

COMMENTARY

A wake-up call for studies of natural selection?

L. E. B. KRUIK* & D. GARANT†‡

Institute of Evolutionary Biology, School of Biological Sciences, University of Edinburgh, Edinburgh, UK†Département de Biologie, Faculté des Sciences Université de Sherbrooke, Sherbrooke, QC, Canada**‡Edward Grey Institute, Department of Zoology, University of Oxford, Oxford, UK*

Mark Blows' (2007) excellent Tale of Two Matrices could make alarming reading for anyone interested in quantifying natural selection. Many of the well-meaning regressions of fitness on one or two phenotypic traits that have accumulated in the literature (e.g. Endler 1986; Kingsolver *et al.*, 2001) may be at best uninformative, and at worst misleading, if selection does not act directly on those traits but instead targets particular combinations of traits. However, although the approaches Blows advocates may well overturn current wisdom in several areas, there are a few caveats that we would like to draw attention to.

Blows, and others before him (Phillips & Arnold, 1989), argue for the use of matrix analysis to reveal how selection may target particular combinations of quantitative traits. Selection on pairwise combinations can be quantified through correlational selection gradients, the interaction between two traits in their effect on fitness. Unfortunately, despite the fact the analysis of correlational selection was first suggested many years ago (Lande & Arnold, 1983), few studies to date have ever ventured this far – possibly because interpreting the large number of parameters involved is a daunting prospect. However, a re-orientation of the axes of measurement can provide a much more efficient description of the relationship between phenotype and fitness. Specifically, a canonical analysis of the matrix of nonlinear selection coefficients, γ , uses linear algebra to distil out the key characteristics of the relationship between multiple phenotypic traits and fitness. The eigenvectors of γ define new combinations (the 'canonical axes') of the original traits, with corresponding eigenvalues that describe the quadratic (stabilizing or disruptive) selection acting on the combinations (Phillips & Arnold, 1989). This is useful, firstly, because there are far fewer parameters to interpret and, secondly, because of the simple observation that a negative eigenvalue indicates convex (stabilizing) selection, whereas a positive value indicates concave (disruptive) selection. Furthermore, the new combinations may contain contributions from multiple

traits, providing a more comprehensive integration of multivariate effects than the pairwise relationships described by γ . Although the procedure requires a grasp of the basics of linear algebra, implementation is relatively straightforward using standard statistical packages.

So why might any of this be alarming? As Blows points out here and in previous work (see Blows & Brooks, 2003), the great majority of previous studies have considered only the diagonal elements of the multivariate selection matrix and so have potentially misrepresented many of the most interesting aspects of the fitness surface. Of the 63 studies listed by Kingsolver *et al.* (2001) in their survey of the literature on selection coefficients, only seven reported correlational selection on three or more traits, and of these only two tested for nonlinear selection along the canonical axes (Blows & Brooks, 2003). Whereas the quadratic selection acting on individual traits (the diagonal elements of γ) might not be statistically significant, a re-orientation of the fitness surface will often reveal substantial nonlinear selection along some of the canonical axes: a re-analysis of the seven studies with correlational selection found stronger selection on the canonical axes than on the original traits in every case. This renders analyses of nonlinear selection that have only considered the diagonal elements of γ effectively incomplete. Without including the off-diagonal information in the picture, we can say little about the prevalence or absence of anything other than directional (linear) selection.

Identification of the main axes of nonlinear selection in a population could therefore provide new angles on several prominent puzzles of evolutionary biology. Many people were probably surprised by the discovery that, amongst the vast wealth of selection gradients in the literature, stabilizing selection is generally weak, and nonlinear selection, in general, is less prevalent than directional selection (Kingsolver *et al.*, 2001). The above arguments suggest that we may simply have been looking in the wrong place. There is also the puzzling observation that additive genetic variance remains abundant for traits that are apparently under strong directional selection, for example through sexual selection (Pomiankowski & Møller, 1995; Rowe & Houle, 1996), and also that tests for microevolution in action in wild populations rarely observe changes that correspond with the predicted responses to directional natural selection (Merilä *et al.*, 2001). Although there may be several possible explanations for each issue, such as insufficient statistical power in many studies (Hersch & Phillips, 2004), a failure to identify the correct targets of selection, or the full potential of stabilizing or disruptive selection, must inevitably generate misleading expectations.

