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# Evolvability

## A Unifying Concept in Evolutionary Biology?

**Edited by:** Thomas F. Hansen, David Houle, Mihaela Pavličev, Christophe Pélabon

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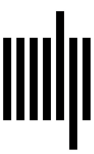

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# 8 The Genotype-Phenotype Map Structure and Its Role in Evolvability

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The potential for evolutionary change is deeply anchored in the kind and amount of heritable phenotypic variation that organisms can produce, and thus in the way that genetic predispositions translate into the phenotype. That translation is the genotype-phenotype (GP) map. We first explain the two common conceptualizations of GP map: the global correspondence map and the local mechanistic map, and how they relate to each other. We focus on the structural aspects of the GP mapping, as summarized in the notions of pleiotropy and epistasis, and argue that their effect on evolvability is not captured sufficiently in the current theory. One way to approach this problem is to address mechanistic, causal mapping explicitly and explore systematically the variational properties of various well-known biochemical or regulatory processes. This may allow us not only to better account for the effects of pleiotropy and epistasis, but also to potentially complement these summarizing concepts themselves with notions that better capture the underlying mechanisms—thus adding the mechanistic aspect to the global GP map.

## 8.1 Introduction

The genotype-phenotype (GP) map captures the translation of genetic predispositions into phenotypic traits and is thus intimately associated with the potential to evolve (the evolvability). Despite the shared general notion, the GP map concept is used with various distinct meanings in biological literature; it is therefore paramount to explain how these meanings relate to one another and be specific about our use of the concept in the present chapter. Most generally, a GP map establishes a mere correspondence between a set of possible genotypes and a set of phenotypes (Lewontin 1974). We refer here to the ensuing theoretical space as a *global* GP map (figure 8.1). A global map is not thought to itself evolve; instead, evolution is conceptualized as movement of a population on this map. Populations and species inhabit (more or less) distinct portions of the map. Referring to the global map does not imply any intention to address the explicit mechanistic nature of mapping or its change as the population moves through the map. When studying the genotype-phenotype relations in a population in the context of the global map, the nonlinearities, or the lack of one-to-one mapping, which naturally arise in the mechanisms of development and physiology, are subsumed into variational concepts, such as pleiotropy or epistasis.

In contrast, when the concept of genotype-phenotype map is applied to individual genotypes, it refers to mapping of a single genotype to a single phenotype through all the organismal processes, including morphogenesis, growth, and physiology (figure 8.1). To

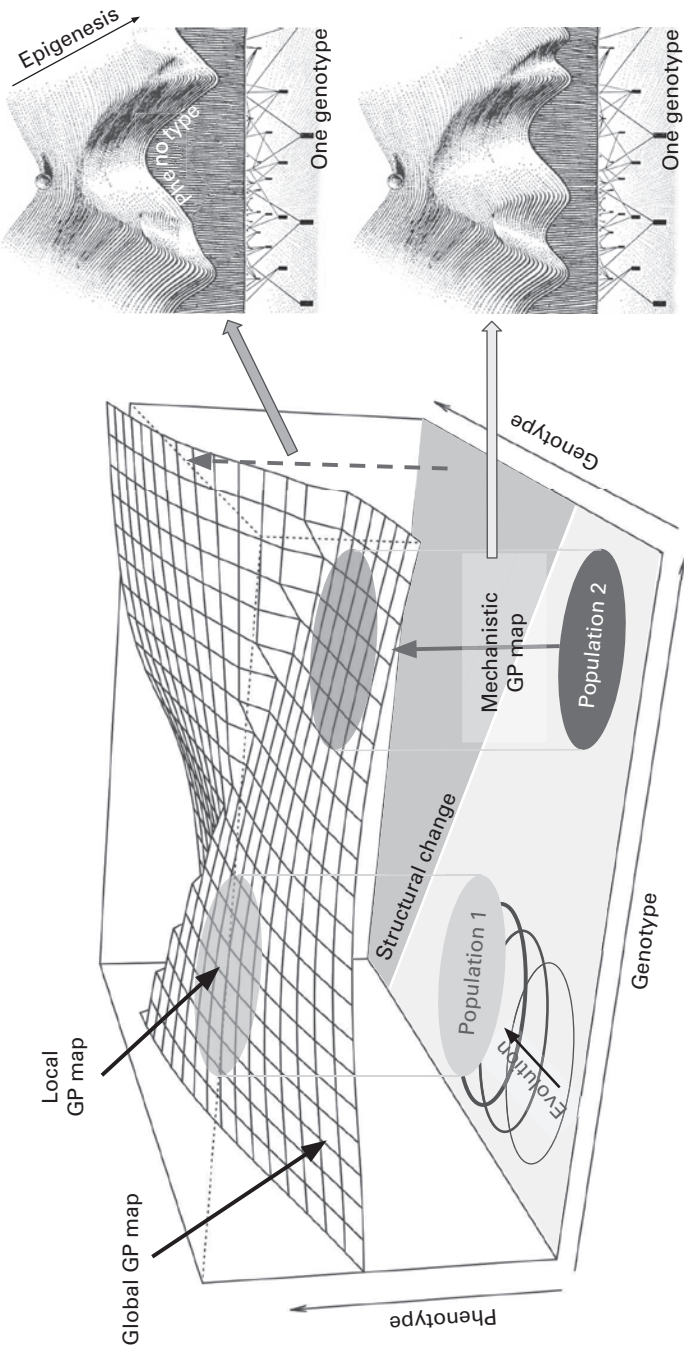


**Figure 8.1**
Three types of GP maps. GP maps describe the relationship between the genotype space (represented by 2 dimensions) and the phenotype space (here 1 dimensional). The entire surface represents a hypothetical global GP map, relating all possible genotypes to the phenotype. Ellipses illustrate local GP maps, which can be deduced based on variation in populations. The GP map concept can also be extended to the description of physiological, developmental, and functional mechanisms by which genotypes direct the epigenesis of phenotypes (mechanistic GP map). Populations evolve by moving in the genotype space, which may change the phenotype and the structure of the local GP map. In this figure, the global map shows a discontinuity in phenotype space, such that genotypes on either side of the boundary labeled "structural change" can show a discrete difference in phenotype. These differences are represented by the two panels on the right. In these panels, the process of epigenesis proceeds like a ball rolling downhill on each landscape (Waddington 1957). Maps in front of the 'structural change' boundary will reliably produce one of the four phenotypes at the bottom of each channel, shown in the lower right panel, while those on the other side of the boundary produce just two possible phenotypes. According to this definition, the global GP map is constant and cannot evolve, the local GP map evolves along with genetic changes in the population, and the mechanistic GP map evolves when populations cross the structural change limit.

each genotype, *a local map* is thereby attributed, represented by Waddington's (1957) epigenetic landscapes reproduced on the right side of figure 8.1. This *mechanistic* GP map explicitly aims to capture some aspect of the nature of mapping and may refer to any intermediate or final level of the phenotype (e.g., gene expression, RNA secondary structure, enzyme activity, or the adult femur length). Such mechanistic maps vary greatly in how detailed they are. This local GP map can be thought to evolve as the population moves through the global GP map. We aim to show in this chapter that the summary concepts used to capture the mapping complexity in the global map, such as pleiotropy or epistasis, often insufficiently capture the potential to evolve. We argue therefore that we would profit from rethinking the mapping in the global GP map in terms of mechanistic mapping (as proposed by Alberch 1991).

