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REVIEW ARTICLE

Revealing evolutionary pathways by fitness landscape reconstruction

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Abstract

The concept of epistasis has since long been used to denote non-additive fitness effects of genetic changes and has played a central role in understanding the evolution of biological systems. Owing to an array of novel experimental methodologies, it has become possible to experimentally determine epistatic interactions as well as more elaborate genotype-fitness maps. These data have opened up the investigation of a host of long-standing questions in evolutionary biology, such as the ruggedness of fitness landscapes and the accessibility of mutational trajectories, the evolution of sex, and the origin of robustness and modularity. Here we review this recent and timely marriage between systems biology and evolutionary biology, which holds the promise to understand evolutionary dynamics in a more mechanistic and predictive manner.

Keywords: *Epistasis; fitness landscape; robustness;regulatory networks; evolution*

Introduction

Genomic sequencing has generated a wealth of information on the molecular basis of organisms and their evolutionary relationships (Benson *et al.*, 2004; Otto, 1997). However, we remain largely ignorant about the interactions between genes that are central to organismal functions and phenotype (Blattner *et al.*, 1997). Information on how phenotypes depend on these interactions is not only relevant for understanding the architecture of cellular functions (Schuldiner *et al.*, 2005; Collins *et al.*, 2006; 2007), but also has profound implications for their evolutionary origin (Lunzer *et al.*, 2005; Zhu *et al.*, 2005; Miller *et al.*, 2006; Dean and Thornton, 2007).

The concept of epistasis provides an elementary description of genetic interactions that are involved in function or fitness (Wright, 1932; Kauffman and Levin, 1987; Arnold *et al.*, 2001). About 100 years ago, William Bateson introduced this term to describe phenotypic deviations from Mendelian segregation ratios due to genes masking the effects of others (Bateson, 1907). Broadly defined, epistasis denotes cases in which the effect of a mutation depends on the genetic background in which it occurs (Poelwijk *et al.*, 2007). For instance, a mutation that is beneficial in one genetic background can be neutral or deleterious in another. Epistasis has played a central role in many evolutionary theories, including those that address speciation (Orr and Turelli, 2001), the evolution of sex (Kondrashov, 1988; Kondrashov and Kondrashov, 2001; Kouyos *et al.*, 2006; de Visser and Elena, 2007; Gandon and Otto, 2007) and adaptation (Wright, 1931; Masel, 2005; Martin *et al.*, 2007; Poelwijk *et al.*, 2007).

Owing to novel experimental approaches, epistatic interactions can now be studied in an increasingly systematic manner. These efforts address a diverse array of biological systems and scientific questions. Some have a predominantly functional perspective, while others are motivated by evolutionary questions. In some cases the focus is on epistatic interactions within a single gene (intra-genic epistasis), while other studies consider larger networks of interacting genes (inter-genic epistasis). The aim of this review is to sketch these recent developments, by giving a number of illustrative examples of these diverse directions. In doing so, we hope to provide an

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overview of the current possibilities and limitations, and to identify new questions within this exciting new field.

Intra-genic epistasis

The nature of the epistatic interactions within a biological system is intrinsically linked to its evolutionary origin and potential. For instance in the absence of epistatic interactions between loci, genetic changes at these loci contribute independently to fitness (Figure 1A). Note that for a Malthusian fitness parameter such as the bacterial growth rate, independence implies that fitness effects are additive, while when fitness is defined as the number of offspring, the individual contributions to fitness multiply. Starting from a sub-optimal phenotype, all adaptive trajectories towards the optimum then rise monotonically in fitness. Consequently, these trajectories are all equally probable to be followed during adaptation. In contrast, when required genetic changes exhibit sign-epistatic interactions (Figure 1A), some trajectories contain fitness decreasing steps, making them much less probable, though other trajectories do exhibit monotonously increasing fitness. We note that any type of epistatic interaction (not only sign epistasis) will result in some difference in the probabilities for different paths. Fitness landscapes are an intuitive concept to consider multiple possible trajectories between two points, and in both previous cases the landscape is smooth and single-peaked. However, landscapes can also be rugged and have more than a single fitness peak. Adapting from one peak to the other then requires two or more simultaneous genetic changes, which is denoted as reciprocal sign epistasis (Figure 1A).

