Multivariate Quantitative Genetics

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Key Points

- While individual traits can be studied separately, a multivariate perspective is essential for understanding how organisms vary and evolve, as the relationships between multiple traits significantly affect their variation, inheritance, and evolution.
- The genetic architecture of complex traits is shaped by pleiotropy (single genes affecting multiple traits) and linkage disequilibrium (non-random associations between alleles), which contribute to the structure of the genetic covariation between traits and can constrain evolutionary trajectories along "genetic lines of least resistance."
- A puzzling alignment exists between patterns of standing variation (G-matrix) and long-term evolutionary divergence across species, despite theoretical models suggesting the G-matrix should only have a temporary effect on evolutionary divergence.

Glossary

Additive genetic effects (a) The linear component of an allele's effect on a trait, measured as half the difference between the phenotypic values of the two homozygous genotypes.

Animal model A statistical method that uses all available information about genetic relationships between individuals in a population to estimate genetic parameters, including genetic variances and correlations.

Breeding design Experimental setups used to estimate genetic parameters by measuring traits in related individuals, such as half-siblings or full-siblings.

Dominance genetic effects (d) The non-additive component of genetic effects, measured as the deviation of the heterozygote phenotype from the average of the two homozygotes.

Epistatic genetic effects Non-additive genetic effects that occur when the effect of one allele on a trait depends on the presence of one or more other alleles, representing interactions between different genetic loci.

G-matrix The additive genetic covariance matrix that describes the genetic relationships between multiple traits in a population, crucial for predicting evolutionary responses to selection.

Genetic architecture The underlying genetic basis of phenotypic traits, including the number and effects of genes involved, their interactions, and their relationship with environmental factors.

Genetic covariation The tendency of different traits to vary together due to shared genetic influences, which can arise from pleiotropy (single genes affecting multiple traits) or linkage disequilibrium (non-random associations between alleles at different loci).

Genetic lines of least resistance Trait combinations that have high amounts of genetic variation and thus represent directions in phenotype space along which evolution can proceed most easily.
Internal selection The constraint on genetic variation imposed by developmental processes that must produce viable organisms, limiting the amount of variation available for natural selection.
Linkage disequilibrium The non-random association between alleles at different loci, which can contribute to genetic covariance between traits.
M-matrix The mutational covariance matrix that describes the pattern of new genetic variation introduced by mutations.
Mapping models Statistical methods used to identify genetic loci that influence phenotypic traits. These include Quantitative Trait Locus (QTL) mapping, which identifies genetic regions associated with trait variation in experimental crosses, and Genome-Wide Association Studies (GWAS), which identify genetic variants associated with traits in natural populations. Both approaches can be adapted for single or multiple traits.
P-matrix The phenotypic covariance matrix that describes the observable relationships between multiple traits, including both genetic and environmental sources of variation.
Pleiotropy The phenomenon where variation in a single genetic locus affects multiple phenotypic traits.
Selection gradient (β) A vector describing the strength and direction of selection on multiple traits, calculated as the partial derivatives of fitness with respect to the traits.
Standing variation The genetic variation currently present in a population, available for selection to act upon.
The Cheverud conjecture The observation that phenotypic covariances (P-matrix) often serve as reasonable proxies for genetic covariances (G-matrix), especially for morphological traits.
The Lande equation A fundamental equation in quantitative genetics ($\Delta z = G\beta$) that predicts the evolutionary response of multiple traits to selection (Δz) will be given by the product of the G-matrix by the selection gradient acting on all the traits.

Abstract

Understanding how organisms vary and evolve requires a multivariate perspective that considers the relationships between multiple traits simultaneously. These relationships are captured by various covariance matrices that describe different aspects of trait variation. The phenotypic covariance matrix (P) describes observable trait relationships, while the genetic covariance matrix (G) captures the inherited component of this variation. The mutational covariance matrix (M) describes how new variation enters populations through mutation. Here, we review the theoretical foundations of these matrices and their roles in evolutionary biology. We discuss how genetic effects such as pleiotropy and linkage disequilibrium contribute to the G-matrix, and examine various methods for estimating these matrices in natural and experimental populations. The G-matrix plays a central role in predicting evolutionary responses to selection through the Lande equation, potentially constraining evolutionary trajectories along genetic lines of least resistance. While the influence of the G-matrix on short-term evolution is well established, its role in macroevolution remains debated. Recent evidence suggests that patterns of standing variation often align with long-term evolutionary divergence, even across deep evolutionary time. This alignment presents a puzzle, as theoretical models suggest the influence of the G-matrix should be temporary and not affect long-term evolutionary divergence. We explore potential resolutions to this paradox, including the role of internal selection in constraining variation and the possibility that G-matrix structure reflects past selective pressures. This synthesis highlights the ongoing importance of multivariate quantitative genetics in understanding both micro- and macroevolution.