When we wish to study how any system works, we observe the consequences of perturbing it. Similarly, we learn about how nature works by observing its variation. In evolving populations, important variation arises by genetic mutations in single individuals. When sequence changes have effects that percolate through the developmental and physiological mechanisms of the individual GP map to change phenotypes of individuals, they cause heritable phenotypic differences in the population, thus constituting the raw material for selection. How the mutations affect the phenotype in individuals (i.e., which characters vary or covary across individuals) is a consequence of the mechanisms constituting GP mapping. This mechanistic GP map is thus not a description of how heritable phenotypic variation manifests, it is instead a description of the underlying processes that shape its manifestation. The observed pattern of variation is not itself a GP map, but a consequence of the variation interacting with a structure of the GP map—in the same way that a shadow of an object is not an object itself, but a consequence of light interacting with an object.

It is important to emphasize that the GP map is not dependent on the presence of variation, it applies regardless of whether all the genotypes actually exist. We thus distinguish between a GP map of traits and what we call the *genetic architecture of traits*. The latter term refers to a statistical population variation summary focusing on observed genetic variation and thus is limited to the traits and mechanisms that vary in the population. The genetic architecture is influenced by the GP map, but also by effects of allele frequency and linkage. Thus, changes to the latter factors can change the genetic architecture of traits, even when the mechanistic GP map remains constant.

We will frequently refer to the *structure* of the mechanistic GP map. Two aspects of GP map structure will be addressed: the involvement of genes in multiple traits, and the dependency of the genetic effect on the genetic background (i.e., corresponding to pleiotropy and epistasis). In both aspects, we do not refer to the statistical concepts (except when explicit; see Hansen, chapter 5)[1] but to the structures necessary for them to arise. For example, roles of two genetic sequences must depend on each other for the statistical interaction to arise when the underlying sequences vary (neglecting linkage disequilibrium, which is transient). Similarly, a gene must be involved in generating different body parts for these parts to covary when mutation arises (variational pleiotropy). We consider such mechanistic pleiotropy and epistasis to describe the general GP map structure. Within this structure, other evolutionary

1. References to chapter numbers in the text are to chapters in this volume.

changes, such as changes of mutational rate or effect size, are thought to occur. Singling out the GP structure as we do here is based on the assumption that it evolves more slowly than the changes within a given structure (note that this assumption underlies the existence of pleiotropic constraints). In other words, we consider that the global GP map consists of *structurally neutral* regions. Moving in these structurally neutral regions entails phenotypic modifications, but not changes of the structural aspects of the mechanistic GP maps. Addressing this topic thoroughly would merit a separate chapter, so to support the notion of separating conceptually structural changes from changes within a given structure, we merely point to the pattern of trait evolution being hierarchical, with structural aspects (e.g., body plan traits, homologies) evolving more slowly than modifications within given structures (e.g., relative sizes of given parts).

These clarifications of assumptions and the use and interpretation of GP maps will help us explain the full role of the (mechanistic) GP map in evolution. Because we consider GP structure to be invariant across subregions of the global GP map (i.e., the *structurally neutral* regions), it can be treated within this region as an *a priori*. In this space, the extant variation as well as the variation to be encountered by the same system due to future mutations (variability sensu Wagner and Altenberg 1996) percolate through the same structure, restricting in the same way the range of patterns of variation and covariation that can be generated by mutation, recombination, and segregation. Genetic variation thus makes the underlying system's structure visible and can be used to explore the GP map structure as well as its effects on the intermediate- and short-term responses to selection. The evolvability in this context is not based solely on the variation currently segregating, but refers to the propensity of the system itself to generate variation.

In this chapter, we focus on the mechanistic GP map structure and its consequences for the general propensity to vary, and thus for the ability or inability of a population to respond to selection on trait means. Addressing the evolution in the space of the *structurally* neutral variation of the GP map corresponds to what is often referred to as short- and intermediate-term evolution. Whether this level of change fully corresponds to within-species evolution is an empirical question, as the GP map structure may differ between species for some processes or body parts but not for others.

The chapter has the following organization. We first explain why the evolvability of complex organisms is an intriguing phenomenon, how this involves the GP map, and based on that, what GP map one might expect in evolvable organisms in section 8.2. Next, we elaborate in more detail the consequences of the GP map structure for evolvability in section 8.3. We point to the difficulties that the statistical approach to the GP map encounters when addressing evolutionary prediction. This leads us in section 8.4 to recognize the need to integrate the mechanistic detail of GP maps, rather than only distribution of extant variation, to predict evolutionary response. We explain how this detail can be integrated with the population genetic approach to model the evolution and evolvability of the phenotype, starting from the mechanistic structure of the map rather than starting from the phenotype. Finally, in section 8.5, we explain the principles of assessing GP map structure and the resulting quantitative genetic measurements useful to both quantitative and population genetic approaches.

## 8.2 Evolving Complex Phenotypes

### 8.2.1 Phenotypes Too Complex to Adapt by Chance: Fisher's Geometric Model

We can start to understand the role of the GP map structure by observing how evolvability changes as the complexity of the system increases. Fisher (1930) illustrated this in his "geometric model." Consider the two-trait situation, shown in figure 8.2. The circle of radius $d$ centered at the optimum $O$, encloses all the points that would be closer to the optimum than genotype $P$. Given this situation, Fisher showed that the probability that an arising mutation will be advantageous approaches 1/2 when the mutational step ($e$) is very small relative to $d$ and decreases with increasing mutational effect size.

Extrapolating this logic to increasing number of traits, the more traits that are affected by a mutation, the smaller the probability that a mutation will be advantageous. The expectation based on this simple intuitive model is therefore that with the increase of complexity (assuming increase in pleiotropy), the probability that each mutation has of being advantageous decreases (Orr 2000). This is the problem of complex adaptation. It should be mentioned that in population genetic dynamics, the situation is somewhat more complex: Probabilities of fixation must also be considered (Kimura 1983), and pleiotropy also introduces advantages into the system by increasing the trait's mutational target size (Hansen 2003; Pavličev and Hansen 2011; see section 8.3.3). Orr (2000) thus used Fisher's geometric model to support an important intuition about the problem of evolvability set by complex adaptation.

By exploring the implicit assumptions of this restricting model, one can understand the conditions that enabled complexity to nevertheless evolve. For example, note that Fisher uses a very particular GP map structure; namely, that of an extreme form of pleiotropy, in which each mutation affects all traits with equal probability and effect size, as represented here by a continuous circle, is replaced by various fragments of the circle (representing the mutationally accessible directions), or by an ellipse (representing asymmetrical mutational sizes), this becomes a different GP map, with differing predictions. We will address such GP maps next.

