Comparative quantitative genetics: evolution of the G matrix

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Quantitative genetics provides one of the most promising frameworks with which to unify the fields of macroevolution and microevolution. The genetic variance–covariance matrix (G) is crucial to quantitative genetic predictions about macroevolution. In spite of years of study, we still know little about how G evolves. Recent studies have been applying an increasingly phylogenetic perspective and more sophisticated statistical techniques to address G matrix evolution. We propose that a new field, comparative quantitative genetics, has emerged. Here we summarize what is known about several key questions in the field and compare the strengths and weaknesses of the many statistical and conceptual approaches now being employed. Past studies have made it clear that the key question is no longer whether G evolves but rather how fast and in what manner. We highlight the most promising future directions for this emerging field.

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Organisms are complex systems comprising interacting characters underlain by shared functional, developmental and genetic processes. Within quantitative genetics (the study of inheritance at the phenotypic level), these relationships are summarized in the additive GENETIC VARIANCE–COVARIANCE MATRIX **G** (see Glossary). The usefulness of the quantitative genetic approach to long-term evolution depends, to a large extent, on whether **G** remains constant or evolves in a predictable manner. For this reason, quantitative geneticists have increasingly turned their attention to the evolution of **G.** Together with natural selection (the ADAPTIVE LANDSCAPE) it determines the direction and rate of evolution.

The most productive approach to the study of evolutionary change is dictated by the importance of genetic details in determining the nature of that change. In some cases, genetics might be irrelevant, and evolution might be best approached as an optimization problem [1]. In other cases, only genetic mechanisms might be worth studying [2]. Quantitative genetics is useful for intermediate cases where genetics matters, but where genetic details do not. The basic quantitative genetic model (Box 1) captures the influence of genetics through **G** and indirectly through the selection gradient, which depends on the PHENOTYPIC MATRIX **P** [3]. If **G** is stable, it can be used to predict the evolutionary potential of a population or to reconstruct the form of selection that has led to divergence among populations [4] (Box 1). Quantitative genetic parameters can also be integrated with phylogenetic information within a likelihood framework to test more precisely for adaptation [5]. If stochastic events, such as genetic drift, fluctuating adaptive landscapes and rare mutations, are more important, then quantitative genetics might not be informative and macroevolution might be decoupled from microevolution. Resolution of this issue is crucial to evolutionary biology as a whole.

Until recently, the usefulness of a quantitative genetic approach to evolution has been asserted or rejected mostly on faith. Neither the high-quality data nor the analytical tools to evaluate possible changes in **G** have been available. Here, we highlight recent advances that are beginning to allow informative comparisons of **G** matrices and discuss the questions of if, how, how fast and why **G** might evolve. We suggest that a new field of study has emerged, COMPARATIVE QUANTITATIVE GENETICS, which has built upon traditional comparisons of genetic variances and covariances but which is distinguished by incorporating phylogenetic information using the comparative method and an emphasis on covariation among traits.

Does G evolve?

Yes. With some important statistical caveats in mind (Box 2), there are clearly some cases where **G** matrices, or some of their elements, are unequal [3,6–8]. The significant changes in **G** sometimes detected by rather small studies imply that real differences are frequently large. Laboratory studies have demonstrated significant divergence at the population level given strong selection [3,9] and/or drift [10,11]. Although matrix correlations do not test the hypothesis of inequality, nonsignificant matrix correlations can be interpreted as evidence for departures from equality, if one has confidence in the precision of the estimates. Although comparable studies at multiple systematic levels are few, comparisons among rodent genera [12,13] have shown nonsignificant correlations, whereas comparisons among and within species were significantly correlated [14] (see reviews in [7,8,15]). Comparisons within species usually show significant correlation or insignificant differences [7,8,15]. Comparisons of **P** matrices find significant differences even more frequently [16–19]. In summary, one cannot assume that **G** is constant [6,20].

How do G matrices differ?

Although **G** can change, understanding those aspects of **G** that change could allow many informative predictions about evolution to be made. For example,

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Box 1. Introduction to the G matrix and quantitative genetics

Quantitative genetics provides a means for predicting the evolution of suites of traits given information about directional selection and the degree of resemblance among relatives. When only one character is selected, say z_1, the response to selection is predicted by the familiar breeder's equation (Eqn I),

$$\Delta\bar{z}_1 = (G_1/P_1)\,S_1 \qquad \text{[Eqn I]}$$

where $\bar{z}_1$ is the population mean; G_1 is the additive genetic variance in trait 1 and sums up the degree of resemblance between relatives; P_1 is the phenotypic variance, and S_1 is the covariance between z_1 and fitness [a]. Alternatively, this equation can be represented as (Eqn II),

$$\Delta\bar{z}_1 = G_1(S_1/P_1) = G_1 b_1 \qquad \text{[Eqn II]}$$

where b_1 is the slope of the regression of fitness on trait 1. If z_1 is genetically correlated with any other traits, then the change in frequencies of genotypes affecting z_1 will also affect these other traits. This indirect response of another trait, say z_2, to selection on z_1 is $\Delta\bar{z}_2 = G_{12}b_1$, where G_{12} is the additive genetic covariance between z_1 and z_2.

In general, directional selection can affect more than one trait, so our focal trait is affected both directly by selection on that trait and by the selection on all other traits correlated with it. The result is a complicated bookkeeping problem solved by means of matrix algebra. The vector of responses to selection is (Eqn III)

$$\Delta\bar{z} = \mathbf{GP}^{-1}\mathbf{S} \qquad \text{[Eqn III]}$$

where **G** is the additive genetic variance–covariance matrix, **P** is the phenotypic variance–covariance matrix, and **S** is the vector of covariances between traits and fitness. Variance–covariance matrices are square symmetric matrices with as many rows and columns as there are traits under study. The diagonal entries are the variances, and the off-diagonal elements give the covariances between traits. Equivalently (Eqn IV),

$$\mathbf{P}^{-1}\mathbf{S} = \beta \qquad \text{[Eqn IV]}$$

where β is the vector of partial regression coefficients of fitness on the traits. The elements of β give the relationship of each trait to fitness, holding the values of other traits constant. Lande [b,c] extended this multivariate approach and was the first to apply it to evolutionary problems.