Introduction

Organisms can be described in terms of their phenotypes, and most phenotypes of commercial or scientific interest show continuous variation. Quantitative genetics is the study of these continuous traits. While it is possible to study traits individually, organisms are composed of many such traits, and the relationship between traits can markedly affect their variation and evolution (Lande, 1979; Lande and Arnold, 1983). Therefore, a multivariate view of quantitative traits is fundamental for a complete understanding of biological organisms, their evolution, and their diversification.

Multivariate quantitative genetics is concerned with the processes that lead to the expression of multiple traits, and how these traits are related to each other and inherited together. Biological populations harbor some degree of genetic variation, in the form of different alleles segregating at individual loci that ultimately responsible for the inheritance of complex traits. These genetic variants have phenotypic effects that are produced though development (Snell-Rood and Ehlman, 2023) and manifest in the organism's phenotype. The underlaying genetic basis for the production of the phenotype is what we call the genetic architecture of the trait (Hansen, 2006), and this genetic architecture can limit and influence the variational structure and evolution of complex traits. For example, one fundamentally multivariate phenomenon is the response to directional selection because the covariation structure produced by the genetic architecture introduces dependencies across individual traits, linking their inheritance and evolutionary trajectories.

Multivariate quantitative genetics attempts to describe these complex relationships through various covariance matrices, each capturing different aspects of trait variation and evolution. In this review, we first explore the fundamental covariance matrices—the phenotypic (P), genetic (G), and mutational (M) matrices—and their theoretical foundations. We then examine how genetic processes such as pleiotropy and linkage disequilibrium contribute to the G-matrix, and discuss various methods for estimating these matrices in natural and experimental populations. Next, we consider how these matrices, particularly the G-matrix, influence evolutionary trajectories through the Lande equation and genetic lines of least resistance. We explore both short-term evolutionary responses in wild populations and the debated role of the G-matrix in macroevolution, including the puzzling alignment between patterns of standing variation and long-term evolutionary divergence. Finally, we discuss the apparent paradox between theoretical predictions of G-matrix evolution and empirical observations of its stability, considering potential resolutions through concepts like internal selection and the role of fluctuating directional selection.

Covariance Matrices

Multivariate quantitative genetics attempts to describe the relationship between traits by quantifying their variational properties and summarizing these relationships using covariance matrices. A covariance matrix is a table of numbers that in which each row and column corresponds to a trait and each off-diagonal position corresponds to the associated covariance between traits. Accordingly, the diagonal positions represent the covariance of each trait with itself, which is the variance of the trait. The covariance matrix is a symmetric matrix, and the diagonal elements are always positive. The off-diagonal elements can be positive or negative, and their magnitude represents the strength of the relationship between traits. To characterize the different sources of variation that compose the total observed phenotypic variation in a population, quantitative genetics uses several different covariance matrices, each one capturing a source or type of variation (Caballero, 2020; Falconer and Mackay, 1996; Lynch and Walsh, 1998).

The P-Matrix

The P-matrix is the simplest one to understand, and is the direct measure of variation in the phenotype, the outcome of all the processes that make individuals different from each other. On the diagonal, we have the variances of each trait under consideration, and in the off-diagonal elements, we have their covariances. For a set of traits $z_1, z_2, \cdots, z_n$, the P-matrix is given by:

$$\mathbf{P} = \begin{bmatrix} P_{11} & P_{12} & \cdots & P_{1n} \\ P_{21} & P_{22} & \cdots & P_{2n} \\ \vdots & \vdots & \ddots & \vdots \\ P_{n1} & P_{n2} & \cdots & P_{nn} \end{bmatrix} \tag{1}$$

where each element P_{ij} is the covariance between traits z_i and z_j, given by:

$$P_{ij} = \frac{1}{N}\sum_{k=1}^{N}\left(z_{ik} - \bar{z}_i\right)\left(z_{jk} - \bar{z}_j\right) \tag{2}$$

where N is the number of individuals in the population, z_{ik} is the value of trait z_i in individual k, and $\bar{z}_i$ is the mean value of trait z_i in the population.