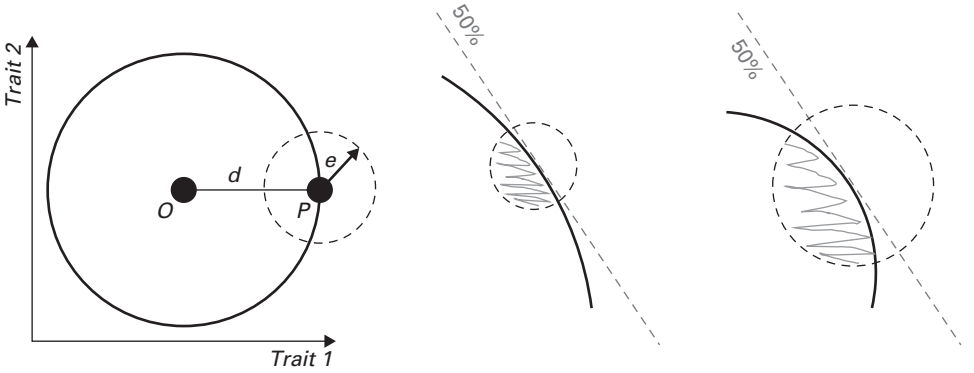


**Figure 8.2**
Fisher's geometric model. The two axes represent two phenotypic traits. $O$, optimal phenotype; $P$, mean phenotypic value of a genotype or population; $d$, distance of $P$ from the optimum; $e$, mutational effect size. Note that as the mutational step size increases, the proportion of possible mutational outcomes that are closer to the optimum than the present phenotype (shaded part of the small circles in the middle and right panels) decreases. Adapted from Pavličev and Wagner (2012).

### 8.2.2 GP Structures Increasing Evolvability: Modularity and Robustness

Since complex organisms do evolve, what then are the GP structures that make organisms evolvable? Here we briefly explain two major structural principles: modularity and robustness (see A. Wagner, chapter 11).

In Fisher's model, universal pleiotropy leads to decreased frequency and size of beneficial mutations. Pleiotropy, the single mutation causing change in multiple phenotypic traits, causes covariation between these traits at the population level. To avoid this effect of pleiotropy, *modularity* of the GP map restricts mutational effects of single loci to sets of traits with common function or development (figure 8.3A, a module sensu Wagner and Altenberg 1996). At the population level, such a GP map will generate covarying clusters of traits, which covary less with other trait clusters.

Why is this structure considered to promote evolvability? In the short term, evolvability is proportional to the availability of heritable genetic variation in the direction of selection, which is not entangled with variation in other phenotypic traits, as these traits may encounter different selection. Note that evolvability in Fisher's model was diminished, because, with increasing complexity, the dimensionality of mutational effect also increased; it is unlikely that changing all traits simultaneously will be advantageous for all traits. Modularity allows independent selection responses of modules without interference due to correlation between modules—an aspect that increases the evolvability by reducing pleiotropic constraint.

Note, however, that the idea of modularity, as expressed most influentially by Wagner and Altenberg (1996), is not only about decreasing the dimensionality of mutational change. Instead, it also involves a functional aspect: The proposed modules are focused on structures with common function or common development. Thus the traits integrated in a module will most likely be selected together, due to *internal selection* (Schwenk and Wagner 2000). Internal selection means that only some directions of variation maintain the initial functionality of the organism, while others don't, regardless of the environment. For example, the mutations that perturb heart function or disable reproduction will be unconditionally deleterious to fitness in any environment. Such selection occurs within the organism. Modules are thus thought to be aligned with the directions of internal selection, therefore reducing the probability of deleterious mutations and focusing the variation in the directions likely to be selected, regardless of external selection. In contrast to external selection, the direction of internal selection is predictable, as it is based on the organism's structure. Although this function-preserving structural aspect was essential in the original concept (Olson and Miller 1958; Riedl 1975), it commonly has been disregarded in the vast

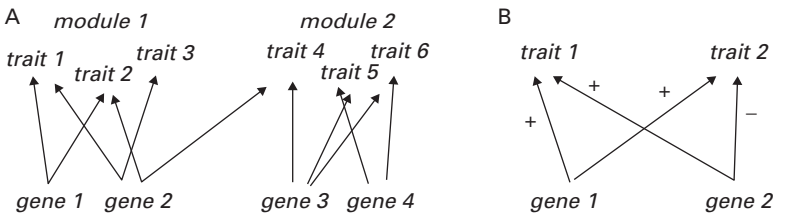


**Figure 8.3**
Two structures of the GP map generate reduced population covariance. (A) Modular pleiotropy. (B) GP map structure that may result in hidden pleiotropy, depending on effect sizes and allele frequencies.

subsequent literature on modularity. This led to a variety of misleading conclusions concerning the effects of modularity on evolvability (Jablonski, chapter 17).

The assessment of pleiotropy will be discussed in section 8.5. Mechanistically, we know from developmental and variational studies that while most genes are reused in development of multiple traits, each mutation does not affect all traits of an organism (e.g., G. Wagner et al. 2008; Wang et al. 2010). Note that recent work in disease genetics has drawn a different conclusion, all genes affecting all traits (*omnigenic model;* Boyle et al. 2017). Yet this discrepancy is not a contradiction. Disease is not a complex trait in the sense that the vertebrate forelimb is a complex trait; it is not an individualized, evolved, and evolvable biological unit but a consequence of a pathological variant thereof (Pavličev and Wagner 2022). It is therefore problematic to use the insights from disease genetics to draw conclusions in evolutionary genetics directly. As biological traits are embedded in an organism, there are many more possibilities to cause a defect than to generate variation relevant for evolution. With respect to biological traits, then, pleiotropy is ubiquitous but not universal, so each mutation does not contribute to variation in all biological traits. Because mutational effects percolate through the developmentally and physiologically organized GP map, the distribution of pleiotropic effects of genes on traits does not follow a random play of chance but instead affects the sets of genes from functionally interacting pathways that are active in specific cells, tissues, and organs, and are not invoked in others. Therefore, we can expect that variation will, however partially, reflect these patterns.

Note that the principle of modularity does not imply that population variation consists of entirely independently varying blocks of traits. There will be covariance even when the traits belong to different modules. This is not surprising, as many fundamental pathways are shared among traits, tissues, and cell types. To the extent that the genes in these pathways vary, they will produce covariation between modules.

*Robustness* is another structural property of GP maps that can increase evolvability. We will address only genetic (not environmental) robustness here. We have seen that the concept of modularity suggests a specific arrangement of mutational effects with respect to the direction in the phenotypic space. In contrast, genetic robustness is about the distribution of mutational *effect sizes*. A GP map is robust if many mutations do not change the phenotype. Therefore, those cryptic mutations may accumulate in the population. If one imagines genotypic space as a network of all possible genetic sequences that are a single mutation away from each other, then in a robust population, a subnetwork of such connected genotypes map to the same phenotype (the so-called *neutral network* of genotypes; Schuster et al. 1994; Fontana 2002). For example, an RNA molecule may be able to fold into its secondary structure and remain functional despite several mutational changes in its sequence.