G is useful for predicting which kinds of evolutionary changes are most readily accomplished. **G** deflects the response to selection toward those trait combinations that have more genetic variation. **G** will therefore affect the amount of time required to reach a novel state and could determine which state the population will ultimately achieve [b,d] (Fig. I). Persistent absence of additive variation for particular combinations of phenotypes would suggest that evolution in certain directions in phenotype space is not possible [b,e]. If **G** and the adaptive landscape are indeed constant over long periods, **G** might be used to predict the evolutionary potential of a population or to reconstruct the form of selection that has led to divergence among populations.

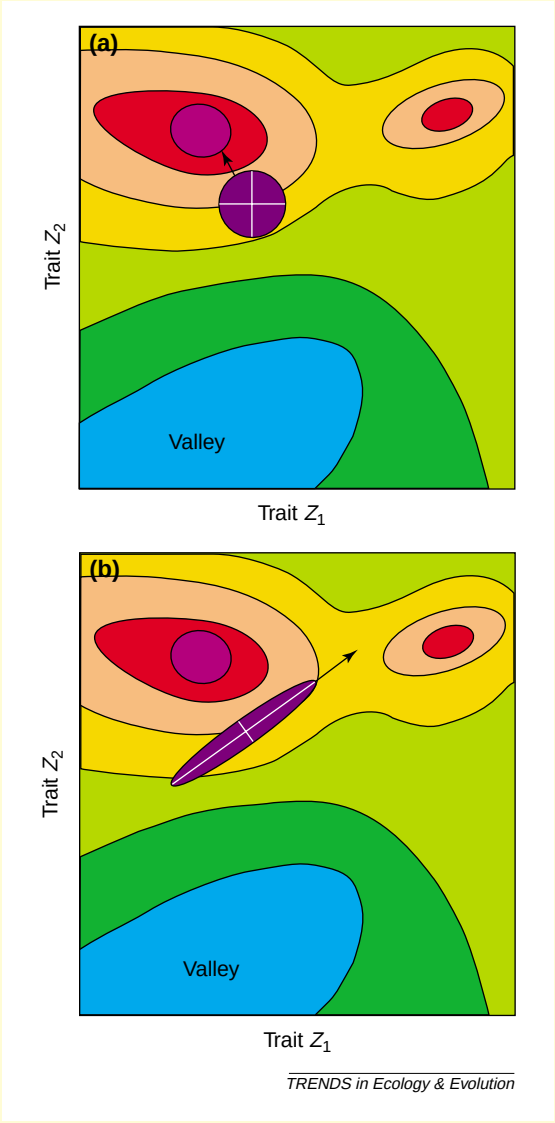

Fig. I. Genetic constraints because of **G** on adaptation. (a) A population in purple sits in an adaptive landscape with two local optima or peaks (red) and a valley (blue). The nearer peak on the left has higher fitness (as indicated by the magenta color) than does the peak on the right. The population has moderate genetic variation for both traits and no correlation or covariance between traits, as indicated by the circular dispersion. The selective gradient (**β**) is a measure of the strength of selection and in this graphical metaphor is equal to the slope of the landscape. Populations will evolve uphill owing to selection. In (a) this population would evolve directly up the fitness peak, which is the global optimum for this region of character space, as shown by the arrow. (b) In this example, the only difference is the strong covariation between traits Z_1 and Z_2 (e.g. Z_1, length of forelimbs; Z_2, length of hind limbs), producing the acute ellipse. The **G** matrix describes the size and shape of the ellipse. Both traits have similar amounts of variation to the example in Fig. Ia, but in this example, there is almost no covariation in the direction that selection would move the population. Instead, the population is likely to move across the slope in the direction of greatest variation (arrow) and could cross the shallow saddle, after which selection would be in the same direction as the main axis of variation. It would then evolve quickly up to the local optimum on the right. This example illustrates that even complete knowledge of the selective forces might not allow one to predict an evolutionary outcome without knowledge of **G**.

References

a Falconer, D.S. and MacKay, T.F.C. (1996) *Introduction to Quantitative Genetics*, Longman Group Ltd

b Lande, R. (1979) Quantitative genetic analysis of multivariate evolution, applied to brain:body size allometry. *Evolution* 33, 402–416

c Lande, R. and Arnold, S.J. (1983) The measurement of selection on correlated characters. *Evolution* 37, 1210–1226

d Price, T. *et al.* (1993) Peak shifts produced by correlated response to selection. *Evolution* 47, 280–290

e Kirkpatrick, M. and Lofsvold, D. (1992) Measuring selection and constraint in the evolution of growth. *Evolution* 46, 954–971

Box 2. Comparing matrices: power and model dependence

A major issue for any comparative quantitative genetic study is statistical power. The genetic variance components that comprise **G**, the genetic variance–covariance matrix, have large sampling errors [a], and so measurements of hundreds of families are usually necessary to provide reasonable power for comparisons. By contrast, sample size for **P**, the phenotypic variance–covariance matrix, is the number of individuals, where sample size for **G** depends on the number of families, usually far less than the number of individuals. Estimating **G** normally requires controlled breeding programs; **P** does not. Because most **G** matrix studies use fewer families, studies are biased towards confirming the null hypothesis. Interpretation is, however, complicated by the use of diametrically opposed null hypotheses, common among older methods of comparing **G** matrices. Matrix correlations test the null hypothesis of no similarity between matrices [b], whereas maximum-likelihood [c] or element-by-element comparisons test the null hypothesis that matrices or a subset of their elements are equal. Few studies have adequately addressed limitations of power when trying to compare covariances [d,e], and the power of more versatile methods, such as common principal components analysis (CPCA), is currently unknown [f].

