Opinion

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Pleiotropy, apparent stabilizing selection and uncovering fitness optima

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Evolutionary theory has emphasized that the evolution of single traits cannot be understood in isolation when pleiotropy is present. Widespread pleiotropy causes the appearance of stabilizing selection on metric traits owing to joint effects with fitness, and results in the genetic variation being concentrated in relatively few combinations of the measured traits. In this review, we show how trait combinations with high levels of genetic variation can be used to uncover fitness optima that are defined by apparent stabilizing selection. Defining fitness optima in this way could provide one avenue by which researchers can overcome the problem posed by measuring the myriad of traits that must influence fitness, or by measuring total fitness itself.

Multivariate evolution and the problem of measuring fitness

A fundamental challenge in evolutionary biology remains the characterization of multivariate phenotypes [1], their underlying genetic basis [2–4] and how selection acts on these complex phenotypes [5,6]. Difficulties in measuring complex, high-dimensional multivariate phenotypes and in determining their genetic basis, have contributed to an empirical focus on single traits and their bivariate relationships. This limited focus has led to incomplete genetic and evolutionary interpretations of empirical data [7,8], and to a disconnect between empirical data and theoretical models of evolution, which explicitly emphasize the importance of multivariate relationships among traits [3,4,9–12]. Recent developments in characterizing multivariate phenotypes and genetic variation are beginning to equip empiricists with the means to bridge this divide [1,8,13,14].

The need to describe multivariate phenotypes adequately is part of the greater challenge in evolutionary biology posed by the problem of measuring fitness [15,16]. Fitness is highly complex, with numerous component traits contributing to lifetime reproductive success. Measuring the fitness of individuals is, and probably will remain, beyond the logistical capability of most empirical systems. Developmental instability [17], heterozygosity [18,19] and condition [20,21] have each been suggested as measures that capture genome-wide aspects of fitness variation, circumventing the need to measure the fitness of individuals directly. However, there is weak or equivocal evidence that individuals with high developmental instability [22], low heterozygosity [23] or low condition [15,24] have lower fitness than do other individuals in the population. Understanding of the evolution of focal traits ultimately depends on their genetic covariation with fitness [25]. Therefore, new, general approaches, that are applicable across a range of empirical systems, are required to characterize how traits co-vary genetically with fitness.

Here, we outline the theoretical justification for, and empirical application of, a new approach to the study of how traits co-vary with fitness and the subsequent characterization of fitness optima. Our approach rests on recognizing two general consequences of widespread pleiotropy that underlie theoretical explanations of the maintenance of genetic variance [3,4,9,10]. First, pleiotropy causes the appearance of stabilizing selection on metric traits (see Glossary) as a consequence of joint effects on these metric traits and on traits that directly affect fitness [3]. Second, pleiotropy restricts genetic variance in traits to a subspace of the multivariate phenotype, causing most of the genetic variance to be associated with just a few combinations of the measured traits [10,14]. We argue that these two general consequences of pleiotropy cause the trait combinations associated with the most multivariate genetic variance to be informative metrics of how individuals deviate from the fitness optimum. We detail empirical approaches that can be taken to undercover fitness optima through consideration of these trait combinations.

Glossary

Apparent stabilizing selection: a measured trait does not directly affect fitness and, therefore, is not directly under selection; however, pleiotropic covariation with traits that do directly affect fitness generates the appearance of selection on that trait; also referred to as indirect stabilizing selection.

g_{max}: the first eigenvector of the additive genetic variance–covariance matrix, **G**. The coefficients of this vector represent an index of the linear combination of measured traits associated with the greatest genetic variance, and can be applied to either phenotypic trait values or breeding values to generate phenotypic or genetic scores.

Hidden pleiotropic effects: the pleiotropic relationship between a measured trait and an unmeasured trait, which can generate the appearance of stabilizing selection on the measured trait even in the absence of any direct effect of the trait on fitness.

Metric trait: a measured trait that exhibits continuous variation.

Multivariate phenotype: a phenotype composed of multiple traits.

Mutation bias: the propensity of mutations to change the phenotype in a particular direction.

Real stabilizing selection: variance-reducing selection acting directly on the measured trait; also referred to as direct stabilizing selection.

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Pleiotropy and apparent stabilizing selection

The extent of mutation bias is expected to differ between metric traits and fitness itself. Mutations will tend to increase or decrease the value of a metric trait with equal frequency, but will decrease fitness more often than they increase it [26]. When mutations have pleiotropic effects on the focal metric trait and on fitness, the metric trait will appear to be associated with fitness even when it does not make a direct phenotypic contribution to lifetime reproductive success. The deleterious fitness effects of pleiotropic mutations thus generate apparent stabilizing selection on focal metric traits [3,4,9]. That is, genotypes with extreme values of the metric trait are likely to carry more mutations, each with deleterious pleiotropic effects on fitness, resulting in selection for intermediate values of the metric trait. A substantial proportion of the selection observed on a focal metric trait is therefore likely to have been generated through hidden pleiotropic effects on other traits under selection and, ultimately, on fitness itself [3,5].

