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Orientation of the Genetic Variance-Covariance Matrix and the Fitness Surface for Multiple Male Sexually Selected Traits

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ABSTRACT: Stabilizing selection has been predicted to change genetic variances and covariances so that the orientation of the genetic variance-covariance matrix (**G**) becomes aligned with the orientation of the fitness surface, but it is less clear how directional selection may change **G**. Here we develop statistical approaches to the comparison of **G** with vectors of linear and nonlinear selection. We apply these approaches to a set of male sexually selected cuticular hydrocarbons (CHCs) of *Drosophila serrata*. Even though male CHCs displayed substantial additive genetic variance, more than 99% of the genetic variance was orientated 74.9° away from the vector of linear sexual selection, suggesting that open-ended female preferences may greatly reduce genetic variation in male display traits. Although the orientation of **G** and the fitness surface were found to differ significantly, the similarity present in eigenstructure was a consequence of traits under weak linear selection and strong nonlinear (convex) selection. Associating the eigenstructure of **G** with vectors of linear and nonlinear selection may provide a way of determining what long-term changes in **G** may be generated by the processes of natural and sexual selection.

Keywords: genetic variance, fitness surface, sexual selection, genetic variance-covariance matrix, lek paradox.

The additive genetic variance-covariance matrix (**G**) is a fundamental parameter in microevolutionary theory (Lande 1979; Agrawal et al. 2001). The **G** matrix will determine the rate and direction in which a population may respond to a given selection regime on a multivariate suite of traits (Lande 1979). The predictive equation for the change in means, $\Delta \mathbf{z} = \mathbf{G}\boldsymbol{\beta}$, will hold for more than one generation only if the genetic basis of the traits under selection, represented by **G**, does not change. However, selection may change **G** as a consequence of the generation of linkage disequilibrium (Bulmer 1980) or as allele frequencies change since genetic variances (Barton and Turelli 1987) and covariances (Bohren et al. 1966; Turelli 1988; Shaw et al. 1995) are dependent on allele frequency. Although changes in **G** under selection attributable to linkage disequilibrium (the Bulmer effect) have been well characterized (Bulmer 1980; Shaw et al. 1995), the problem of how allele frequency change results in changes in **G** remains unsolved.

How the genetic variance may change under selection as a consequence of allele frequency change has eluded a predictive theory because unknown genetic details such as the number of loci, the number of alleles at each locus, and their distribution of effects can have a dramatic influence on the response of the genetic variance (Barton and Turelli 1987). The effect of selection on the genetic basis of traits under selection has usually been described under two alternative sets of genetic assumptions. First, the genetic variance of a single trait may be the result of many loci, each of which have numerous alleles with a Gaussian distribution of effects (Lande 1980). As the trait responds to directional selection, allele frequency change will be minimal, and the change in genetic variance will be small, perhaps on the order of less than 20% (Reeve 2000). Alternatively, the distribution of allelic effects may be leptokurtic as a consequence of the variance of new mutations being far greater than the variance of standing allelic effects at a locus, resulting in most of the genetic variance of the trait being a consequence of a few probably rare alleles (Turelli 1984). Now as the trait responds to directional selection, the increase in frequency of rare alleles may dramatically increase the genetic variance (Barton and Turelli 1987), perhaps by as much as sixfold for some traits (Reeve 2000). There is surprisingly little data on how genetic variances respond to directional selection (Barton and Turelli 1987; Keightley and Hill 1989), but at least one experiment has indicated that a change in selec-

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tion under laboratory and field conditions may increase genetic variance in a manner consistent with the latter set of assumptions (Blows and Higgie 2003).

When multiple traits are considered, predicting the effects of selection on **G** becomes very difficult (Turelli 1988). Under the first set of assumptions above, **G** may evolve the same orientation as the fitness surface if a suite of traits experience a constant pattern of multivariate stabilizing selection (Lande 1980, 1984; Cheverud 1984). However, with the addition of strong pleiotropic mutation, the orientation of **G** could deviate considerably from that of the fitness surface (Arnold 1992). Under a wide range of genetic assumptions, genetic correlations will change under linear selection, particularly as the distribution of allelic effects becomes more skewed (Slatkin and Frank 1990) or as the strength of linear selection increases (Turelli 1988), although changes in **G** may be transitory if the response to selection is based on single genes of major effect (Agrawal et al. 2001). While experimental studies have suggested that genetic drift (Phillips et al. 2001) or selection (Shaw et al. 1995; Blows and Higgie 2003) can change **G** relatively quickly, comparative studies suggest that **G** may be similar between phenotypically similar populations of the same species but progressively more different as divergence increases (Steppan et al. 2002).

Although some studies suggest that **G** may change as a consequence of selection, there have been few attempts to determine the association between how **G** changes under selection and the form of selection. Brodie (1992) found a qualitative association between the sign of correlational selection for and the genetic correlation between two traits of a garter snake. However, for cases when more than two traits are involved, pairwise comparisons of the sign and magnitude of correlational selection gradients and genetic correlations are unlikely to reveal how selection changes **G**. Ideally, one would like to orientate **G** with respect to the directional selection gradient ($\boldsymbol{\beta}$) and the fitness surface defined by the matrix of quadratic and correlational selection gradients ($\boldsymbol{\gamma}$). Here we develop approaches for the direct comparison of the orientation of **G** with vectors of linear and nonlinear selection. First, we use the projection of $\boldsymbol{\beta}$ onto a subspace of **G** to determine the association between linear selection and the orientation of **G**. Second, we employ the method of Krzanowski (1979) to simultaneously determine the critical angles between the principal components (PCs) of **G** and the fitness surface defined by the principal components of $\boldsymbol{\gamma}$, resulting in a quantified measure of the similarity of the orientation of **G** and the fitness surface.

One area of evolutionary biology in which the effect of selection on the genetic variance has been particularly controversial is the consequence of sexual selection for levels of genetic variance in male display traits. If females gain genetic benefits from choosing among males that use display traits as indicators of genetic quality, then both natural and sexual selection may operate in the same direction to greatly reduce the genetic variance in those male display traits. If genetic variance is low for male display traits, then benefits accruing to females for making a choice will also be low, raising the question of why females continue to choose (the "lek paradox"; Kirkpatrick and Ryan 1991). Attempts to resolve the lek paradox have centred on mechanisms that may maintain high levels of genetic variance in male display traits (Pomiankowski and Møller 1995; Rowe and Houle 1996). Although comparative analyses across traits suggest that levels of genetic variance in male sexually selected traits may be high, there have been few attempts to determine whether genetic variance in multiple male sexually selected traits actually exists in the direction of sexual selection.