Findings of matrix similarity are also highly dependent on the model being tested. Principal components analysis (PCA), the parent technique on which CPCA depends, transforms the data from the space of the original variables, which are correlated, to a set of vectors that are uncorrelated. It captures all of the variation in the original data, whilst concentrating the variation explained in a few vectors. The forte of PCA is therefore summarizing high-dimensional data with fewer, uncorrelated variables. Flury developed CPCA to summarize multigroup data in as few vectors as possible [g–j], but evolutionary biologists often have the loftier goal of diagnosing and understanding the differences between matrices, and the method has significant shortcomings for this purpose [f].

The default implementation of CPCA orders vectors to be compared by the amount of variance explained. If these first vectors differ, matrices are declared unrelated. It is biologically plausible that populations might differ in the first vector, often size in morphological data sets, but have similarities in other aspects of variation. CPC vectors can be considered in any order, and the Phillips software [k] allows such reordering. PCA also constrains all of the vectors to be orthogonal (uncorrelated), so the vectors with large amounts of variation constrain the directions of all other vectors. Flury [h,l] proposed a more general approach, called common space analysis, which, in principle, allows any set of vectors to be compared. We know of no other implementations of common space analysis. Alternative methods of finding hidden similarities in matrix structure are needed.

References

a Lynch, M. and Walsh, B. (1998) *Genetics and Analysis of Quantitative Traits*, Sinauer Assoc., Inc.
b Lofsvold, D. (1986) Quantitative genetics of morphological differentiation in *Peromyscus*. I. Tests of homogeneity of genetic covariance structure among species and subspecies. *Evolution* 40, 559–573
c Shaw, R.G. (1991) The comparison of quantitative genetic parameters between populations. *Evolution* 45, 143–151
d Klein, T.W. (1974) Heritability and genetic correlation: statistical power, population comparisons, and sample size. *Behav. Genet.* 4, 171–189
e Phillips, P.C. (1998) Designing experiments to maximize the power of detecting correlations. *Evolution* 52, 251–255
f Houle, D. *et al.* (2002) Interpretation of the results of common principal components analyses. *Evolution* 56, 433–440
g Airoldi, J-P. and Flury, B.K. (1988) An application of common principal component analysis to cranial morphometry of *Microtus californicus* and *M. ochrogaster* (Mammalia, Rodentia). *J. Zool.* 216, 21–36
h Flury, B. (1988) *Common Principal Components and Related Multivariate Models*, John Wiley & Sons
i Klingenberg, C.P. *et al.* (1996) Ontogeny and individual variation: analysis of patterned covariance matrices with common principal components. *Syst. Biol.* 45, 135–150
j Badyaev, A.V. and Martin, T.E. (2000) Individual variation in growth trajectories: phenotypic and genetic correlations in ontogeny of the house finch (*Carpodacus mexicanus*). *J. Evol. Biol.* 13, 290–301
k Phillips, P. (1998) *CPC – Common Principal Component Analysis Program*, http://darkwing.uoregon.edu/~pphil/software.html
l Flury, B.K. (1987) Two generalizations of the common principal component model. *Biometrika* 74, 59–69

absence of variation for some phenotypic combinations would predict that those phenotypic combinations cannot evolve [21]. Conversely, those combinations with the most genetic variation might be more likely to evolve [22]. Roff [15] has focused attention on whether matrices remain linearly related to each other, because an expectation is that, if drift is the only force causing differences in **G**, all elements of **G** would tend to increase or decrease in concert. Several statistical techniques that allow more subtle questions about **G** to be addressed have recently been implemented (Table 1). We focus our discussion on common principal component analysis (CPCA), because of its recent surge in popularity.

Maximum-likelihood methods [23] can be used to test a wide variety of hypotheses about variance–component matrices as well as about equality. With a well-estimated data set, this method permits statistically precise statements about which parts of the matrix differ and by how much, usually through the separate analysis of submatrices. This approach deserves wider application.

CPCA, and the subsequent use of the Flury hierarchy of hypothesis tests [24], has recently been adapted for use with variance–component matrices, such as **G** [25]. The Flury hierarchy determines how many of the principal components-based vectors differ among matrices. The method can thus discriminate matrices with a wide variety of levels of shared structure, including equality (not significantly different), proportionality (unequal, but the hypothesis of proportional eigenvalues is not rejected), CPC, partial CPC, and unrelated (no shared structure; see Box 3 and previous reviews [16,25]). Publicly available software [26,27] has now made CPCA the method of choice for comparing **P** matrices [19] and **G** matrices [8].

