

Evolution of Genetic Variance-Covariance Structure

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Introduction

One of the features of organisms that makes the study of biology so compelling is their apparent complexity. Molecular, cellular, developmental, physiological, neurological, and behavioral systems are each fascinating in and of themselves, but it is their interaction that generates what we see as the organism as a whole. By necessity, biologists have a tendency to break down organisms into their component parts to see how they work. Yet, no trait is an island (Dobzhansky 1956). In particular, we need to consider the organism in its entirety when looking at evolutionary change. This perspective is necessitated simply by the fact that it is the whole individual, not specific traits or particular alleles, that lives and dies. The whole individual is therefore the central unit of selection (Lewontin 1970). How can we deal with the complexity inherent in trying to integrate biological function and evolutionary change across the whole organism? Quantitative genetics provides an attractive potential framework because it attempts to summarize important features of the genetic system while focusing firmly at the level of the phenotype. The multiple trait (multivariate) formulations of quantitative genetics that we will discuss here can be seen both as a conceptual framework for integrating multiple systems at many levels and as a tool for making quantitative predictions about evolutionary change. Whether it succeeds on either count is the subject of active debate (Barton and Turelli 1989). Ways of successfully building upon the traditional statistical framework of quantitative genetics is one of the exciting areas of future growth within the field of evolutionary genetics.

A preoccupation of many evolutionary biologists is the prediction of future response to selection and/or the retrospective estimation of the selection pressure responsible for contemporary phenotypes. In trying to reconcile experimental simplicity with the reality that selection acts on the whole organism, we are generally concerned with multiple traits, each of which has a polygenic basis and varies continuously (not discretely). For these suites of traits, past and future evolution can be explored using the multivariate extension of the breeders' equation (Lande 1979; Lande and Arnold 1983; Ch. 4.1):

$$\Delta\mathbf{z} = \mathbf{G}\boldsymbol{\beta} \quad (1)$$

Response to selection (the vector $\Delta\mathbf{z}$) depends jointly on the strength and direction of selection (the vector $\boldsymbol{\beta}$), and the matrix of additive genetic variances and covariances, \mathbf{G} (Ch. 4.1) (Those unfamiliar with the concept of covariance should consult the brief exposition in Box 1). The contribution of \mathbf{G} to evolution is non-trivial, influencing not just the response of traits directly under selection, but also the rate and direction of evolution in traits that genetically covary with selected traits (Fig. 1).

As the name suggests, the breeders' equation has been used extensively by plant/animal breeders to select for stock with particular attributes. Typically, breeders are interested in response to selection in the short term. In contrast, evolutionary biologists are frequently interested in long-term responses. The utility of the breeders' equation in evolutionary biology depends on $\boldsymbol{\beta}$ and \mathbf{G} remaining relatively stable over the time frame of interest. Gain and loss of traits through evolutionary time is sufficient to demonstrate that \mathbf{G} changes. However, we know very little about *how* \mathbf{G} evolves, which makes it

difficult to predict *when* \mathbf{G} will evolve, and thus when we can or cannot assume constancy and employ Equation (1) in the analysis of evolution.

The \mathbf{G} -matrix evolves through processes that alter allele frequencies: mutation, migration, selection, and drift (Arnold 1992; Barton and Turelli 1987; Lande 1980). Conditions under which \mathbf{G} might remain relatively constant have been predicted both from theory (Turelli 1988) and computer simulations (Jones et al. 2003), but we do not know how often these conditions are met in natural populations. Empirically, the data suggests \mathbf{G} might remain relatively stable over the short term, but does not always do so (reviewed by Jones et al. 2003). Overall there is little support for researchers assuming a stable \mathbf{G} in their system. Thus, understanding the evolution of \mathbf{G} remains an outstanding issue in quantitative genetics.

Quantitative geneticists will continue addressing these issues by conducting computer simulations of evolution and by estimating \mathbf{G} from populations under a range of conditions. An emerging mechanistic approach to tackling these same issues is built upon the fact that \mathbf{G} summarizes large amounts of information about the physical genetic and developmental bases of traits. \mathbf{G} is estimated from observations on the phenotypic similarity among relatives, and we have historically been unable to directly observe what underlies \mathbf{G} . Recent advances in developmental and molecular genetics are bringing us to a position where we break down a \mathbf{G} -matrix so as to consider the contributions of each locus to each trait. This interface between quantitative and molecular genetics may provide insight into the evolutionary processes shaping \mathbf{G} , thereby expanding the framework within which we can understand the evolution of organisms as an integrated whole.

Concepts

What makes \mathbf{G} ?

In many ways, the \mathbf{G} -matrix can be thought of as a nexus connecting genetic information with evolutionary processes. On one hand, the structure of \mathbf{G} is determined by the functional and developmental interactions that generate pleiotropy and by the genomic features that characterize linkage relationships among loci. On the other, \mathbf{G} determines the rate and direction of the response to selection, as well as the pattern of divergence among populations. \mathbf{G} is a statistical abstraction stuck between these levels, seeking to sufficiently summarize the molecular details at one end so that it can serve as an adequate predictor of evolutionary response at the other. Lofty goals. The elements underlying these aims bear closer scrutiny.

The essential genetic underpinnings \mathbf{G} can be appreciated by a careful study of Lande (1980)—which is somewhat akin to saying that the essential underpinnings of subatomic physics can be appreciated by a careful study of a piece of fruitcake¹. Things are not always as self-evident as they could be. Notational difficulties aside, the fundamental concepts are actually relatively straightforward. The most important step toward understanding what \mathbf{G} summarizes at the genetic level is to take a very general view of allelic effects at a given locus (Box 2). First, imagine that a large set of alleles at a single locus might generate a continuous distribution of effects on a given trait. Next, note that

each allele can also affect more than one trait (pleiotropy), such that different alleles at a locus will have different levels of direct and pleiotropic effects on the suite of traits under study. We can represent the effect an allele at locus i has on trait p as x_{ip} . The whole array of pleiotropic effects at the locus is then represented by the vector \mathbf{x}_i (Box 2). Lande (1980) in fact uses only this vector notation for compactness. If we really wanted to proliferate subscripts, we could have included another subscript (say k) to indicate that x_{ikp} is the effect of the k th allele from locus i on trait p . Instead, x_{ip} serves more like a random variable that indicates the particular value that we have drawn from the infinite set of possible allelic effects at locus i . In practice, we often want to average over all of these effects. We represent this averaging using the expected-value function, $E[\cdot]$, as in $E[x_{ip}] = \bar{x}_{ip}$. This expectation is taken over all of the individuals in the population and so is dependent on the underlying frequencies of the alleles.

