landscapes. *Am. Nat.* **174**, S15–S30. (doi:10.1086/599081)
- 48 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. (doi:10.1126/science.1203801)
- 49 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. (doi:10.1126/science.1203799)
- 50 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. (doi:10.1534/genetics.109.112458)
- 51 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. *Nat. Genet.* **43**, 487–490. (doi:10.1038/ng.795)
- 52 Kvitek, D. J. & Sherlock, G. 2011 Reciprocal sign epistasis between frequently experimentally evolved adaptive mutations causes a rugged fitness landscape. *PLoS Genet.* **7**, e1002056. (doi:10.1371/journal.pgen.1002056)
- 53 MacLean, R. C., Perron, G. G. & Gardner, A. 2010 Diminishing returns from beneficial mutations and pervasive epistasis shape the fitness landscape for Rifampicin resistance in *Pseudomonas aeruginosa*. *Genetics* **186**, 1345–1354. (doi:10.1534/genetics.110.123083)
- 54 Rokyta, D. R., Joyce, P., Caudle, S. B., Miller, C., Beisel, C. J. & Wichman, H. A. 2011 Epistasis between beneficial mutations and the phenotype-to-fitness map for a ssDNA virus. *PLoS Genet.* **7**, e1002075. (doi:10.1371/journal.pgen.1002075)
- 55 Salverda, M. L. M., Dellus, E., Gorter, F. A., Debets, A. J. M., Van der Oost, J., Hoekstra, R. F., Tawfik, D. S. & de Visser, J. A. G. M. 2011 Initial mutations direct alternative pathways of protein evolution. *PLoS Genet.* **7**, e1001321. (doi:10.1371/journal.pgen.1001321)
- 56 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. (doi:10.1371/journal.pone.0000096)
- 57 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 Evol. Biol.* **2**, 19. (doi:10.1186/1471-2148-2-19)
- 58 Kryazhimskiy, S., Tkacik, G. & Plotkin, J. B. 2009 The dynamics of adaptation on correlated fitness landscapes. *Proc. Natl Acad. Sci. USA* **276**, 18 638–18 643. (doi:10.1073/pnas.0905497106)
- 59 Lehner, B. 2011 Molecular mechanisms of epistasis within and between genes. *Trends Genet.* **27**, 323–331. (doi:10.1016/j.tig.2011.05.007)
- 60 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.
- 61 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. *Mol. Syst. Biol.* **3**, 121. (doi:10.1038/msb4100155)
- 62 Szappanos, B. et al. 2011 An integrated approach to characterize genetic interaction networks in yeast metabolism. *Nat. Genet.* **43**, 656–662. (doi:10.1038/ng.846)
- 63 Dean, A. M., Dykhuizen, D. E. & Hartl, D. L. 1986 Fitness as a function of β -galactosidase function in *Escherichia coli*. *Genet. Res.* **48**, 1–8. (doi:10.1017/S0016672300024587)
- 64 Kacser, H. & Burns, J. A. 1973 The control of flux. *Symp. Soc. Exp. Biol.* **32**, 65–104.
- 65 Szathmáry, E. 1993 Do deleterious mutations interact synergistically? Metabolic control theory provides a partial answer. *Genetics* **133**, 127–132.
- 66 Trindade, S., Sousa, A., Bivar Xavier, K., Dionisio, F., Godinho Ferreira, M. & Gordo, I. 2009 Positive epistasis drives the acquisition of multidrug resistance. *PLoS Genet.* **5**, e1000578. (doi:10.1371/journal.pgen.1000578)
- 67 Agrawal, A. F. & Whitlock, M. C. 2010 Environmental duress and epistasis: how does stress affect the strength of selection on new mutations? *Trends Ecol. Evol.* **25**, 450–458. (doi:10.1016/j.tree.2010.05.003)
- 68 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. (doi:10.1126/science.1101786)
- 69 Burch, C. L. & Chao, L. 2004 Epistasis and its relationship to canalization in the RNA virus ϕ 6. *Genetics* **157**, 559–567. (doi:10.1534/genetics.103.021196)
- 70 Fisher, R. A. 1958 *The genetical theory of natural selection*. New York, NY: Dover.
- 71 Martin, G., Elena, S. F. & Lenormand, T. 2007 Distributions of epistasis in microbes fit predictions from a fitness landscape model. *Nat. Genet.* **39**, 555–560. (doi:10.1038/ng1998)
- 72 DePristo, M. A., Weinreich, D. M. & Hartl, D. L. 2005 Missense meanderings in sequence space: a biophysical view of protein evolution. *Nat. Rev. Genet.* **6**, 678–687. (doi:10.1038/nrg1672)
- 73 Wang, X., Minasov, G. & Shoichet, B. K. 2002 Evolution of an antibiotic resistance enzyme constrained by stability and activity trade-offs. *J. Mol. Biol.* **320**, 85–95. (doi:10.1016/S0022-2836(02)00400-X)
- 74 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. (doi:10.1126/science.287.5457.1479)
- 75 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. (doi:10.2307/2409029)
- 76 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.