Drosophila serrata individuals use cuticular hydrocarbons (CHCs) for mate recognition (Blows and Allan 1998), and CHCs have been shown to respond to both natural selection on mate recognition (Higgie et al. 2000) and sexual selection (Blows 2002). In particular, female *D. serrata* have a strong preference for certain combinations of male CHCs (Hine et al. 2002), and females may gain genetic benefits from exercising choice (Blows 2002; Hine et al. 2002). The *D. serrata* mate-recognition system therefore provides an opportunity to determine how sexual selection may change the genetic variance in male display traits. Here, we conduct further analyses on two experiments first reported in Hine et al. (2002) to determine the orientation between **G** and the fitness surface for male CHCs. The first experiment was a half-sib genetic experiment, which we use here to estimate **G** for the set of male CHCs under sexual selection. The second experiment was a mate choice experiment, which was used to estimate the strength of linear sexual selection by Hine et al. (2002). This experiment enabled the estimation of the sexual selection fitness surface of male CHCs for our current purpose by determining the quadratic and correlational selection gradients of the $\boldsymbol{\gamma}$ matrix.

Methods

Genetic Analysis of Cuticular Hydrocarbons

A half-sib experiment (Hine et al. 2002) was used to determine the genetic basis of male CHCs. The CHCs included in the analysis have been identified in order of their retention times as Z,Z-5,9-$C_{24:2}$, Z,Z-5,9-$C_{25:2}$, Z-9-$C_{25:1}$, Z-9-$C_{26:1}$, 2-Me-C_{26}, Z,Z-5,9-$C_{27:2}$, 2-Me-C_{28}, Z,Z-5,9-$C_{24:2}$, and 2-Me-C_{30} (Howard et al. 2003). Briefly, 66 sires were each mated to three virgin females, and two male progeny from each of the resulting 198 families had their CHCs

assayed on the gas chromatograph. The standard nested ANOVA model for a half-sib breeding design was used to estimate the additive genetic components of variance of the logcontrasts of relative concentrations of male CHCs. When analyzing the multivariate set of *Drosophila serrata* CHCs, logcontrasts of the relative concentrations of individual CHCs have first been taken to break the unit-sum constraint in this set of proportions (Blows and Allan 1998; Higgie et al. 2000; Hine et al. 2002). Logcontrasts were calculated by dividing all other proportions by an arbitrarily chosen proportion, after which the log was taken of each of these ratios (Aitchison 1986) and were standardized before analysis. The price one pays by using this transformation is the loss of one variable (the divisor), in this case Z,Z-5,9-$C_{24:2}$, but the choice of divisor does not affect the outcome of subsequent analyses (Aitchison 1986, p. 78). The resulting log ratio covariance matrix Σ is well suited to analyses such as multiple regression used in the selection analyses below, because it is nonsingular. This is in contrast to the alternative method of transformation to log ratios, which maintains all variables in the analysis but results in the centered log ratio covariance matrix, which is singular (Aitchison 1986).

Measurement of Sexual Selection on Cuticular Hydrocarbons

Sexual selection on male CHCs was measured in a mate choice experiment described in Hine et al. (2002). Briefly, 123 virgin females were each allowed to choose between two males, and after each female made a choice indicated by successful intromission, the two males were immediately prepared for analysis on the gas chromatograph. To investigate the form of sexual selection on male CHCs, we estimated the linear selection gradient ($\boldsymbol{\beta}$) and the matrix of quadratic and correlational selection gradients ($\boldsymbol{\gamma}$) using multiple regression (Lande and Arnold 1983). Standardized logcontrasts were used in the regressions to allow standardized selection gradients to be estimated. Linear and nonlinear selection gradients were estimated in separate regressions to provide unbiased estimates of the partial linear regression coefficients in $\boldsymbol{\beta}$ (Brodie et al. 1995).

The $\boldsymbol{\gamma}$ matrix was subjected to two transformations. First, a canonical analysis of $\boldsymbol{\gamma}$ was conducted to generate new axes that were aligned with the major axes of the quadratic response surface (Phillips and Arnold 1989; Blows and Brooks 2003). The coefficients that related the new canonical axes back to the original variables are summarized in the **M** matrix and may be interpreted in the same fashion as in principal components analysis. The eigenvalues of these new canonical axes (the eigenvectors in **M**) then allowed the shape of the response surface to be interpreted. Second, the $\boldsymbol{\omega}$ matrix was calculated by taking the negative inverse of $\boldsymbol{\gamma}$ (Arnold et al. 2001). This transformation of the fitness surface reverses the order of the eigenvalues and their associated eigenvectors. So, for example, the first principal component of $\boldsymbol{\gamma}$ ($\boldsymbol{\gamma}_{\mathbf{max}}$) is the direction on the fitness surface with the greatest curvature, whereas the first principal component of $\boldsymbol{\omega}$ ($\boldsymbol{\omega}_{\mathbf{max}}$) may be interpreted as the line of least curvature or selective resistance (Arnold et al. 2001). If stabilizing selection results in the orientation of **G** conforming to the fitness surface, then it is likely that the first few eigenvectors of $\boldsymbol{\gamma}$ will be associated with the orientation of **G**. Conversely, the association between $\boldsymbol{g}_{\mathbf{max}}$ and $\boldsymbol{\omega}_{\mathbf{max}}$ has been considered an important empirical issue, as if the two coincide; evolution along lines of least genetic resistance (Schluter 1996) and least selective resistance are confounded explanations for the divergence between populations (Arnold et al. 2001).

