ESTIMATING NONLINEAR SELECTION GRADIENTS USING QUADRATIC REGRESSION COEFFICIENTS: DOUBLE OR NOTHING?

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The use of regression analysis has been instrumental in allowing evolutionary biologists to estimate the strength and mode of natural selection. Although directional and correlational selection gradients are equal to their corresponding regression coefficients, quadratic regression coefficients must be doubled to estimate stabilizing/disruptive selection gradients. Based on a sample of 33 papers published in *Evolution* between 2002 and 2007, at least 78% of papers have not doubled quadratic regression coefficients, leading to an appreciable underestimate of the strength of stabilizing and disruptive selection. Proper treatment of quadratic regression coefficients is necessary for estimation of fitness surfaces and contour plots, canonical analysis of the γ matrix, and modeling the evolution of populations on an adaptive landscape.

Understanding the strength, mode, and frequency of selection in the wild continues to be an important challenge for evolutionary biologists (Hoekstra et al. 2001; Kingsolver et al. 2001; Stinchcombe et al. 2002; Geber and Griffen 2003; Hereford et al. 2004; Kingsolver and Pfennig 2007). A particularly influential approach to addressing these questions has been the application of regression-based techniques for estimating selection in contemporary or experimental populations (Lande and Arnold 1983). The Lande–Arnold approach has revolutionized microevolution-

ary studies of selection over the last 25 years, and offers investigators the potential to estimate directional, stabilizing/disruptive, and correlational selection on phenotypic traits using relatively simple statistical models.

Here we draw attention to an area of confusion in the application of regression-based methods for estimating the strength of nonlinear selection. In their original paper and subsequent developments of these approaches, Lande and Arnold (1983) stated that selection coefficients could be estimated through regression

techniques. However, the subtle differences that exist between selection gradients, as formulated by Lande and Arnold (1983), and standard regression coefficients have often not been appreciated in the literature. Since 1983, many authors have assumed, or at least implied, that selection coefficients are exactly equal to regression coefficients. Simply put, stabilizing and disruptive selection gradients are equal to twice the corresponding quadratic regression coefficients. Only directional and correlational selection gradients are equal to their corresponding regression coefficients.

TWO FORMS OF QUADRATIC REGRESSION

Relative fitness of individuals within a population may be a complex function of phenotypic values. Lande and Arnold (1983) argued that this individual selection function can often be approximated by a quadratic regression in which relative fitness, w, is a function of linear and squared (quadratic) trait values

$$w = \alpha + \boldsymbol{\beta}^T \mathbf{z} + \frac{1}{2} \mathbf{z}^T \boldsymbol{\gamma} \mathbf{z} + \varepsilon \qquad (1a)$$

(see also Phillips and Arnold 1989). Here, $\mathbf{z} = \{z_1, z_2, \ldots, z_n\}^T$ is the column vector of phenotypic values for n traits, standardized so that their means are zero, α and ε are intercept and error terms, $\boldsymbol{\beta} = \{\beta_1, \beta_2, \ldots\}^T$ is the column vector of directional selection gradients, and $\boldsymbol{\gamma}$ is the matrix of nonlinear selection gradients

$$\boldsymbol{\gamma} = \begin{bmatrix} \gamma_{11} & \gamma_{12} & \cdots & \gamma_{1n} \\ \gamma_{12} & \gamma_{22} & \cdots & \vdots \\ \vdots & \vdots & \ddots & \vdots \\ \gamma_{1n} & \cdots & \cdots & \gamma_{nn} \end{bmatrix},$$

where γ_{ii} is a stabilizing/disruptive selection gradient for trait i, and γ_{ij} is a correlational selection gradient for traits i and j.