The pattern of association of alleles both within and between loci will generate the pattern of genetic variance and covariance for the traits. For now, we will use a system with only two traits for simplicity². Following Lande (1980), there are three possible sets of allelic association that need to be considered. First, there are associations among the effects of alleles segregating at a *single locus*. Each allele at locus i can have a particular influence on each of the two traits (Box 2), so we will collect their effects together in a single matrix, \mathbf{C}_{ii} :

$$\mathbf{C}_{ii} = E \left[\begin{pmatrix} (x_{i1} - \bar{x}_{i1})^2 & (x_{i1} - \bar{x}_{i1})(x_{i2} - \bar{x}_{i2}) \\ (x_{i1} - \bar{x}_{i1})(x_{i2} - \bar{x}_{i2}) & (x_{i2} - \bar{x}_{i2})^2 \end{pmatrix} \right] = \begin{pmatrix} \sigma_{ii1}^2 & \sigma_{ii12} \\ \sigma_{ii12} & \sigma_{ii2}^2 \end{pmatrix}, \quad (2)$$

where σ_{ii1}^2 is the usual per-locus additive allelic or genic variance affecting trait 1 and σ_{ii12} is the additive covariance between traits 1 and 2 contributed by pleiotropic effects of alleles at locus i (Box 2). These latter terms will be generated when alleles at a given locus tend to affect more than one trait, either directly (e.g., a transcription factor turning on a similar set of genes within two different tissues) or indirectly (e.g., a gene that affects metabolic efficiency influencing both growth rate and egg production). *Big picture*: \mathbf{C}_{ii} give the single-locus-affecting-multiple-traits perspective that is the hallmark of pleiotropy and that is frequently the focus of most functional interpretations of genetic covariance structure.

Second, there can be an association between alleles at *different loci* within the same gamete, summarized by \mathbf{C}_{ij} :

$$\mathbf{C}_{ij} = E \left[\begin{pmatrix} (x_{i1} - \bar{x}_{i1})(x_{j1} - \bar{x}_{j1}) & (x_{i1} - \bar{x}_{i1})(x_{j2} - \bar{x}_{j2}) \\ (x_{i2} - \bar{x}_{i2})(x_{j1} - \bar{x}_{j1}) & (x_{i2} - \bar{x}_{i2})(x_{j2} - \bar{x}_{j2}) \end{pmatrix} \right] = \begin{pmatrix} \sigma_{i1j1} & \sigma_{i1j2} \\ \sigma_{i2j1} & \sigma_{i2j2} \end{pmatrix}. \quad (3)$$

Each of the terms in this matrix is a covariance of effects across loci within a gamete (i.e. *cis* acting effects) and are the associations that we would expect to be primarily generated by physical linkage along a chromosome. *Big picture*: linkage between loci (or between genetic elements within genes) contribute to genetic correlations because these linked

¹ With apologies to Douglas Adams—and Russ Lande.

² It will be rough going for a bit here. Try to stay with the big picture point of view at first. You can always come back for the details if interested.

effects tend to be inherited together. As one breaks down the elements of a gene (say a the per nucleotide level), the distinction between linkage and pleiotropy becomes somewhat arbitrary.

Third, the effects from alleles of loci located on *different gametes* (i.e., *trans* acting effects) can be correlated with one another, as in C'_{ij} :

$$C'_{ij} = E \left[\begin{pmatrix} (x_{i1} - \bar{x}_{i1})(x_{j1} - \bar{x}_{j1}) & (x_{i1} - \bar{x}_{i1})(x_{j2} - \bar{x}_{j2}) \\ (x_{i2} - \bar{x}_{i2})(x_{j1} - \bar{x}_{j1}) & (x_{i2} - \bar{x}_{i2})(x_{j2} - \bar{x}_{j2}) \end{pmatrix} \right] = \begin{pmatrix} \sigma_{i1j1} & \sigma_{i1j2} \\ \sigma_{i2j1} & \sigma_{i2j2} \end{pmatrix}. \quad (4)$$

The prime indicates the allele is from the other gamete. How can this be? How can loci from different gametes with effects on different traits possibly matter? If the loci have a tendency to be inherited together, say by assortative mating, then they can indeed influence the correlation across seemingly unrelated traits. Terms in this matrix are therefore especially important in models of sexual selection that depend on a build-up of genetic covariance between traits like male display and female preference. *Big picture*: correlations across gametes (contributed separately by a mother and father) can contribute to a genetic correlation if the effects of assortative mating can overcome the natural tendency for independent segregation among gametes. In most quantitative genetic models, except those explicitly concerned with sexual selection, these effects are usually assumed to be negligible.

The G-matrix itself is the sum of these three potential sources of covariance of allelic effects,

$$\mathbf{G} = 2 \sum_{i=1}^n \sum_{j=1}^n (C_{ij} + C'_{ij}), \quad (5)$$

with the sum extending over allelic covariances for all n loci (Lande 1980). This is a little easier to interpret by writing the elements of \mathbf{G} , say for traits p and q , after separating the terms from Equation (5) based on their definitions from Equations (2-4):

$$G_{pp} = 2 \sum_{i=1}^n \sigma_{ip}^2 + 2 \sum_{i=1}^n \sum_{j=1}^n \sigma_{ipjp} + 2 \sum_{i=1}^n \sum_{j=1}^n \sigma_{ip'jp} \quad (6a)$$

$$G_{pq} = 2 \sum_{i=1}^n \sigma_{ipiq} + 2 \sum_{i=1}^n \sum_{j=1}^n \sigma_{ipjq} + 2 \sum_{i=1}^n \sum_{j=1}^n \sigma_{ip'jq}. \quad (6b)$$

Here G_{pp} is the additive genetic variance for trait p , and G_{pq} is the additive genetic covariance between traits p and q . This breaks the composition of the G-matrix into three separate (and fairly comprehensible) pieces. In each case, the first term refers to the pleiotropic effects of each locus, the second term refers to the variance or covariance generated by linkage disequilibrium, and the third term refers to the covariance among alleles that can be generated by non-random (or assortative) mating.

G-matrices thus represent covariances of covariances. Pleiotropic effects of alleles at a single locus, linkage of alleles with similar effects at multiple loci, and associations of alleles across gametes generated by non-random mating can all generate covariance in genic effects, which in turn generate the genetic covariances observed in \mathbf{G} . The actual

values of the covariances in \mathbf{G} will depend on the frequencies of the underlying alleles. Like all quantitative genetic models, this approach sums over the individual per-locus effects so that we deal solely with the summed variances of genic effects. The genetic covariances therefore average over many individual genic effects, which can even potentially cancel each other out (e.g., Gromko 1995). Ultimately, it is the genic effects themselves that harbor the interesting information regarding the functional basis for the genetic coupling between traits (Box 2), but it is the overall genetic effects that we can observe in the resemblance between relatives.

