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Why and how to study genetic changes with context-dependent effects

Yuichi Eguchi, Gaurav Bilolikar and Kerry Geiler-Samerotte

The phenotypic impacts of a genetic change can depend on genetic background (e.g. epistasis), as well as other contexts including environment, developmental stage, cell type, disease state, and higher-order combinations thereof. Recent advances in high-throughput phenotyping are uncovering examples of context dependence faster than genotype–phenotype maps and other core concepts are changing to reflect the dynamic nature of biological systems. Here, we review several approaches to study context dependence and their findings. In our opinion, these findings encourage more studies that examine the spectrum of effects a genetic change may have, as opposed to studies that exclusively measure the impact of a genetic change in a particular context. Studies that elucidate the mechanisms that cause the effects of genetic change to vary with context are of special interest. Previous studies of the mechanisms underlying context dependence have improved predictions of phenotype from genotype and have provided insight about how biological systems function and evolve.

Address
Center for Mechanisms of Evolution, School of Life Sciences, Arizona State University, Tempe, AZ 85287, United States

Corresponding author:
Geiler-Samerotte, Kerry (Kerry.samerotte@asu.edu)

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Introduction

The phenotypic impacts of genetic changes can depend on context [1•,2•,3••,4,5••,6•]. For example, the impacts of mutation can vary with genetic background (i.e. epistasis) and also with environment, age, sex or cell type. These types of dependencies are often treated as distinct phenomena, but they are all similar in that they raise an important question: How can we predict traits from genetic data when the same mutation can have different impacts depending on its context?

Why quantitative geneticists should care about context dependence

A major goal of modern biology is to use genetic data to make phenotypic predictions, including predictions about an individual's predisposition to disease, which crops will grow in extreme temperatures, and which infectious microbes will develop drug resistance. Increased availability of whole genome data improves power to identify rare genetic variants that have small impacts of phenotype (IMN Wainschtein *et al.* 2019 bioRxiv doi: https://doi.org/10.1101/588020). But genetic variation with context-dependent effects continues to complicate predictions about the phenotypes that we care about [6•]. For example, the causative alleles underlying cystic fibrosis, amyotrophic lateral sclerosis, and Fanconi anaemia do not predict disease severity; instead, genetic variation at other loci modulates their impacts [7–9].

Context dependence is a tricky problem because it is unlikely to be solved by advances in sequencing technology that provide more genetic data at lower cost. Imagine assessing how the impact of every genetic variant depends on every other; this would require consideration of many combinations of variants. Even if we could collect enough data, analyzing this many combinations represents a significant computational challenge [6•,10]. Incorporating environmental and other non-genetic contexts further increases the difficulty of the combinatorics problem. Therefore, comprehensively mapping genotype to phenotype may require new strategies. One such strategy is to incorporate information about the mechanistic basis of context dependence when making phenotypic predictions. For example, previous studies quantify how a common cost of mutation scales non-linearly in order to predict the combined impact of multiple mutations [11].

Why evolutionary geneticists should care about context dependence

Identifying the mechanisms underlying context dependence, in particular mechanisms that buffer the phenotypic impacts of genetic change, is an important goal in the field of evolutionary biology [12]. Computational models demonstrate that buffering mechanisms could protect small populations [13] or tumors [14] from deleterious mutations. But the identity of mechanisms that increase robustness to mutation, and whether or not mechanisms have evolved to increase the robustness of complex traits, is a topic of major debate [2•].