In the univariate (single trait) case equation (1a) reduces to

$$w = \alpha + \beta_i z_i + \frac{1}{2}\gamma_{ii} z_i^2 + \varepsilon \qquad (1b)$$

or in the bivariate case,

$$w = \alpha + \beta_i z_i + \beta_j z_j + \frac{1}{2}\gamma_{ii} z_i^2 + \frac{1}{2}\gamma_{jj} z_j^2 + \gamma_{ij} z_i z_j + \varepsilon. \quad (1c)$$

Following Lande and Arnold (1983), quadratic fitness surfaces have been represented by several authors using these equations (e.g., Kalisz 1986; Rausher and Simms 1989; Brodie 1992; O'Neil 1997; Arnold et al. 2001; Kingsolver and Pfennig 2007; Garant et al. 2007).

Regression analyses as performed by common statistical software are based on a slightly different formulation of the relationship between the dependent and independent variables—i.e., between relative fitness (w) and traits (z). The representation of quadratic regression in standard statistical texts (e.g., Box and Draper 1987, eq. 10.3.3) is given by

$$w = a + b^T \mathbf{z} + \mathbf{z}^T \mathbf{Q} \mathbf{z} + \varepsilon, \qquad (2a)$$

where a represents the intercept in the regression model and $\mathbf{b}$ is a column vector of linear regression coefficients. In this case, $\mathbf{Q}$ is the matrix of quadratic regression coefficients

$$\mathbf{Q} = \begin{bmatrix} q_{11} & \frac{1}{2}q_{12} & \cdots & \frac{1}{2}q_{1n} \\ \frac{1}{2}q_{12} & q_{22} & \cdots & \vdots \\ \vdots & \vdots & \ddots & \vdots \\ \frac{1}{2}q_{1n} & \cdots & \cdots & q_{nn} \end{bmatrix},$$

where q_{ii} and q_{ij} are the quadratic and cross-product regression coefficients. Writing equation (2a) in bivariate form yields the equation commonly found in statistical texts (e.g., Draper and Smith 1981, eq. 5.1.4)

$$w = a + b_i z_i + b_j z_j + q_{ii} z_i^2 + q_{jj} z_j^2 + q_{ij} z_i z_j + \varepsilon. \quad (2b)$$

Standard statistical packages report estimates for the parameters a, b_i, q_{ii}, and q_{ij}. The expression for fitness in terms of selection gradients in equation (1) is only equivalent to the expression for fitness defined by classical regression (eq. 2) when $\alpha = a$, $\beta_i = b_i$, $\gamma_{ij} = q_{ij}$, and $\gamma_{ii} = 2q_{ii}$. In other words, quadratic regression coefficients (q_{ii}) obtained from statistical software must be doubled if they are to be reported as stabilizing/disruptive selection gradients. Likewise the standard errors should also be doubled, although neither P-values nor the statistical power of the regressions will be affected.

ORIGIN OF THE LANDE–ARNOLD FORMULATION

Mathematically, neither formulation (1) nor (2) is more correct than the other; the two forms are simply different. However, the selection gradients in form (1) lead to some useful interpretations, and because of this, it is helpful to understand the origin of the $^1/_2$ in the Lande-Arnold formulation. The factor of $^1/_2$ enters into the selection gradient formulation (eq. 1) so that coefficients of stabilizing selection will be equivalent to partial derivatives of fitness with respect to squared trait values. This consideration applies to coefficients of stabilizing/disruptive selection (γ_{ii}) but not to coefficients of correlational selection (γ_{ij}, $i \neq j$).