However, although we agree fully with Blows' emphasis on the need to consider selection on entire phenotypes rather than single traits, we believe it is worth raising a few points with regard to interpretations of a canonical analysis of γ .

Correspondence: L. E. B. Kruuk, Institute of Evolutionary Biology, School of Biological Sciences, University of Edinburgh, Edinburgh, EH9 3JT, UK. Tel.: +44 131 650 5515; fax: +44 131 650 6564; e-mail: loeske.kruuk@ed.ac.uk

Firstly, stronger quadratic selection on at least one of the new combinations, when compared with the original traits, is algebraically inevitable. This is because the eigenvalues of a real symmetrical matrix (such as γ) span its diagonal elements (Mercer & Mercer, 2000), so there will always be at least one canonical axis for which the quadratic selection is greater than for the original traits – the only exception being the case where γ is already diagonal (no correlational selection), and so the original traits are simply the canonical axes. Similarly, there will also always be at least one canonical axis with less quadratic selection. This has implications for how we phrase questions as to the prevalence of quadratic selection. It is not biologically very interesting simply to identify a new composite trait with stronger quadratic selection: what matters is its statistical significance and its form (stabilizing or disruptive).

Second, following on from the previous point, it may well prove the case that a re-orientation of axes does little to change our *qualitative* impression of the way selection works in a particular system, in particular with respect to the presence or not of statistically significant quadratic selection. Blows and co-workers have published studies of several systems in which a canonical analysis did generate markedly different conclusions (see for example Blows *et al.*, 2003; Chenoweth & Blows, 2005). Although the strongest nonlinear selection on the canonical axes was greater than on the original traits in the seven studies taken from Kingsolver *et al.*'s (2001) survey (Blows & Brooks, 2003), no comparison of statistical significance of the new axes was possible. A canonical analysis of γ will not improve the accuracy of selection coefficients: errors on the canonical coefficients will be the same order of magnitude as the quadratic selection coefficients on the original traits (Phillips & Arnold, 1989). Given the low power of most studies of linear selection in the wild (Hersch & Phillips, 2004), and bearing in mind that such power will even be lower when considering nonlinear selection (Lande & Arnold, 1983; Kingsolver *et al.*, 2001), it may turn out that canonical analyses do not provide a very different picture of selection in natural populations unless sample sizes are supplemented. Furthermore, Phillips & Arnold (1989) first pointed out the power of analyses of the eigenstructure of the nonlinear selection coefficient matrix very eloquently many years ago. It is therefore puzzling why the approach has been so unpopular, in comparison with the analysis of linear or quadratic selection gradients (Lande & Arnold, 1983; Arnold & Wade, 1984). One possible explanation for the apparent lack of uptake is that exploratory analyses failed to show anything different, and so researchers stuck with simpler presentations of analyses of the original traits.

Two recent studies of wild bird populations provide examples of situations where a canonical analysis did not