Orientation of the Genetic Variance-Covariance Matrix and the Fitness Surface

Linear Selection. Although $\boldsymbol{g}_{\mathbf{max}}$ represents the direction of greatest genetic variance, associating this eigenvector with directions of divergence, or linear selection as in the present case, has limited appeal (Blows and Higgie 2003), because much of the genetic variance may be excluded from such a comparison depending on the distribution of eigenvalues of **G**. Alternatively, determining the association between the orientation of **G** and the direction of linear selection may be accomplished by determining what is the closest vector (or projection) of genetic variance to the vector of linear selection $\boldsymbol{\beta}$. A principal components analysis of **G** will result in n new orthogonal axes (where n = the number of traits) that describe a decreasing amount of the genetic variance. Let a subspace of **G** be defined by a subset of principal components of **G** that form linearly independent columns of a matrix **A**. Projection of $\boldsymbol{\beta}$ onto the subspace of **A** is accomplished by first calculating the projection matrix **P** (Strang 1998):

$$\mathbf{P} = \mathbf{A}(\mathbf{A}^{\mathrm{T}}\mathbf{A})^{-1}\mathbf{A}^{\mathrm{T}}. \quad (1)$$

The projection ($\boldsymbol{p}$) that is closest to $\boldsymbol{\beta}$ is then calculated as

$$\boldsymbol{p} = \mathbf{P}\boldsymbol{\beta}. \quad (2)$$

It is important to note that not all the principal components of **G** can be included in **A**. This is because when there are n dimensions, a set of n linearly independent vectors will span the space, and every vector in the space will be a combination of these vectors (Strang 1998). Therefore, inclusion of the n principal components in **A** will produce the identity matrix for the projection matrix

in equation (1), and $\boldsymbol{\beta}$ will simply be recovered from equation (2). The choice of how many of the $n - 1$ principal components to include in a particular analysis will depend on the distribution of the eigenvalues of **G**. The more principal components included, the greater the number of directions in multivariate space that will be explored, and it is likely that the angle between the projection and $\boldsymbol{\beta}$ will become smaller. However, if **G** is nonsingular, the last few principal components may explain diminishingly small proportions of the total genetic variance. In many evolutionary studies, it may be of interest to determine how the direction of linear selection is orientated with respect to the majority of genetic variance rather than finding projections of genetic variance that are the consequence of the inclusion of eigenvectors of **G** that explain very small proportions (say, <1%) of the genetic variance (we note that the multivariate breeders equation is the ideal tool for associating the entire space of **G** and $\boldsymbol{\beta}$ to result in a predicted response to selection). We include the first four principal components (of a total of eight) in our analysis, which together explain 99% of the genetic variance.

Nonlinear Selection. The matrix **G** has been predicted to evolve to become aligned with the fitness surface under certain conditions (Lande 1980; Cheverud 1984; Arnold 1992), although to our knowledge an explicit test of this has not been attempted. Arnold et al. (2001) suggested that the comparison of $\boldsymbol{g}_{\max}$ and $\boldsymbol{\omega}_{\max}$ would be the first step in investigating the orientation of **G** and $\boldsymbol{\omega}$. While this approach has some intuitive appeal, it is not a valid approach to the comparison of subspaces defined by multiple principal components (Cohn 1999). The existence of a large angle between a corresponding pair of principal components (e.g., PC1 of **G** and $\boldsymbol{\omega}$) does not indicate that the two sets of principal components describe different k-dimensional subspaces, where k = number of principal components that describe the subspace (Krzanowski 1988). For instance, PC1 of **G** may be perfectly aligned with PC2 of $\boldsymbol{\omega}$ while being orthogonal to PC1 of $\boldsymbol{\omega}$. Before such angular comparisons are meaningful, the two sets of principal components first need to be rotated to find the best-matching set of orthogonal axes.

Krzanowski (1979) described a method for the comparison of two k-dimensional subspaces that calculates the angles between the best-matched pairs of orthogonal axes. Let a subset of the principal components of **G** again be represented by **A** as above and those of $\boldsymbol{\gamma}$ be represented in a matrix **B**. The eigenvectors in **A** and **B** are first normalized by dividing the coefficients of each eigenvector by the square root of the sums of squares of the coefficients of the respective eigenvector, as is usual for any angular comparison of vectors. The two sets of principal components can then be compared by defining a matrix **S** as

$$\mathbf{S} = \mathbf{A}^{\mathrm{T}}\mathbf{B}\mathbf{B}^{\mathrm{T}}\mathbf{A}. \tag{3}$$

The matrix **S** effectively finds the minimum (or critical) angles between an arbitrary set of orthogonal vectors in the subspace of **A** and a set of orthogonal vectors closest to the same directions in the subspace of **B**. These arbitrary vectors are termed the principal vectors in the subspaces of **A** and **B**. Note that equation (3) differs from the expression in theorem 1 of Krzanowski (1979) because the matrices **A** and **B** have the principal components as columns to be consistent with the projection analysis above, whereas Krzanowski (1979) starts with two matrices containing the principal components as rows.

The eigenvalues of **S** may then be used to determine the similarity between the two subspaces. The smallest angle between any pair of orthogonal axes of **A** and **B** is then defined as $\cos^{-1}\sqrt{\lambda_1}$, where λ_1 is the largest eigenvalue of **S**. The square roots of the inverse cosines of the remaining eigenvalues of **S** will give the remaining set of angles in increasing order of size. Of particular use here is that the sum of the eigenvalues of **S** equals the sum of squares of the cosines of the angles between the two sets of orthogonal axes. This sum will lie in the range 0 to k, as all eigenvalues of **S** will have values between 0 and 1, which equate to critical angles between 0° and 90°. The sum of the eigenvalues of **S** therefore represents a convenient measure of the similarity of the two subspaces because it is bounded within a range of values that have a straightforward interpretation (Krzanowski 1979). If the sum is close to 0, the two subspaces are dissimilar and are approaching orthogonality, while a sum equal to k would indicate that two original matrices (**G** and $\boldsymbol{\gamma}$ in our case) share the same orientation. Again, it is important to note that k cannot equal n in this analysis, since including more than half of the n principal components will constrain the analysis to recover common dimensions (i.e., angles of 0°), and if all n principal components are included, the two subspaces will coincide exactly (W. J. Krzanowski, personal communication). We again include the first four principal components of **G** and $\boldsymbol{\gamma}$ in this analysis, where >99% of the variation contained in both matrices were explained by these principal components.

To determine how the original traits contribute to the similarity between subspaces once two subspaces have been compared, the eigenvectors of $\mathbf{S}[\boldsymbol{a}_i]$ that correspond with each eigenvalue λ_i may be projected onto the subspace of **A** by

$$\boldsymbol{b}_i = \mathbf{A}\boldsymbol{a}_i, \tag{4}$$

where $\boldsymbol{b}_i$ is a principal vector and may be interpreted in the same fashion as any principal component with reference to the coefficients that relate it back to the original

traits. This expression again differs from that in theorem 2 of Krzanowski (1979), since **A** has columns of principal components, not rows. To determine the principal vector compared in the other subspace of **B**, $\mathbf{BB}^{\mathrm{T}}\boldsymbol{b}_i$ is used. For our comparisons of the subspaces of **G** and $\boldsymbol{\gamma}$, we report the principal vectors in the subspace of $\boldsymbol{\gamma}$ because this allowed a determination of whether the strength of nonlinear selection was associated with the similarity between **G** and $\boldsymbol{\gamma}$.