The G-Matrix

The G-matrix, also called the additive genetic covariance matrix, is the result of the genetic effects of all the loci that influence the multiple traits under consideration. The G-matrix is the key to understanding the evolutionary potential of a population, as it captures the amount and structure of the genetic variation that is available for selection to act upon. The G-matrix also captures the expected pattern of inheritance of quantitative traits, and is an estimate of the covariation between parents and offspring when the source of the similarity between generations is genetic (Rice, 2004; Walsh and Blows, 2009). To understand the origin of the genetic covariation captured by the G-matrix, we must first understand how the genetic effects of loci are related to the phenotypic effects of the traits they influence.

The Genetic Architecture of Complex Traits

The value a continuous trait assumes in an individual depends on the individual's genetic make-up (which alleles the individual carries) and the environment the individual is exposed to. This relation is usually represented by $z = g + e$, where z is the phenotypic value of the trait in the individual, g is the genetic component and e is the environmental component. The environmental effect can be assumed to be, on average, zero, and so the mean phenotype $\bar{z}$ is equal to the mean genotypic value $\bar{g}$ in the population. The genotypic value of a particular trait, the expected value of the individual's phenotype, is the result of the sum of all the allelic effects

of the genetic variants the individual carries. The effect of a particular locus on a trait can be expressed as a function of the additive (a) and dominance (d) allelic effects, which capture the linear and residuals components of the average effect of an allele substitution (α) on the phenotype. The average effect of an allele substitution is the expected effect of the exchange of (say) a B to an A allele on the phenotype of a random individual in the population. For a locus k with two alleles (labeled A_k and B_k), the additive and dominance effects on a trait are given by:

$$\begin{aligned} a_k &= \frac{1}{2}\left(g_{A_kA_k} - g_{B_kB_k}\right) \\ d_k &= g_{A_kB_k} - \frac{1}{2}\left(g_{A_kA_k} + g_{B_kB_k}\right) \end{aligned} \tag{3}$$

where $g_{A_kA_k}$, $g_{A_kB_k}$ and $g_{B_kB_k}$ are the genotypic values (average phenotype) of the trait in individuals with genotypes AA, AB, and BB at locus k, respectively. More clearly, a is simply half the difference between the genotypic values of the homozygotes, and d is the difference between the genotypic value of the heterozygote and the average of the homozygotes. If the allele frequencies of A_k and B_k are given by $p(A_k) = p_k$ and $p(B_k) = q_k$, then the average effect of an allele substitution at locus k (α_k) is given by $\alpha_k = a + d(q_k - p_k)$, and depends on both the genetic effects (a property of each locus) and the allele frequency of each allele at that locus. If the alleles are at equal frequency, the dominance effect does not contribute to the average effect of an allele substitution. If one allele is more common, the dominance effect becomes important. These effects add up on the phenotype, and the genetic value of the trait in an individual is a function of average effects of all the alleles in all the loci that influence the trait (Walsh and Blows, 2009).

Contributions to the G-Matrix

When we consider multiple traits, the genetic effect of a locus (a_k and d_k) will be given by vectors, representing the genetic effects on each trait. Likewise, the average substitution effect at locus k on t different traits will be a vector given by:

$$\alpha_k = \begin{bmatrix} \alpha_{k(1)} \\ \vdots \\ \alpha_{k(t)} \end{bmatrix} = \begin{bmatrix} a_{k(1)} + d_{k(1)}(q_k - p_k) \\ \vdots \\ a_{k(t)} + d_{k(t)}(q_k - p_k) \end{bmatrix} \tag{4}$$

Because each locus can influence multiple traits, a phenomenon we term pleiotropy, these additive and dominance effects will contribute to the covariation between traits (Melo et al., 2019). The genetic covariance between traits z_i and z_j can be expressed as the sum of the covariances of the additive and dominance effects of all loci that influence both traits. If we have K loci to consider, the genetic covariance between traits z_i and z_j is given by:

$$G_{ij} = \sum_{k=1}^{K} 2p_kq_k\alpha_{k(i)}\alpha_{k(j)} + \sum_{k=1}^{K}\sum_{x=1}^{K}\left[2\lambda_{kx}\alpha_{k(i)}\alpha_{x(j)}\right]_{k\neq x} \tag{5}$$

The first term is due to the pleiotropic effects of each locus: if the additive and dominance effects of a locus affect two traits at the same time, they will contribute to their genetic covariance. This effect will be greater when the two alleles are in equal frequency ($p_k = q_k = 0.5$), or when the substitution effects are large. The second term is due to the covariance in the allelic states at different loci, measured by the linkage disequilibrium λ_{kx}, which is the covariance in the allelic states of loci k and x. If the allelic states of loci k and x are independent, the second term is zero. If they are in gametic disequilibrium, and the state at one locus is correlated with the state at the other, the second term will be non-zero and will contribute to the genetic covariance between traits (Melo et al., 2019). These are only the additive and dominance contributions to the genetic covariance, and other genetic effects, like epistatic, imprinting, or maternal effects, can also contribute to the genetic covariance between traits.

