Molecular Ecology (2006) **15**, 883–896 doi: 10.1111/j.1365-294X.2006.02809.x

INVITED REVIEW

Studying phenotypic evolution using multivariate quantitative genetics

KATRINA McGUIGAN
Center for Ecology and Evolutionary Biology, 5289 University of Oregon, Eugene, OR 97403, USA

Abstract

Quantitative genetics provides a powerful framework for studying phenotypic evolution and the evolution of adaptive genetic variation. Central to the approach is G, the matrix of additive genetic variances and covariances. G summarizes the genetic basis of the traits and can be used to predict the phenotypic response to multivariate selection or to drift. Recent analytical and computational advances have improved both the power and the accessibility of the necessary multivariate statistics. It is now possible to study the relationships between G and other evolutionary parameters, such as those describing the mutational input, the shape and orientation of the adaptive landscape, and the phenotypic divergence among populations. At the same time, we are moving towards a greater understanding of how the genetic variation summarized by G evolves. Computer simulations of the evolution of G, innovations in matrix comparison methods, and rapid development of powerful molecular genetic tools have all opened the way for dissecting the interaction between allelic variation and evolutionary process. Here I discuss some current uses of G, problems with the application of these approaches, and identify avenues for future research.

Keywords: adaptive landscape, additive genetic variance, eigenanalysis, G-matrix, mutational variance, selection

Received 15 July 2005; revision accepted 20 October 2005

Introduction

Neutral molecular markers (e.g. microsatellites or allozymes) provide valuable insight into historical and contemporary population dynamics. However, variation at marker loci is unlikely to be an accurate predictor of genetic variation at loci contributing to phenotype, that is, the adaptive variation (Hard 1995; Reed & Frankham 2001; Moss *et al*. 2003). Adaptive variation is of interest because it both reflects historical evolution and determines the population's future phenotypic response to evolutionary processes. Although adaptive variation can be directly targeted with molecular tools, those tools are taxonomically restricted, and the complete characterization of adaptive variation in complex (polygenic) traits remains highly resource intensive (Barton & Keightley 2002; Erickson *et al*. 2004). Quantitative genetics provides an alternative approach for studying adaptive variation. Applied at the phenotypic level, quantitative genetic tools contrast with molecular tools in being both inexpensive and taxonomically widely applicable. However, the power and scope of the quantitative genetic approach can be improved through integration with neutral molecular tools. Synthesis of molecular and quantitative tools has already led to the Q_{ST} vs. F_{ST} relative rates test for inferring the evolutionary process driving phenotypic diversification (reviewed by Merilä & Crnokrak 2001). Also, molecular marker methods for inferring relationships could improve the power of parameter estimation methods (e.g. animal models: Lynch & Walsh 1998; Kruuk 2004).

The basic quantitative genetic parameter describing adaptive variation is the additive genetic variance (V_A: Box 1). Scaled by the total phenotypic variance to give the narrow sense heritability (h^2) the additive genetic variance predicts the rate of evolutionary response (R) to selection (S) (breeder's equation: see Falconer & Mackay 1996),

$$R = h^2 S.$$

However, selection generally acts on whole organisms, not on individual traits in isolation (Dobzhansky 1956; Lewontin

Correspondence: Katrina McGuigan, Present address: School of Integrative Biology, University of Queensland, St Lucia, Qld 4072, Australia. Fax: +61-7-3365-1655; E-mail: kmcguigan1@uq.edu.au

Box 1 Definitions of relevant quantitative genetic terms

Phenotypic variance: V_P, the variance of the population about the trait mean.

Additive genetic variance: V_A, the variance about the population trait mean due to the average additive effects of all contributing alleles at all contributing loci (Falconer & Mackay 1996; Lynch & Walsh 1998).

Heritability: h^2, the proportion of phenotypic variance due to additive genetic variance (V_A/V_P).

Additive genetic covariance: Cov_A, the additive genetic covariation of traits in the population. Cov_A is due to pleiotropic alleles (which contribute to multiple traits), and to linkage disequilibrium among alleles within a gamete or among gametes (Lande 1980; see also Phillips & McGuigan in press).

G: the matrix of additive genetic variances and covariances (Fig. 1). **G** is a symmetrical, square matrix with one row and one column per trait.

Eigenanalysis: a mathematical operation on a square, symmetric matrix. This analysis generates new variables, *eigenvectors*, which are linear combinations of the original traits (for **G**, the variances and covariances). These vectors have length, their *eigenvalue* (λ), which describes the (additive genetic) variance associated with that vector.

M: a symmetrical, square matrix summarizing the mutational variances and covariances for a suite of traits. **M** summarizes the (co)variance introduced into the population by novel mutations.

Adaptive landscape: Describes the relationship between average fitness and average phenotype (see Arnold *et al*. 2001; Fig. 2). The adaptive landscape can be described by a square, symmetric matrix and subject to eigenanalysis.

β: The selection gradient, which is estimated as the partial regression coefficients of fitness on the traits (Lande & Arnold 1983).

γ: The matrix describing the curvature of the individual fitness surface. This matrix can be subject to eigenanalysis to generate linear vectors of selection (Arnold *et al*. 2001).

ω: the negative inverse of γ (Arnold *et al*. 2001).

D: the matrix of variances and covariances among a suite of traits as estimated from trait means in each population (Lande 1979). **D** can also be extracted as variance components by MANOVA (McGuigan *et al*. 2005). **D** can also be subjected to eigenanalysis. The eigenvalues of **D** describe the distance (a relative measure of the amount of divergence) between populations and eigenvectors describe the direction of divergence. Schluter (1996) defined the first eigenvector of **D** as *z*. However, given the historical use of *z* as the symbol for an individual's multivariate phenotype it is recommended that *d* be the symbol for divergence vectors. **D** has as many rows and columns as traits, but the eigenanalysis can only recover as many vectors as the degrees of freedom at that level (in an unnested design, the number of taxa minus 1).

1970). Also, traits often share some of their genetic basis and the additive genetic covariation among traits (Box 1) prevents them from evolving independently (Lande 1979; Lande & Arnold 1983; Phillips & Arnold 1989; Schluter & Nychka 1994; Blows & Brooks 2003). Investigations of the evolutionary role of genetic covariation have generally focused on the trade-off between pairs of traits whereby traits are either selected in opposite directions while covarying positively, or are selected in the same direction but have negative genetic covariance (Dickerson 1955; Lande 1979, 1982; Rose 1982). Trade-offs among pairs of traits are conceptually tractable. However, studying pairs of traits might provide little improvement over the univariate approach. In reality, whole suites of traits interact both genetically and during selection. Also, genetic covariance can affect both the rate and direction of phenotypic evolution in the absence of either antagonistic selection or antagonistic pleiotropy (Lande 1979, 1980; Cheverud 1984; Zeng 1988; Björklund 1996; Schluter 1996; Arnold *et al*. 2001; Blows & Hoffmann 2005).

Integral to multitrait quantitative genetics is **G**, the matrix of additive genetic variances and covariances for a suite of traits (Fig. 1). **G** is not conceptually different from the parameters discussed above: each element of **G** is estimated as for individual traits (V_A) or pairs of traits (Cov_A) (Fig. 1). As with other quantitative genetic parameters, **G** is a statistical abstraction, estimated from phenotype without direct observation of the underlying details such as the number of contributing loci and alleles, modes of gene action, or individual allelic affects. Nonetheless, **G** is determined by allelic frequencies in the population and by the distribution of the additive phenotypic effects of those alleles (Lande 1980; see Phillips & McGuigan in press). The genetic information summarized by **G** is connected to evolutionary process through a number of relationships, particularly the multivariate breeder's equation (Lande 1979; Lande & Arnold 1983),

$$\Delta\bar{z} = \mathbf{G}\beta$$

A

$\mathbf{V}_{A1}$	$Cov_{A1,2}$	$Cov_{A1,3}$
$Cov_{A1,2}$	$\mathbf{V}_{A2}$	$Cov_{A2,3}$
$Cov_{A1,3}$	$Cov_{A2,3}$	$\mathbf{V}_{A3}$

B

	1	2	3	4	5	6
1	**2.833**	2.690	3.214	3.980	2.246	3.538
2	2.690	**2.816**	2.990	3.704	1.493	3.493
3	3.214	2.990	**2.396**	3.971	1.840	2.809
4	3.980	3.704	3.971	**5.709**	1.718	4.225
5	2.246	1.493	1.840	1.718	**4.419**	1.662
6	3.538	3.493	2.809	4.225	1.662	**3.111**

C

	PC1	PC2	PC3	PC4	PC5	PC6
λ	3.4E-06	1.9E-06	4E-07	2.9E-08	5E-09	1.5E-10
% variance	59.27	33.26	6.88	0.50	0.09	0.00
Trait 1	0.066	0.063	0.570	−0.147	1.698	34.220
Trait 2	0.146	−0.021	0.626	2.532	0.153	−31.691
Trait 3	0.083	0.278	−0.417	0.558	8.696	−5.593
Trait 4	0.301	0.306	0.194	−4.223	−3.978	−25.937
Trait 5	−0.555	0.715	0.746	0.353	−2.861	−20.358
Trait 6	0.130	0.249	−0.475	2.343	−6.229	21.429