A number of alternative methods are available to assess principal component subspaces (Flury 1988; Cohn 1999). We have chosen the method of Krzanowski (1979) because it alone among those methods reviewed by Cohn (1999) offers a readily interpretable scale with which one can assess the similarity between subspaces. While alternative methods have a lower bound of 0 in the presence of coincident subspaces, these test statistics do not have an upper bound, making it difficult to determine how different two subspaces might be if the null hypothesis is rejected. Common principal component (CPC) models (Flury 1988) in particular have become a popular and effective tool in evolutionary studies as a method of matrix comparison (Phillips and Arnold 1999). We have avoided the use of CPC models in the current context for two reasons. First, CPC was developed specifically for product-moment-based covariance matrices, a data structure that neither **G** nor $\boldsymbol{\gamma}$ strictly satisfies. The simple geometric approach of Krzanowski (1979) may be applied to the comparison of subspaces without regard to this restriction. Second, although Flury's (1988, p. 134) approach elegantly includes all eigenvectors of the two covariance matrices, the common space hypothesis testable under this model allows similarity between matrices to be driven by similarity between principal components, with small eigenvalues in one matrix and large eigenvalues in the other. In our case, we are specifically interested in eigenvectors of **G** that account for substantial amounts of the genetic variance.

The Krzanowski method does come, however, with two related disadvantages. First, as discussed above, no more than half of the principal components can be included in the subspace comparison. Selection of a subset of principal components is therefore required. Here, we have chosen that subset of the principal components that explain the greatest amount of the total variance in each matrix, an approach that ensures the dominant multivariate relationships in the data will be represented in the analysis (Cohn 1999). In our case, 99% of the total variance in each matrix is represented in the subspace comparison, but such a fortuitous distribution of eigenvalues may not always occur. When substantially less variation is explained by the half of the eigenvectors with the largest eigenvalues, alternative criteria for selecting principal components might be considered. For instance, choosing between principal components that have similar eigenvalues on the basis of a strong contribution from an original trait of particular interest might have merit in some cases. Alternatively, original variables could be removed from the analysis to change the distribution of eigenvalues, perhaps after using a variable selection technique for multiple regression to determine if some traits are not necessary to explain variation in fitness.

Selection of principal components is also at the center of the second disadvantage of Krzanowski's method; the generation of the bootstrapped distribution of the test statistic (the sum of the eigenvalues of **S**) is presented in the appendix and computer code data are available as downloadable files in the online edition of the *American Naturalist* and from the second author on request. If **G**, for instance, has principal components that have eigenvalues that are close in magnitude (i.e., they explain similar amounts of the genetic variance), repeated sampling will tend to produce divergent bootstrap replications, resulting in highly variable critical angles between principal vectors and thus values of the test statistic. Such a situation might commonly arise with principal components that explain small amounts of the total variance. Some alternative methods inversely weight the contribution of each angle to the test statistic by its variance, reducing the effect of such eigenvector instability. However, weighting is computationally demanding, requiring the inversion of a covariance matrix to produce these test statistics. The instability of the eigenvectors could be addressed again by the judicious selection of the principal components that enter the analysis (Cohn 1999).

Results

Genetic Analysis of Cuticular Hydrocarbons

The additive genetic variance-covariance matrix (**G**) of the set of eight male CHCs is presented in table 1. Visual inspection of the genetic correlations given above the diagonal in table 1 indicated the three 2-methylalkanes (2-Me-C_{26}, 2-Me-C_{28}, 2-Me-C_{30}) were almost perfectly positively genetically correlated with each other, suggesting that the same genes contributed to the variation in the relative concentration of these three CHCs. The group of 2-methylalkanes were weakly genetically correlated with Z,Z-5,9-$C_{25:2}$, which is the major component of hydrocarbons on the cuticle of *Drosophila serrata* and typically accounts for about 60% of all hydrocarbon. The other major feature of **G** was the strong genetic correlations between Z,Z-5,9-$C_{29:2}$ and all but one other CHC (Z-9-$C_{25:1}$). The degree of structure in **G** may be quantified by conducting a principal components analysis of the covariance matrix in table

Table 1: Additive genetic variance-covariance matrix (**G**) for standardized logcontrasts of eight male cuticular hydrocarbons

	h^2	Z,Z-5,9-$C_{25:2}$	Z-9-$C_{25:1}$	Z-9-$C_{26:1}$	2-Me-C_{26}	Z,Z-5,9-$C_{27:2}$	2-Me-C_{28}	Z,Z-5,9-$C_{29:2}$	2-Me-C_{30}
Z,Z-5,9-$C_{25:2}$	.242	**.06050**	.777	.781	.456	.767	.364	.933	.329
Z-9-$C_{25:1}$	.514	**.06846**	**.12849**	.799	.357	.591	.511	.390	.669
Z-9-$C_{26:1}$	.212	**.04429**	**.06607**	**.05316**	.479	.815	.484	1.133	.383
2-Me-C_{26}	.596	**.0432**	**.04942**	**.04265**	**.14902**	.499	.999	1.327	1.001
Z,Z-5,9-$C_{27:2}$	.568	**.07115**	**.07991**	**.07090**	**.07266**	**.14224**	.422	.995	.321
2-Me-C_{28}	.262	**.02291**	**.04689**	**.02854**	**.09862**	**.04073**	**.06544**	1.232	1.013
Z,Z-5,9-$C_{29:2}$	.204	**.05204**	**.03170**	**.05923**	**.11609**	**.08510**	**.07143**	**.05138**	.943
2-Me-C_{30}	.145	**.01542**	**.04569**	**.01683**	**.07362**	**.02311**	**.04934**	**.04071**	**.03629**

Note: Genetic variances and covariances are in boldface below the diagonal, and genetic correlations are displayed above the diagonal. Heritabilities (h^2) are given in the first column.

1. The first principal component of **G**, $\boldsymbol{g}_{\max}$ (Schluter 1996; Arnold et al. 2001), accounted for 51.9% of the genetic variance in the set of eight CHCs. The coefficients of $\boldsymbol{g}_{\max}$ indicated that the strong positive relationships between the 2-methylalkanes and Z,Z-5,9-$C_{29:2}$ contrasted to the other CHCs was primarily responsible for the dominance of this major axis.