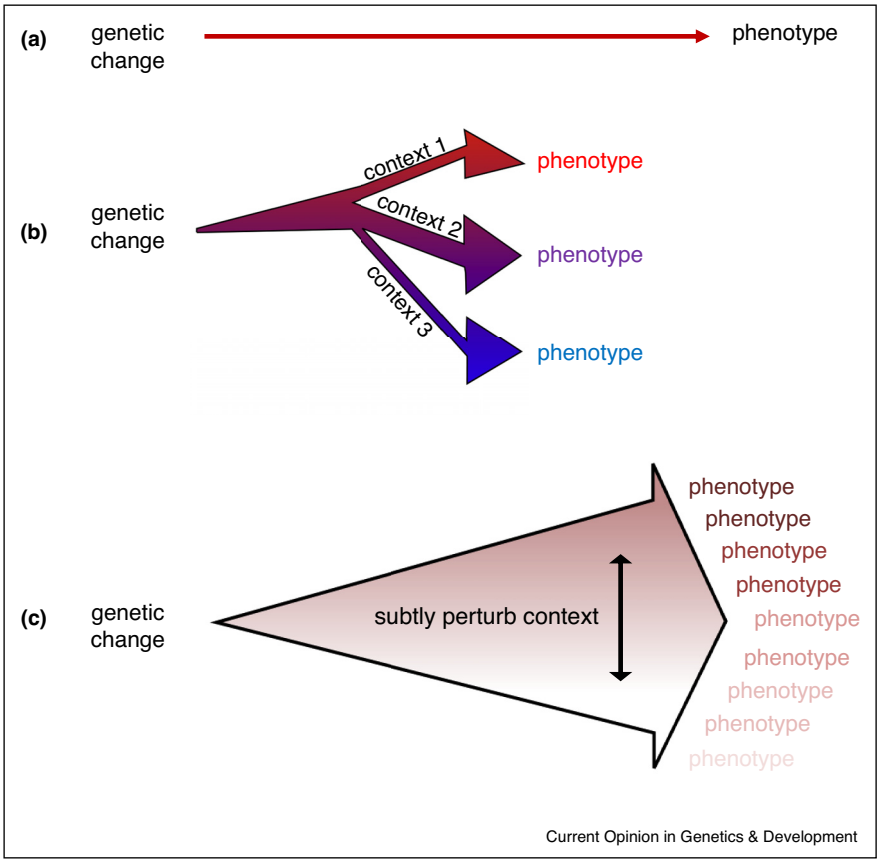
Deeper investigation of the mechanisms driving context dependence is needed.

Other branches of evolutionary biology would also benefit from a deeper consideration of context dependence. Many ideas are centered on the notion that genetic changes have fixed, rather than context-dependent, effects. For example, consider 'evolutionary traps,' which aim to trap infectious microbes or tumors by selecting for mutations that encourage resistance to some drugs at the expense of others. These traps will fail if cells can escape via contextual changes that disrupt the relationship between resistance and susceptibility [15]. For another example, consider the 'cost of complexity', the idea that the more traits a mutation affects, the greater the chance that one of these effects will be deleterious such that the mutation will be purged by selection [16]. Understanding which mutations persist over evolutionary time is more complicated if mutations affect many traits in some contexts and few traits in others. Many other issues, including the long-standing debate about whether biological systems tend to be modular [17] or are 'omnigenic' (i.e. are organized such that every gene has the potential to affect many traits) [18], need reframing in light of emerging data suggesting that whether a mutation affects one trait or many depends on context [5••] (IMH Geiler-Samerotte *et al.* 2019 bioRxiv doi: https://doi.org/10.1101/700716).

Increased focus on context dependence is critical in quantitative and evolutionary biology

Given the difficulty of phenotyping large numbers of individuals, it has been challenging to comprehensively study how the impact of a genetic change varies across genotypes, environments, and other contexts. Here we review recent studies that tackle this challenge by utilizing high-throughput approaches. These studies demonstrate that context dependence contributes to diverse traits in diverse organisms. These studies also highlight how context dependence complicates prediction of phenotype from genotype.

Figure 1



An expanded conceptualization of the genotype-to-phenotype map that includes mutations with context-dependent effects. **(a)** A genotype-to-phenotype map where a genetic change has a fixed effect on phenotype. **(b)** A genotype-to-phenotype map where a genetic change can result in qualitatively different phenotypes depending on the context. **(c)** A genotype-to-phenotype map where a genetic change can result in a spectrum of quantitatively different phenotypes depending on the context.