Evolution of \mathbf{G}

Although the pattern of genetic covariation among traits can play an important role in the evolutionary response of those traits, as exemplified by Equation (1) (Fig. 1), it is equally interesting to ask how \mathbf{G} itself will evolve. Extrapolating from Lande (1980), the deterministic changes in \mathbf{G} can be modeled using the equation shown in Box 3. From a theoretical perspective, we can consider both how deterministic processes, such as selection and mutation, and stochastic processes, such as drift, change \mathbf{G} .

Selection.—Directional selection (β) alone is expected to cause an erosion of genetic variation, but the pattern of correlational selection (γ) can potentially mold the pattern of genetic covariation. The term $(\gamma - \beta\beta^T)$ in Box 3 is actually the curvature of the adaptive landscape (Phillips and Arnold 1989), so one interesting prediction from this model is that \mathbf{G} should evolve to match the orientation of the adaptive landscape (Box 2, Cheverud 1984; Jones et al. 2003). However, the actual pattern of \mathbf{G} will depend on the balance of selection with other evolutionary forces.

Mutation and recombination.—The potential importance of mutation and recombination in the evolution of \mathbf{G} remain relatively unexplored. The influence of recombination is usually ignored because selection ordinarily needs to be fairly strong in order to overcome the ability of even small amounts of recombination to eliminate linkage disequilibrium. Intermittent admixture of isolated populations can generate substantial amounts of linkage disequilibrium, however, as will strong assortative mating among similar genotypes. The relative importance of linkage disequilibrium will ultimately depend on the genomic organization of genes influencing suites of correlated traits. Traditional population genetic models have tended to treat loci as being equivalent to distinct genes, which are usually assumed to be loosely linked. Emerging insights into molecular genetics may require changes in these assumptions. For instance, if one treats the regulatory regions of genes separately from their translational products, then these parts of a “gene” need to be treated as two distinct, tightly linked loci. In more explicit models of developmental regulation, considering the influence of tight linkage between separate factors will become more important.

There is an even more pressing need to consider the influence of mutational covariance on the evolution of \mathbf{G} . Over the last few decades, evolutionary geneticists have speculated extensively on the potential role of genetic correlations as an evolutionary constraint (Barton and Turelli 1989). The true nature of these constraints are most likely to be revealed in the pattern of mutational covariance, since it is against this background of variance that selection can act and the structure of the G-matrix is determined (Box 2;

Jones et al. 2003). It remains an open question whether or not patterns of developmental interactions among genes will influence new mutations to adhere to constrained pleiotropic pathways or whether most loci are instead capable of a wide array of possible phenotypic effects (Box 2). Any such mutational biases are bound to have an important influence on the long-term evolution of **G**. As difficult as accurately estimating the elements of **G** can be, estimating the components of **M** is much harder and unfortunately still leaves us far removed from the distribution of the mutational effects themselves.

Genetic drift—Stochastic variation in gametic frequencies generated by genetic drift will influence both the evolution of the G-matrix itself (Phillips et al. 2001) and the direction of phenotypic evolution, which is mediated by **G** (Lande 1979). Drift can change **G** both through sampling alleles with differing pleiotropic effects and through a build-up (or change in the pattern) of linkage disequilibrium (Eqs. 6-7). The former effects might be expected to be more persistent than the latter, although linkage can potentially decay fairly slowly (Whitlock et al. 2002). Unfortunately, we do not have a firm theoretical handle on the importance of drift through either of these processes (Phillips et al. 2001). Within a population, drift is expected to influence all genetic variances and covariances similarly; they should decline at a rate of $(1 - 1/2N_e)$ per generation, where N_e is the effective population size. If mutation restores some of this variation every generation, then we would expect the long-term mutation-drift equilibrium for **G** to be $2N_e\mathbf{M}$ (after Lynch and Hill 1986). Thus, we expect both the short-term and long-term effects of drift to lead to proportional changes in **G** (Box 1; Phillips et al. 2001). As we discuss below, however, there can be tremendous sampling variation around this expectation, such that for any given population the orientation of the G-matrix can change substantially due to drift (Phillips et al. 2001), especially over extended periods of time (Whitlock et al. 2002).

When populations diverge through drift, it is predicted that the orientation of population means will match the orientation of **G** (Lande 1979; Phillips et al. 2001). This is the same expectation as for the correlated response to selection on a set of traits (Lande 1979), which unfortunately means the observation that divergence is aligned with the major direction of genetic covariation (Schluter 1996) cannot be used to distinguish whether drift and selection has been responsible for the divergence among populations (although see McGuigan et al. 2004).

Assumptions and Complications

The equations and predictions given above are only first approximations of the true complexity underlying the evolution of genetic associations among traits. First of all, the equations describing the evolution of the G-matrix are derived under the assumption that the effects of alleles (both within and between loci) are additive. This is the only way to easily write the genetic covariances in terms of the deviation of the effects of a particular allele from effects of other alleles at the locus. If that deviation depends on genetic context, either because of dominance of other alleles at the same locus or by interactions between that allele and alleles at other loci (epistasis), then things can get much more complicated. Epistasis, particularly when combined with varying levels of linkage, could potentially have a large impact on the apparent pattern of pleiotropy at a given locus.

Imagine, for example, an enhancer region that modulates expression of a given gene in different tissues. The pattern of pleiotropy will depend on changes in the enhancer, on changes in the structural gene, or most likely, on some coordinated set of changes shared between them. To some extent, complications caused by non-linear interactions among genetic factors can be statistically accounted for, especially for predictions of changes in trait means (Ch. 4.1), but their effects on variances are complex (Whitlock et al. 1995) and their influence on genetic covariances is virtually unexplored (see Lopez-Fanjul et al. 2004).

An equally complex but more subtle complication is caused by differences in the distribution of allelic effects (Box 2). These distributions do not affect the definition and interpretation of **G** in a static sense (Eqs. 2-6), but even if we assume all effects at a locus are additive, the distribution of allelic effects can have an important influence on the evolution of the elements of **G**. When allelic effects are normally distributed, as assumed by Lande (1980), Equation (7) can be used to describe changes in **G**. The essential underlying result here is that even as the elements of the allelic covariance matrices change, under normality, the linear relationship between the allelic effects and the phenotypic traits is not (Lande 1980). When the distribution of effects is non-normal, however, there is a complex interplay between the higher moments of the allelic effects and changes in the moments of the traits themselves (Barton and Turelli 1987). For example, if there is skew in the distribution of allelic effects, changes in the trait mean will lead to changes in the genetic variance of that trait, irrespective of changes in variation in the underlying alleles (Barton and Turelli 1987). More complex approaches that explicitly include allelic dynamics provide a strong direction for further work (e.g., Kirkpatrick et al. 2002), although a general set of results, especially with regards to genetic covariances, remains elusive. In the end, all of these complications suggest that we need to know much more about the specific nature of the genetic effects of interest before we will be able to determine what level of theoretical abstraction is appropriate.