The idea that genetic robustness confers evolvability is based on the observation that if the individuals are distributed across a large neutral network, many would reside at its outer edges, close to the border and just a single mutation away from the neighboring neutral network, conferring a different phenotype (A. Wagner 2008). Put differently, a particular incoming mutation in such cases occurs on a large range of different genetic backgrounds, potentially with different outcomes. The phenotypic effects of the mutation differ in the network due to epistasis, leading to heritable phenotypic variation where there was none initially (Hermisson and Wagner 2004; Richardson et al. 2013; Geiler-Samerotte et al. 2019). New phenotypes are not necessarily advantageous, but a large network increases the probability that some will be.

Note that robustness does not monotonically increase evolvability for several reasons. First, strong robustness disables the evolutionary process altogether by suppressing variation. Second, the general association between large neutral network and high evolvability depends on the population size; small populations maintain only a few genotypes and thus cannot realize the advantages of large neutral networks. More interestingly, robustness also depends on the details of the individual GP map structure and the accessibility of the alternative phenotypes (Draghi et al. 2010; Mayer and Hansen 2017). Even large neutral networks may only have access to a small number of alternative phenotypes, and vice versa. Human genetic disease may be considered as an example. Genetic variants that cause human disease by perturbing specific buffered pathways do not release random phenotypic variation but result in rather specific, recurring, disease phenotypes. Thus, the phenotypes arising due to perturbation are constrained to a specific phenotypic space by the rest of the organismal regulation. To what extent the range of the accessible phenotypes is correlated with the degree of robustness depends on the systemic structure into which the trait in question is embedded.

In summary, the intuition behind the role of specific GP map structures in increasing evolvability is that they generate the kind of variational distribution that, more likely than random distributions, allows for a viable and even advantageous response to selection. Let us next take a closer look at the general relation between the GP map and the measure of evolvability—the amount of heritable genetic variation.

## 8.3 Variational Consequences of GP Maps in the Short and Long Term

In this section, we explain how GP map structure affects variation in general and what consequences these effects have for evolvability in the short and long terms. For interested readers, effects and their estimations are described in detail in the section 8.5 (see also Hansen, chapter 5). The general approach used by evolutionary biologists to understand the underlying mechanisms governing evolution is that of forward modeling. We use models predicting how the patterns of variation influence future response to selection. We thus derive expectations about future phenotypes (e.g., in artificial breeding) or about processes that must have acted in the past, to explain what we see in extant species or in the paleontological record. Importantly, the discrepancies between the predictions and reality can reveal that our models do not sufficiently capture the underlying causal processes we aim to understand.

### 8.3.1 Response to Selection

The immediate response to directional selection is driven by the standing additive genetic variation in the population, summarized in a genetic variance matrix **G**. When several phenotypic traits are considered, the phenotypic response can be predicted by the multivariate breeder's equation $\Delta\mathbf{z} = \mathbf{G}\beta$ (Lande and Arnold 1983), where $\Delta\mathbf{z}$ is a vector of changes in mean phenotypic traits in one generation, and $\beta$ is the selection gradient (strength and direction of selection). **G** measures the short-term evolvability of the population, describing the amount and the structure of the genetic variation available to respond to selection.

The standing genetic variation is fueled by mutations, whose contribution can be measured by another variance matrix, **M** (how both matrices are measured is described in section 8.5). This mutational variance matrix depends heavily on the GP map, as it quantifies the statistical distribution of phenotypes resulting from genetic mutations. The size of

**M** depends on the sensitivity of the phenotype to changes in the underlying genotypes, while the shape of **M** depends on the correlation of different traits to the same genetic change. The standing genetic variation **G** is conditioned both by the influx of mutations (**M**) and by the recent history of the population and is thus linked to the GP map. But genetic variation rarely accumulates generation after generation without being affected by environmental or demographic events. Stabilizing or directional selection can indeed erode the genetic variance in some specific directions of the phenotypic space; genetic drift in small populations may also affect the geometry of the genetic covariance (Chantepie and Chevin 2020).

Standing genetic variation fuels short-term response to selection. Once initial existing variation is exhausted, genetic evolution relies on new mutations and is thus more directly affected by the **M** matrix and the underlying GP map (Lande 1980; Turelli 1985; G. Wagner 1989; Slatkin and Frank 1990).

### 8.3.2 Evolution of Variance Matrices

The short-term evolvability of a population depends on **G**, which is largely influenced by recent patterns of selection, gene flow, and drift in the population. Nevertheless, genetic variation is ultimately produced by mutation, and the evolution of **G** is influenced, to some extent, by **M**. The structure of **M**, however, may not be constant. When GP maps are complex and nonlinear, epistatic patterns can drive the evolution of **M** along with the evolution of the genotype. For instance, some genetic backgrounds can be robust (when the local GP map is *flat*), while other backgrounds can be more sensitive to genetic change (when the map is *steep*). Consequently, depending on the structure of the GP map, the mutational pattern **M** may change when the genotypes change in the population. Ultimately, various aspects of the evolvability of a population rely directly and indirectly on the GP map: This map conditions the evolution of **M**, which contributes to the evolution of **G**, which determines the evolution of the population.

### 8.3.3 Correspondence between GP Map and Genetic (G) or Mutational (M) Variance Matrices

We have seen how the distribution and dynamics of variance and covariance in **G** affects evolvability in the short-term, while **M** affects evolvability in the long term. The genetic variance and covariance between phenotypic traits in the **G** and **M** matrices are sums of contributions at many polymorphic genetic locations (loci). Unlike variance, which is always positive, covariance between traits due to a single polymorphic locus can also be negative. The covariance contributions of loci thus can also cancel out, depending on the exact structure of the GP map and the allele frequencies. An example in Figure 8.3B shows a minimal map with full pleiotropy. Note that the effects of genes 1 and 2 can potentially cancel each other, resulting in no covariance between traits 1 and 2, given a specific combination of effect sizes and allele frequencies. Such pleiotropic effects that are not reflected in the variance matrix are called hidden pleiotropy (Gromko 1995; Baatz and Wagner 1997). Hidden pleiotropy may have advantages compared to modular pleiotropy, as such GP structure provides greater mutational domains per trait on average (Hansen 2003). However, Baatz and Wagner (1997) have shown that hidden pleiotropy can cause a constraint, because despite the lack of covariance, selection on single traits does affect the variance of other traits, which may be under stabilizing selection. It can be easily shown that this effect strengthens as the

