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COMMENTARY

The biology of multivariate evolution

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Evolution is best viewed as a multivariate process making a working knowledge of linear algebra a great aide in our understanding of this process (Lande & Arnold, 1983; Manley, 1985; Endler, 1986; Phillips & Arnold, 1989). We therefore are in general agreement with the main thrust of Blows' (2007) argument – a fuller appreciation of multivariate methods, particularly those that employ the diagonalization of the **G** and γ matrices, can aid our understanding of microevolutionary change. Nevertheless, the approach advocated by Blows (2007) is not a panacea that solves all problems in multivariate evolutionary analyses, and there are circumstances where the diagonalization of the **G** and γ matrices is either inappropriate or inferior to alternatives. In our opinion, the utility of matrix diagonalization is largely determined by the particular question of interest and the biology of the system examined. In support of our arguments, we discuss how matrix diagonalization can obscure the biological interpretation of a complex data set. We then discuss how the benefits of matrix diagonalization in evolutionary studies vary with the specific question addressed.

Biology and symmetric matrices

Organisms are made up of many interacting parts that are often shaped by selection to function as integrated units (or character) (Cheverud, 1982, 1996). In order to understand the evolution of such complex multidimensional characters, we must simplify the system to an understandable level that allows for biological interpretation. One of the major strengths of the multivariate approach is that it summarizes the entire pattern of (co)variation in a series of successive orthogonal axes, thereby removing the need to interpret single traits on an element-by-element basis. Given that a symmetric matrix with k traits contains $k(k + 1)/2$ elements, it can be a formidable task to interpret individual elements even when k is not particularly large.

Unfortunately, biology is rarely as simple as the above statistical procedures would dictate. Although matrix diagonalization is mathematically convenient, there are circumstances where it obscures biological interpretation. First, it is unlikely that causal factors will always have orthogonal effects on the phenotype – Houle *et al.* (2002, pp. 434–435) describe such a cautionary tale. Complex phenotypes are composed of many different measurable components, but it is unlikely (if not impossible) that each of these components will have exactly the same function. Consequently, we often do not know how to define a 'functionally related' character and, therefore, to decide on the appropriate unit of selection (Wagner & Laubichler, 2000). Thus, a misguided solution is to measure everything possible and then use matrix diagonalization to identify the dimensions of variation and define the character. Although each orthogonal axis represents a new 'trait' that can be defined by its eigenvector (Phillips & Arnold, 1989), such axes do not necessarily correspond to multivariate patterns of functional variability (Cheverud, 1982; Mitchell-Olds & Rutledge, 1986). In such instances, matrix diagonalization may actually disrupt or obscure the biologically meaningful units of variation.

Consider the case where understanding pleiotropic relationships between functional units of a character, such as those in the mouse mandible (Schwenk, 2001), is the goal. The mouse mandible has clearly identifiable functional units that are genetically and developmentally integrated with a modular structure (Cheverud, 2001). However, despite the modular structure of variation, there are still very large genetic covariances between the modular functional units of the mandible due to whole-mandible pleiotropic effects (Cheverud, 2001). If one diagonalizes **G**, the first eigenvalue contains significant variation from all functional units due to these pleiotropic effects. In such instances, it is preferable to extract eigenvalues from each of the individual modules and then directly estimate genetic parameters in these dimensions (Kirkpatrick & Meyer, 2004; Meyer & Kirkpatrick, 2005). This reduces the number of dimensions to be examined but keeps the modular structure intact. Alternatively, where the nature of the covariance structure is known *a priori*, confirmatory factor analysis and/or structural equation modelling may be a more promising approach (Houle, 2001; Houle *et al.*, 2002).

A second issue, as noted by Lande & Arnold (1983), is that selection analyses based on orthogonal transformations of the data [such as principle components (PCs)] can obscure the relationship between the individual traits and fitness. This occurs because, in the eigenvectors of the PCs that define the new composite 'traits' created by the transformation, traits that are subject to selection are associated with traits that are not experiencing selection (Lande & Arnold, 1983). Whenever traits that are the targets of selection have a substantial phenotypic covariance with traits unrelated to fitness, the use of orthogonal transformations may dilute the ability to detect the effect of selection (Mitchell-Olds & Shaw,

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1987). Thus, selection analyses performed on orthogonal transformations, particularly when associated with substantial multicollinearity in the data set, should always be interpreted with caution (Lande & Arnold, 1983; Mitchell-Olds & Shaw, 1987). The value of orthogonal transformations may be more as a first step for deriving hypotheses that can be followed up with manipulative experiments (Manley, 1985; Endler, 1986; Mitchell-Olds & Shaw, 1987).