Case Studies

There are two broad empirical approaches to determining how **G** evolves: manipulative laboratory experiments and comparative field experiments. Each approach has its advantages and disadvantages (Table 1). Generally, laboratory studies can be used to generate predictions about which evolutionary process generate what patterns in **G**, while comparative studies of natural populations can identify what patterns (and therefore processes) occur in the wild. Table 1 suggests the choice of natural populations is more difficult than the choice of taxa for laboratory systems. Meeting the stringent criteria for natural populations makes it possible to extrapolate results of the study to other natural systems. Here, we use case studies to discuss the attributes and methods of comparative field investigations of **G** versus manipulative laboratory studies of the evolution of **G**.

Comparative study

Butterfly Wing Patterns

Butterflies have the short generation time and limited space requirements that, when coupled with extensive natural variation in size, shape, color and pattern of their wings

(Fig. 2), make them attractive for quantitative genetic studies. Despite this variation, basic pattern elements are relatively easy to identify on most wings, facilitating comparison among taxa (reviewed by Nijhout 1991). Wing pattern divergence (Δz) has been investigated by trying to determine the causative selective force (β) acting upon them, as well as by studying their genetic basis (\mathbf{G}). It has often proven difficult to determine the function of some wing pattern elements (e.g., Lyytinen et al. 2003) and therefore the nature of their selection, but the genetic basis of the traits are amenable to investigation.

Artificial selection to increase or decrease the size of one eyespot in *Bicyclus anynana* resulted in evolution (in the selected direction) of other eyespots (Monteiro et al. 1994). By inference, eyespot size genetically covaries positively among eyespots. This inference was supported by the calculation of genetic correlations among eyespots in two species of butterfly, *Junonia (Precis) coenia* and *J. evarete* (Paulsen 1996). All bivariate genetic correlations among eyespot sizes were positive. Paulsen (1996) also included other wing pattern traits in the \mathbf{G} , with an overall conclusion that the size of similar pattern elements (e.g., eyespots) tended to positively covary, whereas size among different pattern elements (e.g., wing bands) showed no covariation of size. Similarly, there was little correlation between size and position of the pattern elements. The pattern of genetic correlations appeared similar between Paulsen's two species, and a similar pattern of correlation among replicates of the same element, but not among different elements, has been observed in other butterflies (e.g., Kingsolver and Wiernasz 1991; Monteiro et al. 1994). Is the genetic basis of wing pattern in butterflies similar across taxa and can we use the relationships in the breeders' equation to determine the nature of selection responsible for current patterns of diversity?

Using both matrix-wide and an element-by-element approaches, Paulsen (1996) tested the hypothesis that \mathbf{G} did not differ between the closely related *J. coenia* and *J. evarete*. No differences between matrices were detected; we can conclude that \mathbf{G} has not significantly diverged between these *Junonia* species. Can we then assume the genetic relationships among traits are the same for all other butterfly species? To ascertain how broadly applicable the result is we need to consider why \mathbf{G} remained stable. The first hypothesis to consider is that *J. coenia* and *J. evarete* began diverging too recently for differences in allele frequency (due to mutation, selection or drift) to accumulate. Putative divergence time is positively correlated with divergence in phenotypic covariance matrices (\mathbf{P}) (e.g., Baker and Wilkinson 2003; Stepan 1997), suggesting this as a plausible hypothesis. Since we do not know how long *J. coenia* and *J. evarete* have been diverging, we cannot infer what a reasonable time frame for assuming stability of \mathbf{G} might be. The best way to determine if the observed stability is due to recent divergence is to compare these \mathbf{G} -matrices to those of other butterflies within the framework of a known phylogeny. Using a phylogenetic comparative approach, if we observe more recently speciated butterflies to have divergent \mathbf{G} , we can ask whether stabilizing selection has maintained the ancestral pattern of genetic covariation among traits in our two species.

What generates/maintains the genetic interrelationships among these traits? The orientation of \mathbf{G} will be determined by an interaction between genetic variation, selection, and drift (Box 3). If functional relationships among traits generate patterns of correlational selection, then the \mathbf{G} should reflect these relationships. Similarly, developmental

relationships among traits will influence the pattern of mutational input into \mathbf{G} , and selection should also shape development itself. \mathbf{G} may therefore be an interesting place to look for a signature of the interaction between function and development. Kingsolver and Wiernasz (1991) estimated \mathbf{G} for melanin patches on the wings of the butterfly *Pieris occidentalis*. Using data from previous studies, they generated a matrix that described the hypothetical thermoregulatory functional relationships among melanin patches and a matrix that described the hypothetical developmental relationships among patches. Comparing each of these to \mathbf{G} , they concluded the observed patterns of genetic covariation were due to both functional and developmental relationships among traits (Kingsolver and Wiernasz 1987).

Hypotheses about the function of eyespots suggest different roles for dorsal eyespots, which are not exposed at rest, and ventral eyespots, which are exposed at rest (e.g., Breuker and Brakefield 2002; Lyytinen et al. 2003). The existence of a positive genetic covariation of eyespot size across both wing surfaces therefore cannot be generated by a functional relationship between these traits. Although developmental hypotheses have not been formally tested, similar developmental mechanisms (namely response to a morphogen gradient) appear to operate in the formation of all eyespots (reviewed by Brakefield 2001), suggesting \mathbf{G} is influenced by the developmental relationships. If this assessment of the relative influences of development and function is correct, we can predict change in the function of eyespots will have little impact on \mathbf{G} , whereas changes in the underlying developmental relationships among traits will result in evolution of \mathbf{G} .

Thus far, we have interpreted similarity of \mathbf{G} between species as evidence of stability. However, similarity of \mathbf{G} in *J. coenia* and *J. evarete* might be due to convergent or parallel evolution; similar selection regimes generating the same \mathbf{G} independently in each species. This hypothesis cannot be addressed in a two species system, but instead requires a broader phylogenetic context to infer the direction of evolution. An alternative hypothesis is that phenotypic divergence between the species was accompanied by divergence in \mathbf{G} , but that this divergence was not maintained (see Agrawal et al. 2001; Reeve 2000). Agrawal et al. (2001) demonstrated genes of major effect could dramatically, but transiently change \mathbf{G} during directional evolution. Mutagenesis experiments with *B. anynana* has indeed revealed some loci with alleles that dramatically affected eyespot development (Monteiro et al. 2003). Questions about transient instability of \mathbf{G} could be addressed with independent natural populations that have colonized the same habitat at different times, or through temporally fine-scale sampling of \mathbf{G} during experimental evolution.