The linear and quadratic selection gradients, $\boldsymbol{\beta}$ and $\boldsymbol{\gamma}$, respectively describe the average slope and curvature of the individual selection surface. For the linear selection gradient, this averaging of slope is given by

$$\boldsymbol{\beta} = \int p(\mathbf{z}) \frac{\partial w}{\partial \mathbf{z}} \, d\mathbf{z}, \qquad (3a)$$

where $p(\mathbf{z})$ is the phenotype distribution, the gradient operator is defined as $\partial/\partial \mathbf{z} = (\partial/\partial z_1, \partial/\partial z_2, \ldots, \partial/\partial z_n)^T$, and the integral is taken over all z_i (Lande and Arnold 1983, eq. 9). Similarly, the nonlinear selection gradients, $\boldsymbol{\gamma}$, are equivalent to the average

curvature of the relative fitness surface,

$$\boldsymbol{\gamma} = \int p(\mathbf{z}) \frac{\partial^2 w}{\partial \mathbf{z}^2} \, d\mathbf{z}, \tag{3b}$$

where the curvature operator is defined as the Hessian matrix

$$\frac{\partial^2}{\partial \mathbf{z}^2} = \begin{bmatrix} \frac{\partial^2}{\partial z_1 z_1} & \cdots & \frac{\partial^2}{\partial z_1 z_n} \\ \vdots & \ddots & \vdots \\ \frac{\partial^2}{\partial z_n z_1} & \cdots & \frac{\partial^2}{\partial z_n z_n} \end{bmatrix}$$

(Lande and Arnold 1983, eq. 14b). The factor of $^1/_2$ in estimation equation (1) is necessary to achieve this equivalence between γ as a selection coefficient (eq. 1) and γ as a descriptor of curvature (eq. 3b).

The Lande and Arnold estimation equation (eq. 1) can also be viewed as a second-order Taylor series approximation of the individual fitness surface around the mean phenotype. In a Taylor series expansion, each second derivative term is multiplied by a factor of $^1/_2$. Because squared terms such as z_i^2 appear once in the expansion, but cross-product terms such as $z_i z_j$ appear twice, the $^1/_2$ remains with the former but drops out of the latter, that is,

$$\left(\frac{1}{2} \frac{\partial}{\partial z_j} \left(\frac{\partial w(z)}{\partial z_i} \right) + \frac{1}{2} \frac{\partial}{\partial z_i} \left(\frac{\partial w(z)}{\partial z_j} \right) \right) z_i z_j$$
$$= \frac{\partial^2 w(z)}{\partial z_i \partial z_j} z_i z_j = \gamma_{ij} z_i z_j$$

.
The factor of $^1/_2$ in the estimation of γ also insures that the role of γ is straightforward in other connections that depend on its identity as second derivative. In that role, $\boldsymbol{\gamma} - \boldsymbol{\beta}\boldsymbol{\beta}^T$ describes the curvature of the adaptive landscape (where the adaptive landscape is defined as $\ln(\bar{W}(z))$; Lande 1979; Estes and Arnold 2007) and allows an estimation of the loss of genetic variation that occurs each generation due to selection (given by $\Delta \mathbf{G} = \mathbf{G}(\boldsymbol{\gamma} - \boldsymbol{\beta}\boldsymbol{\beta}^T)\mathbf{G}$; Lande 1980a; Lande and Arnold 1983; Phillips and Arnold 1989). That role also leads directly to approximation of the selection surface with a Gaussian function (Lande 1979, 1980a,b) and canonical analysis of the selection surface (Phillips and Arnold 1989; Blows and Brooks 2003), both of which we discuss below.

WHICH FORM OF QUADRATIC REGRESSION HAS BEEN USED IN THE EMPIRICAL LITERATURE?

Lande and Arnold (1983) were, of course, aware of this difference between selection gradients and regression coefficients. In their example using the Bumpus (1899) data, *t* the reported value for the stabilizing selection gradient was estimated as twice the quadratic regression coefficient given by standard statistical packages, although this was not explicitly stated. Although various other authors have pointed out the need to double quadratic coefficients when estimating stabilizing/disruptive selection gradients (e.g., Endler 1986; Wolf and Brodie 1998), and a few empirical

studies have explicitly stated that they did so in their analyses of selection (e.g., Widen 1991; Conner et al. 1996; Winn 2004), the application of this convention does not appear to have been widely reported. In a collection of 30 published papers on selection gradient analysis reviewed for a separate purpose (A. F. Agrawal and J. R. Stinchcombe, unpubl. ms.), we found three papers that specifically stated that quadratic regression coefficients were doubled to estimate stabilizing/disruptive selection gradients. Although some of the remainder may have done so without including an explicit statement to that effect, it is difficult or impossible for the reader to know. In at least one study, the authors reported halving, rather than doubling, quadratic regression coefficients to obtain γ_{ii} values.