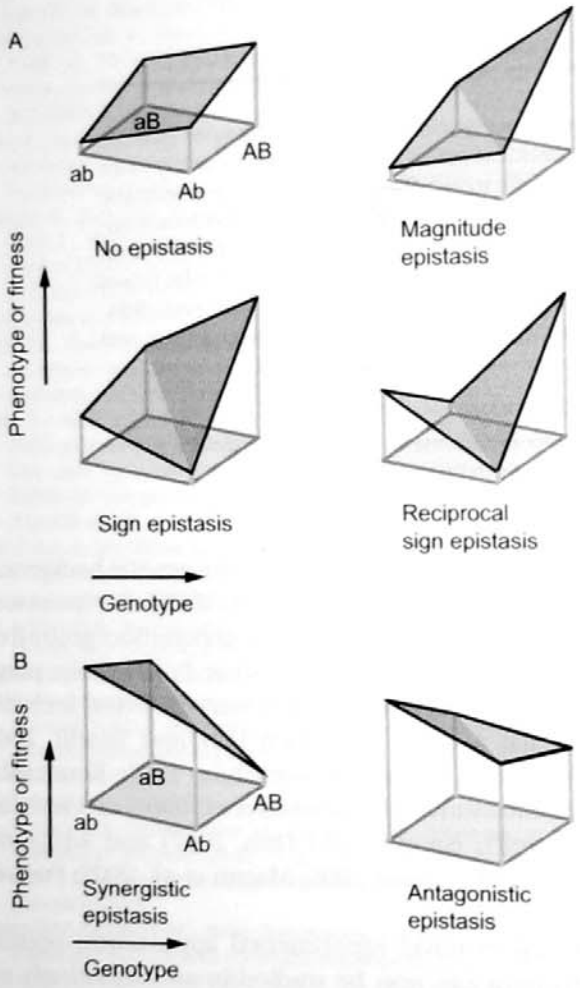

Figure 1. Two classifications of epistatic interactions. A. Paths composed of two mutations are considered, from an initial sequence "ab" towards the optimum sequence "AB". When there is no epistasis, mutation "a" to "A" yields the same fitness effect for different genetic backgrounds ("b" or "B"), while for magnitude epistasis the fitness effect may differ in magnitude, but not in sign. For sign epistasis, the sign of the fitness effect changes. Consequently, some paths become inaccessible. Finally, such a change in sign of the fitness effect can occur for both mutations; this is denoted as reciprocal sign epistasis, and is required for having multiple peaks in the fitness landscape. B. Two types of epistasis that distinguish possible interactions between two genes. Paths are considered from the initial optimal sequence "ab" towards the double knockouts "AB". If the fitness effect of the double knockout is larger than expected from the sum of their individual effects is denoted as synergistic epistasis, while a smaller than additive effect is termed as antagonistic epistasis.

What are the shapes of actual fitness landscapes? This longstanding question is now starting to be addressed. Weinreich and colleagues focused on the protein β-lactamase in *Escherichia coli*. One variant was known to confer resistance to penicillin, while adding five mutations conferred resistance to the newer antibiotic cefotaxime. To gain insight in all possible mutational trajectories between these two variants, all $2^5 = 32$ possible intermediates were constructed and assayed on survival ability in cefotaxime, which is taken as a measure of fitness. The data revealed that a majority of the trajectories contained fitness decreasing or neutral steps, resulting in much reduced chances of being followed by natural selection (Weinreich *et al.*, 2006).

Sign-epistatic interactions underlie these landscape features. For example, Gly238Ser in wild-type background increases the resistance, even though it increases protein aggregation by lowering the thermodynamic stability. This loss of stability is rescued by Met182Thr which alone modestly reduces resistance. Paths that fix Gly238Ser before Met182Thr are therefore plausible, but the reverse order is not. Such a balancing between functional and structural benefits is a more general evolutionary mechanism (DePristo *et al.*, 2005; Bloom *et al.*, 2006) and provides a mechanistic rationale for sign epistasis (Poelwijk *et al.*, 2007; Dean and Thornton, 2007).

One may consider epistasis in fitness or at the functional level. The relation between the two was investigated using isopropylmalate dehydrogenase (IMDH) as a model system. IMDH is involved in biosynthesis of the amino acid leucine, and uses the coenzyme

nicotinamide adenine dinucleotide (NAD^+) as a hydride acceptor during an oxidative decarboxylation. Upon six mutations IMDH exchanges this coenzyme for another, nicotinamide adenine dinucleotide phosphate ($NADP^+$) which is also used by a highly divergent paralog isocitrate dehydrogenase (IDH) (Zhu *et al.*, 2005). The construction of a large number of IMDH intermediates, and analysis of their enzymatic activity *in vitro*, showed a lack of epistasis: all investigated mutations contributed roughly additively to the enzymatic activity. Thus, the genotype–phenotype relation for IMDH coenzyme use is a single featureless peak (Lunzer *et al.*, 2005).

Assays of the same mutants *in vivo*, in which the corresponding growth rates were measured, yielded insight into the relation between genotype and fitness. Within this landscape, many – but not all – mutational trajectories from $NADP^+$ to NAD^+ usage exhibited a fitness dip, which indicates sign epistasis. This introduction of epistasis can be understood from the nonlinear relation between enzymatic activity and growth rate, in combination with the competition between two coenzymes: the fitness decreases when the recognition of $NADP^+$ is broken down, and rises again when NAD^+ recognition is built up. However, because apparently some mutations exist that simultaneously decrease $NADP^+$ interaction and increase NAD^+ interaction, monotonously increasing trajectories are also possible. Thus although the genotype–phenotype map that depends on the functional activity of an enzyme can be free of epistasis, the corresponding phenotype-fitness map can contain (sign) epistatic features.