Experimental studies

Effect of Drift on Genetic Covariation of *Drosophila* Wing Shape

Phillips et al. (2001) designed a laboratory experiment to test the theoretical prediction that random genetic drift does not change the genetic covariation among traits (i.e., the orientation). Rather, because it erodes genetic variance, drift will scale \mathbf{G} by one minus the inbreeding coefficient (see above). Changes in the orientation of \mathbf{G} will affect the direction in which evolution proceeds (Fig. 1), whereas changes in the variance will affect the rate. Exploiting the experimental benefits of *Drosophila melanogaster*, Phillips

et al. (2001) generated a large data set consisting of six wing-shape traits measured for eight daughters from about 90 families in each of 52 inbred lines (4680 families total) and from 1945 families across six control (outbred) lines. The large sample generated here facilitated accurate estimation of **G**. This laboratory data set is equivalent to estimating **G** for 52 neutrally diverging populations (founded by one female and her brother), as well as estimating a known ancestral **G**.

At first glance, the results of Phillips et al. (2001) supported the theoretical prediction that drift reduces genetic variance but does not change the genetic covariation of traits. Common principal components analysis supported proportionality of the average inbred **G** (across the 52 lines) to the outbred (ancestral) **G** (Fig. 3). However, using proportionality of **G** as a signature of drift depends on how drift operates within single populations rather than on the average behavior of drifting populations. In this case, the 52 inbred lines varied considerably in both the orientation of **G** and the level of genetic variance (Fig. 3). Therefore, although theory predicted the average outcome across all populations, it could not predict what would occur within any individual population. Implicit in this result is the conclusion that **G** can diverge through drift over very short periods of time, although whether this occurs frequently in nature has yet to be determined. Following these populations for an additional twenty generations after the initial bottleneck demonstrated that the drift-induced changes in **G** were not transient (Whitlock et al. 2002).

Phillips et al. (2001) noted that the impact of drift on genetic covariance varied from trait to trait. Changes induced by drift evidently depended on the underlying genetic details. Differences in cell lineage and gene expression patterns have led researchers to propose that anterior and posterior portions of *D. melanogaster* wings are separate developmental units, although this hypothesis has been questioned by Klingenberg and Zaklan (2000) based on their observation that only a small proportion of variation among wings is due to region-specific effects. Unfortunately, it is difficult to determine how traits measured by Phillips et al. (2001) fall across these morphogenic regions, making it difficult to assess whether **G** changed more within or between developmental units. In separate analyses of anterior and posterior regions of *D. melanogaster* wings, Zimmerman et al. (2000) detected multiple quantitative trait loci (QTL) associated with wing shape, a first step toward more finely dissecting the underlying genetic basis of these traits. There appears to be significant epistatic genetic variance for wing shape (Gilchrist and Partridge 2001), suggesting that interactions among loci contributing to shape might be complex. Knowing the number of loci contributing to traits in **G** and the interactions among loci is a step toward understanding if variation among traits in these genetic parameters cause the genetic relationships among some traits (as summarized in **G**) to change rapidly, while others to remain stable. This also highlights the fact that the choice of a particular set of traits and the way in which those traits are measured might strongly influence both the interpretation of results and our ability to correlate causal factors across levels of genetic organization.

Distinguishing Pleiotropy from Linkage

If not all aspects of **G** respond in the same way to a specific process evolutionary processes, the proximal cause must be differences in the underpinnings of **G**. Observed

genetic correlations are usually expected to be caused by pleiotropy because correlations due to linkage should break down relatively quickly (Lande 1980). That is, theory predicts that genetic covariance through pleiotropy will be more stable than covariances generated by linkage, which is also consistent with computer simulations that indicate a strong stabilizing effect of pleiotropic mutations on the structure of **G** (Jones et al. 2003). Similarly, Mezey and Houle (2003) have shown that shared similarity in G-matrix structure is likely to be preserved only in cases in which developmental modules have a shared underlying pleiotropic basis.

Pleiotropy is considered ubiquitous, but is necessarily difficult to distinguish from tight linkage. For example, floral traits in plants are tightly coupled both functionally and developmentally and also show substantial genetic correlations. Are these correlations caused by pleiotropy or linkage? Conner (2002) enforced nine generations of random mating in the wild radish (*Raphanus raphanistrum*) and observed no change in covariance of six highly correlated floral traits. Forced random mating should have moved the population closer toward linkage equilibrium, especially if the linkage was generated by either correlational selection or assortative mating (Box 3). This study therefore strongly suggests a pleiotropic basis to the genetic covariances. Of course, very tight linkage may take substantially longer to decay (Conner [2002] showed that the genes influencing these traits would need to have average recombination rates of less than 0.01-0.05 to retain substantial linkage disequilibrium over this time period). When one considers extremely tight linkage, say between different control regions within a gene, then the difference between linkage and variability in pleiotropic effects becomes somewhat semantic (Box 2).

Future Directions

Comparative approaches

The recent, rapid acquisition of data on the evolution of **G** (Steppan et al. 2002) places us in a position to refine experimental design and identify approaches that will yield the most information. We now know that although **G** can diverge greatly over few generations (e.g., Phillips et al. 2001), but that it does not always do so (e.g., Paulsen 1996). Therefore, comparisons of natural populations must contribute more than yes, **G** has evolved, or no, **G** has not evolved. Comparative analyses are still the only way we can estimate the direction and rate of evolution of **G** in natural populations. However, comparisons must be made within a robust phylogenetic framework to achieve this aim.

Although still in its infancy, computer simulations have the potential to generate expected distributions of G-matrices under particular evolutionary scenarios (e.g., Jones et al. 2003). Empirical data from laboratory experiments (e.g., Phillips et al. 2001) are also expanding our understanding of the patterns generated by particular evolutionary processes. This information on relationships between pattern and processes will assist in the interpretation of patterns observed in phylogenetic comparative studies. At the very least, we need to develop null models (e.g., drift) against which other evolutionary hypotheses (e.g., selection) can be tested. The phylogenetic framework serendipitously facilitates identification of taxa whose G-matrices have evolved more or less than expected

based on information from the other taxa. We can then address the questions of why and how do these taxa differ in population parameters, selection pressure, *etc.*

Comparisons within a rigorous phylogenetic framework will also help us assess whether functional or developmental relationships among traits affect stability of **G**. Integration theory, which predicts developmentally or functionally related traits will be pleiotropically controlled (Cheverud 1984), and computer simulations, which show that pleiotropic mutations can stabilize **G** (Jones et al. 2003), lead to the prediction that the portion of the G-matrix that describes functionally and/or developmentally related traits will be more stable than the portions describing unrelated traits. For example, in the case of butterfly wings, Paulsen (1996) detected a marginally insignificant difference ($P = 0.056$) between G-matrices from *J. coenia* and *J. evarete* for the full set of 29 traits, including eyespot size, position and wing vein measurements, whereas a comparison of the submatrix of **G** including just the developmentally related eyespot diameters provided no support for divergence between the species ($P = 0.273$). Further comparisons within a robust phylogenetic framework can determine whether, (a) functionally or developmentally connected traits are characterized by strong genetic covariation, and whether, (b) if G-matrices of these traits are more stable than **G** of unconnected traits.