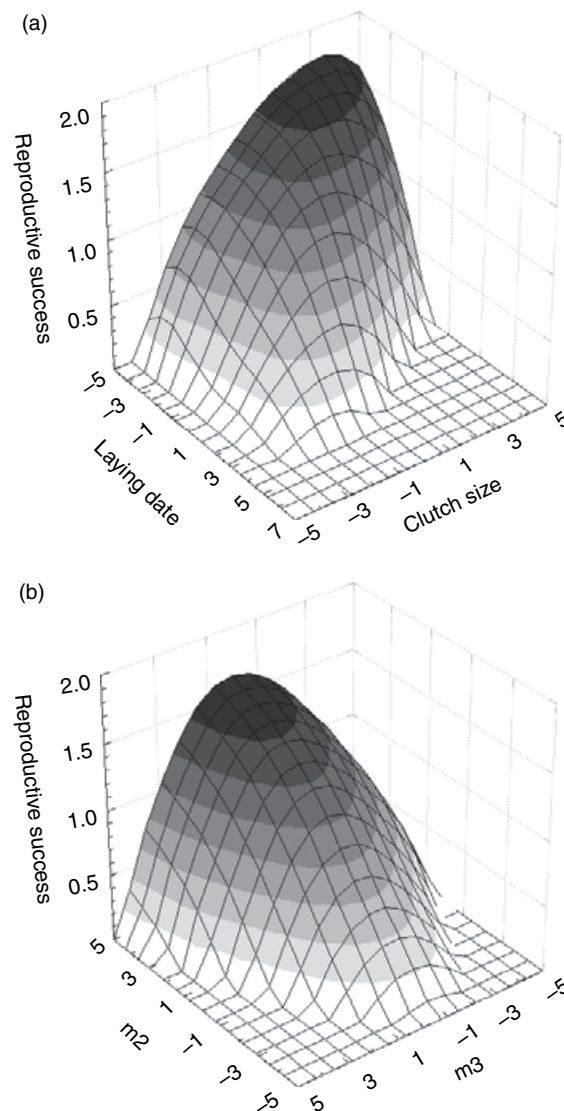


Fig. 1 Fecundity selection in female great tits (*Parus major*) in Wytham Woods, Oxfordshire, from an analysis of breeding time (laying date), clutch size and egg weight. Figures show cubic spline surfaces using either: (a) standardized original traits, laying date and clutch size; or (b) the first two main axes of selection obtained from canonical rotation. (a) Significant nonlinear selection was detected on breeding time and clutch size at both quadratic ($-0.027 \pm 0.014SE$ and $-0.057 \pm 0.014SE$ respectively) and correlational ($-0.057 \pm 0.022SE$) levels; (b) Significant nonlinear selection was detected at both the m3 and m2 canonical axes of selection (eigenvalues -0.074 and -0.012 respectively). m3 contributed 84.9% of the variation and was largely representative of clutch size and laying date (eigenvector loadings 0.857 and 0.514 respectively) whereas m2 contributed 13.8% of the variation and represented all three traits (eigenvector loadings: clutch size 0.480, laying date -0.759 , egg weight 0.440). Sample size $n = 2862$ individuals; (D. Garant, L. Kruuk, R. McCleery & B. Sheldon, unpublished data).

radically change qualitative conclusions. In an analysis of selection on three reproductive traits (clutch size, breeding time and egg size) in female great tits (*Parus major*), equivalent conclusions were reached for the original traits (Fig. 1a) as for canonical axes [Fig 1b; D. Garant, L. Kruuk, R. McCleery & B. Sheldon (unpublished data)]. For both, there was significant convex selection on two out of the three traits (details in Fig. 1 legend), and patterns of nonlinear selection were consistently stronger for fertility selection than for viability selection. Frentiu (2004) presents an analysis of six morphological measures on an island population of the Capricorn silvereye (*Zosterops lateralis chlorocephalus*), in two different years: one of the original traits showed significant quadratic selection in one year, whereas one of the canonical axes was significant in one year and another in another year. These two examples suggest the need for many more comparisons before we can conclude that the canonical analysis will radically change current estimates of the prevalence of significant quadratic selection.

Third, canonical rotation might even reduce the apparent prevalence of quadratic selection. This would be the case in a scenario whereby two traits both showed significant quadratic selection (so that the frequency amongst original traits was $2/n$, where n traits have been measured), but whose effects could be combined into a single canonical axis, and all other canonical axes showed no significant nonlinear selection (so the frequency amongst canonical axes was $1/n$). There is also no reason for canonical analyses to change Kingsolver *et al.* (2001)'s equally unexpected conclusion that disruptive selection is as common as stabilizing selection: for example, in Blows & Brooks (2003) re-analysis of studies reporting correlational selection, the strongest nonlinear selection along the canonical axes consistently took the same form as the strongest nonlinear selection on the original traits.

Fourth, as Blows acknowledges, care will need to be taken to ensure sensible interpretation of the canonical axes, and to avoid lengthy paragraphs of discussions given over to explaining the contortions of the original traits suggested by loadings on the principal components. Although not a perfect solution to problems of interpretation, using a thin-plate spline surface to visualize the major axes of nonlinear selection is certainly a helpful complement to canonical analyses (Fig. 1).

Finally, and very obviously, reaching useful conclusions from a canonical analysis of selection still requires data on informative traits, so that the principal axes of selection can be recovered as linear combinations of these traits. No amount of algebraic wizardry will be able to detect selection acting on aspects of the phenotype that are entirely independent of the measured traits. A lack of significant stabilizing or disruptive selection on the canonical axes is still only a null result with regard to the contents of the data-set – canonical analysis is not a

panacea to solve the requirement of measuring relevant phenotypic traits.