Measurement of Sexual Selection on Cuticular Hydrocarbons

The standardized partial regression coefficients comprising $\boldsymbol{\beta}$ are given in table 2. Hine et al. (2002) previously estimated the strength of directional sexual selection on male CHCs using discriminant function analysis (Endler 1986). The discriminant function provided a univariate description of male CHCs, which best distinguished between chosen and rejected males, and the standardized linear selection gradient was large ($\boldsymbol{\beta} = 0.756$) and significant. The discriminant function and multiple regression approaches are closely related, and when there are two groups involved as in the present case, the discriminant function coefficients and partial regression coefficients that comprise $\boldsymbol{\beta}$ will be proportional (Endler 1986). Here, our goal was not to retest the partial regression coefficients of each individual CHC for significance but rather to associate the orientation of the fitness surface with the genetic basis of the CHCs. Employing the regression approach of Lande and Arnold (1983) provided estimates of selection that could be directly associated with the orientation of the genetic variance-covariance matrix. The partial regression coefficients of $\boldsymbol{\beta}$ (table 2) suggested strong directional selection on 2-Me-C_{28} and to a lesser extent on Z,Z-5,9-$C_{29:2}$ and linear selection in the opposite direction on 2-Me-C_{30}.

None of the quadratic or cross-product coefficients in the $\boldsymbol{\gamma}$ matrix were significant (table 2), suggesting at first glance that there was little nonlinear selection acting on male CHCs, although some of the correlational selection gradients are quite large. Quadratic surfaces with large correlational selection gradients are difficult to interpret from the $\boldsymbol{\gamma}$ matrix alone (Phillips and Arnold 1989). A canonical transformation of $\boldsymbol{\gamma}$ provides a more straightforward way to interpret the form of selection operating on male CHCs, because it rotates the axes until the correlational selection gradients are eliminated to find the major axes of the quadratic response surface (Box and Draper 1987). Canonical axes and their associated eigenvalues are displayed in table 3. The eigenvector that accounted for the most curvature on the fitness surface, $\boldsymbol{m}_8$, contrasted 2-Me-C_{26} with Z,Z-5,9-$C_{25:2}$ and 2-Me-C_{28}. The eigenvector with the second largest eigenvalue, $\boldsymbol{m}_1$, had a strong contribution from 2-Me-C_{28}, which was opposed by Z,Z-5,9-$C_{25:2}$. Considerable nonlinear selection was indicated by the size of the eigenvalues for each of these axes (0.394 and -0.595, respectively), which equate to standardized quadratic selection gradients (Blows and Brooks 2003). Significance of nonlinear selection along the major axes was determined by placing all major axes back into a quadratic regression (Blows and Brooks 2003). Parametric significance testing was appropriate here as the binomial distribution closely approximates the normal distribution when the number of observations is large, and the two outcomes have equal probability under the null hypothesis, both of which are satisfied here. Significant quadratic selection was indicated on this set of traits by the partial F-test considering the contribution of all axes simultaneously ($F = 3.43$, df $= 8, 202$, $P < .001$); however, nonlinear selection along no single axis reached significance in this analysis.

A nonparametric visualization of the sexual selection surface using a thin-plate spline (fig. 1*A*) that does not constrain the visualization of the relationship between the CHCs and fitness to be quadratic (Blows et al. 2003) suggested that there was little curvature to the surface, which is instead dominated by the strength of linear selection (the slope of the plane). The area of high fitness represented primarily large values of 2-Me-C_{28} as it is only this variable that had large coefficients in $\boldsymbol{m}_1$ and $\boldsymbol{m}_8$ with the

Table 2: Vector of standardized directional selection gradients ($\boldsymbol{\beta}$) and the matrix of standardized quadratic and correlational selection gradients ($\boldsymbol{\gamma}$)

	$\boldsymbol{\beta}$	Z,Z-5,9-$C_{25:2}$	Z-9-$C_{25:1}$	Z-9-$C_{26:1}$	2-Me-C_{26}	Z,Z-5,9-$C_{27:2}$	2-Me-C_{28}	Z,Z-5,9-$C_{29:2}$	2-Me-C_{30}
Z,Z-5,9-$C_{25:2}$	−.061	−.016							
Z-9-$C_{25:1}$	−.034	.168	−.016						
Z-9-$C_{26:1}$	.082	−.029	.035	.021					
2-Me-C_{26}	−.115	.173	.081	.100	−.124				
Z,Z-5,9-$C_{27:2}$	−.082	.061	−.082	−.044	−.314	.04			
2-Me-C_{28}	.481	−.536	−.163	−.079	.377	.359	.027		
Z,Z-5,9-$C_{29:2}$	.189	−.043	.115	−.009	.228	−.05	−.164	−.001	
2-Me-C_{30}	−.287	.299	.054	.005	−.306	−.112	.085	.011	−.062

Note: Linear and nonlinear selection gradients were estimated in separate regressions.

correct combination of signs (table 3), consistent with the linear selection analysis (table 2). It should be noted, however, that the area of extreme high fitness on the predicted surface is not supported by any individuals with that phenotype, probably as a consequence of a strong genetic constraint that exists between these two variables.

Orientation of the Genetic Variance-Covariance Matrix and the Fitness Surface

Linear Selection. To investigate the nature of the association between directional selection and **G**, we first calculated the angles and their 95% bootstrapped confidence intervals (CIs) between the first four principal components of **G**, denoted $\boldsymbol{g}_{max}$, $\boldsymbol{g}_2$, $\boldsymbol{g}_3$, and $\boldsymbol{g}_4$ in decreasing order of their eigenvalues, and $\boldsymbol{\beta}$. Resampling for the bootstrapped confidence intervals was conducted by resampling with replacement sire families from the half-sib experiment for genetic eigenvectors and mating pairs for the linear selection gradients. The angles (lower 95% CI, upper 95% CI) between $\boldsymbol{g}_{max}$, $\boldsymbol{g}_2$, $\boldsymbol{g}_3$, $\boldsymbol{g}_4$, and $\boldsymbol{\beta}$ were 84.3° (74.2°, 94.2°), 79.3° (68.3°, 111.1°), 86.6° (70.8°, 107.6°), and 81.5° (70.5°, 109.7°), respectively. Although these angles suggest a lack of association between the direction of selection and the presence of genetic variance, projection of $\boldsymbol{\beta}$ onto the subspace of **G** defined by these four principal components is required to identify the direction of genetic variance most similar to $\boldsymbol{\beta}$. The angle between the projection ($\boldsymbol{p}$) and $\boldsymbol{\beta}$ of 74.7° (50.5°, 82.4°) indicated that the direction favored by sexual selection was considerably divergent from the directions in which the vast majority of genetic variance currently lies.