If all traits could be measured, real (direct) and apparent stabilizing selection could be readily distinguished using multivariate selection analysis (Box 1). However, in the more general situation where relatively few traits are measured, selection acting on unmeasured traits that directly affect fitness, and are correlated with the focal (measured) traits, will be misconstrued as real stabilizing selection acting directly on the focal traits. Owing to the intractability of measuring large numbers of traits [1], this expectation of widespread apparent (rather than real) stabilizing selection remains largely untested (but see [27]).

The presence of widespread pleiotropy predicts another aspect of selection on multiple traits that is not obvious from estimates of the strength of selection for each trait in isolation (i.e. the quadratic selection gradients along the diagonal of $\boldsymbol{\gamma}$, Box 1) [4]. Correlational selection between pairs of traits (the off-diagonal elements of $\boldsymbol{\gamma}$) indicates that selection on one trait is not independent of selection on another. If real stabilizing selection occurred on all phenotypic traits independently, individuals would deviate from the optimum phenotype for many reasons and, consequently, overall fitness (summed across all traits) would be unrealistically low [10]. The degree of non-independence of selection on multiple traits is revealed by the eigenvectors of $\boldsymbol{\gamma}$ [6], which describe the independent linear trait combinations that are under strong (or weak) selection. Such analyses support the expectation that selection cannot be acting independently on individual traits; stabilizing selection is stronger on some trait combinations than on others, and much stronger on such multivariate trait combinations than on individual traits [28] (in the example in Box 1, selection on the major axis of $\boldsymbol{\gamma}$ is 2.5 times greater than on individual traits).

Box 1. Apparent stabilizing selection on metric traits

A change in the phenotypic variance of a trait (z_i) as a consequence of stabilizing (or disruptive) selection can be measured using the quadratic selection differential (Equation I) [5]:

$$C_i = \sigma^2_{z_i^\bullet} - \sigma^2_{z_i} + s^2, \qquad \text{[Eqn I]}$$

correcting the difference between phenotypic variance before ($\sigma^2_{z_i}$) and after ($\sigma^2_{z_i^\bullet}$) selection for the effect of directional selection on the variance (s^2). An alternative form can be given in terms of the squared deviation of each individual from the mean of the population (μ) (Equation II):

$$C_i = \sigma[w,(z_i-\mu_i)(z_i-\mu_i)] \qquad \text{[Eqn II]}$$

where C is now the covariance between relative fitness (w) and the squared deviations for each individual from the population mean, $(z_i - \mu_i)^2$.

In the presence of a second trait, the deviation from the mean in the first trait $(z_i - \mu_i)$ for an individual can co-vary with the deviation of the second trait from its own mean $(z_j - \mu_j)$ (Equation III):

$$C_{ij} = \sigma[w,(z_i-\mu_i)(z_i-\mu_i)] \qquad \text{[Eqn III]}$$

and the change in variance of both traits owing to selection acting directly on the variances can be represented in matrix form as Equation IV:

$$\mathbf{C} = \begin{pmatrix} C_i & C_{ij} \\ C_{ij} & C_j \end{pmatrix}. \qquad \text{[Eqn IV]}$$

The strength of nonlinear selection on each trait (γ_{ij}), corrected for selection on each of the other traits, can be estimated using quadratic regression [5] (Equation V):

$$\boldsymbol{\gamma} = \mathbf{P}^{-1}\,\mathbf{C}\,\mathbf{P}^{-1} \qquad \text{[Eqn V]}$$

where $\boldsymbol{\gamma}$ is the matrix of quadratic selection gradients (assuming trait means are standardized to zero), and $\mathbf{P}$ is the phenotypic variance–covariance matrix (Box 2).

As an example of apparent stabilizing selection, imagine three traits (z_i, z_j and z_k) are all under univariate stabilizing selection, with quadratic selection differentials of 0.05, 0.05 and 0.1, respectively, so that z_k experiences twice the amount of stabilizing selection as the other two traits. All traits have the same phenotypic variance, and are phenotypically correlated to each other to the same moderate degree ($|r| = 0.5$). Using (Equation V), and including covariances between the selection differentials, this can be represented as:

$$\begin{pmatrix} -0.024 & 0.036 & 0.011 \\ 0.036 & -0.024 & 0.011 \\ 0.011 & 0.011 & -0.008 \end{pmatrix}$$

$$= \begin{pmatrix} 2 & 1 & -1 \\ 1 & 2 & -1 \\ -1 & -1 & 2 \end{pmatrix}^{-1} \begin{pmatrix} -0.05 & 0.01 & 0.07 \\ 0.01 & -0.05 & 0.07 \\ 0.07 & 0.07 & -0.10 \end{pmatrix} \begin{pmatrix} 2 & 1 & -1 \\ 1 & 2 & -1 \\ -1 & -1 & 2 \end{pmatrix}^{-1}$$