Inter-genic epistasis

Epistatic interactions can occur between different genetic components when they are functionally related within a network. An elementary example is the recognition between a transcription factor and its binding site within a regulatory region. Which types of epistasis underlie such a molecular recognition and which landscape features they present, was investigated using the *lac* repressor and operator as a model system (Poelwijk *et al.*, 2006). Earlier mutational analysis had shown that two repressor residues and four base-pairs in the operator were central to altering the specificity of binding (Lehming *et al.*, 1990). A large set of mutants with substitutions at these loci had been assayed on their repression value, the ratio between repressed and unrepressed expression of the controlled gene. This genotype–phenotype relation exhibited several distinct peaks: optimal repressor–operator combinations whose paths between them contained significant decreases in repression (Poelwijk *et al.*, 2006). The existence of multiple peaks cannot be explained by sign epistasis alone, but requires the more severe reciprocal sign epistasis (Figure 1A). An intuitive rationale for this type of epistasis is the following: mutating one binding partner likely only benefits a new interaction if the other binding partner is mutated first and *vice versa*. The alternative outcome, in which optima are bridged by a "master key" repressor that binds to multiple operators, was not observed in the data.

As the repressor–operator binding landscape contains many sub-optima, they would seem to act as evolutionary traps that hamper adaptation to the global optima. One phenomenon that may affect this outcome is the presence of duplicated genes and their mutational divergence. *E. coli* contains several homologs of the *lac* repressor, which regulate the expression of different operons independently and must therefore have evolved specific repressor–operator recognition. Indeed, the promiscuous binding of functionally unrelated transcription factors on the *lac* operator should provide a selective pressure on binding specificity. This issue was investigated using the *lac* genotype-repression landscape. It was found that for two identical repressor–operator pairs, many mutational trajectories now did not exhibit decrease in fitness. Mutations appeared to exist for which the obligatory decrease in repression for one repressor was offset by a simultaneous decrease in the penalty for promiscuous binding of the other repressor. Such compensations within biochemical networks are ubiquitously observed and may therefore be a more general adaptive evolution mechanism (Francino, 2005). These results also substantiate the suggestion that the robustness of networks may promote evolvability (Conant and Wagner, 2003; Lynch, 2005).

Within the larger web of biological interactions, epistasis originates not only by direct physical recognition, but also through their hidden functional relations. Elena and colleagues explored this issue by creating random single and double mutants in *E. coli*, using Tn10 transposition and P1*vir* transduction (Elena and Lenski, 1997). Epistasis is absent if the fitness effect of the double mutant equals the sum of the fitness effects of the single mutants. However, a larger than additive effect of the double mutant is denoted as synergistic epistasis (Figure 1B), while a smaller than additive effect indicates antagonistic epistasis (Figure 1B). The data showed that epistasis between these random knockouts is not rare, and that antagonistic and synergistic interactions occur almost at the same frequency (Elena and Lenski, 1997; Otto, 1997). This observation is also supported by experimental studies from other organisms (Burch *et al.*, 2003; Elena, 1999; Wloch *et al.*, 2001; de Visser, 1997; Sanjuan *et al.*, 2004; Rowe et al., 2008) and by computational modeling (Lenski *et al.*, 1999; You and Yin, 2002).

The conservation of epistatic interactions across different organisms was addressed by Tischler and colleagues. This study focused on synthetic lethal interactions, a phenomenon in which two non-lethal mutations yield a lethal phenotype when combined. A large number of synthetic lethal interactions between genes are known for *Saccharomyces cerevisiae*, some of which have orthologous pairs in *Caenorhabditis elegans*. These 837 pairs were assayed in *C. elegans* on fitness using RNA interference (RNAi). It was found that a maximum of 5% of synthetic lethal interactions are conserved between *S. cerevisiae* and *C. elegans*. This value is low compared to the conservation of protein–protein interactions (31%) (Tischler *et al.*, 2008). Thus, surprisingly, even though the gene function between worm and yeast are conserved, the epistatic interactions are not.