Fig. 1 What is **G**? (A) The diagonals of **G** indicate how much additive genetic variance underlies each trait, while the off-diagonals summarize the additive genetic covariance between each pair of traits. (B) Quantitative genetics of morphology in a population of the Lake Eacham rainbow fish (*Melanotaenia eachamensis*) (see McGuigan *et al*. 2005). Six traits were measured: (1) standard length (a–f); (2) predorsal length (a–c measured along midline); (3) head depth (vertical distance through b); (4) body depth (vertical distance through c); (5) caudal peduncle length (d–e); and (6) caudal peduncle depth (e–g). **G** was estimated by regression of laboratory-reared offspring on wild-caught parents for 44 families (McGuigan *et al*. 2005). Values on the diagonal (× 1000) are greater than zero (although confidence intervals were not calculated), suggesting additive genetic variance for all traits. Off-diagonals (× 1000) are all positive, suggesting traits positively covary with one another. However, covariances are not equal: covariances involving trait 5 are smaller than for the other five traits, suggesting a difference in the allelic contributions to this trait. Although such patterns might be apparent from visual examination of **G** they are difficult to interpret without formal statistical analysis. (C) Eigenanalyses (Box 1), such as principal components analysis, are useful for interpreting **G** with respect to both the shared genetic basis of component traits, and the capacity of traits to respond individually to selection. The rainbow fish **G** (B) was subjected to a principal components analysis and components extracted from the covariance (rather than the correlation) matrix. The matrix was positive definite as all eigenvalues were greater than zero. However, the first two eigenvectors were considerably longer than subsequent vectors, explaining 92.53% of the variance in **G**. The noted difference among traits in covariance was readily apparent in the relative contributions of traits to the first (longest) eigenvector. Eigenvector 1 contrasts caudal peduncle length (trait 5) with all other traits. Most genetic variation in this population (59%) is for the caudal peduncle length to vary in the opposite direction to other body dimensions, particularly body depth (trait 4). Contributions in opposite directions suggest the presence of antagonistic pleiotropy (rather than absence of pleiotropic alleles) between caudal length and other traits. Selection for longer caudal peduncles would result in a correlated decrease in other traits, particularly body depth. However, caudal length also contributes strongly to the second eigenvector where it contributed in the same direction as other traits. This indicates potential to respond to selection for the simultaneous increase/decrease in all traits. Some synergistic pleiotropic alleles must also affect these traits.

which predicts the adaptive evolution of trait means over one generation based jointly on **G** and β, the directional selection acting on each trait (Box 1). **G** determines both the rate and direction of adaptation (i.e. the particular peak climbed) (Lande 1979, 1980; Cheverud 1984; Zeng 1988; Björklund 1996; Schluter 1996; Arnold *et al*. 2001). **G** also determines the rate and direction of neutral phenotypic divergence (Lande 1979; Arnold *et al*. 2001; Phillips *et al*. 2001). As the number of traits measured (and the size of the matrix) increases, it becomes difficult to interpret patterns of covariation among traits simply by looking at **G**. Eigenanalysis (e.g. principal components analysis) reduces the dimensionality of **G**, facilitating qualitative and statistical assessments of the evolutionary potential or genetic basis of a suite of traits (Arnold 1992; Jones *et al*. 2003; Fig. 1C). In this way, understanding interactions among suites of traits becomes plausible.

Study of **G** has blossomed in recent years to the extent that Steppan *et al*. (2002) proposed the recognition of a new field: comparative quantitative genetics. To date, interest in estimating **G** has focused firmly on the evolution of **G**, rather than on the exploitation of **G** as an evolutionary tool with which to investigate phenotypic evolution. Understanding how adaptive variation for traits is maintained is a crucial question in evolutionary biology. However, the restricted focus also reflects theoretical concerns about the validity of using **G** to predict phenotypic evolution. Accuracy of the multivariate breeder's equation depends on **G** (and β) remaining unchanged over the time frame of interest. Theory has been unable to resolve the evolution of **G** (Turelli 1988; Bürger & Lande 1994; Kirkpatrick *et al*. 2002), and stability of **G** cannot be assured. Comparison among **G** from different taxa has not yet led to clear predictions of when or how **G** will evolve (Roff 2000; Steppan *et al*. 2002). **G** should be further dissected empirically to determine how evolutionary processes and the genetic details affect the evolution of adaptive variation. Theoretical studies have clearly identified parameters (and ranges of parameter values) that influence the evolution of **G**, and we are

eagerly awaiting the appearance of empirical tests of these hypotheses.

Analytical difficulties have undoubtedly contributed to the dominance of univariate or bivariate studies of phenotypic evolution. Computational, methodological and statistical advances in recent years all make multivariate quantitative genetics more accessible. These analytical advances have occurred in conjunction with an increased focus on wild populations. Ecological context in quantitative genetic studies of free-living populations serves to further focus attention on interactions among traits, and to reinforce the necessity of a multivariate approach. Quantitative genetic studies in wild populations complement the traditional laboratory approach, and have opened up a new range of challenging evolutionary questions that we can address through **G**. Explicitly combining studies of the evolution of **G** with studies of phenotypic evolution might further our understanding of how both adaptive variation and phenotype evolve. The purpose of this paper is to discuss what **G** is, to outline why it is an important tool in evolutionary biology, and to highlight avenues of future research that have the potential to markedly extend our understanding of evolution.

Estimating G

There are several different approaches for estimating **G**. These methods, and their strengths and weaknesses, have been discussed elsewhere (e.g. Falconer & Mackay 1996; Lynch & Walsh 1998; Coltman 2005; Garant & Kruuk 2005). Here I complement published discussions of estimation methods by considering general, persistent problems with estimating **G**. Methods for estimating **G** typically depend on knowing the relationships among members of the population (Falconer & Mackay 1996; Lynch & Walsh 1998). This has historically restricted estimates to taxa predisposed to manipulative laboratory studies. That is, taxa with short generation times, limited space requirements and mating systems in which specific breeding designs can be enforced. Development of molecular genetic methods for inferring relationships among members of a population (Queller & Goodnight 1989; Lynch & Ritland 1999; Blouin 2003; Wang 2004) has opened the way for the application of quantitative genetic tools in wild populations (Ritland 1996, 2000; Milner *et al*. 2000; Moore & Kukuk 2002; Kruuk 2004; Garant & Kruuk 2005). Estimating **G** in wild populations could improve the taxonomic scope of quantitative genetics, as well as expanding the repertoire of lifetime traits that can be studied (Boake *et al*. 2002; Moore & Kukuk 2002). To date, molecular tools have rarely been used in the estimation of quantitative genetic parameters in wild populations (Kruuk 2004; Coltman 2005). Problems with both molecular inference of relationships (e.g. Garant & Kruuk 2005) and the partitioning of variance components (Kruuk 2004; see below) might prevent molecular methods from fulfilling expectations.

Obtaining an adequate number of samples is the greatest cost and limitation of quantitative genetic studies. Inadequate sampling leads to large standard errors about estimates of V_A and Cov_A, precluding conclusions about the genetic basis of the traits or their evolutionary responses (Klein *et al*. 1973; Klein 1974; Hill & Thompson 1978; Koots & Gibson 1996; Lynch & Walsh 1998). There are no palliatives for the logistical difficulties associated with sample sizes. Massive sample sizes are not a requisite. However, experiments need to be carefully designed, taking into consideration how many individuals/sires/families need to be sampled to detect a particular level of variance or covariance and whether the experimental question can be addressed with that level of precision (Klein *et al*. 1973; Klein 1974; Lynch & Walsh 1998). The number of traits for which **G** can accurately be estimated also depends on the sample size (see Kirkpatrick & Meyer 2004). In some cases, limitations on housing and maintenance resources in the laboratory might lead to improvements in sample size through the analysis of wild populations.

Quantitative genetic experiments aim to partition phenotypic variation to its contributing sources such that the additive genetic contribution (**G**) can be accurately extracted from nonadditive genetic effects (epistasis or dominance) and environment. However, poor experimental design often confounds additive genetic variance with phenotypic variance from other sources. Although much of the resemblance among relatives is due to additive alleles (Lande 1979; Falconer & Mackay 1996; Lynch & Walsh 1998) nonadditive variance can also contribute. Therefore, preference should be given to experimental designs in which the additive variance can be cleanly partitioned, such as parent–offspring regression, half-sib breeding designs or animal models (Falconer & Mackay 1996; Lynch & Walsh 1998). Full-sib breeding designs in particular confound additive with nonadditive variance, but are still commonly employed (reviewed by Merilä & Crnokrak 2001). How concerned we should be about confounded variance depends on the contribution of nonadditive variance to the suite of traits (i.e. how much the estimate of (co)variance might be inflated).

Epistasis and dominance are probably both common modes of gene action (Wright 1968; Falconer & Mackay 1996). However, modes of gene action might be dissociated from genetic variance. If nonlinear gene action (epistasis or dominance) during an individual's development is to translate into nonadditive genetic variance at the population level, there must be multiple alleles per interacting locus, and variation among alleles in their interactions and phenotypic effects (Whitlock *et al*. 1995; Brodie 2000). Traditional breeding designs have limited power to estimate nonadditive genetic variance; by combining information

from different classes of relatives, animal models might provide a more powerful approach (Lynch & Walsh 1998). Current debate over the evolutionary role of nonadditive variance (e.g. Lynch *et al*. 1999; Whitlock 1999; Lopez-Fanjul *et al*. 2003, 2004; Barton & Turelli 2004; Gibson & Dworkin 2004) highlights the lack of basic data on the prevalence of nonadditive variance.