Although z_k experiences twice the reduction in variance experienced by the other two traits, there is little direct stabilizing selection ($\gamma_{kk} = -0.008$) on this trait. Both z_i and z_j experience three times the level of stabilizing selection that z_k does. The observed reduction in variance in z_k is therefore primarily a consequence of apparent (indirect) stabilizing selection generated by direct selection on z_i and z_j. The covariance structure (both of the quadratic deviations in **C** and the phenotypes in **P**) generates the appearance of stabilizing selection on z_k. This can be more clearly understood by reducing the problem to a univariate combination of traits that represents the first eigenvector of **C**, $C_{max} = [-0.421, -0.421, 0.803]$, with an associated eigenvalue of $\lambda_{\boldsymbol{C}\mathrm{max}} = 0.173$, experiences most (86.5%) of the quadratic selection in **C** and is heavily weighted by z_k. This trait combination has a large phenotypic variance ($P_{C\mathrm{max}} = C^T{}_{max}\mathbf{P}C_{max} = 3.708$; 62% of the total phenotypic variance), and therefore has a correspondingly small quadratic selection gradient ($\frac{\lambda_{\boldsymbol{C}\mathrm{max}}}{P^2_{\boldsymbol{C}\mathrm{max}}} - 0.013$).

The general overestimation of direct stabilizing selection acting on single traits is a probable outcome of the action of such indirect selection [5].

Pleiotropy and g_{max} as a summary of multivariate genetic variance

There are at least two general observations that reveal how pleiotropy undermines the genetic independence of multiple traits, suggesting the potential for simple summary measures of genetic variation to provide insight into fitness. First, the mutation rate for a metric trait is approximately one-tenth of the mutation rate for an individual [10,19]. This high per-trait mutation rate relative to the per-individual rate reveals that many mutations must have pleiotropic effects and, therefore, that there cannot be a large number of traits that are individually affected by non-pleiotropic mutations [10].

Second, widespread pleiotropy results in relatively few genetically independent trait combinations that account for most of the genetic variance in the set of measured traits [10]. The distribution of genetic variance across multiple traits is described by the genetic variance–covariance (**G**) matrix, from which genetically independent trait combinations can be established by diagonalization (Box 2). The eigenvalues of **G**, which are estimates of the genetic variance associated with the trait combination described in the associated eigenvector, typically decline exponentially [14,29]. That is, most genetic variance in **G** is present in the first few eigenvalues, indicating that pleiotropic covariation among traits causes most of the genetic variance to be confined to a subspace of the multivariate phenotype [14].

The first eigenvector of **G**, $\mathbf{g}_{max}$, is a summary metric that has been influential in studies of directional pheno-

Box 2. Genetic analysis of multiple metric traits

Consider the following example, where five sires have each produced two offspring, which were assayed for four traits. Let $z_{i,j,k}$ denote the phenotypic value of the *k*th trait, of the *j*th individual from the *i*th sire:

$$\mathbf{Z} = \begin{pmatrix} z_{1,1,1} & z_{1,2,1} & \cdots & z_{1,j,1} \\ z_{1,1,2} & \ddots & & \\ \vdots & & \ddots & \\ z_{1,1,k} & & & z_{i,j,k} \end{pmatrix}$$

$$= \begin{pmatrix} -1.2 & -1.1 & -1.1 & -0.2 & 0.0 & 0.8 & -0.2 & -0.1 & 0.6 & 0.8 \\ -2.2 & -0.9 & -0.2 & -0.8 & -0.6 & -1.1 & -0.1 & 1.0 & 0.8 & 1.8 \\ -2.3 & 0.1 & -0.8 & -0.3 & -0.3 & -0.4 & 0.1 & 0.2 & 1.0 & 1.5 \\ 1.3 & 0.7 & 0.1 & 0.6 & 0.4 & -1.0 & -0.4 & 0.0 & 0.2 & -1.7 \end{pmatrix}$$

The relationships among the traits can be visualized as bivariate plots (Figure I). In multivariate statistics, these relationships are often represented by the phenotypic covariance matrix (**P**), which contains the variances of the traits along the diagonal, and the covariances between bivariate traits as off-diagonal elements. The covariance structure of **P** can be determined by diagonalizing this symmetrical matrix using $\Lambda = \mathbf{S}^{-1}\mathbf{G}\mathbf{S}$, where Λ is a diagonal matrix containing the eigenvalues, and **S** is a matrix containing the eigenvectors as columns:

$$\mathbf{P} = \begin{pmatrix} 0.54 & 0.46 & 0.50 & -0.45 \\ & 1.19 & 0.94 & -0.56 \\ & & 0.98 & -0.52 \\ & & & 0.64 \end{pmatrix} \Lambda = \begin{pmatrix} 2.67 & & & \\ & 0.40 & & \\ & & 0.18 & \\ & & & 0.10 \end{pmatrix}$$

$$\mathbf{S} = \begin{pmatrix} 0.35 & -0.58 & 0.47 & -0.57 \\ 0.62 & 0.51 & -0.38 & -0.47 \\ 0.57 & 0.21 & 0.54 & 0.58 \\ -0.40 & 0.59 & 0.59 & -0.37 \end{pmatrix}$$

This diagonalization is analogous to principal components analysis of the covariance matrix of multiple phenotypic traits in an unstructured data set. When the data have structure, as here where individuals had different sires, **P** can be decomposed into the observed variance–covariance matrices for each level (in this example, the among-sire variance–covariance matrix and the within sire variance–covariance matrix). The among-sire variance–covariance matrix, which here is equated with **G** (the additive genetic variance–covariance matrix), can then be subjected to the same diagonalization as used above, and the distribution of genetic variance among genetically independent trait combinations determined.

