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The Contribution of Selection and Genetic Constraints to Phenotypic Divergence

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ABSTRACT: Although divergent natural selection is common in nature, the extent to which genetic constraints bias evolutionary trajectories in its presence remains largely unknown. Here we develop a general framework to integrate estimates of divergent selection and genetic constraints to estimate their contributions to phenotypic divergence among natural populations. We apply these methods to estimates of phenotypic selection and genetic covariance from sexually selected traits that have undergone adaptive divergence among nine natural populations of the fly *Drosophila serrata*. Despite ongoing sexual selection within populations, differences in its direction among them, and genetic variance for all traits in all populations, divergent sexual selection only weakly resembled the observed pattern of divergence. Accounting for the influence of genetic covariance among the traits significantly improved the alignment between observed and predicted divergence. Our results suggest that the direction in which sexual selection generates divergence may depend on the pattern of genetic constraint in individual populations, ultimately restricting how sexually selected traits may diversify. More generally, we show how evolution is likely to proceed in the direction of major axes of genetic variance, rather than the direction of selection itself, when genetic variance-covariance matrices are ill conditioned and genetic variance is low in the direction of selection.

Keywords: microevolution, quantitative genetics, sexual selection.

Introduction

When natural populations have diverged in phenotype and the neutral process of genetic drift can be excluded as a cause, divergent selection is viewed as the most likely causal agent of differentiation (Schluter 2000). In the simplest case, divergent selection can mean that high values of a trait are favored in one population while low trait values are favored in another. Divergence will then occur if the trait is heritable in one or both populations. For multiple traits, variation in the direction of selection in

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each population interacts with the genetic basis of those traits in each population in a more complex way in generating the observed phenotypic divergence among populations.

Lande (1979) showed how the response to selection of a multivariate phenotype ($\Delta \mathbf{z}$) will be biased away from the direction of selection ($\boldsymbol{\beta}$) by the pattern of genetic variances and covariances among traits ($\mathbf{G}$), in his formulation of the multivariate breeders' equation, $\Delta \mathbf{z} = \mathbf{G}\boldsymbol{\beta}$. Within a single population, the response to selection can vary substantially from the optimum direction represented by $\boldsymbol{\beta}$ if the $\mathbf{G}$ matrix has much more genetic variance for some trait combinations than others. When multiple populations are experiencing selection, the divergence among populations can then be generated not only by differences among populations in the direction of selection (spatial variation in $\boldsymbol{\beta}$) but also by variation among populations in $\mathbf{G}$. It is therefore a considerable challenge to understand why a set of populations may have diverged in multivariate phenotypes as a consequence of selection.

Although we have yet to fully understand to what extent microevolutionary processes can predict macroevolutionary patterns of phenotypic divergence, the multivariate quantitative genetic theory centered around the breeder's equation offers perhaps our best opportunity to do so (Arnold et al. 2001). There is some evidence from natural populations that multivariate genetic constraints can bias the direction of evolution. Comparisons of the pattern of phenotypic divergence among populations in matrix form with patterns of genetic covariance have found that divergence is closely aligned with major axes of additive genetic variance in some cases (Mitchell-Olds 1996; Schluter 1996) but not others (McGuigan et al. 2005). The major limitation of this approach has been the potential confounding of genetic drift and selection as causes of any association between axes of genetic variance and divergence (Phillips et al. 2001). Under genetic drift, the pattern of phenotypic divergence among populations is expected

to be proportional to the level of genetic variance (Lande 1979), and any association between the major axes of genetic variance and divergence may therefore be the product of neutral divergence.

One way to distinguish between natural selection and genetic drift is to partition divergence into components known to be a consequence of each process (McGuigan et al. 2005); however, this requires a highly specific sampling design based on independently replicated evolutionary events. A more generally applicable solution has recently been developed by Hohenlohe and Arnold (2008), in which the known phylogeny of a set of populations or species is used to correct the observed pattern of divergence among them for shared history, allowing a test of the neutral model of divergence by comparing this corrected pattern of divergence with **G**. Any significant departure from the neutral expectation can then be interpreted as evidence for a constraining or diversifying force (i.e., stabilizing or disruptive selection), the specific nature of which can be inferred from an investigation of how the matrices describing the two patterns differ.

While it is likely that a wide range of taxa could be used in the application of this comparative approach, there remain two underlying causes of divergence among populations in multiple traits that are not directly addressed in this framework. First, since selection itself is unmeasured, variation in selection (divergent selection) is not directly considered but is instead inferred from the direction of divergence itself. Second, the approach uses a single **G** matrix in the comparisons with patterns of divergence. Thus, the potential for variation in the **G** matrices themselves among the populations to influence the pattern of divergence is not accounted for.

In this article, we show how microevolutionary estimates of divergent selection among multiple populations and variation in the pattern of genetic-covariance structure within them can be associated with patterns of phenotypic divergence among populations. We base our approach on a multivariate framework, first developed by Zeng (1988) and independently derived by Felsenstein (1988) and Hansen and Martins (1996), for characterizing variation in selection among multiple populations and determining its role in population divergence. Zeng (1988) developed two models to describe the divergence of multiple traits among populations. The first (model 1) assumed the presence of both directional selection acting on the traits (caused by a shift in optimum, e.g., as a consequence of environmental change) and stabilizing selection acting to maintain the population at the optimum. Zeng (1988) assumed that each population would experience the same pattern of multivariate stabilizing selection, although the vectors of directional selection were allowed to vary among populations. Under this model, divergence among populations

was a consequence of the pattern of correlational selection and variation in directional selection. Zeng (1988) explicitly assumed in this model that genetic constraints would not be important in determining the pattern of divergence in the longer term. In contrast, model 2 assumed that stabilizing selection was absent and that population divergence in multiple traits was instead influenced by the pattern of genetic covariance among traits.