When related individuals share a common environment (including maternal) **G** will include environmental (co)variance. Common environment effects are prevalent, and can substantially inflate **G** (Lynch & Walsh 1998; Kruuk 2004). Common environment is readily accounted for in the laboratory by, for example, replicating families among rearing enclosures and using specific breeding designs such as paternal half-sibs (Falconer & Mackay 1996). In the wild, cross-fostering can be used to effectively partition out common environment effects (e.g. McAdam & Boutin 2003; Forstmeier *et al*. 2004). However, it is not clear how **G** can be accurately estimated in wild populations where environment cannot be manipulated (Kruuk 2004).

Both Ritland's (1996, 2000) regression method and animal models (see Lynch & Walsh 1998; Kruuk 2004) can accommodate terms for common environment effects. However, identifying individuals who shared a common environment during the development of a trait depends on long-term monitoring of the population. Also, statistical partitioning of common environment effects depends on variation and replication: some related individuals must experience different environments while some unrelated individuals must share a common environment. For some environmental parameters, these conditions might not be met in the wild, particularly in populations with limited dispersal and cross-generational sharing of territories. Researchers studying natural populations should be acutely aware of the problem of common environment. All possible sources of confounded variance should be identified and, if they cannot be statistically partitioned from **G**, their potential to affect **G** should be explicitly addressed.

G: a tool to study phenotypic divergence

A general issue in evolutionary biology is the relative roles played by adaptation and random genetic drift in generating phenotypic variation. Quantitative genetic tools have a long history in addressing this question (Lande 1976, 1977). More recently, quantitative and molecular tools have been coupled, increasing the scope of relative rates tests to infer the action of drift or selection (Q_{ST} vs. F_{ST}: Spitz 1993; Merilä & Crnokrak 2001). **G** provides us with a powerful tool to move beyond retrospective analysis, and to address more complex questions about the specific phenotypic effects of different evolutionary process. **G** can identify evolutionary constraints and differences among populations in their potential to evolve and specifically predict the direction and rate of phenotypic divergence (adaptive or neutral) (Lande 1979; Cheverud 1984; Zeng 1988; Arnold 1992; Arnold *et al*. 2001; Phillips *et al*. 2001).

Evolutionary potential and absolute genetic constraints

Predicting how a population will respond to selection or whether populations will respond similarly are general aims in evolutionary biology, as well as forming the basis of decision-making in conservation management. Neutral markers and univariate quantitative genetic estimators have been used to summarize the capacity of a population for future phenotypic evolution (e.g. Houle 1992; Hard 1995). When appropriately scaled (e.g. Houle 1992), the size of **G** (the sum of eigenvalues: $\Sigma\lambda_i$) provides a similar descriptor. Genetic variance can almost be considered ubiquitous (Lynch & Walsh 1998; Barton & Partridge 2000), and estimates of the size of **G** are likewise expected to be greater than zero. However, the size of **G** cannot be used to address such questions as which trait value combinations will evolve most rapidly, or whether some phenotypes are evolutionarily inaccessible. Size of **G** is therefore too crude an estimator of genetic potential to be a useful tool.

Dimensionality of **G** is a potentially informative descriptor of evolutionary potential. **G** has, at most, as many dimensions as traits. In the maximal case, each eigenvector is associated with additive genetic variance and there are no absolute constraints on evolution (Kirkpatrick & Lofsvold 1992; Mezey & Houle 2005). Conversely, if **G** has fewer dimensions than traits, there are phenotypes (trait combinations) without additive genetic variation, and which cannot evolve in the population. Therefore, dimensionality of **G** provides an estimate of how many independent traits **G** summarizes, and what regions of phenotypic space are evolutionarily accessible (Fisher 1930; Dickerson 1955; Kirkpatrick & Lofsvold 1992; Orr 2000; Reeve 2000; Mezey & Houle 2005). Recent methodological papers have outlined two possible approaches for determining the dimensionality of **G**: bootstrapping and factor analytic modelling.

Mezey & Houle (2005) determined dimensionality by estimating bootstrap confidence intervals for each eigenvalue: when eigenvalue confidence intervals exclude zero the associated eigenvector describes a phenotypic space associated with additive genetic variance. Applying this method to wing shape variables in *Drosophila melanogaster* Mezey & Houle (2005) observed statistical support for each of 20 possible dimensions of **G**. This suggests both that different aspects of wing shape have largely independent genetic bases, and that no wing shapes are evolutionarily inaccessible to the population.

Factor analytic modelling is an alternative approach for determining the number of dimensions of **G** (Kirkpatrick & Meyer 2004; Meyer & Kirkpatrick 2005). Meyer &

Kirkpatrick (2005) estimated the dimensionality of **G** for eight carcass traits measured on beef cattle. For this **G** there was statistical support for only six dimensions (Meyer & Kirkpatrick 2005). Further work is necessary to fully explore the properties of both bootstrapping and factor analytic modelling, and to then determine whether absolute genetic constraints are common, whether they are more likely for particular types of traits, whether populations vary in their constraints, and what underlies the constraint (lack of mutational input or fixation of alleles).

When estimated for the same traits similarity among **G** of different taxa can be assessed through direct comparison. Detailed discussions of matrix comparison methods appear elsewhere (Houle *et al*. 2002; Roff 2002; Steppan *et al*. 2002; Blows & Higgie 2003). Currently, the most popular method is common principal components analysis (CPC), which determines whether eigenvectors and associated eigenvalues differ among matrices (Phillips & Arnold 1999; see also Houle *et al*. 2002; Mezey & Houle 2003). **G** might differ only in eigenvalues (proportional change), implying that populations differ in the rate at which they will respond to selection, but no difference in the phenotypes that can evolve. Alternatively, eigenvectors of **G** might differ, implying that a unique set of phenotypes is accessible to each population (although whether these phenotypes actually evolve will depend on selection) (Phillips & Arnold 1999; Steppan *et al*. 2002). Current methods for comparing **G** have certain weaknesses, and developing better methods for statistical matrix comparisons is an ongoing challenge (Roff 2002; Steppan *et al*. 2002; Mezey & Houle 2003; Begin *et al*. 2004; Kirkpatrick & Meyer 2004).

Directional evolutionary constraint or bias

Theoretically, **G** influences the direction of both phenotypic adaptation (Lande 1979; Cheverud 1984; Zeng 1988; Arnold 1992) and drift (Lande 1976, 1979; Arnold *et al*. 2001; Phillips *et al*. 2001). In the decades since Lande (1979) published the multivariate breeder's equation there has been considerable debate over the usefulness of this relationship, which depends on **G** remaining stable over the time frame in question (e.g. Turelli 1988). Schluter (1996) sidestepped this issue, examining not the stability of **G**, but the relationship between **G** and the direction of divergence. Using a novel method to test for an association between **G** and the direction of phenotypic divergence Schluter (1996) demonstrated that **G** was associated with the direction of divergence for at least 4 million years. The direction of phenotypic divergence was defined as the first eigenvector of the divergence variance–covariance matrix, **D** (Box 1). Similarity between **G** and the direction of phenotypic divergence is determined by the angle between major eigenvectors of each matrix (Schluter 1996). Blows & Higgie (2003; Blows *et al*. 2004) extended this approach, comparing the entire phenotypic space described by **G** and by **D**, rather than just their major eigenvectors.

Development of methods for associating **G** with divergence, along with Schluter's (1996) results, encouraged a flurry of empirical tests of directional genetic constraint on evolution. These analyses both supported (Schluter 1996; Arnold & Phillips 1999; Badyaev & Foresman 2000; Begin & Roff 2003, 2004; Blows & Higgie 2003; McGuigan *et al*. 2005) and rejected (Merilä & Björklund 1999; Badyaev & Hill 2000; McGuigan *et al*. 2005) the hypothesis that **G** imposes a directional constraint on phenotypic evolution over typical micro-evolutionary time frames. The contradictory nature of these results focuses attention on factors influencing the association between **G** and **D**. Variation in the stability of **G** is a prime candidate, but I will first discuss several other factors that could influence the association of **G** with **D**.

The influence of **G** on the direction of phenotypic divergence is expected to decay over time as the population comes under the influence of an adaptive peak (Lande 1979; Via & Lande 1985; Zeng 1988). Schluter (1996) explicitly looked for and found evidence that the direction of divergence becomes less similar to major eigenvectors of **G** as time since divergence increases. This result contrasts with that of McGuigan *et al*. (2005) who also compared **G** with directions of divergence over different timescales. At the deepest level of divergence (*c*. 2 million years ago), allopatric species of rainbow fish (*Melanotaenia*) diverged in a direction closely associated with the major eigenvector of **G**. However, conspecific populations isolated for < 1 million years did not diverge in a direction predicted by major eigenvectors of **G** (McGuigan *et al*. 2005). Therefore, time since divergence cannot be the only factor determining the association of **G** with directions of phenotypic divergence.