In light of these findings, we and others suggest a paradigm shift: away from pinpointing the impact of a genetic change in a particular context, and toward examining the spectrum of effects a genetic change may have on a particular phenotype [5••] (IMH Geiler-Samerotte *et al.* 2019 bioRxiv doi: https://doi.org/10.1101/700716). In order to encourage such a shift, it would be helpful to expand the genotype-to-phenotype map concept to include genetic changes that have context-dependent effects (Figure 1). Re-thinking the way the genotype-to-phenotype map is conceptualized may illuminate new avenues for exploration, including questions about the mechanisms that cause some mutations to have context-dependent effects while others do not, and questions about the extent to which we can predict the impacts of mutation across contexts without exhaustive mapping.

The high-throughput studies we review in the next section of this paper suggest that power to predict phenotype from genotype may be gained by understanding the mechanisms driving context dependence. Therefore, in a later section of this paper, we suggest an additional approach to study context dependence that focuses on mechanism: we pursue the hypothesis that basic features of cells (e.g. gene regulatory networks) create generic rules that modify the impacts of mutation in predictable ways. Focusing on ubiquitous mechanisms underlying context dependence may yield insights that improve phenotypic predictions across diverse organisms.

Review of current approaches to investigate context dependence

Measuring the impact of gene deletions across many contexts

Pioneering work in yeast utilizes gene suppression and deletion libraries to survey gene-by-environment interactions [19] and gene-by-gene interactions [20,21] for nearly every gene. These studies identified genetic changes with phenotypic impacts that depend on context. A comprehensive study of 23 million pairs of gene deletions identified one million cases where a deletion's impact on growth is modified by a second deletion (i.e. there were one million cases where the combined effect of both deletions could not be predicted from the individual effects of each deletion) [20]. A follow-up study suggested tri-genic interactions are 100 times as prevalent [22].

Similar studies in organisms ranging from fruit flies [23,24•] to cancers [25–27] also detect genetic changes with impacts that depend on context. Many studies find that pairwise interactions change across genetic backgrounds, environments, or over time [23,24•,28,29]. This is called a higher-order interaction. By demonstrating that it is difficult to predict higher-order from pairwise interactions, these results further highlight how context dependence complicates phenotypic predictions from genetic data.

In some cases, a mechanistic understanding of context dependence improves predictions of phenotype from genotype. For example, genes sharing similar functions tend to interact with other genes in a similar way, which allows prediction of some pairwise interactions from others [20,21] (Figure 2). Other studies suggest that improved predictions of higher-order interactions may be achieved by understanding how the connections among components of a signaling pathway re-wire across environments, cell types or other contexts [23,24•,28].