Estimating G-Matrices and Genetic Effects

One of the main challenges in multivariate quantitative genetics is experimentally measuring the G-matrix and these pleiotropic genetic effects. We consider estimating both these quantities in turn. The G-matrix is usually estimated some sort of breeding design, of which several are available, like half-sib or full-sib, where experimental individuals share one (half-sib) or both parents (full-sib), and the variance among these individuals serve as an estimate of the genetic variation in the trait (Lynch and Walsh, 1998). The idea behind these methods is that related individuals will share some proportion of their genetic information, and therefore their genetic values will be correlated. By measuring the phenotypes of related individuals and assessing how similar they are, we can estimate both the proportion of total variation that is due to genetic differences, and the genetic covariance between traits. A more general method that is not restricted to specific breeding designs the *animal model*, which explores all the information contained in the pedigree or the genetic relatedness across a set of individuals to estimate the proportion of variation that is attributable to genetic effects. The animal model has been leveraged to explore genetic variation in natural populations, with great success (Bonnet et al., 2022; Charmantier et al., 2014). See Lynch and Walsh (1998) for a detailed explanation of these methods and Wilson et al. (2010) for an introduction to the animal model.

As for genetic effects, finding all the traits a particular locus affects is challenging, and most studies focus on single trait models that use association mapping to relate the genotype state of each genetic marker to the variation in a phenotype of interest. The exact method depends on the relatedness structure of the mapping population, but bespoke methods are available for many standard experimental crosses (Broman et al., 2019) or complex outbred populations (Yang et al., 2011; Zhou and Stephens, 2014). The genetic effects of each locus can be estimated by performing a linear regression analysis where the phenotype is the dependent variable and the genotype state (typically coded as 0, 1, or 2 allele copies) is the independent variable (Rice, 2004), and significant association between marker and phenotype is interpreted as a true genetic effect. If individuals used in the regression are related, a mixed model can be used to account for the relatedness between individuals and avoid spurious associations due to the population structure (Eu-Ahsunthornwattana et al., 2014). Usually, these analysis are done using only one trait at a time as the response variable, and many studies attempt to combine the independent mapping results for several traits to find true pleiotropic loci (Kenney-Hunt et al., 2008; Porto et al., 2016). Another strategy is to use principal components to find combinations of traits that are independent in their phenotypic variation, and then map these principal components to the genetic markers (Pallares et al., 2015; Pelletier et al., 2023; Pitchers et al., 2019). These are workable methods, but, ideally, we should strive to map all the genetic effects of a locus on all traits simultaneously, to avoid missing loci with important genetic effects that are only detectable in the multivariate context (Meyer and Birney, 2018). While there have been attempts at true multivariate mapping (Melo et al., 2019; Mitteroecker et al., 2016; Zhou and Stephens, 2014), these methods are still limited to small number of traits (under ~10). Recently, some ingenious approximate methods have made true progress in the goal of multivariate mapping (Hannah et al., 2018; Kemper et al., 2018; Runcie et al., 2021), but the computational challenges are still significant for truly high dimensional traits, like gene expression.

Comparing G and P

Because estimating G-matrices is difficult, resource intensive, and often impossible (such as when working with museum samples), it is common to use the P-matrix as a proxy for the G-matrix. The similarity between the G and P-matrices is referred to as the *Cheverud conjecture*.[1] Cheverud (1988) found that phenotypic correlations were broadly good predictors of genetic correlations, and indeed most differences between them in the morphological datasets he investigated could be explained by the poor estimates of the genetic correlations, which are notoriously difficult to estimate (Morrissey et al., 2019). The P-matrix is the result of the sum of all the genetic and environmental sources of trait covariation, and, in the absence of strong environmental covariation, will be a reasonable proxy of the structure of the G-matrix. Furthermore, Cheverud (1984) also points out that the environmental processes that lead to environmental correlations also percolate through the same developmental pathways that lead to genetic correlations, which would make G and E similar, increasing the structural similarity between P and G. One interesting consequence of these results is that, if sample sizes are low, P might be a better predictor of the underlaying G-matrix than a direct estimate of the G-matrix (Penna et al., 2017). Indeed, while the similarity between P and G is not universal (Hadfield et al., 2007), many tests of the Cheverud conjecture using morphological data suggest that it is a useful approximation (Garcia et al., 2014; Roff, 1995; Sodini et al., 2018; Styga et al., 2018).