A visual impression of the lack of association between the direction of sexual selection and the genetic variance in male CHCs is given in figure 1*B*, where the fitness surface represented by the two major canonical axes $\boldsymbol{m}_1$ and $\boldsymbol{m}_8$ is shown as a contour plot and best linear unbiased predictor estimates of the breeding values of the 66 sires have been placed on the same surface. The breeding values for $\boldsymbol{m}_1$ and $\boldsymbol{m}_8$ are strongly negatively correlated, and the axis of their negative correlation is clearly unaligned in this two-dimensional space, with the major slope of the fitness surface representing the direction and strength of linear selection.

Nonlinear Selection. The comparison of the subspaces of **G** and $\boldsymbol{\gamma}$ defined by the first four principal components resulted in the sum of the eigenvalues of **S** of 1.41, which was more extreme (i.e., smaller) than all 1,000 of the bootstrap replications (appendix and computer code data), indicating that the null hypothesis of coincident subspaces could be rejected at $P < .001$. After taking the negative inverse of $\boldsymbol{\gamma}$ to generate the $\boldsymbol{\omega}$ matrix, principal components analysis of $\boldsymbol{\omega}$ enabled the determination of the line of least selective resistance of the fitness surface, $\boldsymbol{\omega}_{max}$. The line of least selective resistance explained 65.6% of the variance in $\boldsymbol{\omega}$. The first principal components from **G** and $\boldsymbol{\omega}$, $\boldsymbol{g}_{max}$ and $\boldsymbol{\omega}_{max}$, were compared (Arnold et al. 2001), which indicated that the two dominant eigenvectors were at an angle of 54.2°. The subspaces remained quite different when the four principal components of **G** and $\boldsymbol{\omega}$ were compared, resulting in a sum of the eigenvalues of **S** of 2.59 ($P = .013$). Note how the comparisons of the first four principal components of **G** with the first four principal components of $\boldsymbol{\gamma}$ and $\boldsymbol{\omega}$ combine to give a sum of the eigenvalues of **S** equal to 4, as this is equivalent to including all the eigenvectors of $\boldsymbol{\gamma}$ in a single analysis.

To determine which parts of the two subspaces were more similar and to relate these similarities back to the original CHC traits, the eigenvectors of **S** constrained in the $\boldsymbol{\gamma}$ subspace are presented in table 4. The eigenvector that was most similar between **G** and $\boldsymbol{\gamma}$ had the largest contribution from the traits that experienced the weakest linear selection, Z-9-$C_{25:1}$, and a secondary contribution from the trait that experienced the strongest nonlinear (convex) selection, 2-Me-C_{26} (table 2). In contrast, the eigenvector that was most different between **G** and $\boldsymbol{\gamma}$ had the strongest contribution from 2-Me-C_{28}, which experienced the strongest linear selection (table 2).

Table 3: The **M** matrix of eigenvectors from the canonical analysis of $\boldsymbol{\gamma}$

$\boldsymbol{m}_i$	λ_i	Z,Z-5,9-$C_{25:2}$	Z-9-$C_{25:1}$	Z-9-$C_{26:1}$	2-Me-C_{26}	Z,Z-5,9-$C_{27:2}$	2-Me-C_{28}	Z,Z-5,9-$C_{29:2}$	2-Me-C_{30}
$\boldsymbol{m}_1$	.394	.518	.309	.081	−.031	−.358	−.662	.169	.183
$\boldsymbol{m}_2$	.200	−.239	.135	.214	.600	−.481	.142	.391	−.337
$\boldsymbol{m}_3$	.028	.217	.320	−.648	.183	.359	.168	.489	.016
$\boldsymbol{m}_4$	.008	.262	.345	.686	.160	.515	.202	.054	.086
$\boldsymbol{m}_5$	−.016	.165	.113	−.100	.232	−.368	.473	−.282	.676
$\boldsymbol{m}_6$	−.058	−.429	−.026	.188	−.391	−.044	.010	.615	.496
$\boldsymbol{m}_7$	−.094	.405	−.806	.084	.262	.086	.002	.293	.127
$\boldsymbol{m}_8$	−.595	.427	−.021	−.077	−.551	−.318	−.498	−.178	.354

Note: The eigenvalue (λ_i) of each eigenvector ($\boldsymbol{m}_i$) is given in the first column.

Discussion

Genetic Analysis of Cuticular Hydrocarbons

The cuticular hydrocarbons of *Drosophila serrata* have been the subject of a number of genetic experiments (Blows and Allan 1998; Hine et al. 2002; Blows and Higgie 2003), but here we have concentrated on the genetic relationships between individual CHCs for the first time. Heritabilities of the CHCs varied from low (2-Me-C_{30}) to moderately high (Z,Z-5,9-$C_{27:2}$) values, consistent with the demonstration that heritable variation existed in *D. serrata* CHCs through their direct response to selection (Higgie et al. 2000). Of greater importance was that the pattern of genetic covariances seemed to reflect developmental relationships between the eight CHCs. In particular, the block of the 3-methylalkanes that were almost perfectly positively correlated with each other may be a consequence of a shared biosynthetic pathway. The 2-methylalkanes with an even number of backbone carbons are formed by insects using the amino acid valine as the sole source of the methyl groups (Nelson 1993).

Measurement of Sexual Selection on Cuticular Hydrocarbons

Strong linear selection dominated the sexual selection fitness surface of male CHCs, with only limited evidence for the presence of nonlinear selection. Nonparametric visualization of the two major canonical axes of the quadratic response surface indicated that the major feature of the fitness surface was a sloping plane. This orientation of fitness surface suggests that females have a strong preference for an extreme male CHC blend and that male mating success increases in a roughly linear fashion with increasing levels of those CHCs. The CHC shown to be under strongest linear selection was 2-Me-C_{28}, which also was the CHC that contributed most strongly to the fitness peak revealed by the nonparametric visualization of the quadratic response surface. Therefore, increasing relative concentrations of 2-Me-C_{28} are implicated by both analyses as being under strong directional sexual selection.