Comparisons of **G** with **D** have not explicitly distinguished divergence due to different evolutionary processes, such as selection vs. drift. In laboratory experiments where selection is artificially applied, it is straightforward to statistically distinguish variance attributable to drift from that due to selection. Multivariate analysis of variance (MANOVA) can be used to extract variance components specific to each source of experimental variance. For example, **D** can be estimated for the selection treatment ($\mathbf{D}_{\text{selection}}$) and for each replicate (population or line) nested within treatment ($\mathbf{D}_{\text{drift}}$) (McGuigan *et al*. 2005). Nonetheless, **D** has typically been estimated as the variance–covariance matrix of means from each population (e.g. Blows & Higgie 2003).

With natural populations, there are many different aspects of environment and population structure that could be driving phenotypic evolution. The breeder's equation specifically deals with selection, and investigators have generally examined the relationship between **G** and **D** for functionally important traits known or suspected to be

under selection. Langerhans & DeWitt (2004) proposed a nested MANOVA approach to investigate repeated evolution. Using nested MANOVA, phenotypic variance due to selection imposed by dominant aspects of the environment is partitioned from variance due to other processes, such as drift and subtle differences in the position of local selective optima.

McGuigan *et al*. (2005) took a similar approach in their study of rainbow fish, using MANOVA to partition phenotypic divergence between water flow habitat, species, and population. Rainbow fish adapt to still vs. flowing water, evolving in body shape, sustained swimming performance and muscle morphology (McGuigan *et al*. 2003). Colonization of lakes by stream fish has independently occurred several times in different species. By comparing **G** to the **D** estimated for each of the different sources of variance McGuigan *et al*. (2005) determined that adaptation to water flow was less strongly associated with **G** than was divergence among species or among replicate populations. This result indicates the directional constraint imposed by **G** depends on the evolutionary process driving divergence (McGuigan *et al*. 2005). McGuigan *et al*. (2005) hypothesized that conspecific populations experiencing the same water velocity diverged primarily through drift, and reported that **D** estimated at this level (populations nested within species and habitat) was approximately proportional to **G**, consistent with predictions for drift-driven divergence (Lande 1979; Arnold *et al*. 2001). These results suggest drift might be more tightly constrained by **G** than is adaptation (McGuigan *et al*. 2005). There is substantial scope to test these hypotheses, particularly in systems in which drift and selection can be partitioned (Langerhans & DeWitt 2004).

Whether or not **G** persistently influences the direction of divergence will depend on the characteristics of the adaptive landscape (Box 1; Fig. 2) (Cheverud 1984; Lande 1984; Brodie 1992; Jones *et al*. 2003, 2004). If the eigenstructure of **G** is similar to that of the adaptive landscape, then both selection and genetic covariation will drive evolution in the same direction, and **G** will be persistently associated with **D** (Arnold *et al*. 2001; Arnold 2003). That is, selection along major eigenvectors of **G** will result in divergence along major eigenvectors of **G** (Fig. 2B). Conversely, selection orthogonal to major eigenvectors of **G** will cause the association between **G** and **D** to decay with time as the population attains the adaptive peak (Fig. 2A).

Blows *et al*. (2004) estimated both **G** and the fitness surface for male cuticular hydrocarbons (contact pheromones) in *Drosophila serrata*. These hydrocarbons form the basis of the mate choice system in the species, and are under strong directional selection through female mate choice. **G** was not aligned with either γ or ω, matrices describing the curvature of the adaptive landscape (Box 1) (Blows *et al*. 2004). Orthogonality of **G** to divergence (e.g. Merilä & Björklund 1999; Badyaev & Hill 2000; McGuigan *et al*. 2005), and/or the fitness surface (Blows *et al*. 2004) could indicate erosion of genetic variance during adaptation, or reveal a genuine lack of congruence between **G** and the adaptive landscape. Identification of common relationships awaits further empirical investigation.

Finally, variation among taxa and/or traits in the evolutionary stability of **G** might contribute to variability in the

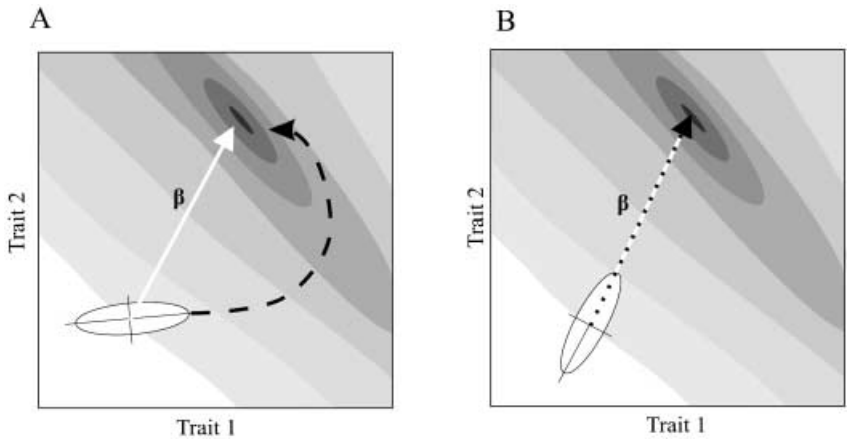

Fig. 2 A hypothetical example of the relationship between the adaptive landscape, **G**, and directions of phenotypic divergence for two traits. The contours on the plot describe regions of equal fitness, with increasing darkness describing increasing fitness (i.e. black is a peak on the landscape). The open ellipse is **G**, with the two eigenvectors drawn as the axes of variation. The population phenotypic mean is the centre of **G**, at the intersection of the eigenvectors. In (A) there is little covariation between Trait 1 and Trait 2, and most of the genetic variation underlies Trait 1 (i.e. the first eigenvector of **G** lies along the Trait 1 axis). Selection (β) favours an increase in both traits simultaneously. The population is initially expected to evolve along the major eigenvector of **G** (i.e. along the broken black curve), making an oblique approach to the adaptive peak. Therefore, once the population evolves to the fitness peak there will be a limited similarity of **G** and **D**. (B) β is the same as in (A), but now **G** is aligned with the adaptive landscape. Therefore, selection acts along genetic lines of least resistance (dashed black line) and a persistent relationship between **G** and **D** is expected.

association of **D** with **G**. Typically, **G** is estimated from a single extant population in a single environment (but see e.g. Begin & Roff 2003, 2004; Blows & Higgie 2003). Point estimates of **G** are implicitly assumed to represent the variation available during divergence. If this assumption is invalid there is little reason to expect **G** to be similar to **D**. The average **G** has been shown to be a better predictor of phenotypic evolution than single estimates of **G**, highlighting the importance of short-term stochastic fluctuations, as well as longer-term evolution (Jones *et al*. 2004). Two issues are involved here: direct environment effects and evolution of **G**.

Environment can directly influence the genetic contribution to a suite of traits (Coyne & Beecham 1987; Riska *et al*. 1989; Houle 1991; de Jong & van Noordwijk 1992; Sgrò & Hoffmann 2004; Weinig & Schmitt 2004). If this is a common phenomenon, **G** estimated in one environment will be unable to accurately predict divergence in a different environment (Turelli 1988; Riska *et al*. 1989). Direct effects on V_A have been extensively studied, revealing common but unpredictable effects of environment (Hoffmann & Merilä 1999; Blanckenhorn 2002). There is less information on direct environmental effects on covariances, but available data suggest a similar story (Sgrò & Hoffmann 2004). Environment might affect the magnitude but not the direction of trait covariation (e.g. Conner *et al*. 2003), suggesting an effect on rate but not direction of evolution. Alternatively, environment might alter relationships among traits (Cano *et al*. 2004; Sgrò & Hoffmann 2004), suggesting potential effects on the direction of evolution. Further research is required to characterize the direct effects of environment on **G**, and how these influence the relationship between **G** and **D**.

Evolution of G

Ultimately, to understand the evolution of phenotype we have to understand how genetic (co)variance evolves (Lande 1975, 1980; Lande & Arnold 1983; Turelli 1985, 1988; Barton & Turelli 1987; Houle 1991; Reeve 2000; Zhang & Hill 2002, 2003). Because **G** depends on alleles, it evolves through processes affecting allele frequencies: drift, selection, mutation and migration. There has been extensive theoretical exploration of the effects of these processes on **G**, but the problem remains analytically intractable (Turelli 1988; Bürger & Lande 1994; Kirkpatrick *et al*. 2002). There are two sources of uncertainty over the evolutionary behaviour of **G**. Firstly, the evolutionary outcome of any process depends on the unknown genetic details underpinning **G** (Barton & Turelli 1987, 1989; Slatkin & Frank 1990; Zhang & Hill 2002; Griswold & Whitlock 2003; Zhang *et al*. 2004). Secondly, the specific parameter values of the evolutionary processes (e.g. rate of drift, strength and type of selection, mutation rate) and the interactions among processes influence the evolution of **G** (Barton & Turelli 1989; Bürger & Lande 1994; Kirkpatrick *et al*. 2002; Jones *et al*. 2003, 2004). Again, there is a dearth of information about evolutionary processes operating in naturally evolving populations, making precise predictions of the behaviour of **G** impossible.