The M-Matrix

So far, we have only discussed the sources of standing variation in a population. But all biological variation, in ultimate terms, is the result of mutation. The M-matrix describes the structure of new variation that enters biological populations as the result of new mutations that change and introduce new genetic effects on the phenotypes. The M-matrix measures the covariance in the changes in the genotypic values of traits due to new mutations. On the diagonal of the M-matrix, we have the mutational variances, which is the increase in additive genetic variance due to mutation, per generation. On the off-diagonal elements, we have the change in additive genetic covariance due to mutation, per generation. Theory predicts that, under stabilizing selection and after equilibrium, the G and M matrix should be proportional, as the influx of new mutations gradually changes the structure of the G-matrix (Cheverud, 1984; Lande, 1980). Simulations have shown that epistatic effects can lead to an alignment between M and the pattern of selection (Jones et al., 2014), but the efficacy of this type of selection in natural populations and for many traits is still unclear (Houle et al., 2017; Melo and Marroig, 2015).

Estimating the M-matrix is quite challenging, usually requiring the use of mutation accumulations lines, in which several inbreed lines are kept at low population sizes and relaxed selection for many generations, to allow mutations to accumulate and quickly fix (Lynch and Hill, 1986). The phenotypes of these lines are then measured, and the covariance in the phenotypic changes between lines gives an estimate of the M-matrix (Caballero, 2020). This method has only been applied to a handful of model organisms like *C. elegans* (Mallard et al., 2023), *Arabidopsis* (Park et al., 2017), *Drosophila* (McGuigan et al., 2014), and others (Bakerlee et al., 2021; Ho et al., 2019). One recent M-matrix estimate used an ingenious experimental design to simultaneously estimate M and G, allowing for a direct comparison between the two matrices (Dugand et al., 2021). This comparison between the structures of M and G suggested that the M matrix is more constrained than the G-matrix, and that some mutational constraints (Cai et al., 2024) are broken during development, leading to the observed G-matrix (Dugand et al., 2021).

[1]Although James Cheverud himself has been known to say that it should be called the *Cheverud law*.

Multivariate Evolution and the G and M Matrices

The G-matrix takes on a central role when we consider the evolution of many traits simultaneously. Under directional selection, the expected response to a selective event is given by the Lande equation Lande (1979):

$$\Delta z = GP^{-1}S = G\beta \tag{6}$$

where S is the selection differential, the covariance between fitness and the traits under selection; P^{-1} is the inverse of the phenotypic covariance matrix; and their product $\beta = P^{-1}S$ is the selection gradient, which, under well-behaved conditions, is the vector of the partial derivatives of the log-fitness surface with respect to the traits (Lande and Arnold, 1983). The expected response to selection is given by the product of the G-matrix and the selection gradient. This equation shows the interaction between standing genetic variation and selection in determining the evolutionary trajectory of a population. The G-matrix is the source of the genetic variation that is available for selection, and the selection gradient is the direction in which the population is being pushed by selection. Unpacking the components of the Lande equation, we see that the expected response of each trait is composed of a direct part and an indirect part, due to selection on and covariation with other traits. For 3 traits, we have:

$$\begin{aligned}\Delta z_1 &= G_{11}\beta_1 + G_{12}\beta_2 + G_{13}\beta_3\\ \Delta z_2 &= G_{21}\beta_1 + G_{22}\beta_2 + G_{23}\beta_3\\ \Delta z_3 &= G_{31}\beta_1 + G_{32}\beta_2 + G_{33}\beta_3\end{aligned} \tag{7}$$

The diagonal terms, of the form $G_{ii}\beta_i$, are the direct responses of each trait to selection, and the off-diagonal terms, of the form $G_{ij}\beta_j$, are the indirect responses of each trait to selection. We see that the evolutionary change of each trait depends on the selection gradient of every trait it is correlated with.