The shape of the fitness surface for male CHCs suggested that female preference for male CHCs may be open-ended. Open-ended female preferences occur when a female's response increases with an increase in the male trait (Kirkpatrick 1987), resulting in preferences for extreme male traits (Ritchie 1996). Open-ended preferences are important in sexual selection theory because they may be more likely to result in rapid coevolution between male traits and female preferences since stabilizing selection on the male trait is weak (Hall et al. 2001). Male and female CHCs have been observed to respond rapidly to the manipulation of sexual selection in hybrid populations (Blows 2002), suggesting that the preferences displayed here may result in rapid evolutionary change in this system.

Orientation of the Genetic Variance-Covariance Matrix and the Fitness Surface

We found evidence against the coincidence between the eigenstructure of **G** and $\boldsymbol{\gamma}$ that has been predicted by quantitative genetic theory (Lande 1980; Cheverud 1984). This is perhaps not surprising given that strong directional selection rather than stabilizing selection is the predominant form of sexual selection that operates on male CHCs in this population. Suggestively, weak linear selection and strong convex selection seemed to be associated with the principal vector that was most similar between the two subspaces. In addition, there was some indication that the principal vector most different between **G** and $\boldsymbol{\gamma}$ was associated with the trait under strongest linear selection. However, a robust test of the effect of nonlinear selection on the orientation of **G** will require a system in which the fitness surface for the set of traits under consideration displays much more curvature. In particular, a system in which a stationary point exists within the sampled space (i.e., the eigenvalues of $\boldsymbol{\gamma}$ would be all negative in the case of a stationary peak) would be the ideal system to test Lande's (1980) hypothesis. Therefore, the type of selection

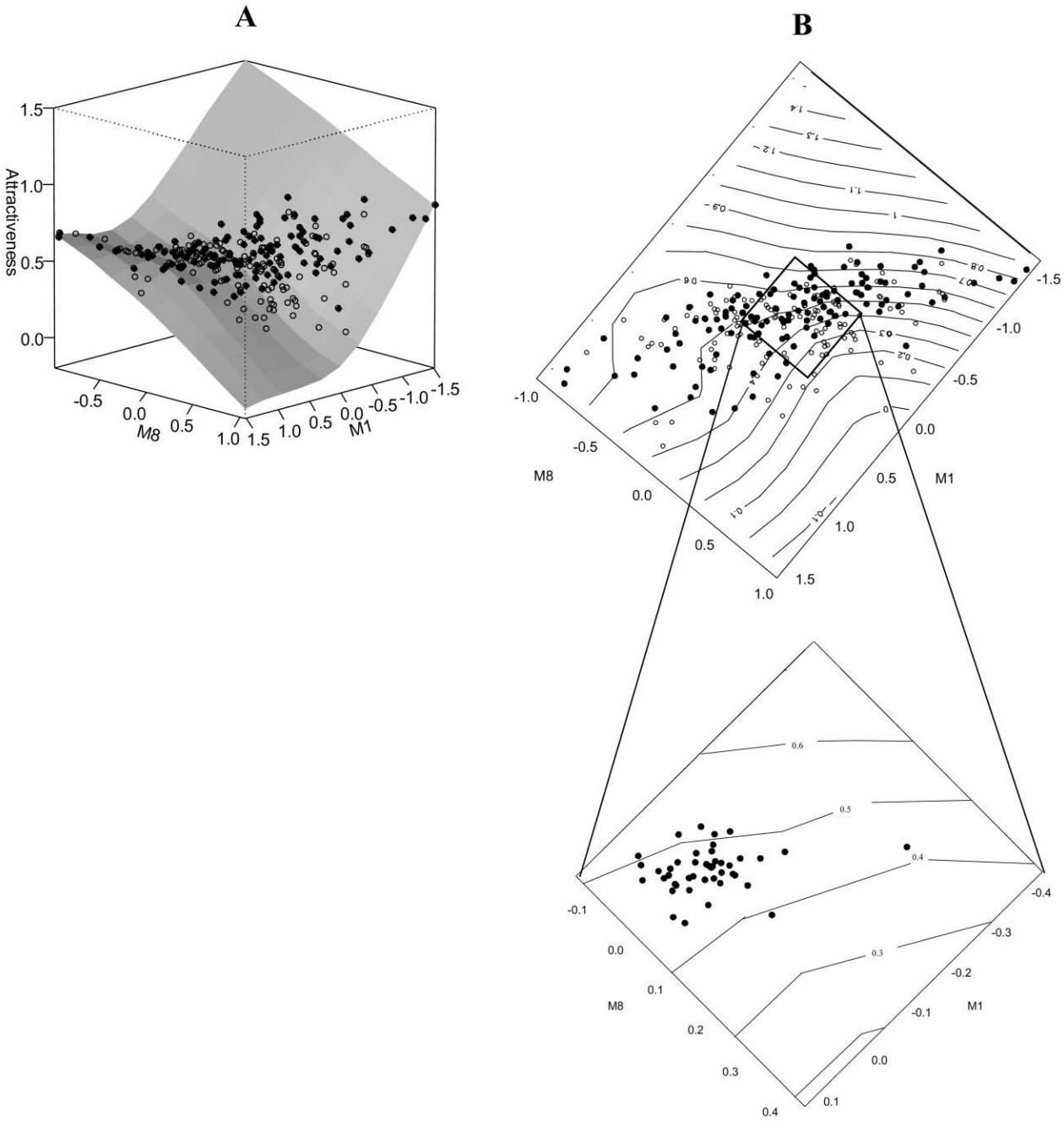

Figure 1: Nonparametric visualization of the fitness surface defined by the canonical axes $\boldsymbol{m}_1$ and $\boldsymbol{m}_8$. The surface has been fitted using a thin-plate spline, the multivariate generalization of the cubic spline, using the SAS TPSPLINE procedure. The value of the smoothing parameter chosen minimized the generalized cross-validation score. *A*, Three-dimensional surface displaying the predicted values of all chosen (*filled circles*) and rejected (*open circles*) males. *B*, Contour plot of the same surface, with the enlarged section of the contour plot displaying the breeding values for each sire estimated from the best linear unbiased predictor values from the linear model for a half-sib breeding design. Note how the major axis of genetic variation (imagine the major axis through an ellipse around the breeding values) is roughly orthogonal to the slope of the fitness surface running from the bottom corner (low fitness) to the top corner (high fitness). The Pearson's product-moment correlation between the breeding values is -0.823 ($P < .001$), which is reduced to -0.770 ($P < .001$) with the removal of the sire with extreme negative values of $\boldsymbol{m}_1$ and positive values of $\boldsymbol{m}_8$.