Using a general linear model (GLM; nested individuals within sires) to decompose **P** into the two observed variance–covariance matrices, **G** and the associated diagonalization for this example are:

$$\mathbf{G} = \begin{pmatrix} 0.51 & 0.62 & 0.52 & -0.42 \\ & 0.99 & 0.77 & -0.50 \\ & & 0.47 & -0.41 \\ & & & 0.11 \end{pmatrix}, \Lambda = \begin{pmatrix} 2.26 & & & \\ & 0.10 & & \\ & & -0.10 & \\ & & & -0.18 \end{pmatrix},$$

$$\mathbf{S} = \begin{pmatrix} 0.46 & -0.77 & -0.31 & 0.32 \\ 0.66 & 0.60 & -0.46 & 0.05 \\ 0.49 & 0.07 & 0.83 & 0.25 \\ -0.34 & 0.22 & -0.09 & 0.91 \end{pmatrix}$$

The first eigenvector of **G**, the linear combination of the original traits that displays the greatest amount of genetic variance, is found in the first column of **S**, and has been termed $\mathbf{g}_{max}$ [31]. We focus here on $\mathbf{g}_{max}$ because the eigenvalues of **G** typically decline exponentially [14,29], but subsequent eigenvectors of **G** might also explain substantial proportions of the genetic variance in some trait sets, and hence be pleiotropically associated with fitness. When the eigenvalues of **G** are similar in magnitude, capturing similar levels of genetic variance, investigation of these other trait combinations contained in other columns of **S** could also be informative of the fitness optimum.

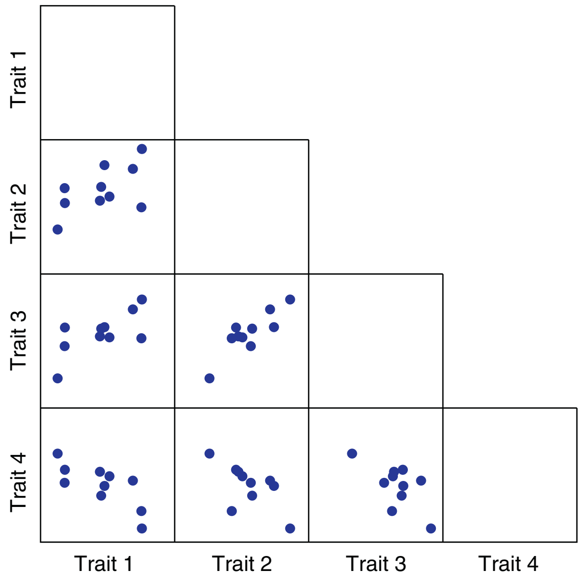

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Figure I. Bivariate scatterplots of the four measured traits for the ten offspring of the five sires.

Box 3. Genetic analysis of $\boldsymbol{g_{max}}$ in the presence of apparent stabilizing selection

Building on the example in Box 2, where the ten sons of five sires were each assayed for four traits, the coefficients of the first eigenvector of **G** represent an index that can be used to generate a phenotypic score for $\boldsymbol{g_{max}}$ for each of the ten individuals using:

$$Z_{g_{max}} = g_{max}{}^{\mathrm{T}}\mathbf{Z} = (-3.6\ -1.2\ -1.0\ -0.9\ -0.7\ -0.2\ 0.0\ 0.7\ 1.2\ 2.8)$$

These phenotypic scores can then be subjected to the same GLM used to estimate **G** to then estimate the genetic variance of $\boldsymbol{g_{max}}$, the genetic correlation between $\boldsymbol{g_{max}}$ and other traits, or the genetic (breeding) values for $\boldsymbol{g_{max}}$. Alternatively, the coefficients of $\boldsymbol{g_{max}}$ can be applied to the breeding values for each of the four traits to estimate the breeding values for $\boldsymbol{g_{max}}$ using $\mathbf{A}_{g_{max}} = g_{max}{}^{\mathrm{T}}\mathbf{A}$, where **A** is the matrix of breeding values.

In addition to the four phenotypic traits, if fitness of the ten individuals was observed to be:

$$W_z = (0.3\ 1.1\ 1.2\ 1.2\ 1.2\ 1.3\ 1.3\ 1.2\ 1.1\ 0.5),$$

$\boldsymbol{g_{max}}$ would be under strong phenotypic stabilizing selection; individuals with intermediate phenotypic scores for $\boldsymbol{g_{max}}$ have the highest fitness (Figure Ia). Despite this, the genetic correlation (r_A) between $\boldsymbol{g_{max}}$ and fitness is negligible (0.24; Figure Ib). However, the genetic correlation between the square of $\boldsymbol{g_{max}}$ and fitness is perfect ($r_A = -1.00$; Figure Ic); that is, it is a genetic relationship that can only be detected if the expectation of apparent stabilizing selection is explicitly considered.