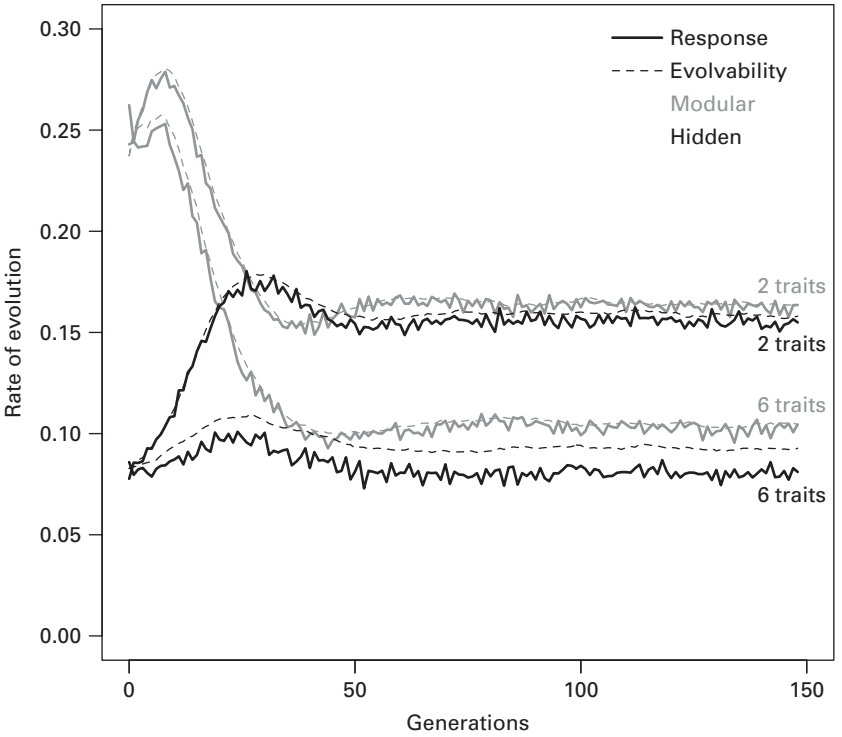


**Figure 8.4**
Simulated focal trait evolution given a hidden pleiotropic (black) and modular (gray) GP map. Corridor evolution (Baatz and Wagner 1997) is applied with directional selection ($\beta = 1$) on focal trait and stabilizing selection (quadratic selection gradient $\gamma = 2$) on all other traits. Both GP maps consist of 6 traits and 20 loci affecting the focal trait, with the same initial genetic variance and no covariance between the focal trait and all other traits. The solid lines show the rate of evolution of the focal trait (the mean across 10 repeats). The dashed lines track the 1-generation prediction based on Lande and Arnold (1983), updated for current **G**. For each GP map, a situation with 1 constraining trait (2 traits total) and 5 constraining traits (6 traits total) is shown. Evolvability with hidden pleiotropy is lower than for the modular pleiotropy case. The figure shows that the hidden pleiotropy imposes cost on evolvability, as the response deviates even in the short term from the predicted response (dashed), and that this deviation increases with the number of traits. Trait number also affects modular GP (due to linkage, not shown). Thus, despite the initially identical **G** values, the response, even in 1 generation, differs from the prediction, and it differs between GP maps. Starting values in this plot differ due to 1000 generations of stabilizing selection on all traits to achieve mutation-selection equilibrium prior to 150 generations of corridor selection (for simulation details, see Hansen et al. 2019).

stabilizing selection on other traits strengthens, and it also strengthens with the number of traits that share loci (Hansen et al. 2019; figure 8.4).

A related aspect to consider is that even though equivalent **G** matrices can be generated by different GP maps, they may not be equally accessible in populations with different GP maps, affecting the selection response. For example, without pleiotropy, the lack of covariance between divergently selected traits is a default (assuming no linkage disequilibrium). In contrast, lack of covariance requires coordinated modifications at many loci when it evolves by matching the single-locus covariance contributions to cancel out. Although this effect appears subtle when considering two traits, it is substantial when matching includes multiple traits and when the mutations are rare but large, leading to erratic covariance change and departures from **G**-based predictions (figure 8.4; based on Pavličev, unpublished data). Linkage, in addition, will generate covariance even in modular maps (figure 8.4).

In summary, the example of hidden and modular pleiotropy shows that no unique correspondence exists between GP structure and **G**/**M**. Nevertheless, the differences among GP maps generating the same **G** affect the response to selection (figure 8.4), which shows that GP map structure cannot be predicted based on the statistical summary matrices.

In addressing how GP map affects variation, we have so far only focused on one aspect of GP structure: pleiotropy. We assumed independence between effects at single loci. However, variation also arises as a consequence of yet another GP map aspect: the interdependencies between effects at different loci. This phenomenon, termed epistasis, defines the shape of the GP map, the presence of hills and valleys in the genotypic landscape, and the ruggedness of the genotype-to-phenotype relationship. Developmentally and physiologically, interdependency of mutational effects is expected (e.g., proteins do not function in isolation; they depend on conformation and abundance of many other proteins to interact with, either directly or indirectly). Effects of mutation in one protein will therefore depend on the genetic sequence of other proteins. Interdependency can affect not only the size of a mutation but also its direction (i.e., which traits are affected), so the interdependency can affect the mutation's contribution to trait variance and the covariance between traits. Analogous to contributions of pleiotropic loci to covariance across loci, the contributions of epistatic interactions to variance and covariance can add up across pairs of loci (directional epistasis; see section 8.5.2) or cancel out. In the later case, the overal contributions of interactions to variance (and covariance) are reduced and effects of single interactions invisible in the final statistical matrices. This aspect of GP structure and its effects on long-term evolvability, especially in multivariate settings, is difficult to assess systematically and has therefore received limited attention. From the above, it can be assumed that the existence of pleiotropy and epistasis is not captured fully in the statistical matrices. When it comes to long-term predictions, or to explaining the past, organismal details not captured in the statistical concepts (**G** and **M**) start to matter. How can we properly integrate them into evolutionary theory?

## 8.4 Turning the Question Bottom-Up: What Variation *Can* Real GP Maps Generate?

The prevailing approach of evolutionary theory is to study the effects of allele changes on statistical population-level assessment of phenotypes (including fitness). As explained above, the differences in structural details of the GP map can affect evolution even when they are not reflected in summary measurements of variation. Therefore, we should turn our attention to these differences to understand how and what kind of mutational variation can arise in the first place, even when the developmental and physiological detail and their evolution may not be our main interest. Placing genotypes at the bottom, and phenotype at the top, as in figure 8.1, we thus ask the bottom-up question: What kind of variation can the encountered physiological and developmental processes generate?

### 8.4.1 Integrating Mechanistic Detail: What Type, How Much, and How?

Mechanistic detail conferring the structure of the genotype-phenotype map should thus be integrated into the existing theory. But what kind of detail matters, how fine-grained does it need to be, and how do we integrate it? In section 8.3, we showed that pleiotropy matters,

yet the GP map affects other aspects of variation besides pleiotropy that may be necessary to predict long-term evolutionary trajectories. The kind and amount of organismal detail needed to better understand and predict evolution are empirical questions. Instead of trying to interpret the variation pattern observed (which can have many causes); we ask: *What kind of variation can familiar organismal processes generate?* In other words, what variational properties do those organismal processes engender?

There are clear precedents for investigating variational properties in earlier attempts to integrate organismal processes (metabolism, enzymatic reactions, and gene regulation) with evolutionary theory. Most prominently, Kacser and Burns (1981) have used this general approach to show why mutations introduced into long enzymatic pathways are mostly recessive, explaining the dominance of the wild type as a systemic property. This work prompted the development of Metabolic Control Theory, which constitutes a major source of explicit GP map models. Metabolic Control Theory paved a way for attempts to bridge the organismal theory of metabolism to population genetic theory (e.g., Keightley and Kacser 1987; Keightley 1989, 1996; Clark 1991; Szathmáry 1993; Frank 1999, 2019a; 2019b; Bagheri et al. 2003; Bagheri and Wagner 2004). Recent use of metabolic GP models enables a plethora of mechanistic insights on sensitivity of metabolic circuits to mutations, on the network structure underlying systemic properties such as homeostasis, as well as prediction of the frequent forms of pathology (e.g., Nijhout et al. 2015, 2019; Reed et al. 2017). The connection of this work to quantitative genetics theory suggests that this work is a highly promising path forward.