Because it was impossible to tell from published works the extent to which the doubling of quadratic regression coefficients had been implemented in empirical studies, and to assess the extent of this problem more systematically, we surveyed all papers published in the journal *Evolution* from 2002 to 2007 inclusively that used the Lande–Arnold approach to estimate coefficients of nonlinear selection. We found 33 papers that reported estimates of stabilizing/disruptive selection gradients (γ_{ii} values). Only one of these studies explicitly stated that quadratic regression coefficients had been doubled to estimate stabilizing/disruptive selection gradients. We contacted the first author of the remaining 32 papers asking for more detail on how γ_{ii} values had been estimated. To ensure candid responses, authors were promised both confidentiality and that the resulting data would be analyzed and presented in a way that made it impossible for a particular method of analysis to be tied to an individual paper. We received responses from all authors, and in 31 cases authors were able to definitively describe the method of analysis, leading to an overall sample of 32 papers for which we had unambiguous confirmation of how the analysis was performed. From this sample, 25 papers (78%) reported unadjusted quadratic regression coefficients as γ_{ii} values, whereas seven papers (22%) doubled the quadratic regression coefficients as γ_{ii} values. Depending on the method of analysis used in the remaining paper for which we lack firm data, the percentage of papers failing to double the quadratic regression coefficients could range between 75% and 81% in this sample of 7 years of papers published in *Evolution*. Several of the authors mentioned in their replies that this issue had been a source of confusion for them and their colleagues. Our recommendation is that authors should double quadratic regression coefficients to estimate γ_{ii} values and that they explicitly state this in their methods.

Our survey results indicate that γ_{ii} values are most often reported as unadjusted quadratic regression coefficients, meaning that stabilizing/disruptive selection coefficients have often been underestimated by a factor of two. In a small fraction of cases, authors have correctly doubled quadratic regression coefficients to estimate γ_{ii} values but, because it was not explicitly stated in

Table 1. Distribution of reported and true disruptive/stabilizing selection gradients (γ_{ii}) published in *Evolution* from 2002 to 2007 in original units.

Percentile	Reported γ_{ii}	True γ_{ii}
Minimum	−2.19	−4.38
25th Percentile	−0.099	−0.13
Median	−0.003	−0.005
75th Percentile	0.06	0.084
Maximum	2.209	4.418
Percentage of Negative Estimates	51.6%	

A total of 673 estimates were tabulated from 32 studies, 25 of which reported uncorrected quadratic regression coefficients. The number of negative estimates, indicative of stabilizing selection, is given in the last row.

their methods, it is impossible for the average reader to know that correct adjustment was done. The percentage change in the point estimates of typical strength of selection from our survey suggests that the failure to multiply quadratic regression coefficients by two leads to an underestimate of the typical strength of nonlinear selection across all studies by 25–40% (Table 1). It remains unclear how many of the estimates of γ_{ii} reviewed by Kingsolver et al. (2001) underestimate the strength of selection by a factor of two.

Many of the reported estimates of nonlinear selection in our sample are not significantly different from zero, and because doubling them does not affect statistical significance or power, the true values of stabilizing and disruptive selection are larger, but still not significantly different from zero. The approximately symmetrical distribution of stabilizing and selection gradients around zero is also similar to the pattern found in the Kingsolver et al. (2001) study, suggesting that stabilizing selection is less common than previously thought (or disruptive selection more common), or

that estimates of stabilizing or disruptive selection are heavily influenced by sampling error (cf. Kingsolver et al. 2001; Knapczyk and Conner 2007).