Taking this approach a step further, the genetic covariance between the squared deviations from the population mean ($\boldsymbol{\mu}$) of two metric traits is related to the mean number of pleiotropic deleterious alleles ($\bar{k}$) carried by individuals by Equation VI [4]:

$$\frac{1}{k} = \frac{\sigma_{(z_i-\mu_i)^2(z_j-\mu_j)^2}}{\sigma^2_{(z_i-\mu_i)}\sigma^2}. \qquad \text{[Eqn VI]}$$

If individuals on average carry a single deleterious allele that affects both traits, then individuals with phenotypes that deviate substantially from the population mean for z_i will also have extreme phenotypes for z_j and the genetic correlation will be high. However, if individuals carry a larger number of deleterious alleles, the genetic correlation will be weaker (e.g. Figure 1c, main text).

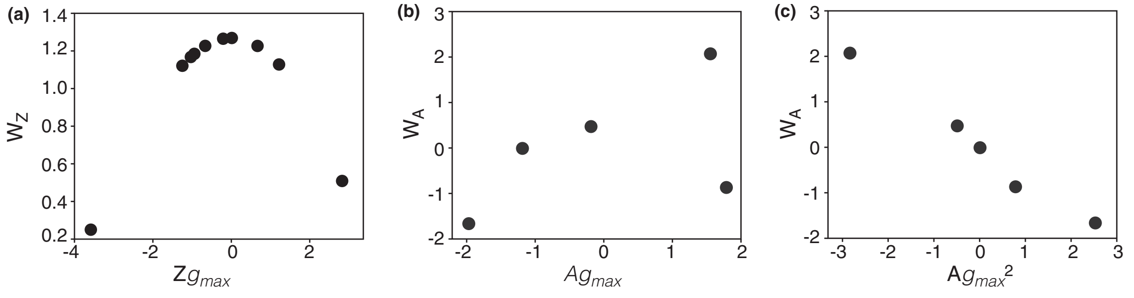

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Figure I. Scatterplots of **(a)** phenotypic $\boldsymbol{g_{max}}$ scores and fitness; **(b)** breeding values for $\boldsymbol{g_{max}}$ and breeding values for fitness and; **(c)** breeding values for the squared deviations of $\boldsymbol{g_{max}}$ and breeding values for fitness. Although all ten individuals have phenotypic $\boldsymbol{g_{max}}$ scores, only the five sires have breeding values for $\boldsymbol{g_{max}}$. Breeding values are mean centered.

typic evolution, describing the direction in multivariate trait space expected to have a substantial impact on the response to selection and, consequently, on phenotypic divergence [13,30–32]. Here, we suggest that $\boldsymbol{g_{max}}$ also provides an informative indirect metric for studying fitness itself. The $\boldsymbol{g_{max}}$ of a set of standard metric traits is itself a metric trait; as illustrated in Box 3, individual phenotypic scores can be generated for $\boldsymbol{g_{max}}$, and these scores can then be analyzed in the same manner as any other metric trait. As with all metric traits, much of the genetic variation captured by $\boldsymbol{g_{max}}$ must ultimately have its source in mildly deleterious mutations occurring across the genome [26]. In comparison with a standard metric trait, such as those measured to estimate **G**, $\boldsymbol{g_{max}}$ is expected to capture a greater portion of the accumulated pleiotropic mutation in a set of traits, including mutations with pleiotropic effects on fitness. Consequently, strong stabilizing selection should be generated on $\boldsymbol{g_{max}}$, providing the opportunity to investigate the genetic basis of fitness using this simple summary statistic.

Although the distinction between real and apparent stabilizing selection is immaterial to the evolutionary fate of alleles [10], it has important consequences for the empirical investigation of selection on $\boldsymbol{g_{max}}$. Given that widespread pleiotropy leads to the expectation that most traits will be under apparent, rather than real stabilizing selection, we expect stabilizing selection on the genetic variance of $\boldsymbol{g_{max}}$ to be a general observation across all the types of metric trait for which $\boldsymbol{g_{max}}$ might be estimated. However, because selection will be apparent (generated through pleiotropic genetic covariation with fitness) rather than real (generated through phenotypic covariation with fitness), detection of stabilizing selection on the phenotypic values of $\boldsymbol{g_{max}}$ depends on at least two other factors: whether the traits that are the direct targets of selection have been included in the analysis (Box 1), and how the environment contributes to phenotypes. We emphasize this important distinction between analyses of phenotypic and genetic variance in $\boldsymbol{g_{max}}$ below.