Simulation studies analysing the behaviour of **G** for specific parameter values are one approach to exploring how genetic (co)variances evolve. Studies of **G** evolving *in silico* have lead to testable hypotheses about the relative effects of different parameters, and the effects of variable parameter values (e.g. Reeve 2000; Jones *et al*. 2003, 2004). Predictions derived from analytical and simulation studies of the evolutionary behaviour of **G** can be tested through manipulative laboratory experiments in which evolutionary parameter values can be controlled (Steppan *et al*. 2002; Phillips & McGuigan in press). However, to determine which processes operate in wild population it is necessary to apply those pattern expectations to the study wild populations.

Drift

Random genetic drift results in loss of alleles and is theoretically predicted to proportionally reduce the total variance, scaling **G** by $1 - F$ (the inbreeding coefficient) (Wright 1951; Lande 1980). If **G** commonly evolves through drift, and if drift affects only the size ($\Sigma\lambda_i$) of **G** (and thus only the rate of evolution), the breeder's equation could still be used to accurately predict directions of phenotypic divergence. Computer simulations support the theoretical prediction that the size of **G** evolves through drift (Jones *et al*. 2003, 2004). Phillips *et al*. (2001) tested the proportionality hypothesis in the laboratory by comparing **G** (of six wing size and shape traits) among 52 inbred *Drosophila melanogaster* lines derived from one generation of brother–sister mating. Although the predicted scaling relationship held on average, **G** of individual lines varied widely about the expectation (Phillips *et al*. 2001). Individual lines deviated from the average not just with respect to the total variance retained (eigenvalues of **G**), but also in trait covariation (eigenvectors) (Phillips *et al*. 2001). Furthermore, when the inbred lines were maintained at a larger population size without further enforced brother–sister mating **G** continued to evolve in a direction that was random with respect to the ancestral **G** (i.e. drift-induced changes were not transient) (Whitlock *et al*. 2002).

The findings of Phillips *et al*. (2001; Whitlock *et al*. 2002) suggest proportionality of **G** is not a useful criterion for inferring the action of drift vs. selection in the wild. Detection of the average response will be unlikely in studies of natural populations because few populations are sampled (both by nature and by the investigator). In addition, looking for an average response makes it difficult to identify populations in which **G** has evolved through other processes.

There are two obvious avenues for improving the effectiveness of the proportionality criterion. Steppan *et al*. (2002) highlighted the phylogenetic approach. By sampling multiple populations within a robust phylogenetic framework, it is possible to infer rate and direction of evolution, and identify **G** diverging through drift vs. those evolving under directional selection (Steppan *et al*. 2002). Comparison of replicate populations from the same selection regime vs. populations from different selection regimes is another powerful method for partitioning the divergence due to drift from that attributable to selection (Blows & Higgie 2003; Langerhans & DeWitt 2004; McGuigan *et al*. 2005).

Selection

Selection on phenotype also drives changes in the frequencies of alleles and might affect the eigenvalues and/or eigenvectors of **G**. Empirically, the relationship between **G** and selection is unresolved. Artificial directional selection changes trait covariation in some, but not all cases (Wilkinson *et al*. 1990; Shaw *et al*. 1995; Blows & Higgie 2003; Phelan *et al*. 2003). Nonproportional changes among **G** of natural populations implicates directional selection (Roff 2000; Steppan *et al*. 2002), but recall that drift could be responsible for such nonproportionality. Theoretically, whether **G** evolves during selection depends on both the molecular genetic details underlying the traits, and on the relationship between **G** and the shape and orientation of the adaptive landscape (Lande 1980; Barton & Turelli 1987, 1989; Turelli 1988; Slatkin & Frank 1990; Reeve 2000; Arnold *et al*. 2001; Zhang & Hill 2002; Griswold & Whitlock 2003; Jones *et al*. 2003, 2004). Specifically, the number of loci and alleles and, most importantly, the distribution of allelic effects determine how selection affects **G** (Barton & Turelli 1987, 1989; Slatkin & Frank 1990; Zhang & Hill 2002; Griswold & Whitlock 2003; Zhang *et al*. 2004). Although the behaviour of **G** under selection has been explored for different models of the distribution of allelic effects (e.g. Turelli 1988; Slatkin & Frank 1990; Reeve 2000; Jones *et al*. 2003), this issue cannot be resolved without empirical characterization of the molecular genetic basis of suites of traits. Improvements in molecular genetic tools have opened the way for such characterizations, making it plausible that models of the genetic basis of **G** can be rephrased to reflect what is known of the development of traits.

Persistent multivariate stabilizing selection (i.e. selection favouring the current trait value combinations) is predicted to lead to a stable **G** that is aligned with the adaptive landscape (Lande 1980, 1984; Cheverud 1984; Arnold 1992). This prediction was confirmed by *in silico* evolution (Jones *et al*. 2003, 2004). Conversely, analytical and simulation results predict reduced stability of **G** on landscapes lacking strong curvature (Arnold *et al*. 2001; Jones *et al*. 2004). Thus, the sensitivity of **G** to selection might depend on whether selection affects just the multivariate mean, or if there is selection for phenotypic covariance (Lande 1979). Recall the *Drosophila serrata* example (Blows *et al*. 2004) in which **G** was dissimilar to γ and ω. Blows *et al*. (2004) suggested this lack of association might be a general result for traits under strong directional, rather than stabilizing selection. Considerably more empirical data are necessary before we can resolve the evolutionary effects of the adaptive landscape. Our understanding of the patterns generated in **G** by different types of selection is currently insufficient to be able to infer when selection might have driven divergence among **G** of wild populations.

Mutation

Mutations can strongly influence the evolutionary dynamics of **G** through the distribution of mutational effects relative to standing genetic variation (Lande 1980; Barton & Turelli 1987; Turelli 1988; Slatkin & Frank 1990), particularly the extent of pleiotropy (Jones *et al*. 2003, 2004; Mezey & Houle 2003). Increasing pleiotropy decreases the evolution of **G** *in silico*, particularly if the distribution of pleiotropic effects (i.e. **M**: Box 1) aligns with the adaptive landscape (Jones *et al*. 2003). The alignment of **M** and the adaptive landscape have been variously discussed, based on the idea that selection favours pleiotropy for functionally related traits (Cheverud 1984; Jones *et al*. 2003). There has been no empirical assessment of the relationship between **M** and the adaptive landscape, reflecting the practical difficulties associated with accurately estimating these parameters.

M can be estimated through mutation accumulation (MA) experiments: lines derived from a single inbred parent population are maintained at very small population sizes and drift fixes different novel alleles in different lines (mutation–drift equilibrium: Lynch & Walsh 1998). Although conceptually simple, MA experiments are logistically challenging. Low natural rates of mutation make it necessary to maintain many replicate lines over many generations before mutations can be statistically detected. Therefore, MA experiments are restricted to laboratory taxa with very short generation times and very limited space requirements. Mutation rates can be accelerated, but biases in the distribution of mutations resulting from mutagens reduce confidence in the generality of results. Additional drawbacks of MA are that subtlety and context-dependency of phenotypic effects make it impossible to observe all mutations. Nonetheless, our current lack of basic data on mutational (co)variance makes these onerous experiments extremely valuable.

Estes *et al*. (2005) estimated the (co)variances of mutations that had accumulated among 67 single-individual *Caenorhabditis elegans* lines after 370 generations. The results of this study were consistent with the hypothesis that mutational input to functionally related traits is pleiotropic

(Estes *et al*. 2005; see also Keightley *et al*. 2000). Induced mutations in *Arabidopsis thaliana* also generated persistent covariance among particular traits, although the functional relationships between these covarying traits were not clear (Camara *et al*. 2000).

Conclusions and future directions

The initial appeal of **G** as an evolutionary tool arises from the elegant simplicity of the multivariate breeder's equation: short-term phenotypic evolution can be predicted without information on the contributing alleles, and, over the short-term, **G** might exert strong directional bias over divergence (Björklund 1996; Schluter 1996). This apparent simplicity belies the complexity of the real world where the genetic underpinnings of **G** and the specifics of the evolutionary process acting on phenotype are expected to affect the evolutionary dynamics of **G**, and the relationship between **G** and **D** (Turelli 1988; Slatkin & Frank 1990; Jones *et al*. 2003). Theoretical reservations about the use of **G** are empirically supported: **G** is a good predictor of the direction of phenotypic divergence in some cases, but not in others. What does this mean? Do we discard **G** as an evolutionary tool?

No. By summarizing a trait's genetic underpinnings, whose inherent complexity and physical inaccessibility make them difficult to directly study, **G** provides us with a powerful tool to investigate the specific effects of evolutionary process. Our understanding of evolution depends on consideration of the connections between standing genetic variance, mutational variance, the adaptive landscape and phenotypic divergence among populations. All of these levels can be understood through their connections with **G**. These issues therefore should be the focus of future research.

*Standing genetic variation: **G***

Empirical attention to date has been focused at this level, assessing the stability of **G** (Roff 2000; Steppan *et al*. 2002). Because the molecular genetic underpinnings of **G** are theoretically predicted to strongly influence its evolution (Turelli 1988; Slatkin & Frank 1990; Jones *et al*. 2003, 2004), it is necessary to consider the evolutionary stability of **G** within the context of the number of loci, the number of alleles, and the distribution of allelic effects (including pleiotropy, additive vs. nonadditive gene action and direct environment). Powerful molecular tools now exist (e.g. Fraser & Marcotte 2004) to explore **G** from the molecular perspective. Combining molecular and quantitative approaches shifts attention from determining how loci contribute to phenotype to determining how allelic variation at those loci contributes to phenotypic variation within the population.