Other approaches to explicit GP map models are the RNA secondary structure models (Schuster et al. 1994; Ancel and Fontana 2000) and transcriptional regulatory models. Among the latter, variational properties have been studied for some of the recurring transcriptional regulatory motifs (Gjuvsland et al. 2007, 2013; Widder et al. 2012). Yet another model of a GP map is the gene network model introduced by A. Wagner (1996; reviewed in Fierst and Philipps 2015). Furthermore, G. Wagner (chapter 10) proposes a similar kind of bottom-up approach for exploring the evolvability properties of various growth scenarios.

In short, the field of explicit GP mapping may not have been at the center stage of evolution and evolvability research, but it is well populated and growing, and we suggest that it carries a high potential for system-level understanding of evolvability. In section 8.4.2, we discuss one set of recent bottom-up approaches to asking the question of the role of the GP map in predicting long-term evolution.

### 8.4.2 System-Level Approaches in Physiology and Development

Population and quantitative genetic models focus either on genotype or phenotype space, respectively. However, the substantial effort to model one of these spaces generally leads to very naive consideration of the other (Lewontin 1974). Omholt (2013) argued that the mainstream framework of evolutionary theory so far lacks causal relations between genotype and phenotype. However, the realistic connections between genotype and phenotype can nowadays be integrated, thanks to intense work on physiological and developmental mechanisms, into the classical modeling framework, thus explicitly integrating causal links. From the 2000s, such models started to emerge under the name of *causally cohesive genotype-phenotype models* (hereafter cGP models; Rajasingh et al. 2008). Most cGP models are individual-based and follow a structure described in figure 8.5, where individuals are

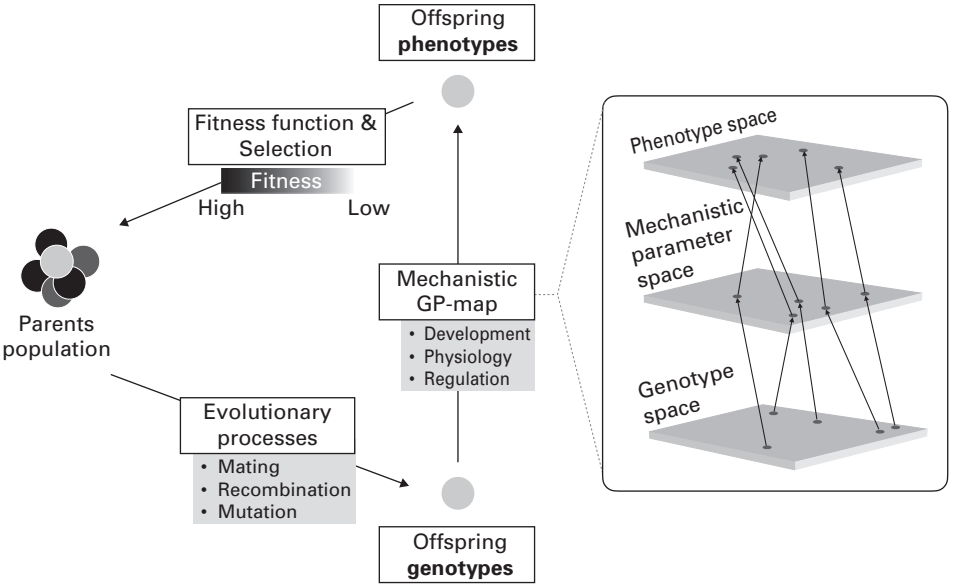


**Figure 8.5**
Building blocks of a cGP model. See text for explanation. This figure is inspired by figures in Omholt (2013) and Milocco and Salazar-Ciudad (2020).

characterized by a genotype determining a physiological mechanism (i.e., a mechanistic GP map) generating the expression of a phenotype. If the model involves evolution, then a fitness function is added, which maps phenotype to fitness. One of the key features of cGP models is the use of explicit physiological parameters, in which genetic variation can be assumed to exist. The variation thus arising in this model is anchored in specific causal biological hypotheses. In the next paragraphs, we first outline steps for developing a cGP model. We then illustrate the application with four studies.

To build a cGP model, five blocks of information must be provided, as listed below. These blocks are reflected on the left side of the flow diagram in figure 8.5 and are constitutive of the subsequent selection step. For this chapter, we consider these blocks as independent, whereas in practice their generation is a strongly interdependent process, with development of one block having consequences for all others, with no unique correct order of addressing them.

*Defining the phenotype:* The goal here is to specify traits that are sufficiently simple that their causal basis is understood and still address the question of interest. Defining a phenotype remains a challenging exercise. A global phenotype can always be segmented into more traits, but beyond a certain point, this will not improve our understanding of evolution (Houle 2001). Moreover, it is highly recommended to study traits produced by well-described underlying mechanisms.

*Determining the mechanistic GP map:* The joint consequence of pleiotropy and polygeny is that many interconnected pathways participate in the expression of a single trait, what Houle (1991) called functional architecture. Because the full complexity of mechanisms involved in the production of the phenotype usually cannot be integrated, the traits to be implemented in the model must efficiently capture the functional link between genotype

and phenotype. A good start is to focus on simple, general pathways that play major roles in the system studied. Due to explicit modeling of causal mechanisms, realistic nonlinear relations between genotype and phenotype can arise; an element frequently neglected in simpler models but known to be a major player in evolution

*Defining the genotype:* This building block sets assumptions about which parts of the model are directly affected by mutation. For example, if the mechanistic GP map is a hormonal or enzymatic system, one could choose to consider abundances or the reaction velocity per molecule, or both, as mutable parameters. This block is critically dependent on the structure set by the mechanistic GP map, limiting the possible evolutionary outcomes. Again, a challenge is to find an appropriate compromise between complexity and abstraction.

*Specifying selection and computing fitness:* The type of selection (directional, stabilizing, or fluctuating) and the associated fitness function may be chosen to mimic reality or to determine the response of the system to a hypothetical scenario.

*Setting parameters of the evolutionary process:* This block determines the population genetic model parameters, such as mutation type, rate, and size, recombination, and population size.

Models based on this cGP approach have been implemented in many different contexts. We present four examples of studies, illustrating the diversity of questions that can be considered using this framework.

To our knowledge, Rajasingh et al. (2008) presented the first cGP model. They studied a species of chinook salmon (*Oncorhynchus tshawytscha*) whose subpopulations exhibit two alternative phenotypes: white-fleshed and red-fleshed. To reproduce empirical data obtained in the crosses, they implemented a model GP map with a system of ordinary differential equations (ODEs) describing the uptake and deposition of carotenoids, a metabolic pathway responsible for the flesh color in salmonids. They compared two genetic architectures (two-locus two-alleles versus single-locus three-alleles) and found that a standard single locus model was best able to explain their observations.