Finally, in systems with a simple biology, interpretation may be obfuscated by orthogonal transformation of the data. We can illustrate this point using Moore's (1990) dataset examining episodes of sexual selection acting on sexually dimorphic traits in the dragonfly, *Libellula luctuosa*. Although the traits in this study were simple linear measurements, it is likely that a large proportion of the biological reality was captured – the wings are virtually flat with a simple shape, there are three (virtually rectangular) brown, white and clear areas on the wings, and the correlation between the width of the coloured patches and their area is high. Although the standard selection gradients significantly under-estimated the strength of nonlinear selection (median $|\gamma| = 0.52$, median $|\lambda| = 0.72$, Wilcoxon test: $Z = -2.201$, $n = 6$, $P = 0.03$), as is commonly the case in studies of selection (Blows & Brooks, 2003), little interpretive value is gained from a canonical analysis of the results. For example, regression analysis of mating success data revealed significant positive directional selection on brown-patch and white-patch size, quadratic (negative γ) selection on body size and positive correlational selection on brown-patch and white-patch size (Table 1; Moore, 1990). Canonical analysis of these data yields two statistically significant axes, $\mathbf{m}_1$ and $\mathbf{m}_4$, but no new insight is gained (Table 2). The axis $\mathbf{m}_1$ describes the pattern of linear and nonlinear selection operating on the size of the brown and white patches, whereas $\mathbf{m}_4$ describes the nonlinear selection operating on body size. The result is an individual selection surface with a multivariate saddle (Fig. 1) that is congruent with intuition based on the sign and magnitude of the β and γ values presented in Table 1.

Table 1 The vector of standardized linear selection gradients (β) and the matrix of standardized quadratic and correlational selection gradients (γ). These gradients were rederived, but also published in Moore (1990).

		γ			
Traits	β	WL	BP	WP	BS
Wing length (WL)	0.157	−0.194			
Brown-patch size (BP)	0.498*	0.207	−0.217		
White-patch size (WP)	0.778**	0.361	0.536*	0.107	
Body size (BS)	−0.293	0.514	−0.135	−0.368	−0.494*

Randomization tests: $^*P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$.

Table 2 The **M** matrix of eigenvectors from the canonical analysis of γ. The linear (θ_i) and quadratic (λ_i) canonical coefficients along each eigenvector are given in the last two columns.

	M				Selection	
	WL	BP	WP	BS	θ_i	λ_i
$\mathbf{m}_1$	0.302	0.460	0.825	−0.125	0.698***	0.350**
$\mathbf{m}_2$	0.804	−0.036	−0.189	0.563	−0.076	−0.060
$\mathbf{m}_3$	−0.085	0.884	−0.458	0.023	0.145	−0.369
$\mathbf{m}_4$	−0.506	0.070	0.269	0.817	0.035	−0.724*

WL, wing length; BP, brown-patch size; WP, white-patch size; BS, body size.
Randomization tests: $^*P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$.

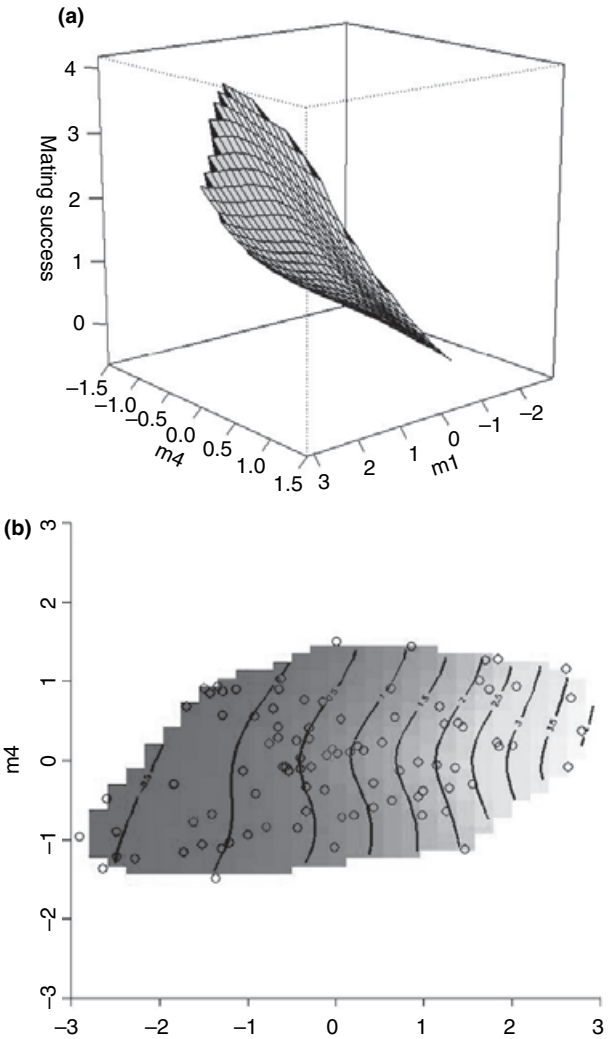

Fig. 1 Thin-plate spline visualization of the individual selection surface representing linear and nonlinear selection on $\mathbf{m}_1$ and $\mathbf{m}_4$. (a) a perspective view, (b) a shaded contour plot with supporting data points. Lighter regions indicate higher fitness.