An example system in which the synthesis of quantitative and molecular genetics is well advanced is the butterfly wing (Beldade & Brakefield 2002; McMillan *et al*. 2002). The abundant and highly visual nature of variation in butterfly wing patterns has long made them the focus of evolutionary research (Nijhout 1991). Reference to molecular toolkits of model organisms facilitated the identification of loci contributing to wing patterns (e.g. Carroll *et al*. 1994; Keys *et al*. 1999; French & Brakefield 2004), and to the detection of allelic variation in phenotypic effect (Beldade *et al*. 2002a). Quantitative genetic experiments on eyespots estimated positive genetic covariance for size among eyespots (e.g. Monteiro *et al*. 1994; Paulsen 1994, 1996). This inference of pleiotropy was supported by the detection of alleles that affected the size of multiple eyespots simultaneously (Brakefield 1998; Beldade *et al*. 2002a). However, genetic correlations among eyespots were less than one (e.g. Monteiro *et al*. 1994; Paulsen 1994, 1996). Additive genetic variance for independent evolution of eyespot size was further revealed by selection experiments that successfully evolved the two dorsal forewing eyespots in opposite directions (Beldade *et al*. 2002b, c) and by the detection of mutations with independent effects on individual eyespots (Monteiro *et al*. 2003). Although these experiments have yet to completely characterize the underpinnings of **G**, they illustrate the potential to dissect **G** using quantitative and molecular genetic tools.

*Mutational input: **M***

Possibly the most challenging issue standing in the way of our understanding of phenotypic evolution is developing an understanding of the input of novel variance. As with standing genetic variation, we should asses mutational input from two perspectives: at the molecular genetic level, and at the level of the summary statistic **M**. Characterization of novel mutations is already firmly entrenched in developmental biology. Although **G** has been estimated from quite a few different taxa and for different suites of traits, there are very few estimates of **M**. Several challenges have to be contended with in order to obtain accurate estimates of **M** (Lynch & Walsh 1998), but such data will be highly informative.

Armed with **M** there are several explicit hypotheses that can be addressed. What is the dimensionality of **M**? If **M** has fewer dimensions than traits, absolute genetic constraints have been identified. Trait value combinations (eigenvectors) without mutational variance are regions of phenotypic space that are evolutionarily inaccessible to the population because mutation cannot generate allelic variation for the phenotypes (although environmental variance might generate these phenotypes). Is **G** aligned with **M**, or does selection constantly weed out mutations, changing the eigenstructure of **G**? If **M** has more dimensions than **G**,

variation for certain trait combinations was depleted through selection or drift.

Adaptive landscape

A large hole in our knowledge of the evolution of genetic (co)variances and of phenotype is the role of the adaptive landscape. It has been proposed that selection will favour the alignment of **G** with the fitness surface (and therefore with **D**: Cheverud 1984; Arnold *et al*. 2001; Arnold 2003; Jones *et al*. 2003, 2004), and possibly also with the mutational variance (Cheverud 1984). Analytical comparison of the fitness surface with other evolutionary parameters is now computationally possible (e.g. Blows *et al*. 2004). In addition to the basic question of whether there is alignment, we need specifically to ask whether the relationship varies among different suites of traits (e.g. life history vs. morphology), whether such differences are associated with differences in shape and orientation of the adaptive landscape (e.g. is it more/less curved for traits more/less closely associated with fitness) or with differences in the molecular genetic basis of the traits. By combining dimensionality analysis with projection analysis (Blows *et al*. 2004), it is possible to test specific hypotheses about the relationship between the phenotypic space described by **G** (or **M**) and the region of phenotypic space associated with high fitness. If peaks on the adaptive landscape are located in regions of phenotypic space underlain by mutational (or genetic) variance then unsupported dimensions of **G** (or **M**) might be evolutionarily irrelevant.

*Phenotypic divergence **D***

For many years there has been a (necessary) disconnect between laboratory and field quantitative genetics. Much research could only be done within the laboratory, where experimenters controlled all parameters and ensured sufficient replication to assess the evolutionary repercussions of individual processes. There is still a strong need for controlled laboratory studies to determine, in particular, how selection affects **G**. We should systematically explore the effects of different adaptive landscapes and of differences in the genetic basis of the traits on the evolution of **G** and of phenotype. At the same time, these experiments can be couched in real biological context through reference to wild populations.

Only by directly assaying wild populations can we get at the processes driving natural phenotypic evolution. The coupling of quantitative genetics with molecular tools has opened access to estimating evolutionarily relevant parameters in natural populations (Garant & Kruuk 2005). These methods need to be put to the test. Improved accessibility of wild populations has occurred in conjunction with increasing recognition that naturally replicated populations are powerful tools for studying evolution (Langerhans & DeWitt 2004; McGuigan *et al*. 2005). We are still awaiting the answer to some basic questions, such as whether populations evolve in the direction of selection, or along the major axes of **G**.

G can also be applied to more practical questions, particularly in conservation management. Use of more appropriate methods for predicting the potential of a population to respond to changes in selection pressure is becoming increasingly important in light of increasing anthropogenic threats to native populations. There has already been acknowledgement that quantitative but not molecular estimates of genetic variation reflect capacity of a population to evolve (e.g. Hard 1995; Reed & Frankham 2001; Moss *et al*. 2003). An organism is built through interactions among gene products, consists of interacting, semi-independent suites of traits, and interacts with its environment throughout its life. Although breaking these interactions into component parts aids our investigations, it is only by considering the whole that can we fully understand evolution. **G** provides a tool that allows us to unify analysis of all component parts.

Acknowledgements

Thanks to F. Frentiu, E. Hersch, E. Hine and M. Blows for their comments on previous drafts of this paper. The manuscript was improved through critical comments from three anonymous reviewers and S. Arnold. The author was supported by P. Phillips and J. Postlethwait.

References

Arnold SJ (1992) Constraints on phenotypic evolution. *American Naturalist*, **140**, S85–S107.

Arnold SJ (2003) Performance surfaces and adaptive landscapes. *Integrative and Comparative Biology*, **43**, 367–375.

Arnold SJ, Pfrender ME, Jones AG (2001) The adaptive landscape as a conceptual bridge between micro- and macro-evolution. *Genetica*, **112**, 9–32.

Arnold SJ, Phillips PC (1999) Hierarchical comparison of genetic variance-covariance matrices. II. Coastal-inland divergence in the garter snake, *Thamnophis elegans*. *Evolution*, **53**, 1516–1527.

Badyaev AV, Foresman KR (2000) Extreme environmental change and evolution: stress-induced morphological variation is strongly concordant with patterns of evolutionary divergence in shrew mandibles. *Proceedings of the Royal Society of London. Series B, Biological Sciences*, **267**, 371–377.

Badyaev AV, Hill GE (2000) The evolution of sexual dimorphism in the house finch. I. Population divergence in morphological covariance structure. *Evolution*, **54**, 1784–1794.

Barton NH, Keightley PD (2002) Understanding quantitative genetic variation. *Nature Reviews Genetics*, **3**, 11–21.

Barton N, Partridge L (2000) Limits to natural selection. *Bioessays*, **22**, 1075–1084.

Barton NH, Turelli M (1987) Adaptive landscapes, genetic-distance and the evolution of quantitative characters. *Genetical Research*, **49**, 157–173.

Barton NH, Turelli M (1989) Evolutionary quantitative genetics: how little do we know? *Annual Review of Genetics*, **23**, 337–370.

Barton NH, Turelli M (2004) Effects of genetic drift on variance components under a general model of epistasis. *Evolution*, **58**, 2111–2132.

Begin M, Roff DA (2003) The constancy of the G matrix through species divergence and the effects of quantitative genetic constraints on phenotypic evolution: a case study in crickets. *Evolution*, **57**, 1107–1120.

Begin M, Roff DA (2004) From micro- to macroevolution through quantitative genetic variation: positive evidence from field crickets. *Evolution*, **58**, 2287–2304.

Begin M, Roff DA, Debat V (2004) The effect of temperature and wing morphology on quantitative genetic variation in the cricket *Gryllus firmus*, with an appendix examining the statistical properties of the Jackknife-MANOVA method of matrix comparison. *Journal of Evolutionary Biology*, **17**, 1255–1267.

Beldade P, Brakefield PM (2002) The genetics and evo-devo of butterfly wing patterns. *Nature Reviews Genetics*, **3**, 442–452.

Beldade P, Brakefield PM, Long AD (2002a) Contribution of distal-less to quantitative variation in butterfly eyespots. *Nature*, **415**, 315–317.

Beldade P, Koops K, Brakefield PM (2002b) Developmental constraints versus flexibility in morphological evolution. *Nature*, **416**, 844–847.

Beldade P, Koops K, Brakefield PM (2002c) Modularity, individuality, and evo-devo in butterfly wings. *Proceedings of the National Academy of Sciences, USA*, **99**, 14262–14267.

Björklund M (1996) The importance of evolutionary constraints in ecological time scales. *Evolutionary Ecology*, **10**, 423–431.