Nevertheless, whilst bearing the above points in mind, we are hopeful that the arguments presented by Blows will serve as a wake-up call for evolutionary biologists studying natural selection, forcing them to pay due attention to multi-trait associations. Furthermore, previous studies of multivariate selection have usually focussed on suites of related traits, such as different aspects of body size. Interesting patterns may lie in more diverse mixes – see for example, Blais' *et al.* (2004) combination of morphological and behavioural traits with measures of parasite resistance and genetic diversity in a study of nonlinear sexual selection in three-spine sticklebacks (*Gasterosteus aculeatus*). Analyses of cross-generational bundles of traits, containing both maternal and offspring phenotype, could provide unique insights into selection acting via maternal effects, for example by quantifying the associations of different phenotypes such as offspring size and offspring number with both mother's and offspring's fitness (Wolf & Wade, 2001).

In conclusion, there is clearly no longer any excuse for splashing around in the shallows with analyses of univariate, or at best quadratic, selection coefficients: studies of natural selection need to take the plunge and wrestle with the canonical re-orientations lurking in the depths.

Acknowledgments

LK is funded by the Royal Society, London. DG is supported by a Discovery Grant from the Natural Sciences and Engineering Research Council of Canada (NSERC) and by New Researchers start-up program grant from the Fonds Québécois de la Recherche sur la Nature et les Technologies (FQRNT). Many thanks to John Taylor for working out a proof of the fact that the eigenvalues span the diagonal elements and to Ben Sheldon for use of the Wytham great tit data.

References

- Arnold, S.J. & Wade, M.J. 1984. On the measurement of natural and sexual selection: theory. *Evolution* **38**: 709–719.
- Blais, J., Rico, C. & Bernatchez, L. 2004. Nonlinear effects of female mate choice in wild three-spine sticklebacks. *Evolution* **58**: 2498–2510.
- Blows, M.W. 2007. A tale of two matrices: multivariate approaches in evolutionary biology. *J. Evol. Biol.* **20**: 1–8.
- Blows, M.W. & Brooks, R. 2003. Measuring nonlinear selection. *Am. Nat.* **162**: 815–820.
- Blows, M.W., Brooks, R. & Kraft, P.G. 2003. Exploring complex fitness surfaces: multiple ornamentation and polymorphism in male guppies. *Evolution* **57**: 1622–1630.
- Chenoweth, S.F. & Blows, M.W. 2005. Contrasting mutual sexual selection on homologous signal traits in *Drosophila serrata*. *Am. Nat.* **165**: 281–289.
- Endler, J.A. 1986. *Natural Selection in the Wild*. Princeton University Press, Princeton.

- Frentiu, F.D. 2004. *Adaptive Morphological Evolution in an Island Bird (Zosterops lateralis chlorocephalus): A Quantitative Genetic Approach*. School of Life Sciences. PhD thesis, University of Queensland, Australia.
- Hersch, E.I. & Phillips, P.C. 2004. Power and potential bias in field studies of natural selection. *Evolution* **58**: 479–485.
- Kingsolver, J.G., Hoekstra, H.E., Hoekstra, J.M., Berrigan, D., Vignieri, S.N., Hill, C.E., Hoang, A., Gibert, P. & Beerli, P. 2001. The strength of phenotypic selection in natural populations. *Am. Nat.* **157**: 245–261.
- Lande, R. & Arnold, S.J. 1983. The measurement of selection on correlated characters. *Evolution* **37**: 1210–1226.
- Mercer, A.M. & Mercer, P.D. 2000. Cauchy's interlace theorem and lower bounds for the spectral radius. *Int. J. Math. & Math. Sci.* **23**: 563–566.
- Merilä, J., Sheldon, B.C. & Kruuk, L.E.B. 2001. Explaining stasis: microevolutionary studies of natural populations. *Genetica* **112**: 119–222.
- Phillips, P.C. & Arnold, S.J. 1989. Visualizing multivariate selection. *Evolution* **43**: 1209–1222.
- Pomiankowski, A. & Møller, A.P. 1995. A resolution of the lek paradox. *Proc. R. Soc. Lond. B* **260**: 21–29.
- Rowe, L. & Houle, D. 1996. The lek paradox and the capture of genetic variance by condition dependent traits. *Proc. R. Soc. Lond. B* **263**: 1415–1421.
- Wolf, J.B. & Wade, M.J. 2001. On the assignment of fitness to parents and offspring: whose fitness is it and when does it matter? . *J. Evol. Biol.* **14**: 347–356.