Empirical approaches for uncovering fitness optima using $\boldsymbol{g_{max}}$

When phenotypic $\boldsymbol{g_{max}}$ scores and a component of fitness are measured for the same individuals there are two ways in which apparent stabilizing selection on $\boldsymbol{g_{max}}$ can be revealed to identify fitness optima. First, less genetic variance in $\boldsymbol{g_{max}}$ among individuals with high (rather than low) values of the fitness component is direct evidence for stabilizing selection on the genetic variance associated with $\boldsymbol{g_{max}}$. Second, genetic analyses involving the squared trait deviations of $\boldsymbol{g_{max}}$ can be used to infer stabilizing selection, and estimate the genetic variance in fitness. We detail these approaches in the following sections.

Reduced genetic variance of $\mathbf{g_{max}}$ *among high fitness individuals*

One straightforward approach to visualizing the selective optimum is to use fitness components that are dichotomous, such as mating success or survival, to classify individuals into high and low fitness groups. Phenotypic scores for each individual for $\boldsymbol{g_{max}}$ of a set of metric traits can then be subject to genetic analysis separately for high and low fitness subsets of the population. Low fitness individuals are expected to have more extreme genetic values of $\boldsymbol{g_{max}}$, resulting in greater genetic variance in scores of $\boldsymbol{g_{max}}$ among the set of low fitness individuals than among the set of high fitness individuals.

Just such a pattern has been observed in a population of *Drosophila bunnanda* [33] (Figure 1a). Male *D. bunnanda* with low fitness (categorized by low competitive mating success) had nearly twice the genetic variance in $\boldsymbol{g_{max}}$ as the subset of the population with high fitness. Furthermore, $\boldsymbol{g_{max}}$ was the trait combination that differed most in genetic variance between the two fitness groups [33]. Estimated breeding values for $\boldsymbol{g_{max}}$ can then be used to visualize the adaptive optimum of $\boldsymbol{g_{max}}$ (Figure 1b).

In our *D. bunnanda* example of stabilizing selection on the genetic variance of $\boldsymbol{g_{max}}$, there was no stabilizing (or directional) selection on the $\boldsymbol{g_{max}}$ phenotypes [33]. This study therefore illustrates the necessity of genetic, not phenotypic, analyses of selection on $\boldsymbol{g_{max}}$. Confounding effects of environment on detecting selection in evolutionary studies are well known [34,35], and it has often been assumed that the environment will generate correlations between traits and fitness, resulting in the detection of selection that has no evolutionary consequence [36]. The contrary pattern, where the environment obscures the genetic correlation with fitness, is also likely to occur, as in our example. In particular, for genotypes close to the adaptive optimum, random environmental effects will shift the phenotype away from the optimum, but for genotypes far from the optimum, the environmental contribution could shift phenotypes toward the optimum as well as further away from it. This is analogous to theory that shows that the probability that a mutation moves a genotype closer to or further away from an optimum is a function of how close the genotype is to the optimum [37]. Irrespective of the specific mechanism masking the association between fitness and $\boldsymbol{g_{max}}$ at the phenotypic level, we suggest that phenotypic values of $\boldsymbol{g_{max}}$ will often