Table 4: Eigenvectors of **S** constrained to be in the subspace of $\boldsymbol{\gamma}$ (the $\mathbf{V}_1$ matrix of principal vectors) that were compared with **G**

Cuticular hydrocarbon	$\boldsymbol{b}_1$	$\boldsymbol{b}_2$	$\boldsymbol{b}_3$	$\boldsymbol{b}_4$
Z,Z-5,9-$C_{25:2}$	−.130	.112	.231	−.012
Z-9-$C_{25:1}$	−.755	−.227	−.053	.008
Z-9-$C_{26:1}$	.100	−.088	.052	−.004
2-Me-C_{26}	.467	−.341	−.024	.030
Z,Z-5,9-$C_{27:2}$	.226	.273	−.104	.015
2-Me-C_{28}	.173	−.020	−.104	−.059
Z,Z-5,9-$C_{29:2}$	.288	−.162	.130	−.009
2-Me-C_{30}	−.001	.194	.028	.033

Note: The $\boldsymbol{b}_i$'s are listed in this table in increasing size of the angle between the $\boldsymbol{b}_i$ and the corresponding vector in the subspace of **G**.

operating on the traits of interest should be used as a guide to determine which analysis is more appropriate for a particular system under consideration.

Directional selection, however, may have had a substantial influence on the eigenstructure of **G**. The vector of directional selection gradients was unaligned with 99% of the genetic variance in male CHCs, suggesting that the strong, open-ended female preferences may have reduced genetic variance in the direction of sexual selection. Such a response of genetic variances and covariances to directional selection would require large changes in allele frequencies, which would need to increase well beyond their symmetrical frequencies to result in large reductions in the level of genetic variance and/or covariance. Although we have observed large increases in genetic variances as a consequence of natural selection on mate recognition in populations of *D. serrata* (Blows and Higgie 2003), we have yet to directly observe the consequence of sexual selection on genetic variances. Nevertheless, the genetic variance in CHCs may be a consequence of a genetic basis (perhaps a few genes of major effect, for example) that may result in large changes in allele frequency under selection.

The maintenance of genetic variance in male sexually selected traits, particularly when those traits may be indicators of fitness as in this case (Hine et al. 2002), has been problematic for sexual selection theory (Turner 1995; Kotiaho et al. 2001). Natural and sexual selection operating in the same direction would be expected to decrease genetic variance, at least until a cost to the expression of the male trait causes the process to reach an equilibrium (Fisher 1930; Kirkpatrick 1987). At least two hypotheses have been put forward to explain the maintenance of genetic variance in male sexually selected traits that predict that genetic variance in these traits will actually increase as a consequence of selection on the variance (Pomiankowski and Møller 1995) or as sexually selected traits evolve to become condition dependent (Rowe and Houle 1996). Indeed, sexually selected traits have been reported to display larger coefficients of genetic variation than life-history traits (Pomiankowski and Møller 1995; Kotiaho et al. 2001).

Our results indicate that simply relying on comparisons of heritability or coefficients of variation across traits may be inadequate to assess the effect of selection on levels of genetic variation in male display traits. Heritabilities in male CHCs were moderate in most cases (table 1), the median coefficient of genetic variation for these traits was 13.5% (which is higher than the median level of 8% for sexually selected traits in other species; Pomiankowski and Møller 1995), and CHCs respond rapidly to natural selection (Higgie et al. 2000), all of which suggest ample genetic variation in this set of sexually selected traits. The point is that virtually none of this genetic variation lies in the direction of sexual selection. Consequently, the predicted response of male CHCs to sexual selection using the equation $\Delta\mathbf{z} = \mathbf{G}\boldsymbol{\beta}$ and the estimates of **G** and $\boldsymbol{\beta}$ from tables 1 and 2 indicates that all males CHCs would change by only about 1% of a phenotypic standard deviation per generation or less. A similarly small predicted response to sexual selection was reported by Brooks and Endler (2001) for a set of eight color and body size traits in male guppies that also had high coefficients of additive genetic variation (a median of 28%, assuming an autosomal mode of inheritance, with a lower limit of 7% if all traits are completely *Y*-linked). Projection of $\boldsymbol{\beta}$ (Brooks and Endler 2001, their table 6) onto the subspace defined by the first four principal components of **G** (Brooks and Endler 2001, their table 2), which explains 97% of the genetic variance in male guppy ornaments, results in an angle between $\boldsymbol{\beta}$ and the closest direction of genetic variance of 50.7°. Again, there appears to be little genetic variation in male display traits left in the direction of sexual selection, in spite of the male traits displaying high levels of genetic variance.

If **G** does evolve in response to the form and strength of selection operating on a set of traits, using **G** from extant populations in evolutionary analyses faces at least two problems. First, using the eigenstructure of **G** as a tool for determining whether populations or species have evolved in a particular direction as a consequence of genetic constraint becomes even more difficult when one considers how **G** might change under selection (Arnold et al. 2001). If **G** and the fitness become aligned as a consequence of a pattern of multivariate stabilizing selection, then it will be difficult to distinguish between the effects of **G** (genetic constraint) and the fitness surface (the position of an optimum) on the direction that a set of populations has evolved in. Second, many retrospective selection analyses are interested in predicting past directional selection gra-

dients based on estimates of **G** from extant populations. If the unaligned nature of **G** eigenstructure and $\boldsymbol{\beta}$ in our population was a consequence of past selection as we suggest, a retrospective selection analysis would fail spectacularly using parameters from this population, since **G** eigenstructure is likely to be a consequence of selection rather than a fixed constraint, as these analyses assume.

The genetic basis of adaptation remains an outstanding question in evolutionary genetics (Orr and Coyne 1992). If many genes with equal effects underlie a set of traits, allele frequency change as a consequence of selection is likely to be slow because selection on each locus will be weak compared with selection on the mean (Barton and Turelli 1989). Alternatively, if numbers of alleles per locus and loci per trait are moderate and the distribution of allelic effects is skewed (Turelli 1984) or if genes with major effects are common (Orr 1998; Agrawal et al. 2001), allele frequencies are likely to change substantially in response to natural and sexual selection. It is then not a question of whether **G** will change under selection but how. While direct experimental tests of changes in genetic variances and covariances under selection can determine changes over the short term and are still needed (Barton and Turelli 1987; Keightley and Hill 1989), associating the eigenstructure of **G** with that of the fitness surface may provide a way of determining what long-term changes in **G** may be generated by the processes of natural and sexual selection.

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