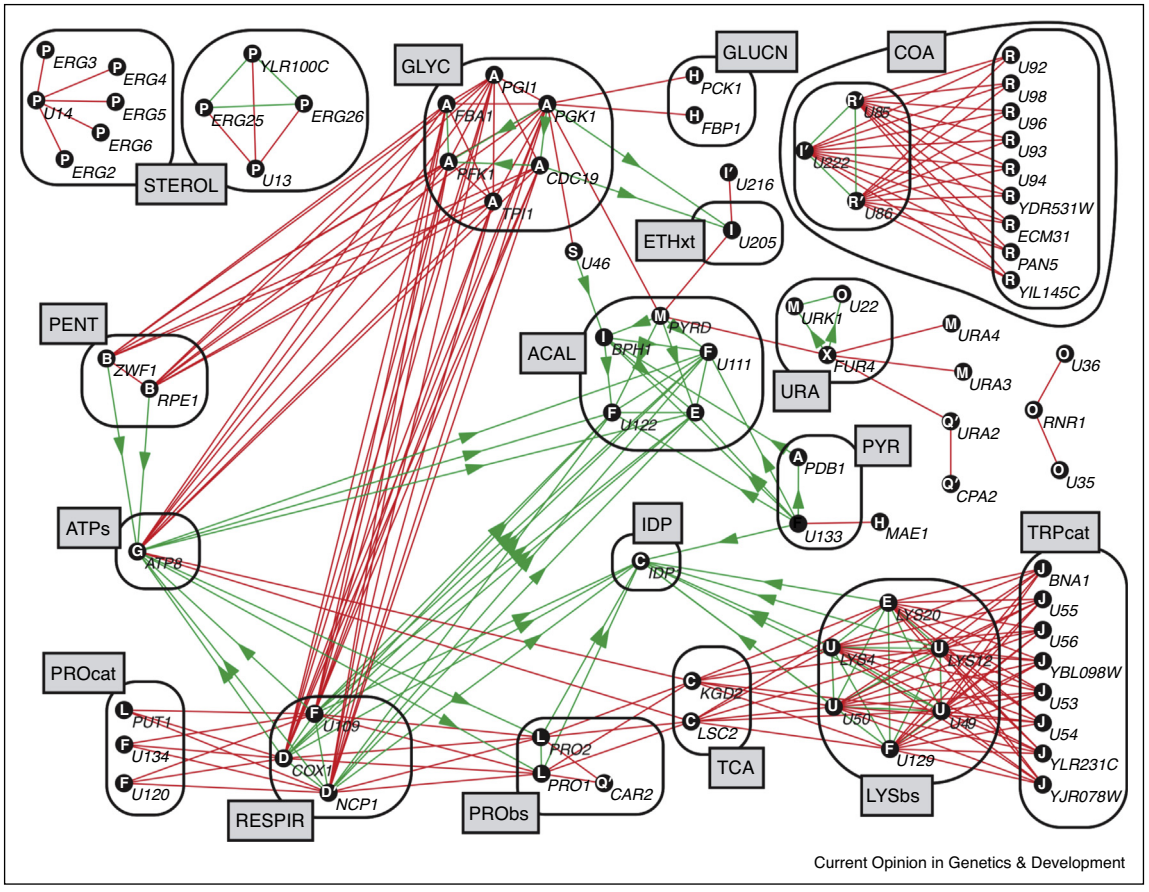
Comprehensively quantifying genetic interactions within a single molecule

Another approach to studying context dependence focuses on how the phenotypic impacts of mutation depend on other mutations within the same protein or ribozyme [3••,11,30•,31–33]. Because these studies are limited in scope to single molecules, they can be very comprehensive. One study engineered all possible combinations of 12 mutations that accumulated during the evolution of an alternatively spliced human exon. Competition between alternative splice sites causes the maximum impact of each mutation to occur in genetic backgrounds with intermediate levels of alternative splicing. This non-monotonic relationship predicts the impact of higher-order combinations of mutations (up to 10) from pairwise interaction data [30•]. Other studies find mutations that improve protein function can be deleterious in genetic backgrounds where protein stability is compromised. Quantitative models of the tradeoff between stability and function improve predictions about how mutational impacts combine [3••,11,32]. Other studies show that interactions between mutations can change across environments [33,34]. Collectively, these single-molecule studies demonstrate that mutations with context-dependent effects are common. They suggest that understanding the mechanisms driving context dependence can improve predictability, though some unpredictability remains [3••,35].

Screening genetic variation found in nature for context-dependent effects

Other approaches seek to understand the degree to which genetic variants found in natural populations exhibit context dependence [6•]. Some studies investigate highly interactive genes called 'genetic modifiers,' demonstrating these genes influence the phenotypic impacts of hundreds of natural genetic variants dispersed across genomes [36,37•,38]. One study examined how each of seven genetic modifiers interacts with a panel of ~1500 natural genetic variants across 10 environments, finding that higher-order interactions (e.g. gene-by-gene-by-environment) are prevalent [37•]. Other studies focus on phenotypes of interest, examining how the impacts of alleles that make major contributions to these

Figure 2



Studies in yeast demonstrate that genes can be organized into functional modules based on pairwise interactions.
Green lines connecting genes signify that double gene deletions have smaller impacts on growth than expected given the impact of single gene deletions. Red lines signify that double deletions have larger impacts than expected. These pairwise interactions are used to group genes (black nodes) into modules (enclosed boxes) that interact with other modules in the same way (i.e. all lines connecting two modules are the same color). The functions of the genes within each module are related, suggesting that genes sharing similar functions interact with other genes in a similar way. This also suggests that the interactions between some genes predict how others will interact. This figure was reproduced with permission from Segrè *et al*. [21].