There are still significant methodological issues to be overcome in order to conduct the proper analysis in a comparative framework (Phillips and Arnold 1999; Steppan et al. 2002). Further, there is little value in conducting studies with insufficient power (Box 1), so the total level of effort required to conduct a complex comparative analysis among a number of different taxa will be substantial—perhaps even daunting.

Manipulative and experimental approaches

Although there is no substitute for comparative natural experiments to determine what processes shape **G** under natural evolution, the valuable role of laboratory experiments is clearly established (e.g., Phillips et al. 2001; Shaw et al. 1995). As we accumulate more data on the behavior of **G** under particular evolutionary scenarios, we can increase the sophistication of experimental studies to include simultaneous action of more than one process and thus toward the consideration of how different processes interact. We can then generate more realistic predictions of the behavior of **G** under natural conditions.

The genetic basis of genetic covariances

There are effectively two levels at which we can attempt to dissect the molecular genetic basis of **G**: detailed analysis of the function and variation of individual genes versus broad analysis identifying all loci contributing to a particular suite of traits. By considering the action of individual genes, researchers investigating butterfly eyespots are moving toward understanding which genes contribute to eyespots, as well as the nature of the interactions among those genes (e.g., Beldade et al. 2002). Taking this approach generally requires *a priori* information about the system (for example, information from related taxa about genes affecting particular traits or processes) but enables very precise consideration of variation at those loci.

Quantitative trait loci (QTL) analyses represent a method taking the other, top down approach to identifying loci associated with phenotypic variation. There are certain advantages to using QTL analyses to dissect **G**: they require no *a priori* knowledge of the genetic basis of traits; they can be used to investigate multiple traits simultaneously, facilitating identification of pleiotropic/linked loci; they can potentially be used to estimate the total number of loci contributing to any one trait; and can be used to identify (candidate) loci for further study. Currently, there are also disadvantages to using QTLs: a QTL covers considerably more of the chromosome than a gene does, making it impossible to ascertain whether a QTL reflects pleiotropic effects of a single locus or linked loci; and, with small genetic differences and small sample sizes, QTL analyses are not very powerful. Both of these problems might result in underestimation of loci and overestimation of pleiotropy. Lack of power could also result in detection of different QTL in different populations, leading us to the erroneous conclusion that the underlying basis of **G** has evolved when in fact it has not (Gibson 2002). Ultimately, we will need the combination of functional specificity coupled with global analysis that is promised by the genomics revolution. In reality, this will still end up demanding a great deal of fairly traditional genetic analysis, but the potential for rapid advance is definitely on the horizon.

The need for this molecular dissection is inevitable because we ultimately need to know much more about the distribution of allelic effects underlying quantitative traits before we can move on to a more sophisticated understanding of the evolution of those traits. Not all mutations (i.e., not all alleles) have the same effect on all traits to which that locus contributes (Box 2; Stern 2000). This highlights a fundamental aspect of **G**, one that is accessible only through developmental genetics. **G** is determined by allele frequencies and, if not all alleles are equally pleiotropic, changes in allele frequency will change the orientation of **G**. The similarity of pleiotropic affects among alleles at a locus might depend on whether the locus codes for structural or regulatory proteins (Stern 2000). Variation at structural loci is likely to equally impact all traits to which that the protein contributes, resulting in similar pleiotropy of all alleles. In contrast, variation at regulatory loci might affect when/where proteins are expressed, thus having different effects on different traits, and thereby generating different levels of pleiotropy among alleles. The different contributions of regulatory versus structural genes to evolution is a subject of ongoing debate in molecular evolution and has implications for our understanding of the evolution of **G**.

G stands at the center of quantitative models that help to describe the pattern of phenotypic change generated by a wide variety of evolutionary processes. **G** also serves as a metaphor for the role integrated genetic systems play in understanding the evolution of the whole organism. It is perhaps fitting that matrices usually have very box-like representations, because for the last several decades the G-matrix has been used as a black box into which tremendous amounts of underlying genetic complexity could be placed while research focused squarely at the level of the phenotype. To move from metaphor to reality, we need to open that box and see what lies inside.

Acknowledgements

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Suggested Readings

- Barton, N. H., and M. Turelli. 1987. Adaptive landscapes, genetic distance, and the evolution of quantitative characters. *Genetical Research* 49:157-174. • A difficult paper that begins a continuing effort to create a general approach for examining the role that allelic distributions play in structuring the pattern of genetic variation and other features of phenotypic evolution.
- Cheverud, J. M. 1984. Quantitative genetics and developmental constraints on evolution by selection. *Journal of Theoretical Biology* 110:155-171. • An interesting paper that strongly asserts the value of a union between developmental and evolutionary genetic thinking for understanding the pattern genetic covariation among traits.
- Lande, R. 1979. Quantitative genetic analysis of multivariate evolution, applied to brain:body allometry. *Evolution* 33:402-416. • The most important paper understanding the multi-trait representation of the evolution of quantitative characters.
- Steppan, S. J., P. C. Phillips, and D. Houle. 2002. Comparative quantitative genetics: evolution of the G matrix. *Trends in Ecology and Evolution* 17:320-327. • A review of many issues and a number of studies that have compared G-matrices.
- Stern, D. L. 2000. Evolutionary developmental biology and the problem of variation. *Evolution* 54:1079-91. • A strong statement of the relationship between developmental gene regulation and the generation of phenotypic variation.

Box 1: What is a Covariance?

Before getting carried away discussing the importance of genetic covariances for the evolutionary process, it is necessary to understand what a covariance is. Statistically, a covariance describes the association between two variables. It is closely related to the more familiar concepts of regression and correlation, which are covariances normalized on different scales. The covariance between X and Y is given by:

$$\sigma_{XY} = E[(X - \bar{X})(Y - \bar{Y})]$$

and can be estimated as:

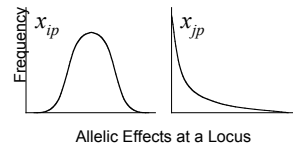
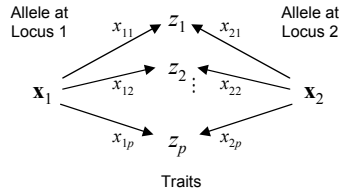
$$\text{Cov}(X, Y) = \frac{1}{n-1} \sum_{i=1}^n (X_i - \bar{X})(Y_i - \bar{Y})$$

The correlation between X and Y is the covariance normalized by the standard deviation of each trait ($\rho_{XY} = \sigma_{XY} / (\sigma_X \sigma_Y)$), while the regression of Y on X is the covariance normalized by the variance of X ($\beta_{XY} = \sigma_{XY} / \sigma_X^2$). The covariance of a variable with itself is simply its variance ($\sigma_{XX} = \sigma_X^2$). Covariances, rather than correlations, are used to describe the evolution of traits because they are not normalized, existing on the same scale as the traits themselves.