Genetic Constraints to Selection

The effect of G in the evolution of multiple traits is profound. Because genetic variation is structured, trait combinations with high amounts of genetic variation effectively function as attractors in evolutionary response. This was first noticed by Dolph Schluter, who termed these trait combinations *genetic lines of least resistance* (Schluter, 1996). Given that selection depends on the available additive genetic variation, the evolutionary trajectory of a population will be biased towards the directions with the most genetic variation. This bias can be so strong that it can lead to the evolution of traits that are not under direct selection, a phenomenon known as *evolutionary constraints* (Arnold, 1992). We can identify this direction of least resistance as the first eigenvector of the G-matrix, which is the direction in phenotype space along which the genetic variation is maximized. The evolutionary trajectory of a population will be biased towards this direction, and the amount of genetic variation will determine the amount of evolutionary change that is possible. In contrast to directions with high amounts of genetic variation that respond quickly to selection, directions in phenotypic space with low amounts of genetic variation can be resistant to selection. In other words, some combinations of traits can theoretically not be changed by selection, a type of absolute constraint on evolutionary change (Walsh and Blows, 2009). Hine et al. (2014), using a multivariate trait in *Drosophila serrata*, attempted to test this prediction that directions with very low variation should not respond to selection by applying artificial selection along every eigenvector of the G-matrix. In their experiments, some of the experimental replicates of the low-variation eigenvectors did not respond to selection, suggesting that the G-matrix can indeed constrain the evolutionary trajectory of a population. However, this effect was not consistent, and some replicates did indeed respond, suggesting that the constraints imposed by the G-matrix are not absolute.

The G-Matrix and the Missing Response to Selection in Wild Populations

While laboratory populations display consistent and often predictable responses to directional selection, wild populations frequently do not (Merilä et al., 2001). Even in cases when both selection and genetic variation, the necessary ingredients for evolutionary response, are present, some wild populations appear to be stable, in a case of what has been called missing response to selection (Pujol et al., 2018). There are several possible explanations for this missing response. In wild populations, estimating the strength of directional selection is challenging, and we rarely have a full picture of the associations between traits under consideration and fitness. Just as challenging is obtaining an unbiased estimate of the additive genetic variance. In wild populations, environmental effects are not controlled, and correlated environmental effects can bias the estimate of additive genetic variance (Caballero, 2020). Even when common garden experiments are available, G-matrix estimates are extremely noisy (Chong et al., 2018; Marroig et al., 2012) and can lead to biased estimates of the expected response to selection. The same applies to other types of genetic effects. Usually, only additive effects are considered, but interaction effects, like dominance, epistasis, and gene by

environment interactions can become confounded with additive variation (Krätschmer and Robinson, 2024), and also contribute to the response to selection. Another possibility is that we are simply not measuring the correct multivariate traits that are relevant to predict the response to selection (Kruuk et al., 2008). If the traits included in the analysis are correlated with other traits, also under selection, it is possible that the selection in the other traits is preventing the response in the traits under consideration. Without a full multivariate picture of the genetic variation and of the relevant traits, we risk making incorrect predictions.

Indirect Selection and Macroevolution

The effect of G on the evolution of multiple traits is well-established in short time scales, but its effect on long-term evolution is less clear. In his original paper, Schluter proposed that the effect of G would fade in longer time scales, biasing the evolutionary trajectory of a population toward its evolutionary optimum, but not its final destination (**Fig. 1**). This expectation has been challenged by several recent results, where the divergence across species and populations is strikingly similar to the within-population patterns of covariation (Holstad et al., 2024; Houle et al., 2017; Machado et al., 2023; Marroig and Cheverud, 2005; Opedal et al., 2023; Rohner and Berger, 2023, 2025). The source of this alignment between within- and between-population divergence is still unclear (Schluter, 2024; Tsuboi et al., 2024), but it is likely that the G-matrix and M-matrix are key players in this process (Cai et al., 2024; Melo et al., 2016). The results are still puzzling for several reasons: (1) a long-term bias in phenotypic change due to selective bias caused by genetic correlations would mean that the amount of genetic variation or the introduction of new variation via mutation would have to be limiting factors in macro-evolutionary divergence, but, usually, divergence is much too small to have been limited by these two sources of variation. In other words, the standing variation and mutational variance we measure is more than enough to fuel much larger divergences in the time these species had to differentiate (Houle et al., 2017); (2) in some instances, the alignment between divergence and standing variation is extremely deep, e.g., 185 million years in Rohner and Berger (Rohner and Berger, 2025). Such a deep alignment is difficult to explain using only constraints, as we would expect that constraints would have to be so strong that they would cause most standing genetic variation to be deleterious, a pattern that (Rohner and Berger, 2025) do not observe.