phenotypes are modulated by natural genetic variation [7–9,39–41]. In many cases, natural genetic variants that interact with major-effect alleles tend to have related functions. For example, the natural genetic variants that modify the impact of three major-effect alleles causing aberrant yeast colony morphology all represent different regulatory inputs into the transcription of a single gene [41]. Other studies survey the number of traits influenced by natural genetic variants, finding that this number varies across contexts [5••] (IMH Geiler-Samerotte *et al*. 2019 bioRxiv doi: https://doi.org/10.1101/700716). Collectively, these studies reveal pervasive context dependence in nature.

Analyzing how context dependence influences evolutionary trajectories

Another approach to investigating context dependence focuses on adaptive evolution. These studies find that adaptive mutations have fitness impacts that depend on genetic background [35,42–47] and environment [45,48,49]. For example, several studies predict the order in which mutations occurred during evolution. They do so by reconstructing all possible orderings, finding that some are less likely because the impacts of mutations change from adaptive to deleterious when present in certain combinations [35,42,43,47]. Other studies find that evolution follows a rule of declining adaptability, such that the benefit of any particular adaptive mutation declines as fitness improves [44,45]. Another reason adaptive mutations may have diminished impacts when combined is if they serve redundant functions [46]. Understanding the mechanisms that diminish the benefit of adaptive mutations in some genetic backgrounds improves power to predict fitness. However, these predictions can be frustrated when the interactions between mutations change across environments.

Promisingly, power to predict higher-order interactions (i.e. gene-by-gene-by-environment) may improve upon considering how the genes that contribute to fitness change across environments [45].

A nuanced approach: focusing on ubiquitous mechanisms driving context dependence

Several studies above suggest that the changing impacts of mutation become more predictable given mechanistic explanations as to why these impacts depend on context. Next, we pursue the idea that features common to many cells can modify the impacts of genetic change in predictable ways. By focusing on ubiquitous mechanisms underlying context dependence, perhaps we can identify guiding principles that allow prediction of phenotype from genotype across diverse organisms. As test cases, we focus on two features that most cells possess: network interactions and costs of protein production. We explore the challenges and potential benefits of understanding how these features contribute to context dependence.

How network structure results in context dependence

Molecules participating in the same biological network or pathway may interact by enhancing or repressing each other's activity or by operating sequentially during biosynthesis. Trends describing how network interactions give rise to context dependence have been observed in simulated network models. These models suggest that transcription factors that activate expression of many genes tend to enhance the impacts of mutation, while transcription factors that repress expression more likely buffer mutational impacts [2•]. Power to study these trends in biological networks is often limited by the number of mutant strains that can be engineered and the throughput with which network output can be measured.

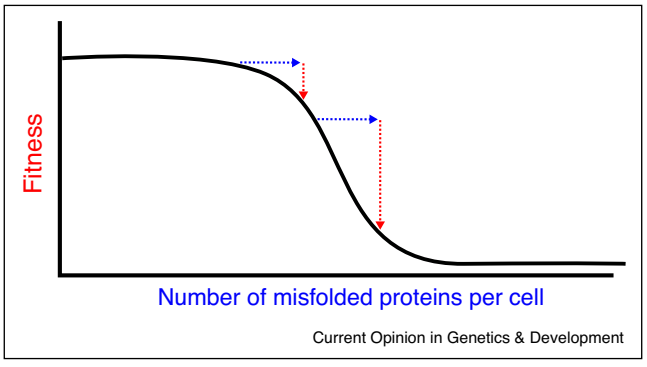
However, recent high-throughput experiments have begun to examine context dependence arising in small biological networks. These studies identify trends that enable predictions about how mutational effects combine. For example, a study of ~5000 double mutants in yeast's galactose pathway demonstrates that their impacts on growth can be predicted with only 55% accuracy from the growth-impacts of single mutants, but with 90% accuracy when considering simple models that capture stereotypical relationships between three network components (New and Lehner 2019 bioRxiv doi:https://doi.org/10.1101/589606). Another study of the lambda bacteriophage switch surveyed the impact of thousands of mutations to either a *trans*-regulatory or *cis*-regulatory component, finding that the combined impact of mutations to both components depends on how the *cis* mutations influence binding of RNA polymerase [50•]. And an earlier study in yeast explains how the impact of pairwise gene deletions can be significantly worse than expected given the impact of single gene deletions, but only in some environments. Redundant biological pathways can reduce the impact of each single deletion, but only in environments where both pathways are active [51].