At the genetic level, a covariance between two traits is generated when alleles affecting both traits tend to be found within the same individual (Box 2). Genetic covariances can be estimated using standard quantitative genetic approaches (Ch. 4.1), for instance, from the regression of offspring values for trait X on the parental values for trait Y (and visa versa). As second-order statistics, covariances require large sample sizes to be accurately estimated. As genetic covariances are subject to sampling variation at more than one level (genetic and environmental), the accuracy of their estimation is even more troubling. Only a few studies in the evolutionary literature are of sufficient sample size to provide more than the crudest estimates of the pattern of genetic covariance among traits.

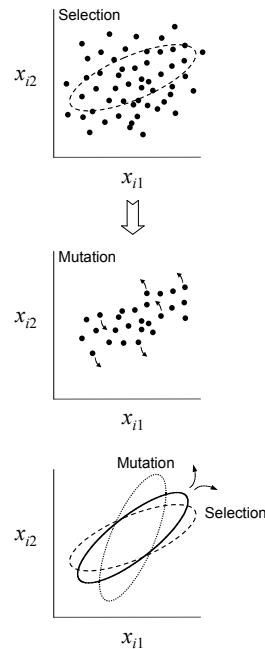
With only two traits, the genetic covariance is easy to interpret, but as we consider more traits it becomes more difficult to interpret the pattern of genetic covariances. Principal components analysis is one statistical tool used to interpret patterns of genetic covariation among traits (Fig. 3; see Phillips and Arnold 1999). Principal components analysis generates new variables (principal components), which are linear combinations of the “traits” (genetic variances and covariances) in \mathbf{G} . As vectors, principal components have direction and length. The first principal component describes the trait combination (direction) for which there is the most genetic variance (length). Subsequent principal components will describe less variation, but unique directions. \mathbf{G} is then interpreted by its orientation (the directions of its principal components) and size (the length of principal components). For example, two matrices that are proportional to one another (an important prediction for genetic drift, for instance) would have identical principal component orientations, but the dimensions along each component would be proportionally expanded or contracted (see Fig. 4).

Box 2: Pleiotropic Effects



We might have a tendency to think about pleiotropic effects in a very diffuse way, perhaps calling forth the traditional definition of a single locus that affects more than one trait. To understand the genetic basis of covariances we need a much more precise view of pleiotropy—one that focuses at the level of the allele rather than the locus as a whole. In particular, some alleles at a given locus might have pleiotropic effects, while others do not. More subtly, the direction and magnitude of the pleiotropic effects on a suite of traits might vary from allele to allele. This requires that every locus be represented by the range of pleiotropic effects generated by each allele, as in the figure above.

The array of all possible allelic effects at a given locus will generate a distribution of effects, as shown to the right. Each locus might have its own distribution, and, in general, we do not really know what these distributions tend to look like. Popular possibilities are fairly “normal” symmetrical distributions (left) and “L”-shaped, highly skewed distributions (right). To assess the pleiotropic contributions of each allele, these distributions must be combined in a bivariate (and ultimately multivariate) view. Alleles at a given locus can potentially have any pattern of pleiotropic effects, including no pleiotropy at all. The effect of correlational selection (Fig. 1) might have on the pattern of pleiotropic effects of the underlying alleles can be visualized by laying a fitness contour on top of the distribution of allelic effects. Correlational selection occurs when fitness depends on a combination of trait values rather than on the individual traits themselves (e.g., on how the upper and lower mandibles fit together to make a single fruit-cake-cracking jaw). Given unconstrained effects, the distribution of allelic effects should evolve to match the pattern of selection. The actual translation between the alleles is mediated through the mapping of the allelic effects onto the traits under selection (Lande 1980). Mutational input can disturb the pattern of pleiotropy in each generation, so



if there is bias in the pattern of mutational input, the distribution of effects will evolve to match that bias to some degree. The equilibrium distribution of effects will therefore depend on a balance between selection and mutation, as well as other factors (Box 3).

Box 3: Evolution of the G matrix

$$\Delta \mathbf{G} = \mathbf{G}(\boldsymbol{\gamma} - \boldsymbol{\beta}\boldsymbol{\beta}^T)\mathbf{G} + 2\mathbf{M} - 2\sum_{i=1}^n \sum_{j=1}^n r_{ij}(\mathbf{C}_{ij} - \mathbf{C}'_{ij})$$

Selection Mutation Recombination

This is a composite equation that shows how different evolutionary forces influence the evolution of the genetic variance-covariance (\mathbf{G}) matrix (mostly based on Lande 1980). The first term describes the influence of natural selection, where $\boldsymbol{\gamma}$ is the average curvature and orientation of the individual selection surface and $\boldsymbol{\beta}$ is the average slope of the individual selection surface (Lande and Arnold 1983). Taken together, these two elements describe the local curvature and orientation of the adaptive landscape (Phillips and Arnold 1989). The response to selection is generated through a balance between the tendency of directional selection to erode genetic variation and the ability of stabilizing and correlational selection to reorient the pattern of among-trait correlation. In this term, the influence of selection is reflected back on change in \mathbf{G} through the lens of the existing pattern of genetic covariance. The second term describes the role that new mutations play in structuring \mathbf{G} . This is summarized by the mutational covariance matrix \mathbf{M} , where $\mathbf{M} = \sum_i \mathbf{M}_i$ is the sum of the mutational covariances generated by the pattern of pleiotropic mutation at each locus. In the absence of other evolutionary forces, \mathbf{G} will tend to match any biases induced through mutational effects guided by, say, developmental processes. Finally, the third term describes the rate of degradation of linkage-induced genetic covariance generated by recombination, where r_{ij} is the recombination rate between loci i and j ($r_{ii} = 0$). Additional terms are needed when the distribution of allelic effects is not normal (e.g., Barton and Turelli 1987).

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Figure Legends.

Figure 1. The response of two genetically correlated traits to correlational selection. The orientation and size of \mathbf{G} is described by the white ellipse, with the narrow oval shape indicating most of the genetic variance is common to both traits, rather than independent (i.e., covariance). The cross-hairs of the ellipse indicate the current population mean for the two traits; the position of the population on the fitness surface. The darker the region of the fitness landscape, the higher the fitness, with black areas indicating local adaptive peaks; regions shaded the same colour have equal fitness. β (white line) describes the most direct approach of the population to the nearest fitness peak. A) Traits are negatively genetically correlated, but there is selection for an increase in both traits; the initial direction of evolution will be away from the fitness peak. The population will eventually ascend the fitness peak, but this will take longer than if \mathbf{G} was oriented in the same direction as the fitness surface (i.e., along β). B) Traits are positively correlated and the population is in the neighbourhood of two fitness peaks, both of which generate selection for an increase in both traits. However, because of the genetic covariance among traits, the population will evolve toward peak 2 rather than peak 1.