- 77 Schoustra, S. E., Debets, A. J. M., Slakhorst, M. & Hoekstra, R. F. 2007 Mitotic recombination accelerates adaptation in the fungus *Aspergillus nidulans*. *PLoS Genet.* **3**, e68. (doi:10.1371/journal.pgen.0030068)
- 78 MacLean, R. C., Bell, G. & Rainey, P. B. 2004 The evolution of a pleiotropic fitness tradeoff in *Pseudomonas fluorescens*. *Proc. Natl Acad. Sci. USA* **101**, 8072–8077. (doi:10.1073/pnas.0307195101)
- 79 Cooper, T. F., Ostrowski, E. A. & Travisano, M. 2007 A negative relationship between mutation pleiotropy and fitness effect in yeast. *Evolution* **61**, 1495–1499. (doi:10.1111/j.1558-5646.2007.00109.x)
- 80 Poon, A. & Chao, L. 2005 The rate of compensatory mutation in the DNA bacteriophage ϕ X174. *Genetics* **170**, 989–999. (doi:10.1534/genetics.104.039438)
- 81 Poon, A. F. Y. & Chao, L. 2006 Functional origins of fitness effect sizes of compensatory mutations in

- the DNA bacteriophage ϕ X174. *Evolution* **60**, 2032–2043.
- 82 Remold, S. K. & Lenski, R. E. 2004 Pervasive joint influence of epistasis and plasticity on mutational effects in *Escherichia coli*. *Nat. Genet.* **36**, 423–426. (doi:10.1038/ng1324)
 - 83 Crow, J. F. & Kimura, M. 1979 Efficiency of truncation selection. *Proc. Natl Acad. Sci. USA* **76**, 396–399. (doi:10.1073/pnas.76.1.396)
 - 84 Hamilton, W. D., Axelrod, R. & Tanese, R. 1990 Sexual reproduction as an adaptation to resist parasites (a review). *Proc. Natl Acad. Sci. USA* **87**, 3566–3573. (doi:10.1073/pnas.87.9.3566)
 - 85 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. (doi:10.1534/genetics.107.084533)
 - 86 Kishony, R. & Leibler, S. 2003 Environmental stresses can alleviate the average deleterious effect of mutations. *J. Biol.* **2**, 14. (doi:10.1186/1475-4924-2-14)
 - 87 Yeh, P. J., Hegreness, M. J., Aiden, A. P. & Kishony, R. 2009 Drug interactions and the evolution of antibiotic resistance. *Nat. Rev. Microbiol.* **7**, 460–466. (doi:10.1038/nrmicro2133)
 - 88 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 Evol. Biol.* **10**, 11. (doi:10.1186/1471-2148-10-11)
 - 89 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. *Proc. Natl Acad. Sci. USA* **91**, 9037–9041. (doi:10.1073/pnas.91.19.9037)
 - 90 Rozen, D. E., Habets, M. G. J. L., Handel, A. & de Visser, J. A. G. M. 2008 Heterogeneous adaptive trajectories of small populations on complex fitness landscapes. *PLoS ONE* **3**, e1715. (doi:10.1371/journal.pone.0001715)
 - 91 Kashtan, N. & Alon, U. 2005 Spontaneous evolution of modularity and network motifs. *Proc. Natl Acad. Sci. USA* **102**, 13 773–13 778. (doi:10.1073/pnas.0503610102)
 - 92 de Visser, J. A. G. M. *et al.* 2003 Perspective: evolution and detection of genetic robustness. *Evolution* **57**, 1959–1972. (doi:10.1554/02-750R)
 - 93 Wagner, A. 2005 *Robustness and evolvability in living systems*. Princeton Studies in Complexity. Princeton, NJ: Princeton University Press.
 - 94 Wilke, C. O. & Adami, C. 2001 Interaction between directional epistasis and average mutational effects. *Proc. R. Soc. Lond. B* **268**, 1469–1474. (doi:10.1098/rspb.2001.1690)
 - 95 Sanjuán, R. & Elena, S. F. 2006 Epistasis correlates to genomic complexity. *Proc. Natl Acad. Sci. USA* **103**, 14 402–14 405. (doi:10.1073/pnas.0604543103)
 - 96 Sanjuán, R. & Nebot, M. R. 2008 A network model for the correlation between epistasis and genomic complexity. *PLoS ONE* **3**, e2663. (doi:10.1371/journal.pone.0002663)
 - 97 Lynch, M. & Conery, J. S. 2003 The origins of genome complexity. *Science* **302**, 1401–1404. (doi:10.1126/science.1089370)
 - 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. (doi:10.1038/35085569)
 - 99 Weinreich, D. M. & Chao, L. 2005 Rapid evolutionary escape by large populations from local fitness peaks is likely in nature. *Evolution* **59**, 1175–1182.
 - 100 Wagner, G. P., Pavlicev, M. & Cheverud, J. M. 2007 The road to modularity. *Nat. Rev. Genet.* **8**, 921–931. (doi:10.1038/nrg2267)
 - 101 Watson, R. A., Weinreich, D. M. & Wakeley, J. 2011 Genome structure and the benefits of sex. *Evolution* **65**, 523–536. (doi:10.1111/j.1558-5646.2010.01144.x)
 - 102 Hayden, E. J., Ferrada, E. & Wagner, A. 2011 Cryptic genetic variation promotes rapid evolutionary adaptation in an RNA enzyme. *Nature* **474**, 92–95. (doi:10.1038/nature10083)