APPLICATIONS OF QUADRATIC SELECTION COEFFICIENTS

One of the major contributions of the Lande–Arnold approach was the approximation of fitness surfaces using multiple regression (e.g., Arnold et al. 2001; Arnold 2003; Hereford et al. 2004), which provides several useful tools. First, the fitness surface can be visualized by plotting the fitness function for a set of traits (Brodie et al. 1995). Second, one can predict the fitness of other phenotypes in the population (e.g., O'Neil 1999). Both applications require the correct application of the partial regression coefficients. Either equations (1) or (2) can be used to plot a fitness surface but the corresponding coefficients must be appropriately applied (i.e., γ_{ii} or q_{ii}, respectively). We recommend that authors explicitly report which equation has been used to generate fitness contour plots and surfaces to remove any ambiguity for readers (e.g., Arnold and Bennett 1988; Brodie 1992).

The potential for confusion in the interpretation of selection surfaces can be illustrated with a two-trait fitness contour plot (Fig. 1). As described by Phillips and Arnold (1989), if γ_{ij}^2 is greater than $\gamma_{ii} \times \gamma_{jj}$ there will be a saddle in the fitness surface (when γ_{ii} and $\gamma_{jj} < 0$), but a fitness peak will exist on the surface if $\gamma_{ij}^2 < \gamma_{ii} \times \gamma_{jj}$. If one incorrectly assumes that q_{ii} from equation (2) are equivalent to γ_{ii}, it is easy to find a case in which $\gamma_{ij}^2 > q_{ii} \times q_{jj}$ but $\gamma_{ij}^2 < 4 \times q_{ii} \times q_{jj}$. (Recall that $4 \times q_{ii} \times q_{jj} = \gamma_{ii} \times \gamma_{jj}$). In these instances, the incorrect use of a quadratic regression coefficient instead of a selection gradient would lead to the mistaken inference that a saddle existed in the fitness surface, when in fact a fitness peak was present (Fig. 1).
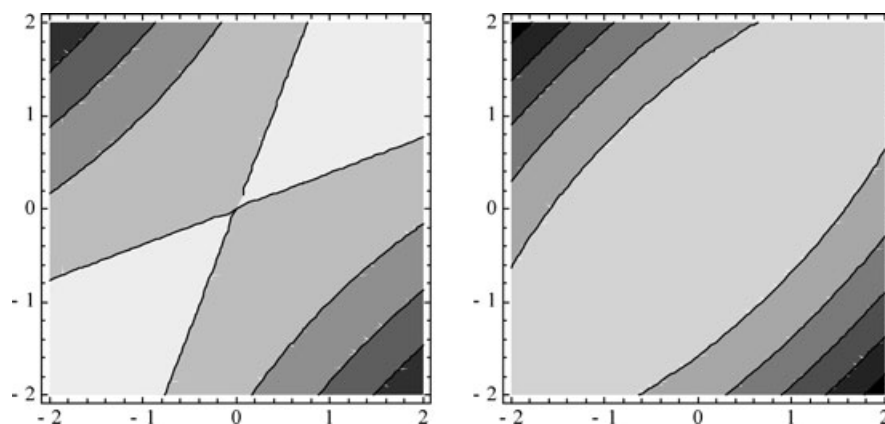

Figure 1. Misinterpretation of regression analysis can lead to qualitatively incorrect inference of the selection surface. In this example, we imagine a case in which regression analysis returns quadratic coefficients of $q_{11} = q_{22} = -0.10$, and $q_{12} = -0.15$ (and all directional selection gradients are zero). If one wrongly assumes that $\gamma_{ii} = q_{ii}$, then the selection surface predicted by equation (1) is a saddle (contour plot of selection surface shown in left panel). In reality, $\gamma_{ii} = 2q_{ii}$, and the correct selection surface is a peak (right panel). See text for details.