Any conclusions regarding matrix similarity depend strongly on the model being tested (Box 2). For all its advantages, the CPCA method makes sequential comparisons of orthogonal vectors. A finding that two matrices are 'unrelated' in a CPCA does not mean that there are no similarities, but rather that all of the tested null hypotheses fit less well than do the alternative hypothesis of no similarity. For example, Steppan's [16] CPCA of **P** matrices never found common structure among leaf-eared mice *Phyllotis* spp. By contrast, sample-size-adjusted matrix correlations averaged 0.93 among species, indicating that matrices were still very similar. Studies of **P** typically show a complete loss of CPC structure (e.g. dropping from proportional to

Table 1. Methods for matrix comparison

Method	Approach	Strengths	Weaknesses	Refs[a]
Element x element	t-test	Detailed, isolates specific elements	No synthesis, ignores nonindependence of elements	[6,55]
Matrix correlation	Correlation, permutation tests	Overall measure of similarity	Does not distinguish among many types of difference; ignores proportional changes; pairwise comparisons only; easily influenced by a few shared values; improper hypothesis-testing framework	[12–14,54, 56]
Matrix regression	Regression	Estimates of proportionality	Can be strongly influenced by outliers (especially in covariance matrices); improper hypothesis-testing framework	[4,15]
Disparity		Overall measure of difference, most easily applied to phylogenetic data	No clear metric; no integral statistical model	[33]
Maximum likelihood	Likelihood	Statistical power, applicable at several levels	Pairwise comparisons only, does not compare matrix structure	[3,6,23,57]
CPCA	Principal components	Statistical power, hierarchy of models; multiple comparisons	Orthogonality of components might not reflect biology; does not incorporate nonindependence owing to phylogeny	[8,11,16–19, 24,25, 29–31,40]
CPC for dependent vectors	Principal components	Based on CPC method; takes extra covariance patterns (e.g. growth, environment) into account	Same limitations as CPC approach, with even more restrictions on the pattern of shared relationships across covariance sets; has yet to be extended for genetic covariance components	[58,59]
Matrix pattern	Correlation	Nonparametric model of matrix structure derived from functional and/or developmental models	Shares all of the problems of matrix correlation; unclear how to compare different models	[60]
Confirmatory factor analysis	Factor analysis; linear models	Tests explicit structural models that can be biologically motivated; allows hypothesis testing of different models	Not well developed for comparative analyses; has yet to be extended to genetic covariance components	[42]

[a]Please note that the references are examples and are not necessarily comprehensive.

unrelated) with more inclusive clades, rather than a sequential loss of the smaller EIGENVECTORS. That is, PC1 often differs among very similar matrices, leading CPCA to declare them 'unrelated'. The development of more general models of similarity is needed (Table 1). An alternative approach is to explore the dimensionality of **G** (Box 4), which might be able to detect conservation of underlying structure that the CPCA model might miss.

How fast do G matrices change?

This now appears to be the crucial question given the observation that **G** can evolve. The diversity of methods used in published studies makes comparison of their results difficult. Comparisons among closely related populations most frequently show no significant differences [7]. Considering **P** as well as **G** in the increasing number of CPCA studies (only three sets of studies [8,25,28,29] have applied CPCA to **G**), findings have ranged from proportionality [19,29] to no shared structure [18,30], to intermediate conditions with several CPCs [11,16,18,31]. In some cases, the degree of shared CPC structure depends strongly on the method of data standardization [32]. Above the species level, most studies [7] find significant differences in **G**. In addition, no published study (of **G** or **P**) among subspecies or at more inclusive taxonomic levels has accepted shared structure at more than the first two to three eigenvectors [16,17,19,28]. Thus, **G** or **P** are usually not significantly different among phenotypically similar populations, but statistically significant differences are the norm among phenotypically divergent populations (e.g. subspecies and species [33]). Comprehensive multitaxon studies are needed to confirm these tentative conclusions about the rates of **G** matrix evolution.

The rate of **G** matrix evolution is best determined through the comparative approach. When characters evolve slowly relative to cladogenesis, the COMPARATIVE METHOD is needed to account for phylogeny (to avoid correlations among observations [34]) – in this case matrices – and to estimate more accurately the direction and rate of evolution. Just as several methods can be used to compare two matrices, there are several approaches to structuring comparative analyses: single-pair comparisons, hierarchical multigroup comparison and ancestral reconstruction.

All but one [14] study of **G** have compared just two taxa . Such comparisons contain no phylogenetic information and therefore cannot determine direction of change.

A second approach is the hierarchical application of matrix-comparison methods, particularly those that can analyse multiple matrices, such as CPCA. In CPCA, for example, all members of a clade are analysed together for shared structure, and the analysis is repeated for all clades [16]. Another application is to conduct all pairwise comparisons among members of a clade, taxon, or taxonomic

category, [16] and partition the comparisons among categories or ranks. However, this incorporates minimal phylogenetic information and the degrees of freedom must be reduced to reflect the multiple comparisons. Interpretation of hierarchical analyses becomes more difficult as the number of lineages increases (Box 3).

The third and potentially most powerful method is ancestral reconstruction, which allows change to be partitioned among branches of a phylogeny [33] (Box 3). Once ancestral matrices are estimated, any of the matrix-comparison methods (Table 1) can be employed. Unfortunately, significant error can also arise in estimating ancestors [35], and that problem is likely to be exacerbated with correlated multivariate data. Uncertainty can be accommodated in a likelihood or bayesian framework [36]. In addition, although comparative studies of individual characters can sometimes verify ancestral conditions from fossils, such verification will be difficult for variances, which are properties of populations. **G** will almost always be impossible to measure for ancestors (but see [37]), but **P** can sometimes be estimated. The most commonly used application of this general approach, independent contrasts [34], is unlikely to be appropriate to the questions asked by quantitative geneticists, because it tests evolutionary correlations over time rather than decomposing the nature of changes. Maximum likelihood methods, already applied to univariate data [35], can be modified for correlated multivariate data, although the errors involved in those estimates can be very high for biologically interesting features that vary significantly.

Why do G matrices change?