Evolution of the G-Matrix

Another difficulty in this debate is that the G-matrix is not immutable (Pavlicev and Cheverud, 2015). As **Eq. (5)** clearly shows, G is the product of the sum of genetic effects (which can change due to mutation) and the allelic composition of the population (which can change due to drift, selection, mutation, and migration). Furthermore, the developmental changes can also change the

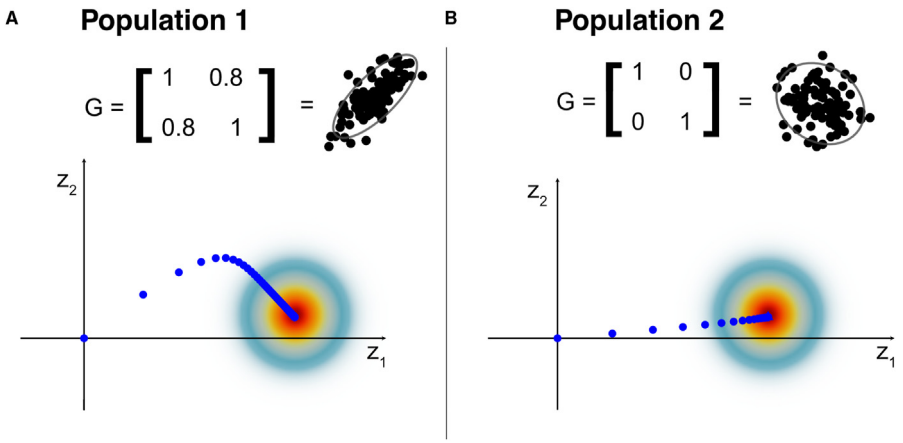

Fig. 1 The effect of genetic covariation on multivariate evolutionary trajectories. In both panels, the populations start at the origin and respond to the selective pressure imposed by the single peak selective surface, represented by the red-blue gradient (red values correspond to higher fitness). Each generation is represented by a point in blue, and the populations move towards the adaptive peak. A random sample of individuals from each population is shown next to the G-matrix to illustrate the relation between the two traits. Each black point is an individual, and the elipse represents a 95% confidence region for phenotypes of the individuals. (A) Population 1 has a genetic correlation value of 0.8, and therefore, the initial response to directional selection is biased towards the direction of high-genetic variance, the genetic line of least resistance. After an initial biased response, the population turns towards the adaptive optimum, slowly making its way to the peak. (B) Population 2 has practically no genetic correlation between traits, and they are freer to respond independently, moving directly o the optimum. After sufficient time, the effect of the G-matrix dissipates, as both populations head to the same optimum.

phenotypic effect of variants, which can change the genetic covariance between traits. Several models of G-matrix evolution have shown that the structure of genetic variation can be affected by changes in the pattern of pleiotropic effects, changes to the genotype-phenotype map, or changes in the M-matrix (Jones et al., 2007, 2014; Melo and Marroig, 2015; Milocco and Salazar-Ciudad, 2022; do O and Whitlock, 2023). These models that show a G-matrix that can evolve with relative ease are in stark contrast to the empirical observation that in many groups covariation patterns tend to be remarkably stable (Henry and Stinchcombe, 2023; Houle et al., 2017; McGlothlin et al., 2018; Porto et al., 2009). The reconciliation of these two observations is still an open question, but it is likely that the G-matrix is not as mutable as the models suggest, and that the constraints on the G-matrix are stronger than we currently understand (Cai et al., 2024; Melo et al., 2016). The idea of internal selection has been proposed as a solution to this apparent contradiction between the mutability of the G-matrix and its stability (Cheverud, 1984; Renaud et al., 2006; Riedl, 1978). Internal selection is the idea that the genetic variation that is manifested in the phenotype is under strong constraint by the developmental processes that must generate a functional organism, in which all the parts fit together. This constraint is so strong that it can limit the amount of genetic variation that is available for selection, and can bias the evolutionary trajectory of a population. This idea is still controversial, but it is a promising avenue to reconcile the stability of the G-matrix with the mutability of the genetic effects that compose it (Rohner and Berger, 2025).

Given this dual nature of the G-matrix, being both a product of the evolutionary history of the population and a constraint on its subsequent evolution, we must also consider the possibility that the alignment between divergence and standing variation is not due to the constraining nature of the G-matrix, but that the structure of the G-matrix is a product of the selective pressures that lead to the divergence between populations. Indeed, directional selection has been shown to be capable of increasing the variation in the direction of selection, which would contribute to the alignment between divergence and standing variation (Assis et al., 2016; Jones et al., 2004; Melo and Marroig, 2015; Pavlicev et al., 2011; Penna et al., 2017). Recently, Holstad et al. (2024) have proposed that the alignment between divergence and standing variation is the product of the interaction between the G-matrix and the pattern of fluctuating directional selection that a population is subjected to in a changing environment. Under their model, as the environment changes, the mean of the population is pushed in a direction, and the response is biased by the genetic covariance structure, causing more evolutionary change along the lines with the most genetic variation. This process would then lead to a detectable long-term relation between variation and divergence (Uyeda and McGlothlin, 2024). Indeed, recent estimates of selection have shown strong short-term variation in selective patterns (Stroud et al., 2023).