Another use of selection gradients is in modeling the evolutionary trajectory of the population mean on an adaptive landscape (Lande 1979). In this context, the individual selection surface is commonly represented as a Gaussian function

$$w(\mathbf{z}) = \exp\left\{-\frac{1}{2}(\mathbf{z} - \theta)^T \omega^{-1}(\mathbf{z} - \theta)\right\}, \qquad (4)$$

where the column vector θ is the location of a selective maxima on the surface (Lande 1979). The strength of nonlinear selection is given by the symmetric matrix ω, which is analogous to a variance–covariance matrix. The adaptive landscape (average population fitness as a function of average trait values) corresponding to the individual selection function (4) is likewise Gaussian with an optimum at θ and a selection matrix given by $\omega + \mathbf{P}$, where $\mathbf{P}$ is the within-population phenotypic variance–covariance matrix before selection (Lande 1979). The selection coefficients ω can be easily calculated from γ and β (Estes and Arnold 2007). Quadratic regression coefficients estimated using form (2) should be doubled before conversion to ω.

Multivariate analyses of nonlinear selection are also affected by confusing the two forms of quadratic regression. Canonical analysis of the matrix of nonlinear selection gradients allows an interpretation of stabilizing and disruptive selection to be made on trait combinations that describe the greatest amount of nonlinear variation on fitness surfaces (Phillips and Arnold 1989; Simms 1990; Simms and Rausher 1993; Blows and Brooks 2003). Although canonical analysis has been advocated for > 15 years (Phillips and Arnold 1989; Simms 1990), its potential utility in empirical studies and meta-analyses has only recently become more widely appreciated (Björklund and Senar 2001; Blows and Brooks 2003; Blais et al. 2004; Brooks et al. 2005; Bentsen et al. 2006; Holleley et al. 2006; Blows 2007a,b; Garant et al. 2007; Hunt et al. 2007; Kruuk and Garant 2007).

Canonical analysis can proceed through either of two approaches: the use of response-surface methodology implemented in common statistics packages or eigenanalysis of an estimated γ matrix. The former involves a diagonalization of $\mathbf{Q}$ (Bisgaard and Ankenman 1996; Blows and Brooks 2003) whereas the latter involves a diagonalization of γ (Phillips and Arnold 1989). Because $\gamma = 2\mathbf{Q}$, the normalized eigenvectors of γ and $\mathbf{Q}$ will be the same but the corresponding eigenvalues of γ will be twice those of $\mathbf{Q}$. In other words, doing the analysis in either way is equivalent in the sense that the orientation of the eigenvectors and the relative sizes of the eigenvalues are unaffected. However, the eigenvalues (and their standard errors) obtained from Proc RSREG or similar procedures should be doubled to obtain the eigenvalues of γ. Eigenvectors will not be correct, however, if they are simply calculated from a matrix of unadjusted regression coefficients estimated as in equation (2b) (i.e., if the off-diagonal elements are not halved).

Conclusions

The application of the Lande–Arnold framework has been instrumental in allowing empiricists to measure the strength and form of selection on a wide variety of taxa in a formal and consistent manner that can be related to equations for evolutionary change. The form and strength of nonlinear selection, particularly the strength of stabilizing selection, are empirical issues that are fundamental to evolutionary biology (Endler 1986; Kingsolver et al. 2001; Kingsolver and Pfennig 2007), and underlie much of quantitative genetics theory (Johnson and Barton 2005). The unfortunate confusion in the literature with regard to the estimation of quadratic selection gradients is likely to have resulted in a general underestimation of the strength of stabilizing selection by up to one half. Because of this confusion we have been retreating from rather than advancing toward the solution to one of the classic problems in evolutionary biology, the maintenance of genetic variation in the presence of strong selection (Johnson and Barton 2005).

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