Given the many evolutionary forces that are expected to buffet **G**, the observed differences in **G** matrix structure are not surprising. Mutation, selection, genetic drift and migration are all expected to affect **G** [20,38]. A more productive focus might therefore be on cases in which **G** might be expected to retain shared structure over time. Genetic drift provides an obvious starting point, because drift in a population of reduced effective size is expected to cause a proportional shrinking of all elements in **G**. This expectation has been proposed by Roff as a way to distinguish the effects of drift and selection [15]: proportional changes in **G** matrix structure are ascribed to drift and nonproportional changes to selection. Although appealing, this dichotomy has a flaw. Although proportionality is the theoretical expectation for drift, a great deal of variation around this expectation is likely. A large study on the effects of drift on wing morphology in *Drosophila* has demonstrated the extent of this variation [11]. Given enough time, any pattern of divergence among **G** matrices would probably be compatible with the hypothesis of drift. More generally, matrices may diverge by drift even when the effective sizes of the populations are equal. We note that CPCA and

Box 3. Evolution of matrices

Figure I illustrates the hierarchy of models in common principal components analysis (CPCA). Compared to the root ancestor A, ancestor C and descendants 1, 2 and 3 are unequal but proportional. The eigenvectors (orientation of axes) are the same, whereas the eigenvalues (variances along each axis) all differ by a scalar amount. Descendant 5 shares the eigenvectors with ancestor A, but the eigenvalues for the two axes do not differ by the same amount. Thus, they share a common principal component (CPC) structure (proportionality is a special case of CPC). Descendant 4 differs by both orientation and relative variances and therefore for these two dimensions, shares no common structure with A (unrelated). If, however they did share other axes for dimensions not plotted here, then descendant 4 and ancestor A would share partial CPC (PCPC). Thus, several levels in the hierarchy are portrayed by taxa in reference to the root ancestor A; equality (B), proportionality (C, 1–3), CPC (5) and unrelated (4). Further discussion of the CPC hierarchy can be found in [a,b].

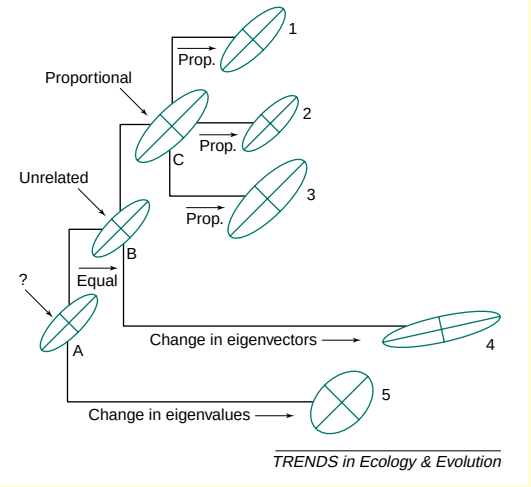

Fig. I. Hypothetical evolution of genetic variance–covariance matrices on a phylogeny. Text below branches summarizes changes along a lineage (branch). Length of each branch is proportional to the magnitude of change in covariance structure as estimated by, for example, matrix disparity (except for the branch from A to B, which should have zero length but is expanded for visual clarity). Ancestors A, B, and C and descendents 1, 2, and 3 all share proportional matrices. The total variation among them has changed, but the pattern of covariation has not. Text above the nodes indicates degree of shared principal component structure expected to be revealed by CPCA given the changes along the branches. Ancestral matrices are shown here but cannot be observed directly in most real groups. Estimation of ancestral matrices, like that of any character, can be difficult under bias or lineage variation in rates [c]. For example, the ancestor associated with arbitrary CPC model (B) would not be estimated accurately; the information from the highly divergent descendant would skew the estimate. Also, although three of four extant taxa in the clade share proportionality with the ancestor, CPCA would probably detect no shared structure among the four because of the one divergent taxon, 4.

References

a Phillips, P.C. and Arnold, S.J. (1999) Hierarchical comparison of genetic variance–covariance matrices. I. Using the Flury hierarchy. *Evolution* 53, 1506–1515

b Roff, D. (2000) The evolution of the **G** matrix: selection or drift? *Heredity* 84, 135–142

c Cunningham, C.W. *et al.* (1998) Reconstructing ancestral character states: a critical reappraisal. *Trends Ecol. Evol.* 13, 361–366

Box 4. Dimensionality of G

An alternative perspective on the role of the genetic variance–covariance matrix, **G**, as an evolutionary constraint is to examine its dimensionality. The data from a quantitative genetic study exist in a space with axes defined by each trait in the study – if many traits are studied, the phenotype space has many dimensions. If one could plot all the breeding values from a study, they might fall in a subspace of this phenotype space. For three traits, phenotype space has a dimensionality of three, but we might find that all the points fall on a plane, so the data reside in a 2D space. One would then predict that evolution would be restricted to the plane. A matrix in which the dimensionality of the data is lower than the dimensionality of the phenotype is called a singular matrix.

CPC provides one framework to explore dimensionality, but Kirkpatrick *et al.* [a] proposed and implemented several approaches to estimate explicitly the dimensionality of genetic variation. Their techniques were developed for the more complex case where one is interested in genetic variation in a biological function, such as a growth trajectory, but they could easily be applied to the typical point estimates of genetic parameters. Application of these techniques to data sets on growth in several mammals suggests that only a few aspects of growth and form could be shown to be genetically variable [b]. Kirkpatrick *et al.*'s techniques are mostly modifications of more widely used techniques in multivariate analysis and might not represent optimal solutions to this statistical problem. More research on this problem is needed. Surprisingly little empirical attention has been paid to dimensionality of **G**, even though it is frequently mentioned in the literature on constraints [c].