The mechanistic GP map incorporated into a cGP model can be a single physiological pathway or an extremely complex system, such as that used by Vik et al. (2011) to simulate in detail the functioning of a whole cell. They modeled a mouse heart cell, drawing on a large body of empirical literature and previously developed mathematical models. Existing partial models were combined to constitute a complex GP map consisting of 35 ODE with 175 parameters. Resulting action potentials and ion concentrations represent phenotypes. The model was able to reproduce ion circulation into and out of the cells. Deviations in genetic bases for ion currents (as genotypic values) were examined for their ability to reproduce disease phenotypes. Such heavily parameterized models that describe cellular processes at a fine-grained level represent promising tools to predict the proximate determinants responsible for disease symptoms.

The aim of Milocco and Salazar-Ciudad (2020) was to compare predictions based on the multivariate linear breeder's equation to those based a nonlinear mechanistic GP map modeled as a cGP model. To build the cGP, they associated an existing developmental model of mammalian teeth with 21 genetically variable developmental parameters with a population genetics model. The resulting individual phenotypes were characterized by a complex 3-dimensional structure (the tooth morphology). Phenotypes evolved for 400 generations under stabilizing selection that selected for an optimal tooth shape different

from the initial tooth shape. They demonstrated that the bias between predictions from the breeder's equation and their results arose when populations were located in a nonlinear region of the global GP map. Hence, the study of models considering a realistically complex model GP map is justified by the limits of classic quantitative genetics tools, which cannot fully account for variation in development, as argued by Polly (2008).

Bourg et al. (2019) built an evolutionary model to study the evolution of the shape of a trade-off between life-history traits. Their mechanistic GP map incorporated a dynamic hormonal system that determines allocation of resources. They coupled this system with a classic population genetic model. Individuals were described by genotypes composed of genes coding for the expression of hormones and their receptors. Depending on the hormone-receptor affinities and their respective concentrations, a limited energetic resource was distributed differentially between two abstract vital functions (or traits). As a result, they obtained individual phenotypes corresponding to two abstract trait values that represented an internal energetic compromise. They let populations evolve under directional selection over 100,000 generations. Trade-offs expressed at the level of the population were not necessarily linear and could evolve due to a change in the local GP map.

These models illustrate how modeling explicit GP maps in mechanistic detail can be a useful avenue to better understand the potential for evolutionary change. We next turn to a brief overview of approaches to detect and assess GP map structure.

## 8.5 Detecting and Measuring Structure

In the previous sections, we described the structural aspects of the GP map and the statistical parameters that are estimated at the population level. Both these aspects are still relevant when the question is asked bottom up, as we have seen. Here, we want to briefly describe how these aspects of structure are detected and measured, and the limitations that measurement may face.

### 8.5.1 Direct Measurement of GP Maps

The most straightforward way to access the GP map structure is to measure an exhaustive set of genotypes directly (i.e., to generate many genotypes differing by one or a very few mutations and then measure the corresponding phenotypes). In most species, such an approach would be highly impractical, very costly, or simply not feasible. Yet combining recent technology (e.g., nucleic acid synthesis, new generation sequencing) with high-throughput phenotyping methods in micro-organisms makes it possible to explore the complexity of the GP map by generating thousands of mutants in the vicinity of wild-type sequences for single proteins (Jacquier et al. 2013; Bank et al. 2015) or noncoding RNAs (Li et al. 2016). At the molecular level, the GP map exploration generally reveals complex epistatic interaction patterns. Most combinations of single mutations do not interact, but some have strong interactions. These strong interactions can have more than two loci (Domingo et al. 2018; Poelwijk et al. 2019). In these cases, interactions are often strong enough to create sign epistasis, where the identity of the variant favored by selection depends on the genetic background it is in. This generates a fitness landscape with multiple fitness peaks. It is usually unclear whether a succession of nondeleterious mutants exists that would allow natural selection to push a population from one peak to another.

Quantitative trait locus (QTL) detection has been exploited for decades to identify molecular markers associated with quantitative trait differences among individuals. QTL detection can be attempted on specific populations generated by a controlled experimental cross design or on a sample from natural populations, including humans. With affordable and efficient new sequencing technologies, the latter have become increasingly popular under the term GWAS (for genome-wide association studies). In principle, estimating the statistical effect of marker genotypes on quantitative traits could lead to a satisfactory approximation of the underlying GP map. Whether this is achievable in practice remains unclear (Manolio et al. 2009; Young 2019; Uricchio 2020).

Detecting QTLs that display epistatic interactions is, in theory, a straightforward extension of the classical QTL detection methods: Instead of looking for markers with a significant association to the phenotype, the focus needs to be on pairs of markers with a significant interaction effect on the phenotype (Carlborg and Haley 2004; Carlborg et al. 2006). The statistical power of such analyses remains limited, even in large samples, as the number of marker pairs to test is huge. A particularly interesting form of epistasis creates variation in pleiotropy. When two phenotypic traits are affected by the same genetic variants, they share a (partially) common genetic basis, and a QTL affecting one trait is expected to affect the other trait. Genetic variants that modify pleiotropy will change the relative magnitude of those effects, which makes it possible to detect pleiotropy modifier QTLs (referred to as relationship QTLs, or rQTLs) by the presence of an interaction term between their effects on both traits (Cheverud et al. 2004). Here again, the statistical power to detect such interactions is lower than for traditional QTL mapping, and detection issues will rapidly increase with the number of phenotypic traits.

### 8.5.2 Measuring Statistical Properties of Genetic Architectures

Quantitative genetics aims to describe genetic architectures of traits from a statistical point of view rather than via the specific influences of identified biochemical or regulatory pathways. Genetic and phenotypic diversity on which selection acts, fueling evolution, is then measured as the additive genetic variance of quantitative traits. Here we briefly review how the structure of genetic architectures is described statistically through two distinct (although subtly related) kinds of interactions: the fact that genes may influence several characters (pleiotropy), which translates into statistical covariances among traits (in **G** and **M** matrices), and the fact that the effect of single substitutions does not add up to produce total variation (epistasis), which arises because the genetic effects depend on the genetic background in which the mutation takes place.

Mutational variances and covariances, reflecting the rate, size, and pleiotropic effects of mutations before selection, are key features of any long-term quantitative genetics theoretical prediction (Jones et al. 2007). Yet they are notoriously challenging to estimate from empirical data: Mutations are individually rare, and their effects are usually small enough that they cannot be recognized against the background of existing phenotypic variation. Typical mutational heritabilities (mutational variance relative to the phenotypic variance) are in the range of $10^{-4}$ to $10^{-3}$, usually less than 1% of the heritable genetic variance. The most common experimental design to measure **M** matrices consists of deriving a highly inbred genotype and then maintaining replicate copies as mutation accumulation lines. The increase in phenotypic (co)variance among lines is the estimator of the **M** matrix.

**M** matrices have been estimated in a small number of species, most of which are short-lived organisms. Mutational covariance studies consistently report large positive correlations among fitness components and life-history traits (in *C. elegans:* Estes et al. 2005; Keightley et al. 2000, in *Daphnia:* Lynch 1985, or in *Drosophila:* Houle et al. 1994), and moderate correlations among morphological traits (Houle and Fierst 2013). There is also consistent evidence that **M** matrices may differ among close genotypes or populations (Fernández and López-Fanjul 1996), suggesting that the structure of **M** matrix is evolvable.