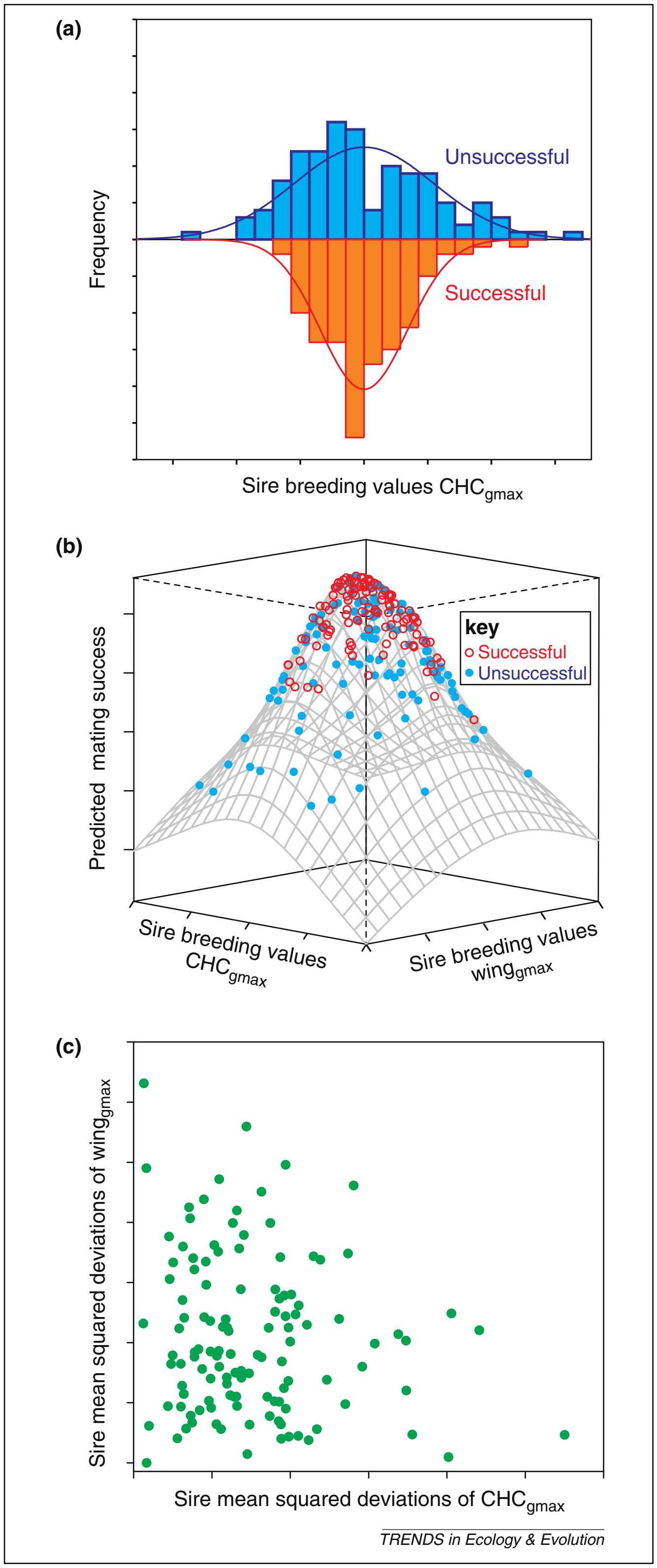

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Figure 1. Examples of empirical visualization of fitness optima through the genetic analysis of $\boldsymbol{g_{max}}$. Male *Drosophila bunnanda* from a half-sib quantitative genetic breeding design were assayed for a dichotomous fitness component, competitive mating success, nine cuticular hydrocarbons (CHCs; used as sexual signals [33]) and for nine wing traits (not used as sexual signals) [55]. The major axis of genetic variance was estimated for these two trait sets: CHC$_{gmax}$ [33] and Wing$_{gmax}$ [55] and used to generate individual scores for each $\boldsymbol{g_{max}}$. **(a)** Differences in genetic variance of CHC$_{gmax}$ in high versus low fitness (i.e. successful versus unsuccessful in gaining mates) males, indicating that variance-reducing (stabilizing) selection is acting on $\boldsymbol{g_{max}}$. **(b)** Thin-plate spline representations of the stabilizing selection acting on genetic values (mean centered breeding values) of both CHC$_{gmax}$ and Wing$_{gmax}$, for successful (open circles) and unsuccessful (closed circles) males, illustrating the position of the selective optimum for both $\boldsymbol{g_{max}}$ trait combinations. **(c)** The distribution of sire means for the squared deviations from the optimum of the $\boldsymbol{g_{max}}$ for the two trait sets. The covariation between two $\boldsymbol{g_{max}}$ can indicate whether alleles affecting $\boldsymbol{g_{max}}$ are either common or rare. The weak association here suggests that the specific pleiotropic mutations affecting fitness and $\boldsymbol{g_{max}}$ differed between the two trait sets. No sire means appear in the top right-hand quadrant of this space, which represents phenotypes that are far from the fitness optimum on both $\boldsymbol{g_{max}}$. The absence of genetic variants affecting both Wing$_{gmax}$ and CHC$_{gmax}$ suggests that either pre-adult selection against widespread pleiotropic mutations is strong or the mutation rate for alleles with widespread effects is low.

be uninformative of the fitness optimum, and emphasize that if researchers are to understand fitness optima, experimental designs must enable selection to be detected on the genetic variance. That is, it is unlikely to be sufficient to take the index for g_{max} from one population where a breeding design has been conducted, and apply this to the phenotypic values of another population to look for associations between fitness and g_{max} in the second population.

Genetic analysis of squared trait deviations

The second type of approach using g_{max} involves the genetic analysis of the squared deviations of g_{max} to determine both the presence of a fitness optimum and the genetic variance in fitness, as first suggested by Wright [38]. Squared deviations from the population mean underpin phenotypic analyses of stabilizing selection (Box 1), but their role in genetic analyses has not been widely appreciated. There are three distinct analytical approaches that are applicable to the squared deviations of g_{max}, providing different insights into the nature of the fitness optimum.

First, fitness components measured on a continuous (rather than dichotomous) scale can be genetically correlated with the square of the g_{max} scores of individuals. We illustrate this approach with a hypothetical example in Box 3. This example again illustrates the distinction between phenotypic and genetic analyses of g_{max} scores. The phenotype of g_{max} is under strong stabilizing selection (Box 3, Figure Ia), whereas the genetic correlation of this trait with fitness is small (Box 3, Figure Ib). That is, the standard quantitative genetic approach, based on the genetic analysis of g_{max} scores, failed to detect the strong association with fitness because low fitness was associated with both high and low g_{max} scores owing to individuals deviating in both directions from the optimum. In this case, the detection of stabilizing selection at the genetic level depends on the analysis of squared deviations; the genetic correlation between the squared deviations of g_{max} and fitness was perfect and negative (Box 3, Figure Ic). Genotypes with g_{max} scores far from the optimum have lower fitness than do genotypes closer to the optimum (with smaller squared deviations). We are unaware of any empirical examples of this approach.