Matrix diagonalization and biological questions

Given the importance of **G** and γ to evolutionary theory (Lande & Arnold, 1983), it is not surprising that many questions in evolutionary biology require an intimate knowledge of their structure. If one is trying to describe the overall variation in **G** or γ, or even how the dominant axis of **G** (or $\mathbf{g}_{max}$) relates to the β vector to examine the importance of genetic constraints (Schluter, 1996), matrix diagonalization is clearly a useful technique. There are, however, a number of questions in evolutionary biology where matrix diagonalization is not useful.

If an examination of the causal relationship between traits and fitness is the goal, it is unlikely to be answered convincingly with an observational approach that characterizes patterns of variation in β or γ (Mitchell-Olds & Shaw, 1987). The biological understanding of selection is ultimately the only way to determine how selection acts on the phenotype (Manley, 1985; Endler, 1986). To this end, manipulative studies that either break the functional relationships between traits (e.g. Brooks *et al.*, 2005; Bentsen *et al.*, 2006) or extend phenotypes beyond their natural range (e.g. Anholt, 1991; Sinervo & Basolo, 1996) have been particularly useful. In systems that are not as amenable to experimental manipulation, path analysis may provide an alternative (Scheiner *et al.*, 2000), particularly because it enables analytic models to be constructed (and tested) that are based specifically on the biology of the organism being examined.

Sometimes estimating important genetic parameters of evolutionary change, such as the mean phenotypic response to selection ($\Delta\bar{\mathbf{Z}} = \mathbf{G}\boldsymbol{\beta}$) or the change of **G** due to selection within a generation [$\Delta\mathbf{G}=\mathbf{G}(\gamma-\beta\beta^{T})\mathbf{G}$], is the goal. In the former case, matrix diagonalization is unnecessary because the vector of mean phenotypic response to selection (i.e. the multivariate breeders' equation) uses **G** as a transformation matrix to relate selection within a generation (β) to changes in trait means across generations. This gives a concise way of relating the space of **G** and β in the $\Delta\bar{\mathbf{Z}}$ vector without the need for matrix diagonalization. In the later case, the diagonalization of γ does not provide the correct characterization of the form of nonlinear selection because it is the curvature of the adaptive landscape (i.e. $\gamma-\beta\beta^{T}$) that determines how selection shapes **G**, not the curvature of the individual selection surface (i.e. γ) (Phillips & Arnold, 1989). In most cases (when the trait distribution is approximately multivariate normal), the adaptive landscape and the individual selection surface have the same β, but the curvature of the adaptive landscape often differs considerably from γ because it is weighted (and smoothed) by the phenotypic distribution (Phillips & Arnold, 1989). Thus, to understand the relationship between the structure of **G** and patterns of selection, analyse the structure of $\gamma-\beta\beta^{T}$ not γ.

Although Blows (2007) gives a cursory discussion to issues regarding linear selection, nonlinear components of selection need not be emphasized at the cost of linear selection. We reiterate the point made eloquently by Phillips & Arnold (1989): one cannot interpret the consequences of γ without β. For example, the diagonalization of γ may reveal a negative eigenvalue for the major canonical axis suggesting 'stabilizing' selection towards an optimum. However, if there is a strong linear component along this axis, the pattern of selection should be seen as linear selection that has curvature (Lande & Arnold, 1983). Phillips & Arnold (1989) describe how to characterize both linear and nonlinear selection within canonical analysis (the 'A canonical form'). When linear selection is minimal an alternative involving an additional transformation ('B canonical form') allows a full description of selection using the curvature of the individual fitness surface, but additional restrictions and assumptions are associated with the use of form B (Phillips & Arnold, 1989).

Finally, if questions of generality and/or comparability are being addressed, then matrix diagonalization may complicate the direct comparison of two or more matrices; e.g. when comparing multiple individual selection surfaces for a common set of phenotypic traits (e.g. Chenoweth & Blows, 2005). Because the axes derived from an orthogonal transformation occupy a unique multivariate subspace that often differs considerably from the original trait space, the canonical rotation of the individual fitness surfaces places the data in different subspaces. Consequently, any biologically meaningful comparison of the **M** matrices between the sexes requires projection into a common subspace. It is conceptually and computationally simpler to use sequential model building procedures (Draper & John, 1988) to compare selection in the untransformed space.

Summary

Although we agree that the multivariate approach advocated by Blows (2007) often provides insights into current selection, it should not be viewed as a panacea that solves all problems in evolutionary biology. Although a multivariate view of evolution clearly prevails, the difficulty in defining characters, the plethora of legitimate research goals, and the potential loss of generality or interpretability can supersede the statistical sophistication of matrix diagonalization. By identifying some of the limitations of this methodology we hope that we clarify when its use is appropriate. Our general recommendation is that matrix diagonalization should be used if possible and appropriate – but as always, biological questions must take precedence over statistical techniques.

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