Mutational correlations due to pleiotropy may induce genetic correlations, which condition the evolvability of populations. As genetic drift and selection can also cause genetic correlations through linkage disequilibrium, the **G** variance matrix cannot be deduced directly from **M** and must be measured independently. The concept of additive genetic variance (and covariance) is rooted in the decomposition of genetic variances (Fisher 1918), in which heritable and environmental components can be distinguished based on the phenotypic covariance among relatives; genetically related individuals will share part of their genotype, while the residual effects will remain independent (Hansen, chapter 5). Progress in statistical methods has made it possible to use information from all related individuals in a multigeneration pedigree simultaneously and to estimate genetic variance and covariance components underlying the structure of the genetic correlations for many traits in the population.

**G** matrices for different traits are known from a wide variety of organisms (Roff 1996). Positive correlations among life-history traits and morphological traits are regularly reported, but they are not strong or systematic enough to define clear general rules (Pélabon et al., chapter 13). Knowledge of **M** in addition to **G** enables us to understand which portion of segregating variational pattern is due to inherent structure of the GP map, and which portion may be a consequence of the current selection. When both **G** and **M** matrices are measured on the same population, they sometimes match convincingly (Houle et al. 2017), which has two possible explanations: (1) natural selection is weak, and **G** is mostly shaped by **M**; and (2) **M** has evolved to match the pattern favored by selection. This latter hypothesis remains controversial, due to the lack of clear theoretical and empirical support (Jones et al. 2014).

In contrast to **G**, the phenotypic variance matrix **P** is considerably easier to estimate, as it can be estimated from phenotypic measurements in the population. As the environmental (nongenetic) sources of variation are often expected to dominate the phenotypic structure, evolutionary predictions from phenotypic covariances are theoretically dubious. Yet in practice, environmental covariances are often remarkably similar to the **G** matrix. This observation, sometimes referred to as the Cheverud's conjecture (after Cheverud 1988), has received a substantial amount of empirical confirmation (Roff 1996; Kruuk et al. 2008; Dochtermann 2011), although the reasons that phenotypic covariances match genetic covariances need to be clarified (Noble et al. 2019; Chevin et al 2021).

When measured in a reference genotype, epistatic effects are usually referred to as *functional* (or *physiological*) effects (Cheverud and Routman 1995). They correspond to a local description of the curvature of the GP map. When averaged over all genotypes in a population, weighted by genotype frequencies, epistatic effects are called *statistical.* Statistical and functional estimates of epistasis are complementary descriptions of the structure of the GP map, and each can be calculated from the other if we have a detailed understanding of which loci are involved (Álvarez-Castro and Carlborg 2007). The question of whether an evolutionarily relevant, global measurement of epistasis exists is not

straightforward to answer. A traditional way to quantify epistasis in populations is based on an extension of the decomposition of phenotypic variance (Cockerham 1954; Lynch and Walsh 1998). Yet the epistatic contribution to the phenotypic variance carries little information about the underlying genetic architecture (Álvarez-Castro and Le Rouzic 2015). When it comes to predicting the response to directional selection, directional epistasis, a measurement of the average curvature of the GP map in the population, is a better alternative (Hansen and Wagner 2001). Measurement of directionality of epistasis attempts to capture the evolutionarily relevant part of epistasis—that is, which has the potential to speed up (positive epistasis) or slow down (negative epistasis) the evolution in the context of directional selection (Carter et al. 2005). Positive directional (or synergistic) epistasis indicates that allelic effects tend to reinforce one another, while negative (or antagonistic) epistasis describes a situation where allelic effects cancel one another. Directional epistasis can be estimated from various datasets, including line crosses, targeted mutations, or selection responses (Pavličev et al. 2010; Le Rouzic et al. 2011; Le Rouzic 2014). Epistasis is thus another structural aspect of the GP map which shows that different structures can produce the same variational distributions.

Directional epistasis has seldom been measured directly on quantitative traits, in spite of its theoretical relevance. Systematic directional epistasis is expected for traits that do not scale linearly with an underlying physiological function. For instance, growth traits may scale exponentially and thus display positive epistasis for trait increases. However, the few existing empirical measurements are not consistent. For example, chicken body size shows positive epistasis (Le Rouzic 2014), while mouse size-related traits show negative epistasis (Pavličev et al. 2010). Directional epistasis on multiplicative fitness has important theoretical consequences for the evolution of sex, recombination, and mutation rate (Phillips et al. 2000), and has thus been under intense scrutiny. Overall, there is solid empirical evidence for negative epistasis on fitness (the effects of beneficial mutations tend to cancel out), at least in microorganisms (Martin et al. 2007).

## 8.6 Conclusion

The way that epistemic entities are conceptualized in any theory constrains the range of problems that can be addressed using the theory—and this is no different for evolutionary theory. The specific statistical concepts of evolutionary quantitative genetics conveniently lower the dimensionality of data, yet thereby obscure important aspects of organismal complexity, which matter in particular for long-term evolvability. But to address evolvability, which aspects of real organisms and which types of detail do we need to include? Can those be integrated into existing approaches? These are, foremost, empirical questions. The nascent field of GP map studies cannot yet answer them.

Here we suggest (not for the first time) that the answers may require us to complement the existing theory by turning to the relevant epigenetic (sensu Waddington 1957) processes and address the question from the bottom-up: What mutational variation *can* these processes generate? What is the space of **M** matrices that can arise? How does this **M** space change during the evolution of the processes? What is the structure of the GP map at its various intermediate levels? Are there important principles on a less coarse-grained scale than variational modularity and robustness?

The field of modeling explicit physiological/ developmental processes and their influence on evolution poses challenges. Access to the full GP map is likely infeasible for most complex non-model organisms—but an exhaustive map is hopefully not necessary. Defining informed numerical models of aspects of GP maps (e.g., metabolic and regulatory networks or developmental cascades), around which theory can be built, may be the most feasible path to practical use of the GP map. Deciphering the consequences of the GP map structure on evolvability thus relies heavily on experimental, conceptual, and theoretical progress. This long path will require input from a wide variety of disciplines, including systems biologists; physiologists; developmental biologists; and molecular, quantitative, and population geneticists.

In practical terms, some of the empirical questions to ask are:

- What are the variational properties of specific organismal structures and processes?
- What are the consequences of those properties for evolution?
- Are there dynamical principles with common consequences for evolutionary change, and could these replace the current statistical concepts?
- How do variational properties differ between species?

The expectation is that the study of the structure of GP maps will allow us to see the systemic and molecular changes that contribute to the change in the phenotype and its variation in a context richer than linearly associating specific single nucleotide polymorphisms (SNPs) with specific phenotypic change. Long-term predictions may appear hard to validate, but using comparative studies of physiological and developmental parameters, we can start to understand how our predictions of evolvability based on GP maps correspond to long-term evolutionary change. The comparative approach could thus bridge the existing populational questions to inform the long-term, macroevolutionary change (Jablonski, chapter 17).

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