Finally, one final consideration is that Schluter's initial dismissal of the long-term effect of the G-matrix was contingent on a simple selection pattern: one evolutionary optimum, that was eventually reached, such that only the trajectory to that optimum would depend on the G-matrix. However, if the evolutionary landscape is more complex, with multiple optima, or with a changing optimum, the G-matrix could play a more important role in the long-term evolution of a population (Melo et al., 2016; Schluter, 2024). Under a more complex selective surface, a peak-selection role for the G-matrix could be important, and the alignment between divergence and standing variation could be a product of the G-matrix biasing not only the trajectory of the populations towards a single optimum, but also influencing which optima are reachable (**Fig. 2**, Melo et al. (2016)).

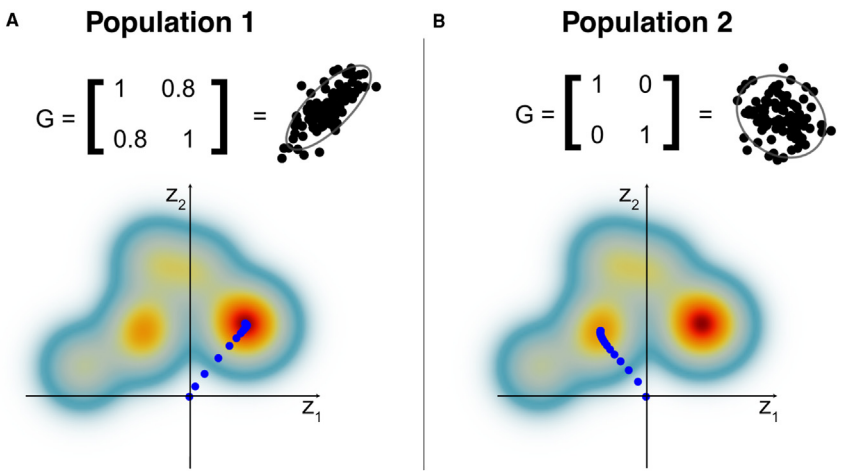

Fig. 2 The effect of genetic covariation on multivariate evolutionary trajectories on complex fitness surfaces. In both panels, the populations start at the origin and respond to the selective pressure imposed by the same multi-peak, complex, selective surface, represented by the red-blue gradient (red values correspond to higher fitness). Each generation is represented by a point in blue, and the populations move towards the adaptive peak. A random sample of individuals from each population is shown next to the G-matrix to illustrate the relation between the two traits. Each black point is an individual, and the ellipse represents a 95% confidence region for phenotypes of the individuals. (A) Population 1 has a high genetic correlation value of 0.8, and therefore, the initial response to directional selection is biased towards the direction of high-genetic variance, the genetic line of least resistance, which makes the population reach the adaptive optima on the right of the surface. (B) Population 2 has practically no genetic correlation between traits, and they are more free to respond independently, moving directly towards the optimum on the left of the surface. The peak the population reaches is different depending on the genetic covariance structure.

Conclusions

The genetic architecture of complex traits lies at the heart of evolutionary biology, shaping how organisms vary and evolve. While it is possible to study individual traits in isolation, organisms are fundamentally multivariate systems, with traits linked through shared developmental pathways and genetic effects such as pleiotropy and linkage disequilibrium. This inherently multivariate nature of biological variation influences how populations respond to selection, potentially constraining evolutionary trajectories and creating interdependencies between traits that can either facilitate or impede adaptation. Understanding these relationships has been greatly advanced by modern statistical and experimental approaches, from detailed breeding designs to sophisticated mapping studies in natural populations, revealing the complex web of genetic effects that underlies phenotypic variation.

The influence of this multivariate genetic architecture on evolution manifests itself across multiple timescales, from short-term responses to selection to long-term evolutionary trajectories. While its role in constraining short-term evolution through genetic lines of least resistance is well established, its influence on macroevolution presents an intriguing paradox. Theoretical models suggest genetic relationships between traits should be evolutionarily labile, yet empirical evidence consistently shows remarkable stability in patterns of trait covariation across deep evolutionary time. Several potential explanations have emerged, including the role of internal selection in constraining variation, the interaction between fluctuating directional selection and genetic architecture, and the possibility that genetic relationships influence which adaptive peaks populations can reach in complex fitness landscapes. Moving forward, integrating these various explanations and testing their predictions against empirical data will be crucial for advancing our understanding of multivariate evolution.

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