References

a Kirkpatrick, M. *et al.* (1990) Analysis of the inheritance, selection and evolution of growth trajectories. *Genetics* 124, 979–993

b Kirkpatrick, M. and Lofsvold, D. (1992) Measuring selection and constraint in the evolution of growth. *Evolution* 46, 954–971

c Maynard Smith, J. *et al.* (1985) Developmental constraints and evolution. *Q. Rev. Biol.* 60, 265–287

Roff's test differ in the definition of proportionality; elements related by a scalar multiplier versus linear regression constrained to pass through the origin as a predictor of elements, respectively. Much more work is needed on the nature of the variance in **G** generated by drift and on identifying the timescales that are actually relevant for divergence, which are especially important for providing a meaningful null hypothesis against which comparative analyses can be tested.

Both selection and mutation can also maintain similarity in **G** matrix structure under certain circumstances [38]. In particular, when the pattern of correlational selection matches the pattern of genetic covariation, selection can maintain this association [38]. Mutation might be the most important player in this process. Long-term evolution of **G** might be dominated by the pattern of pleiotropic mutation. We know little about the nature of pleiotropic mutations [39] (the **M** matrix) and certainly have no observations of the relationship between **M** and variation in **G** among populations. There have been few studies of **M**, but induced mutations can cause significant changes in **M** within species [28] and, consequently, to **G** and **P** as well [40]. Ultimately, the evolution of **G** will be guided by all of the forces that affect the evolution of genetic variance itself. In this case, a comparative approach might actually yield new insights, because the multivariate nature of the data allows one to ask how evolutionary forces are influencing the covariance structure of an entire suite of characters rather than trying to tease apart multiple influences on a single trait.

Comparative quantitative genetics should be strongly influenced by, and potentially influence, the emerging synthesis between functional developmental genomics and studies of quantitative variation (e.g. [41]). First, finer-scale genetic information, especially regarding relationships among traits, is needed for modeling long-term evolution of **G**. Second, developmental models can be turned into statistical models of covariance structure [42], which will be needed for more meaningful comparisons among matrices. Observations about the evolution of **G** might provide insights into the forces affecting **G** and more importantly into the underlying processes that generate the covariance structure in the first place [43]. A comparative approach to **G** matrix evolution should provide insights into macroevolutionary changes in developmental structure.

Are G and P matrices similar?

The elements of **P** can be estimated much more accurately than can those of **G** (Box 2), because sampling errors scale with the inverse of the sample size. This has led some researchers to suggest that the **P** matrix will provide a more precise estimate of the form of **G** [8,44,45] should they be proportional. The **P** matrix is the sum of the **G** matrix and all other sources of covariation, including genetic covariance not contained in **G**, and environmental covariation (**E**). Both genetic and nongenetic causes of covariance can be structured by the functional architecture that underlies the traits. If each hormone or regulatory gene that helps to build a trait provides an opportunity for both genetic and nongenetic effects to occur, then genetic and nongenetic variances will be correlated. This hypothesis can be tested by a direct comparison of **G** and **E** [8]. Consistent with this notion, genetic and phenotypic variances are very highly correlated [46,47]. However, there are many reasons why **G** and **P** might depart from proportionality [48]. Comparisons of **P** have typically found more divergence more frequently than have comparisons of **G**, particularly within species.

Roff [44] tested the correlation of **P** and **G** by a survey of the literature. He found that phenotypic and genetic correlations were as correlated with each other as could be expected if they only differed because of sampling errors. The correspondence was particularly good for morphological traits, which tend to have high heritabilities (i.e. **G** is a large proportion of **P**). Evolutionary forces, such as genetic drift, might be expected to have different effects on **G** and **E**, and thereby lead to divergence in **P** even if these underlying matrices share similarities [11]. Particular phenotypic and genetic correlations certainly differ significantly in some cases, but, overall, there is surprisingly little empirical evidence to reject the hypotheses that **P** and **G** are proportional. Further testing of this conjecture is still needed. Nearly all of the multitaxon comparisons to date have involved **P** matrices [16,17,19,30,33,49]

Glossary

Adaptive landscape: a representation of the forces of natural selection where phenotypic trait values are the X and Y coordinates and mean fitness is the elevation.
Comparative quantitative genetics: the comparative study of quantitative genetic parameters, especially covariance matrices, across populations or species.
Comparative method: the application of phylogenetic information to cross-taxon comparisons.
CPCA: common principal components analysis; a generalization of principal components analysis extended to multiple matrices.
Eigenvectors: latent or characteristic-roots of the variance–covariance matrix; they define the orientation in multidimensional space of the orthogonal axes of maximum variation.
Genetic variance–covariance matrix: a symmetrical matrix that summarizes the additive genetic contribution to the variances of and covariances between phenotypic traits. **G** matrix or **G** covariance matrix are shorthand references.
Phenotypic matrix: phenotypic variance–covariance matrix, measured directly for a population without partitioning out genetic and environmental contributions.

rather than **G** matrices, presumably because of this disparity in ease of estimation.

Conclusions and future directions

Clearly, **G** can evolve. The important questions now are what parts of **G** evolve, what is the rate at which **G** evolves, and how does that rate compare to the rate of speciation, population differentiation and changes in the adaptive landscape? Empirical and theoretical studies are needed, as are new or improved analytical methods. Empirical studies are needed to test assumptions about the relationship between **G** and **P**, for example. Perhaps the greatest need is for studies that robustly estimate **G** for multiple taxa. The most efficient approach might be to study taxa related to those that have already been studied and to build on earlier studies rather than duplicating them. With thoughtful species selection, this approach can also be used to expand morphological diversity. The clade containing the well-studied mouse *Mus* and rat *Rattus*, for example, includes many ecologically and morphologically divergent species, including grazing and earthworm specialists. Greater diversity can also be achieved by including groups outside the model organisms that have been the primary focus of past studies. Developmental [50] and integrative [51] approaches have great potential to provide explicit hypotheses, which would provide stronger theoretical and mechanistic frameworks for the study of changes in **G**.