Second, in the presence of a fitness optimum, the genetic variance in fitness itself can be estimated as the genetic variance in the squared deviations [4]. This representation of the genetic variance in fitness does not depend on the traits underlying g_{max} having a direct effect on fitness. Information on the genetic variance in fitness can therefore be gained from genetic analyses of squared deviations of g_{max} in the absence of any further information on exactly what phenotypic traits are involved in conferring high fitness. A conceptual shift from single-trait-centric approaches toward genetic analyses of g_{max} could provide a useful avenue for furthering understanding of the evolution of fitness.

Third, the genetic covariance between the squared deviations of different traits (Box 3) can help distinguish between two alternative mutational models [4]. If multiple alleles contribute variation to g_{max} [39], additive-by-additive epistasis in fitness will be common [40], and fitness will be affected by combinations of mutations. High non-additive relative to additive genetic variance of squared deviations of g_{max} would support this model [40], as would relatively low genetic covariance between the squared deviations of different traits (Figure 1c), indicating different alleles contributed to g_{max} deviations of different trait sets. Alternatively, a substantial proportion of the genetic variance in g_{max} among low fitness individuals might be due to few mutations of large effect [3]. For example, if individuals carry at most one deleterious mutation, then an individual with an extreme value of one trait will also be extreme for other traits, resulting in a high genetic correlation between the squared deviations of g_{max} traits.

Finally, we suggest that investigations of the mutational variance of the squared deviations of g_{max} could provide a valuable insight into the rate of mutations affecting fitness. Mutational genetic variance increases with trait complexity [41], and major axes of genetic variance might represent larger mutational targets than would individual metric traits. Recent molecular evidence suggests that phenotype-based mutation estimates underestimated the rate of deleterious mutations [42], perhaps through difficulties in characterizing the phenotype. The relationship between g_{max} and fitness optima might lead to greater insight into the mutational input to fitness.

General implications of apparent stabilizing selection on g_{max}

The detection of strong apparent stabilizing selection on the traits that harbor the greatest levels of genetic variance (i.e. g_{max} of trait sets) provides a new empirical focus for investigating the maintenance of genetic variance and the operation of selection. Characterizing fitness optima through the apparent stabilizing selection on g_{max} might provide one way of circumventing the problem of how to measure the myriad of traits that influence fitness. Genetic analysis of the squared deviations of traits under apparent stabilizing selection provides a measure of the genetic variance in fitness, and has the potential to help distinguish between key theoretical models of the maintenance of genetic variation.

An important, but untested, assumption of this approach to revealing fitness optima through stabilizing selection on g_{max} is that information on total fitness is not required. Pleiotropic mutations are expected to result in the simultaneous depression of different aspects of fitness. This expectation is supported by the observation that mutational correlations among life-history traits tend to be large and positive [43] and that inbreeding depression has general effects across life-history traits [44,45]. Therefore, we suggest that many individual fitness components will be informative of the fitness optimum when analyzed in conjunction with g_{max}. We again emphasize that this expectation depends on the genetic rather than phenotypic analysis of g_{max} scores.

Two avenues of empirical investigation are suggested. First, tests are needed of the prediction that apparent stabilizing selection on g_{max} is a general phenomenon. This can be done using the analytical approaches outlined above. An important component of these empirical tests will be to determine how different fitness components are genetically associated with $\boldsymbol{g_{max}}$, testing the expectation that different fitness components will all be informative. Second, empirical tests are needed of the prediction that $\boldsymbol{g_{max}}$ captures much of the genetic load in complex phenotypes. Such tests can be achieved through mutation accumulation experiments; as mutations accumulate, more extreme values of $\boldsymbol{g_{max}}$ should become more common. It might also be informative to address genetic load through studies of inbreeding depression: deviations in $\boldsymbol{g_{max}}$ should be particularly strongly correlated with inbreeding depression.

Finally, analysis of stabilizing selection on $\boldsymbol{g_{max}}$ has the potential to further understanding of specific evolutionary problems that require information on the genetic variance in fitness. For example, the targeting by natural and sexual selection of the same genetic variants has important implications for several evolutionary issues, including the evolution of sex [46,47], local adaptation [48,49], population fitness [24] and sexual selection [50]. Individuals carrying extreme genotypes for $\boldsymbol{g_{max}}$ should have lower fitness under natural selection as a consequence of pleiotropic mutation, and lower fitness under sexual selection if mating success is affected by genetic load [24,50,51]. Evidence is generally lacking that mating success co-varies with the presence of deleterious alleles. A few studies have demonstrated that females can discriminate against artificially introduced deleterious mutations of large effect [52,53], or the more widespread effects of mutation induced by mutagens [54]. The apparent stabilizing selection on $\boldsymbol{g_{max}}$ detected in *D. bunnanda* [33] suggests that females can discriminate against mates carrying greater numbers of the deleterious alleles that segregate naturally in populations. Further studies adopting similar approaches will determine whether this alignment of natural and sexual selection is a general phenomenon.

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