Figure 2. Variation in dorsal eyespots of Nymphalid butterflies. A) *Junonia coenia* (Nymphalinae); B) *J. evarete* (Nymphalinae); C) *Stichopthalma camadeva* (Morphinae); D) *Faunis menado* (Morphinae) and; E) *Taenaris macropus* (Morphinae). Note the difference in size between the two hindwing eyespots in *J. coenia*, but not *J. evarete*. Pictures kindly provided by F. Nijhout based on those originally presented in Nijhout (1991) (c.f. Figure 2.2 and Figure 5.18A and C).

Figure 3. Effects of genetic drift on the genetic covariance between two wing characteristics in *Drosophila melanogaster*. A) A graphical representation of the genetic covariance between two wing-vein angles. The outer ellipse shows the orientation of covariance in the outbred population whereas the grey ellipse shows the average covariance of 52 inbred lines created by brother-sister mating. Note that the ellipse for the inbred lines has shrunk proportionally relative to the outbred population as predicted by theory. B) Estimates of genetic covariance for these two traits for each of the 52 inbred lines. Note that there is a great deal of variation around the average shown in (A) (center box). After Phillips et al. (2001).

Fig. 1.

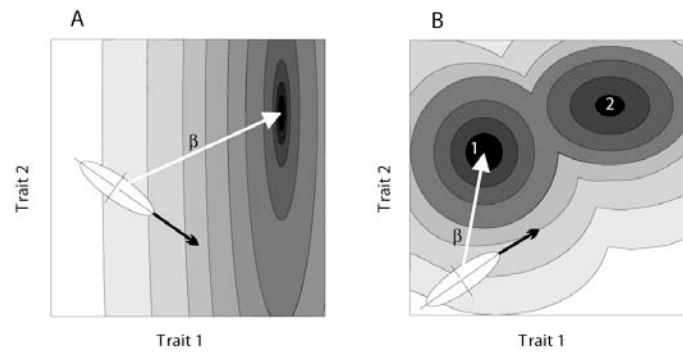


Fig. 2

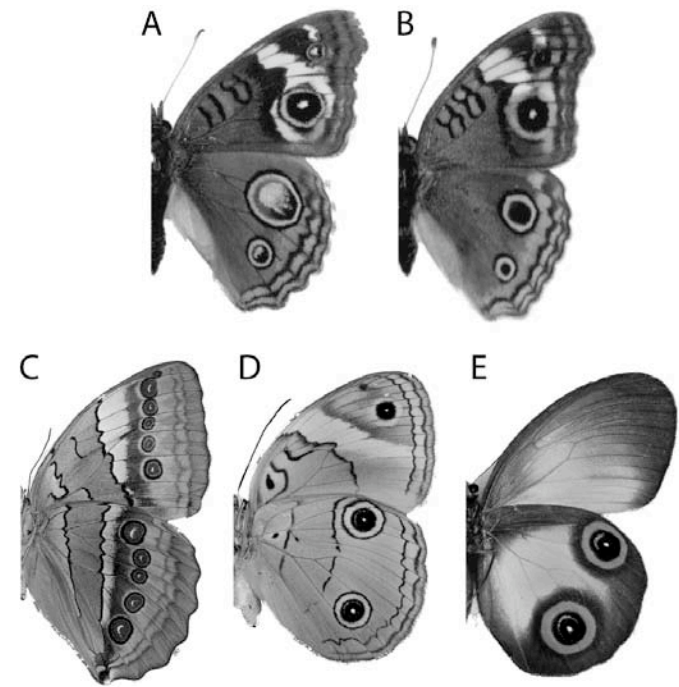


Fig. 3

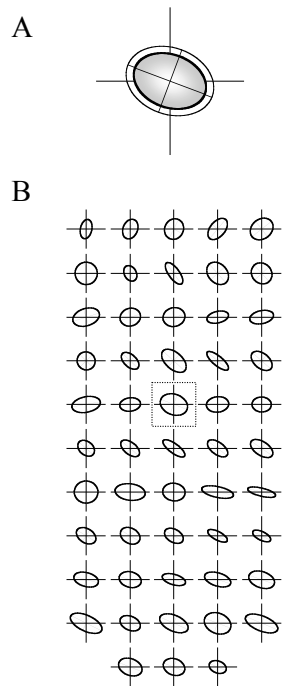


Table 1. Relative attributes of comparative and manipulative experiments to investigate the evolution of **G**.

	Natural Comparative	Manipulative Laboratory
Utility in determining which evolutionary processes act on G and what patterns they generate	<ul style="list-style-type: none"> ▪ Can infer which processes operate in natural populations, and how processes interact ▪ But, difficult to estimate parameters of selection, drift, migration or mutation 	<ul style="list-style-type: none"> ▪ Cannot infer which processes operate in natural populations, nor how they interact ▪ But, can estimate and/or control the strength of selection, mutation, migration and drift, and therefore determine the cause of observed patterns
Limitations on choice of taxa	<ul style="list-style-type: none"> ▪ Quantitative genetic parameters frequently estimated in the laboratory due to difficulties in estimating relationships in wild populations (although the latter is greatly preferable). Thus, usually limited to organisms with easy husbandry and ability to conduct controlled matings/crosses ▪ Generation time less critical because evolution has already occurred ▪ Relationships between the populations should be known from well-supported phylogenies or historical data ▪ Differences (or lack thereof) in selection pressure between populations should be known or inferred ▪ Population parameters that affect drift or response to selection (e.g., population size and generation time) should be estimated 	<ul style="list-style-type: none"> ▪ Need to house in laboratory restricts choice to taxa with easy husbandry and limited space requirements ▪ Generally conducted on taxa with short generation times to keep experimental duration short when populations need to be taken through multiple generations of evolution ▪ Generally begin with a single stock, generate several populations (lines) which are subject to known evolutionary process ▪ Population parameters known and controlled
Replication/sample sizes	<ul style="list-style-type: none"> ▪ Replication limited by time/space of researcher, but also by the availability of wild populations/individuals ▪ Require high replication of populations due to the multitude of evolutionary processes acting (e.g., many selective forces) ▪ Require high replication within populations due to variability in natural habitats 	<ul style="list-style-type: none"> ▪ Replication/sample size usually limited by space and manpower rather than availability ▪ Control the evolutionary processes acting on the population, so do not need sample multiple populations to have the power to estimate processes ▪ Frequently, lower environmental variance, so more accurate estimates of G for a given sample size
Genetic tools	<ul style="list-style-type: none"> ▪ Need to develop genomic resources for interesting natural systems 	<ul style="list-style-type: none"> ▪ Often, molecular genetic and developmental tools are also available for taxa that are tractable for laboratory quantitative genetic analyses