Blanckenhorn WU (2002) The consistency of quantitative genetic estimates in field and laboratory in the yellow dung fly. *Genetica*, **114**, 171–182.

Blouin MS (2003) DNA-based methods for pedigree reconstruction and kinship analysis in natural populations. *Trends in Ecology & Evolution*, **18**, 503–511.

Blows MW, Brooks R (2003) Measuring nonlinear selection. *American Naturalist*, **162**, 815–820.

Blows MW, Higgie M (2003) Genetic constraints on the evolution of mate recognition under natural selection. *American Naturalist*, **161**, 240–253.

Blows MW, Hoffmann AA (2005) A reassessment of genetic limits to evolutionary change. *Ecology*, **86**, 1371–1384.

Blows MW, Chenoweth SF, Hine E (2004) Orientation of the genetic variance-covariance matrix and the fitness surface for multiple male sexually selected traits. *American Naturalist*, **163**, E329–E340.

Boake CRB, Arnold SJ, Breden F *et al.* (2002) Genetic tools for studying adaptation and the evolution of behavior. *American Naturalist*, **160**, S143–S159.

Brakefield PM (1998) The evolution–development interface and advances with the eyespot patterns in *Bicyclus* butterflies. *Heredity*, **80**, 265–272.

Brodie ED III (1992) Correlational selection for color pattern and antipredator behavior in the garter snake *Thamnophis ordinoides*. *Evolution*, **46**, 1284–1298.

Brodie ED III (2000) Why evolutionary genetics does not always add up. In: *Epistasis and the Evolutionary Process* (eds Wolf JB, Brodie EDI, Wade MJ), pp. 3–19. Oxford University Press, Oxford, UK.

Bürger R, Lande R (1994) On the distribution of the mean and variance of a quantitative trait under mutation–selection–drift balance. *Genetics*, **138**, 901–912.

Camara MD, Ancell CA, Pigliucci M (2000) Induced mutations: a novel tool to study phenotypic integration and evolutionary constraints in *Arabidopsis thaliana*. *Evolutionary Ecology Research*, **2**, 1009–1029.

Cano JM, Laurila A, Palo J, Merilä J (2004) Population differentiation in G matrix structure due to natural selection in *Rana temporaria*. *Evolution*, **58**, 2013–2020.

Carroll SB, Gates J, Keys DN *et al.* (1994) Pattern formation and eyespot determination in butterfly wings. *Science*, **265**, 109–114.

Cheverud JM (1984) Quantitative genetics and developmental constraints on evolution by selection. *Journal of Theoretical Biology*, **10**, 155–171.

Coltman DW (2005) Testing marker-based estimates of heritability in the wild. *Molecular Ecology*, **14**, 2593–2599.

Conner JK, Franks R, Stewart C (2003) Expression of additive genetic variances and covariances for wild radish floral traits: comparison between field and greenhouse environments. *Evolution*, **57**, 487–495.

Coyne JA, Beecham E (1987) Heritability of two morphological characters within and among natural populations of *Drosophila melanogaster*. *Genetics*, **117**, 727–737.

Dickerson GE (1955) Genetic slippage in response to selection for multiple objectives. *Cold Spring Harbor Symposia on Quantitative Biology*, **20**, 213–224.

Dobzhansky T (1956) What is an adaptive trait? *American Naturalist*, **90**, 337–347.

Erickson DL, Fenster CB, Stenoien HK, Price D (2004) Quantitative trait locus analyses and the study of evolutionary process. *Molecular Ecology*, **13**, 2505–2522.

Estes S, Ajie BC, Lynch M, Phillips PC (2005) Spontaneous mutational correlations for life-history, morphological and behavioral characters in *Caenorhabditis elegans*. *Genetics*, **170**, 645–653.

Falconer DS, Mackay TFC (1996) *Introduction to Quantitative Genetics*. 4th edn. Longman Group, Essex, UK.

Fisher RAS (1930) *The Genetical Theory of Natural Selection*. Clarendon Press, Oxford, UK.

Forstmeier W, Coltman DW, Birkhead TR (2004) Maternal effects influence the sexual behavior of sons and daughters in the zebra finch. *Evolution*, **58**, 2574–2583.

Fraser AG, Marcotte EM (2004) Development through the eyes of functional genomics. *Current Opinion in Genetics and Development*, **14**, 336–342.

French V, Brakefield PM (2004) Pattern formation: a focus on notch in butterfly eyespots. *Current Biology*, **14**, R663–R665.

Garant D, Kruuk LEB (2005) How to use molecular marker data to measure evolutionary parameters in wild populations. *Molecular Ecology*, **14**, 1843–1859.

Gibson G, Dworkin I (2004) Uncovering cryptic genetic variation. *Nature Reviews Genetics*, **5**, 681–690.

Griswold CK, Whitlock MC (2003) The genetics of adaptation: the roles of pleiotropy, stabilizing selection and drift in shaping the distribution of bidirectional fixed mutational effects. *Genetics*, **165**, 2181–2192.

Hard JJ (1995) A quantitative genetic perspective on the conservation of intraspecific diversity. *American Fisheries Society Symposium*, **17**, 304–326.

Hill WG, Thompson R (1978) Probabilities of non-positive definite between-group or genetic covariance matrices. *Biometrics*, **34**, 429–439.

Hoffmann AA, Merilä J (1999) Heritable variation and evolution under favourable and unfavourable conditions. *Trends in Ecology & Evolution*, **14**, 96–101.

Houle D (1991) Genetic covariance of fitness correlates — what genetic correlations are made of and why it matters. *Evolution*, **45**, 630–648.

Houle D (1992) Comparing evolvability and variability of quantitative traits. *Genetics*, **130**, 195–204.

Houle D, Mezey J, Galpern P (2002) Interpretation of the results of common principal components analyses. *Evolution*, **56**, 433–440.

Jones AG, Arnold SJ, Bürger R (2003) Stability of the G-matrix in a population experiencing pleiotropic mutation, stabilizing selection, and genetic drift. *Evolution*, **57**, 1747–1760.

Jones AG, Arnold SJ, Bürger R (2004) Evolution and stability of the G-matrix on a landscape with a moving optimum. *Evolution*, **58**, 1639–1654.

de Jong G, van Noordwijk AJ (1992) Acquisition and allocation of resources: genetic (co) variances, selection, and life histories. *American Naturalist*, **139**, 749–770.

Keightley PD, Davies EK, Peters AD, Shaw RG (2000) Properties of ethylmethane sulfonate-induced mutations affecting life-history traits in *Caenorhabditis elegans* and inferences about bivariate distributions of mutation effects. *Genetics*, **156**, 143–154.

Keys DN, Lewis DL, Selegue JE *et al.* (1999) Recruitment of a hedgehog regulatory circuit in butterfly eyespot evolution. *Science*, **283**, 532–534.

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

Kirkpatrick M, Meyer K (2004) Direct estimation of genetic principal components: simplified analysis of complex phenotypes. *Genetics*, **168**, 2295–2306.

Kirkpatrick M, Johnson T, Barton N (2002) General models of multilocus evolution. *Genetics*, **161**, 1727–1750.

Klein TW (1974) Heritability and genetic correlation: statistical power, population comparisons and sample size. *Behavior Genetics*, **4**, 171–189.

Klein TW, De Vries JC, Finkbeiner CT (1973) Heritability and genetic correlation: standard errors of estimates and sample size. *Behavior Genetics*, **3**, 355–364.

Koots KR, Gibson JP (1996) Realized sampling variances of estimates of genetic parameters and the difference between genetic and phenotypic correlations. *Genetics*, **143**, 1409–1416.

Kruuk LEB (2004) Estimating genetic parameters in natural populations using the 'animal model'. *Philosophical Transactions of the Royal Society of London. Series B, Biological Sciences*, **359**, 873–890.

Lande R (1975) Maintenance of genetic variability by mutation in a polygenic character with linked loci. *Genetical Research*, **26**, 221–235.

Lande R (1976) Natural selection and random genetic drift in phenotypic evolution. *Evolution*, **30**, 314–334.

Lande R (1977) Statistical tests for natural selection on quantitative characters. *Evolution*, **31**, 442–444.

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

Lande R (1980) The genetic covariances between characters maintained by pleiotropic mutations. *Genetics*, **94**, 203–215.

Lande R (1982) A quantitative genetic theory of life history evolution. *Ecology*, **63**, 607–615.

Lande R (1984) The genetic correlation between characters maintained by selection, linkage and inbreeding. *Genetical Research*, **44**, 309–320.

Lande R, Arnold SJ (1983) The measurement of selection on correlated characters. *Evolution*, **37**, 110–126.

Langerhans RB, DeWitt TJ (2004) Shared and unique features of evolutionary diversification. *American Naturalist*, **164**, 335–349.

Lewontin RC (1970) The units of selection. *Annual Review of Ecology and Systematics*, **1**, 1–18.

Lopez-Fanjul C, Fernandez A, Toro MA (2003) The effect of neutral nonadditive gene action on the quantitative index of population divergence. *Genetics*, **164**, 1627–1633.

Lopez-Fanjul C, Fernandez A, Toro MA (2004) Epistasis and the temporal change in the additive variance-covariance matrix induced by drift. *Evolution*, **58**, 1655–1663.