The greatest need on the analytical side is for improved methods of matrix comparison that are statistically powerful, biologically meaningful, robust and that allow decomposition of the data. Although CPCA has been widely adopted, we see it as an interim method that will remain useful only until more appropriate methods are developed. Modifications of factor analytic methods, such as confirmatory factor analysis [42] or common space analysis [24], which relax the assumption of orthogonality in PCA methods, are potential next steps. Provided that **G** does not evolve quickly with respect to the species and clades of interest to evolutionary biologists, improved methods of ancestral reconstruction for multivariate data should be a focus of comparative studies. The field is also hampered even in formulating scientific questions by the difficulties of visualizing such complex data. New visualization techniques are being developed (e.g. [52]) and could be adapted to, or new ones developed for, evolutionary studies.

Finally, we have only begun to ask some of the most interesting questions. For example, is **G** evolution decoupled from phenotypic evolution? That appears to be the case with **P** matrices in *Phyllotis* [16] and **G** within *Clarkia dudleyana* [53]. The **G** matrix, treated as a character in its own right, can be used to explore the evolution of developmental systems and their role in phenotypic evolution. A comparative quantitative genetic approach should provide a natural linkage between studies concentrating primarily on genetic details and those focusing on long-term phenotypic outcomes.

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References

1 Parker, G.A. and Maynard Smith, J. (1990) Optimality theory in evolutionary biology. *Nature* 348, 27–33
2 Stern, D.L. (2000) Perspective: evolutionary developmental biology and the problem of variation. *Evolution* 54, 1079–1091
3 Shaw, F.H. *et al.* (1995) Changes in genetic variances and covariances: **G** whiz! *Evolution* 49, 1260–1267
4 Arnold, S.J. (1992) Constraints on phenotypic evolution. *Am. Nat.* 140, S85–S107
5 Baum, D.A. and Donoghue, M.J. (2001) A likelihood framework for the phylogenetic analysis of adaptation. In *Adaptation and Optimality* (Orzack, S.H. and Sober, E., eds), pp. 24–44, Cambridge University Press
6 Paulsen, S.M. (1996) Quantitative genetics of the wing color pattern in the buckeye butterfly (*Precis coenia* and *Preceis evarete*): evidence against the constancy of **G**. *Evolution* 50, 1585–1597
7 Roff, D.A. and Mousseau, T.A. (1999) Does natural selection alter genetic architecture? An evaluation of quantitative genetic variation among populations of *Allonemobius socius* and *A. fasciatus*. *J. Evol. Biol.* 12, 361–369
8 Arnold, S.J. and Phillips, P.C. (1999) Hierarchical comparison of genetic variance–covariance matrices. II. Coastal-inland divergence in the garter snake *Thamnophis elegans*. *Evolution* 53, 1516–1527
9 Wilkinson, G.S. *et al.* (1990) Resistance of genetic correlation structure to directional selection in *Drosophila melanogaster*. *Evolution* 44, 1990–2003
10 Bryant, E.H. and Meffert, L.M. (1988) Effect of an experimental bottleneck on morphological integration in the housefly. *Evolution* 42, 698–707
11 Phillips, P.C. *et al.* (2001) Inbreeding changes the shape of the genetic covariance matrix in *Drosophila melanogaster*. *Genetics* 158, 1137–1145
12 Kohn, L.A.P. and Atchley, W.R. (1988) How similar are genetic correlation structures? Data from mice and rats. *Evolution* 42, 467–481
13 Atchley, W.R. *et al.* (1992) Evolutionary divergence, shape change, and genetic correlation structure in the rodent mandible. *Syst. Biol.* 41, 196–221
14 Lofsvold, D. (1986) Quantitative genetics of morphological differentiation in *Peromyscus*. I. Tests of homogeneity of genetic covariance structure among species and subspecies. *Evolution* 40, 559–573
15 Roff, D. (2000) The evolution of the **G** matrix: selection or drift? *Heredity* 84, 135–142
16 Steppan, S.J. (1997) Phylogenetic analysis of phenotypic covariance structure. I. Contrasting results from matrix correlation and common principal component analyses. *Evolution* 51, 571–586
17 Ackermann, R.R. and Cheverud, J.M. (2000) Phenotypic covariance structure in tamarins (genus *Saguinus*): A comparison of variation patterns using matrix correlation and common principal component analysis. *Am. J. Phys. Anthropol.* 111, 489–501
18 Badyaev, A.V. and Hill, G.E. (2000) The evolution of sexual dimorphism in the house finch. I. Population divergence in morphological covariance structure. *Evolution* 54, 1784–1794
19 Dodd, R.S. *et al.* (2000) Evolutionary divergence in the pan-Atlantic mangrove *Avicennia germinans*. *New Phytol.* 145, 115–125
20 Turelli, M. (1988) Phenotypic evolution, constant covariances, and the maintenance of additive variance. *Evolution* 42, 1342–1347