The aspect common to the above studies is that they assess epistasis through measurement of fitness. As the molecular mechanisms that underlie fitness are often poorly understood, inference of epistasis from fitness alone usually does not provide a mechanistic explanation of epistasis. Epistasis can also be detected at lower levels of organization, such as transcription, for which the underlying mechanisms are better understood. One study focused on two glucose-adapted lines of *E. coli* (Cooper *et al.*, 2008) and the ancestral line, as well as *crp* knockouts for each of these lines. *crp* is a key global regulatory gene that itself was not altered in the glucose adaptation (Cooper *et al.*, 2003). Deletion of the *crp* gene appeared more detrimental to growth in the evolved strains compared to the ancestor. The cause of this effect was found in the adapted expression profile: in the ancestral strain, *crp* controls the expression of 171 genes whereas in the evolved strains it controls an additional 115 genes that were not initially *crp*-dependent (Gosset *et al.*, 2004). This study highlights the importance of lower-level phenotypic characterization when aiming to understand the mechanisms that underlie epistasis.

Epistasis is also thought to be related to the occurrence of sexual recombination. In the 1980s, Kondrashov introduced the mutational deterministic hypothesis, which states that the deleterious mutations that are combined during recombination are purged from the population (Kondrashov, 1988). This scenario requires synergistic epistasis, as it makes combined mutations more harmful than expected from their individual effects. The relation between epistasis and sexual reproduction has been investigated in a computational study of networks of transcriptional regulators as found in *Drosophila melanogaster* (Jeager *et al.*, 2004; Azevedo *et al.*, 2006). Individuals who attained stable expression patterns upon variation of their network interactions and selection were considered viable, whereas individuals that failed to reach equilibrium were considered not viable. The simulations showed that synergistic epistasis, and accordingly mutational robustness, indeed increased within the network in a sexually reproducing population while it did not in asexual populations. This effect is more prominent especially if the population experiences a high mutation rate and there are many genetic interactions. It remains to be seen whether these results in turn also provide a rationale for the maintenance of sex despite its costs.

The relation between epistasis and modularity was addressed in a computational study of *S cerevisiae* metabolism. By deleting single and pairs of genes *in silico* and computing the resulting growth rate using flux balance analysis, epistatic interactions could be assessed. Between two functional modules, such as those responsible for respiration and glycolysis, significant epistatic interactions were observed. These epistatic interactions were either consistently synergistic or consistently antagonistic. On the other hand, within a functional module both types of epistasis were observed. These results point to a correlation between network architecture and epistasis, and challenge the common notion that epistasis is stronger within a functional module than between the modules (Moore, 2005; Segre *et al.*, 2005).

In addition to epistatic interactions between genotypes, interactions between genotype and environment are also common. In this case, the effect of a mutation depends on the environment in which fitness is assayed and vice versa. To investigate genotype by environment interactions, random insertion mutants of *E. coli* were assayed in four different environments. It was found that about 40% of the insertions yielded different fitness effects in the different environments, showing that genotype by environment interactions are common (Remold and Lenski, 2001).

Conclusions and perspective

The studies reviewed here illustrate that epistasis plays a central role in a broad array of systems, scientific questions and experimental methodologies. The questions range from being predominantly functional, where epistasis is a powerful tool to unravel functional relations between genes, to mainly evolutionary questions, where epistasis provides a mechanistic explanation or even a prediction of adaptive dynamics. Some research focuses on interactions between residues within a single protein, while others consider the full regulatory networks underlying organismal fitness. These diverse approaches have provided an intriguing insight into hidden correlations within the design and evolutionary potential of biological systems.

However, the current approaches have considerable limitations and only begin to scratch the surface of all relevant correlations. For instance, intra-genic studies have so far only explored base-pair substitutions within restricted parts of genotype space. One may argue that organisms can exploit genetic changes in other regions to achieve the same functionality, thus making observed constraint and epistasis irrelevant. However, phylogenetic analysis shows that evolutionary transitions similar to several studied examples have in fact occurred in evolutionary history. Larger scale genomic rearrangements such as recombination are also known to play an essential role in the evolution of some functions. It would be of interest to explore how recombination events as well as ploidity affect fitness landscapes and the evolutionary trajectories within it. On the other hand, studies of epistasis within larger networks do explore more distant interactions, but in return typically only consider knockouts as the genetic change, and lethality as the response. It would be fruitful to investigate more detailed changes, such as altered expression levels or point mutations resulting in novel functionality, as well as more quantitative fitness measurements.

The complexity of biological and ecological systems makes finding new questions easy. For instance, most fitness landscapes and epistases studied so far have considered straightforward constant environments, even though much of the evolutionary innovation in nature likely results from adaptation in variable environments. The fitness landscape and epistasis concepts hold promise to also address the role of environmental variability in a more mechanistic manner. One may also wonder about the causal relation between epistasis and structure or function. Is biological function and structure predominantly shaped by epistasis, or *vice versa*, are epistatic interactions shaped by structure and function?

Recent decades have brought detailed functional insight at the molecular level. Increased understanding at the network and systemic level now enables a link to fitness, and allows one to address evolutionary dynamics throughout all levels of biological organization in a quantitative and mechanistic manner.

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