Lynch M, Pfrender M, Spitze K *et al.* (1999) The quantitative and molecular genetic architecture of a subdivided species. *Evolution*, **53**, 100–110.

Lynch M, Ritland K (1999) Estimation of pairwise relatedness with molecular markers. *Genetics*, **152**, 1753–1766.

Lynch M, Walsh B (1998) *Genetics and Analysis of Quantitative Traits*. Sinauer Associates, Sunderland, Massachusetts.

McAdam AG, Boutin S (2003) Effects of food abundance on genetic and maternal variation in the growth rate of juvenile red squirrels. *Journal of Evolutionary Biology*, **16**, 1249–1256.

McGuigan K, Franklin CEF, Moritz C, Blows MW (2003) Adaptation of rainbow fish to lake and stream habitats. *Evolution*, **57**, 104–118.

McGuigan K, Chenoweth SF, Blows MW (2005) Phenotypic divergence along lines of genetic variance. *American Naturalist*, **165**, 32–43.

McMillan WO, Monteiro A, Kapan DD (2002) Development and evolution on the wing. *Trends in Ecology & Evolution*, **17**, 125–133.

Merilä J, Björklund M (1999) Population divergence and morphometric integration in the greenfinch (*Carduelis chloris*): evolution against the trajectory of least resistance? *Journal of Evolutionary Biology*, **12**, 103–112.

Merilä J, Crnokrak P (2001) Comparison of genetic differentiation at marker loci and quantitative traits. *Journal of Evolutionary Biology*, **14**, 892–903.

Meyer K, Kirkpatrick M (2005) Restricted maximum likelihood estimation of genetic principal components and smoothed covariance matrices. *Genetics Selection Evolution*, **37**, 1–30.

Mezey JG, Houle D (2003) Comparing G matrices: are common principal components informative? *Genetics*, **165**, 411–425.

Mezey JG, Houle D (2005) The dimensionality of genetic variation for wing shape in *Drosophila melanogaster*. *Evolution*, **59**, 1027–1038.

Milner JM, Pemberton JM, Brotherstone S, Albon SD (2000) Estimating variance components and heritabilities in the wild: a case study using the 'animal model' approach. *Journal of Evolutionary Biology*, **13**, 804–813.

Monteiro AF, Brakefield PM, French V (1994) The evolutionary genetics and developmental basis of wing pattern variation in the butterfly *Bicyclus anynana*. *Evolution*, **48**, 1147–1157.

Monteiro A, Prijs J, Bax M, Hakkaart T, Brakefield PM (2003) Mutants highlight the modular control of butterfly eyespot patterns. *Evolution and Development*, **5**, 180–187.

Moore AJ, Kukuk PF (2002) Quantitative genetic analysis of natural populations. *Nature Reviews Genetics*, **3**, 971–978.

Moss R, Piertney SB, Palmer SCF (2003) The use and abuse of microsatellite DNA markers in conservation biology. *Wildlife Biology*, **9**, 243–250.

Nijhout FH (1991) *The Development and Evolution of Butterfly Wing Patterns*. Smithsonian Institution Press, Washington, D.C.

Orr HA (2000) Adaptation and the cost of complexity. *Evolution*, **54**, 13–20.

Paulsen SM (1994) Quantitative genetics of the butterfly wing color patterns. *Developmental Genetics*, **15**, 79–91.

Paulsen SM (1996) Quantitative genetics of the wing color pattern in the buckeye butterfly (*Precis coenia* and *Precis evarete*): evidence against the constancy of G. *Evolution*, **50**, 1585–1597.

Phelan JP, Archer MA, Beckman KA *et al.* (2003) Breakdown in correlations during laboratory evolution. I. Comparative analyses of *Drosophila* populations. *Evolution*, **57**, 527–535.

Phillips PC, Arnold SJ (1989) Visualizing multivariate selection. *Evolution*, **43**, 1209–1222.

Phillips PC, Arnold SJ (1999) Hierarchical comparison of genetic variance-covariance matrices. I. Using the Flury hierarchy. *Evolution*, **53**, 1506–1515.

Phillips PC, McGuigan K (in press) Evolution of genetic variance-covariance structure (G). In: *Evolutionary Genetics: Concepts and Case Studies* (eds Fox CW, Wolf JB). Oxford University Press, Oxford, UK.

Phillips PC, Whitlock MC, Fowler K (2001) Inbreeding changes the shape of the genetic covariance matrix in *Drosophila melanogaster*. *Genetics*, **158**, 1137–1145.

Queller DC, Goodnight KF (1989) Estimating relatedness using genetic markers. *Evolution*, **43**, 258–275.

Reed DH, Frankham R (2001) How closely correlated are molecular and quantitative measures of genetic variation? A meta-analysis. *Evolution*, **55**, 1095–1103.

Reeve JP (2000) Predicting long-term response to selection. *Genetical Research*, **75**, 83–94.

Riska B, Prout T, Turelli M (1989) Laboratory estimates of heritabilities and genetic correlations in nature. *Genetics*, **123**, 865–871.

Ritland K (1996) Marker-based method for inferences about quantitative inheritance in natural populations. *Evolution*, **50**, 1062–1073.

Ritland K (2000) Marker-inferred relatedness as a tool for detecting heritability in nature. *Molecular Ecology*, **9**, 1195–1204.

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

Roff D (2002) Comparing G matrices: a MANOVA approach. *Evolution*, **56**, 1286–1291.

Rose MR (1982) Antagonistic pleiotropy, dominance, and genetic variation. *Heredity*, **48**, 63–78.

Schluter D (1996) Adaptive radiation along genetic lines of least resistance. *Evolution*, **50**, 1766–1774.

Schluter D, Nychka D (1994) Exploring fitness surfaces. *American Naturalist*, **143**, 597–616.

Sgrò CM, Hoffmann AA (2004) Genetic correlations, tradeoffs and environmental variation. *Heredity*, **93**, 241–248.

Shaw FH, Shaw RG, Wilkinson GS, Turelli M (1995) Changes in genetic variances and covariances: G whiz! *Evolution*, **49**, 1260–1267.

Slatkin M, Frank SA (1990) The quantitative genetic consequences of pleiotropy under stabilizing and directional selection. *Genetics*, **125**, 207–213.

Spitze K (1993) Population structure in *Daphnia obtusa*: quantitative genetic and allozymic variation. *Genetics*, **135**, 367–374.

Steppan SJ, Phillips PC, Houle D (2002) Comparative quantitative genetics: evolution of the G matrix. *Trends in Ecology & Evolution*, **17**, 320–327.

Turelli M (1985) Effects of pleiotropy on predictions concerning mutation–selection balance for polygenic traits. *Genetics*, **111**, 165–195.

Turelli M (1988) Phenotypic evolution, constant covariances, and the maintenance of additive variances. *Evolution*, **42**, 1342–1347.

Via S, Lande R (1985) Genotype–environment interaction and the evolution of phenotypic plasticity. *Evolution*, **39**, 502–522.

Wang J (2004) Estimating pairwise relatedness from dominant genetic markers. *Molecular Ecology*, **13**, 3169–3178.

Weinig C, Schmitt J (2004) Environmental effects on the expression of quantitative trait loci and implications for phenotypic evolution. *Bioscience*, **54**, 627–635.

Whitlock MC (1999) Neutral additive genetic variance in a metapopulation. *Genetical Research*, **74**, 215–221.

Whitlock MC, Phillips PC, Moore FBG, Tonsor SJ (1995) Multiple fitness peaks and epistasis. *Annual Review of Ecology and Systematics*, **26**, 601–629.

Whitlock MC, Phillips PC, Fowler K (2002) Persistence of changes in the genetic covariance matrix after a bottleneck. *Evolution*, **56**, 1968–1975.

Wilkinson GS, Fowler K, Partridge L (1990) Resistance of genetic correlation structure to directional selection in *Drosophila melanogaster*. *Evolution*, **44**, 1990–2003.

Wright S (1951) The genetical structure of populations. *Annals of Eugenics*, **15**, 323–352.

Wright S (1968) *Evolution and the Genetics of Populations*. *I. Genetic and Biometric Foundations*. University of Chicago Press, Chicago, Illinois.

Zeng Z-B (1988) Long-term correlated response, inter-population covariation and interspecific allometry. *Evolution*, **42**, 363–374.

Zhang XS, Hill WG (2002) Joint effects of pleiotropic selection and stabilizing selection on the maintenance of quantitative genetic variation at mutation-selection balance. *Genetics*, **162**, 459–471.

Zhang XS, Hill WG (2003) Multivariate stabilizing selection and pleiotropy in the maintenance of quantitative genetic variation. *Evolution*, **57**, 1761–1775.

Zhang XS, Wang JL, Hill WG (2004) Redistribution of gene frequency and changes of genetic variation following a bottleneck in population size. *Genetics*, **167**, 1475–1492.

K. McGuigan is currently on ARC funded Australian Postdoctoral Fellow at the University of Queensland. She is broadly interested in evolution and genetics. This has led to research into population structure and history, the relationships between morphology and performance, gene family evolution, and quantitative genetics of functional morphology. Her current research revolves around understanding factors affecting the evolution of quantitative genetic variation in Australian *Drosophila*.