A potential criticism of these studies is that understanding context dependence arising within small networks may not improve predictions about complex traits. This criticism recalls the debate about the extent to which biological systems are modular [17] or whether very many genes contribute to each complex trait [18]. Studies of context dependence have shed light on this debate by demonstrating that the genes contributing to complex traits can change across contexts (as can the pairwise interactions between these genes). Continued study of how regulatory or functional relationships between genes result in context dependence may provide further insight about the genetic architecture of complex traits.

How accumulating costs from misfolded proteins result in context dependence

Another generic feature of cells that may cause mutational impacts to vary across contexts is the toxicity of misfolded proteins [52,53]. Computational models predict that the same misfolding mutation would have larger deleterious effects in cells possessing larger numbers of misfolded proteins (Figure 3) [54]. Understanding how costs from protein misfolding change with context could be of wide-spread value given the ubiquity of protein misfolding. Most mutations to coding sequences increase misfolding [55], and the number of misfolded

Figure 3



How accumulating costs from misfolded proteins might result in context dependence.
This toy model shows how the impact of a misfolding-inducing mutation might change depending on the number of misfolded proteins that are already present within a cell. The blue arrows are the same length and represent the number of misfolded proteins that a particular mutation contributes. The fitness impact of these additional misfolded proteins (length of red arrows) depends on context. The relationship between fitness and the cell's misfolded protein burden (black line) is unknown, though computational models suggest fitness declines exponentially [54].

proteins in cells changes with cell type, age, and environment [52,56].

It seems unlikely that there will be a simple trend summarizing how the cost of misfolding grows as the number of misfolded proteins in a cell increases. The impacts of protein misfolding seem to differ for different proteins [57–59]. Further, cells have a variety of systems, such as proteasomes and chaperones, to contend with misfolded proteins [53,56,58]. Upregulation or downregulation of these systems may modify the relationship between the cost of misfolding and the cell's misfolded-protein burden. Still, trends may exist that apply to some proteins. Indeed, a recent study revealed a general rule on the limits of overexpression that applies to typical proteins and identified a subset of proteins that do not follow the rule [60•].

Open questions

How much of the difficulty in mapping genotype to phenotype is due to context dependence?

There is disagreement about whether genetic changes with context-dependent versus very small additive effects create more difficulty in mapping genotype to phenotype [6•]. Further examination of context dependence and the mechanisms driving it may suggest why some complex traits are more influenced by it than others [61].

Do mechanisms that cause dependence on one context cause dependence on others?

In this review we consider dependence on diverse contexts, including genetic background, environment, cell type or time. Whether similar mechanisms contribute to all types of context dependence is an open question, though in some cases this seems likely. For example, the cost of a misfolding-inducing mutation may scale non-linearly with the cell's misfolded protein burden (Figure 3) regardless of whether this burden is increased due to environmental factors or the presence of many other misfolding-inducing mutations.

How often are the mechanisms driving context dependence general versus specific?

Previous studies demonstrate cases where a general mechanism contributing to context dependence, such as the tradeoff between protein stability and function, does not describe how mutations within the same protein sequence interact [3••,43]. The extent to which general features of cells contribute to context dependence, and the extent to which elucidating these contributions will improve the mapping from genotype to phenotype, remain open questions.

Outlook

Recent high-throughput technologies quantify the spectrum of phenotypic impacts a genetic change may have across diverse genetic backgrounds, environments, or other contexts. Continued study of context dependence, especially the mechanisms that cause it, has the potential to improve predictions of phenotype from genotype and to provide a better understanding of how biological systems function and evolve.

Conflict of interest statement

Nothing declared.

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