21 Lande, R. (1979) Quantitative genetic analysis of multivariate evolution, applied to brain:body size allometry. *Evolution* 33, 402–416
22 Schluter, D. (1996) Adaptive radiation along genetic lines of least resistance. *Evolution* 50, 1766–1774
23 Shaw, R.G. (1991) The comparison of quantitative genetic parameters between populations. *Evolution* 45, 143–151
24 Flury, B. (1988) *Common Principal Components and Related Multivariate Models*, John Wiley & Sons
25 Phillips, P.C. and Arnold, S.J. (1999) Hierarchical comparison of genetic variance–covariance matrices. I. Using the Flury hierarchy. *Evolution* 53, 1506–1515
26 Klingenberg, C.P. (1995) *dCPC: Common Principal Components for Dependent Random Vectors*, ftp://life.bio.sunysb.edu/morphmet/dcpc.exe
27 Phillips, P. (1998) *CPC – Common Principal Component Analysis Program*, http://darkwing.uoregon.edu/~pphil/software.html
28 Camara, M.D. and Pigliucci, M. (1999) Mutational contributions to genetic variance–covariance matrices: an experimental approach using induced mutations in *Arabidopsis thaliana*. *Evolution* 53, 1692–1703
29 Pfrender, M.E. and Lynch, M. (2000) Quantitative genetic variation in *Daphnia*: temporal changes in genetic architecture. *Evolution* 54, 1502–1509
30 Pigliucci, M. *et al.* (1999) Evolution of phenotypic plasticity a comparative approach in the phylogenetic neighbourhood of *Arabidopsis thaliana*. *J. Evol. Biol.* 12, 779–791
31 Donohue, K. *et al.* (2000) Density dependence and population differentiation of genetic architecture in *Impatiens capensis* in natural environments. *Evolution* 54, 1969–1981
32 Waldmann, P. and Andersson, S. (2000) Comparison of genetic (co)variance matrices within and between *Scabiosa canescens* and *S. columbaria*. *J. Evol. Biol.* 13, 826–835
33 Steppan, S.J. (1997) Phylogenetic analysis of phenotypic covariance structure. II. Reconstructing matrix evolution. *Evolution* 51, 587–594
34 Felsenstein, J. (1985) Phylogenies and the comparative method. *Am. Nat.* 125, 1–15
35 Schluter, D. *et al.* (1997) Likelihood of ancestor states in adaptive radiation. *Evolution* 51, 1699–1711
36 Huelsenbeck, J.P. *et al.* (2000) Accommodating phylogenetic uncertainty in evolutionary studies. *Science* 288, 2349–2350
37 Cheetham, A.H. *et al.* (1994) Quantitative genetics of bryozoan phenotypic evolution. 2. Analysis of selection and random change in fossil species using reconstructed genetic parameters. *Evolution* 48, 360–375
38 Lande, R. (1980) The genetic covariance between characters maintained by pleiotropic mutations. *Genetics* 94, 203–215
39 Houle, D. *et al.* (1996) Comparing mutational variabilities. *Genetics* 143, 1467–1483
40 Camara, M.D. *et al.* (2000) Induced mutations: a novel tool to study phenotypic integration and evolutionary constraints in *Arabidopsis thaliana*. *Evol. Ecol. Res.* 2, 1009–1029
41 Mackay, T.F.C. (2001) Quantitative trait loci in *Drosophila*. *Nat. Rev. Genet.* 2, 11–20
42 Zelditch, M.L. *et al.* (1990) Variation in developmental constraints in *Sigmodon*. *Evolution* 44, 1738–1747
43 Houle, D. (1991) Genetic covariance of fitness correlates – what genetic correlations are made of and why it matters. *Evolution* 45, 630–648
44 Roff, D.A. (1997) *Evolutionary Quantitative Genetics*, Chapman & Hall
45 Cheverud, J.M. (1988) A comparison of genetic and phenotypic correlations. *Evolution* 42, 958–968
46 Houle, D. (1992) Comparing evolvability and variability of quantitative traits. *Genetics* 130, 195–204
47 Waitt, D.E. and Levin, D.A. (1998) Genetic and phenotypic correlations in plants: a botanical test of Cheverud's conjecture. *Heredity* 80, 310–319
48 Willis, J.H. *et al.* (1991) Can one predict the evolution of quantitative characters without genetics? *Evolution* 45, 441–444
49 Armbruster, W.S. *et al.* (1999) Covariance and decoupling of floral and vegetative traits in nine neotropical plants: a re-evaluation of Berg's correlation–pleiades concept. *Am. J. Bot.* 86, 39–55
50 Nemeschkal, H.L. (1999) Morphometric correlation patterns of adult birds (Fringillidae: Passeriformes and Columbiformes) mirror the expression of developmental control genes. *Evolution* 53, 899–918
51 Chernoff, B. and Magwene, P.M. (1999) Afterword. In *Morphological Integration* (Olson, E.C. and Miller, R.L., eds), pp. 319–348, The University of Chicago Press
52 Meng, Z. and Pao, Y.H. (2000) Visualization and self-organization of multidimensional data through equalized orthogonal mapping. *IEEE Trans. Neural Netw.* 11, 1031–1038
53 Podolsky, R.H. *et al.* (1997) Population structure of morphological traits in *Clarkia dudleyana*. II. Constancy of within-population genetic variance. *Evolution* 51, 1785–1796
54 Cowley, D.E. and Atchley, W.R. (1990) Development and quantitative genetics of correlation structure among body parts of *Drosophila melanogaster*. *Am. Nat.* 135, 242–268
55 Brodie, E.D., III (1993) Homogeneity of the genetic variance–covariance matrix for antipredator traits in two natural populations of the garter snake *Thamnophis ordinoides*. *Evolution* 47, 844–854
56 Spitze, K. *et al.* (1991) The covariance structure of life-history characters in *Daphnia pulex*. *Evolution* 45, 1081–1090
57 Service, P.M. (2000) The genetic structure of female life history in *D. melanogaster*: comparisons amoung populations. *Genet. Res.* 75, 153–166
58 Klingenberg, C.P. *et al.* (1996) Ontogeny and individual variation: analysis of patterned covariance matrices with common principal components. *Syst. Biol.* 45, 135–150
59 Neuenschwander, B.E. and Flury, B.D. (2000) Common principal components for dependent random vectors. *J. Multivar. Anal.* 75, 163–183
60 Cheverud, J.M. *et al.* (1989) Methods for the comparative-analysis of variation patterns. *Syst. Zool.* 38, 201–213