Zeng (1988) considered that divergence among populations in the short term would be influenced by genetic constraints (model 2), a view supported by null-model comparisons of trait and marker divergence (Merilä and Crnokrak 2001; Leinonen et al. 2008), but that over longer periods the pattern of divergence would be influenced only by the fitness functions (model 1). There is some evidence that divergence is consistent with the influence of stabilizing selection over a range of evolutionary time periods (Estes and Arnold 2007), but it remains unknown over what period of time the influence of genetic constraints may dissipate (Schluter 1996). Furthermore, Zeng (1988) assumed the maintenance of genetic variation by mutation-selection balance in traits, which results in the eventual overriding influence of the fitness function. In contrast to this view, recent work on the distribution of multivariate genetic variance has suggested that some phenotypic trait combinations may have very little genetic variance (Kirkpatrick 2009) and that selection may be effective at depleting genetic variance under some circumstances (Blows et al. 2004; Hunt et al. 2007; Van Homrigh et al. 2007). Our main focus in this article is therefore Zeng's model 2, because it explicitly incorporates the possibility that genetic constraints influence the pattern of divergence among populations of a single species. Using data on sexual-selection gradients and genetic-covariance matrices for a set of contact pheromones from nine natural populations of *Drosophila serrata*, we illustrate our approach by estimating the contribution of sexual selection to the observed among-population divergence of these traits in nature. Although we consider model 2 a more appropriate framework from the perspectives of both its direct consideration of genetic constraint and its biological relevance to our case study data set, we present the development of Zeng's model 1 and its application to this data set in appendix A in the online edition of the *American Naturalist.*

Integrating Selection, Genetic Constraints, and Divergence: A Geometric Framework

Our approach differs in three important respects from many previous attempts to understand divergence using microevolutionary parameters in retrospective selection analyses (Lande 1979; Turelli 1988; Arnold et al. 2001). First, rather than inferring the strength and direction of

selection by substituting observed levels of divergence and genetic covariance into the multivariate breeders' equation (Lande 1979), we employ direct, empirical estimates of directional selection gradients independently determined for each population. Second, variation in these directional selection gradients among populations is explicitly incorporated in Zeng's (1988) framework. Although Zeng originally developed his models with natural selection in mind, the example we use concentrates on sexual selection alone. Finally, we incorporate heterogeneity in the genetic-covariance structure among populations by extending Zeng's (1988) approach to generate population-specific predicted responses to selection.

Quantifying Multivariate Divergence in Phenotypes and Selection

The phenotypic divergence among a set of populations for multiple traits can be represented by the interpopulation variance-covariance matrix of population means **D** (Lande 1979; Zeng 1988; Blows and Higgie 2003; McGuigan et al. 2005):

$$\mathbf{D} = \begin{pmatrix} \sigma^2(\mu_1) & \sigma(\mu_1, \mu_2) & \sigma(\mu_1, \mu_n) \\ & \ddots & \vdots \\ & & \sigma^2(\mu_n) \end{pmatrix}, \qquad (1)$$

where the diagonal elements, $\sigma^2(\mu_n)$, are the variances among the k population means for the n traits and the off-diagonal elements are the covariances among population means for each bivariate trait combination. To determine how much of the among-population phenotypic divergence was a consequence of variation in selection, Zeng (1988) showed how variation among populations in directional selection gradients (Lande and Arnold 1983) on the n traits (β_n) could be characterized by the interpopulation variance-covariance matrix **B**:

$$\mathbf{B} = \begin{pmatrix} \sigma^2(\beta_1) & \sigma(\beta_1, \beta_2) & \sigma(\beta_1, \beta_n) \\ & \ddots & \vdots \\ & & \sigma^2(\beta_n) \end{pmatrix}, \qquad (2)$$

where the diagonal elements, $\sigma^2(\beta_n)$, represent the variance among populations of the directional selection gradients on the nth trait and the off-diagonal elements represent the covariance among populations of directional selection gradients for each bivariate trait combination.

The genetic-covariance structure for a suite of traits can dramatically influence the response to selection (Lande 1979), causing substantial deviations from the direction of selection under a wide range of conditions (Hansen and Houle 2008). When this covariance structure is taken into

account, the level of among-population divergence generated by variation in directional selection is given by equation (23) in Zeng (1988):

$$\mathbf{R}_{\mathbf{G_w}} = \mathbf{G_w} \mathbf{B} \mathbf{G_w}, \qquad (3)$$

where $\mathbf{R}_{\mathbf{G_w}}$ is the interpopulation variance-covariance matrix for responses to selection generated by directional selection and $\mathbf{G_w}$ is a single, common genetic variance-covariance matrix for all populations. It is important to note for what follows that $\mathbf{R}_{\mathbf{G_w}}$ can also be calculated by estimating the single-generation predicted response to selection within each population with the multivariate breeders' equation and then calculating the variances and covariances among the elements of the response vectors from each population.

Zeng (1988) assumed that **G** did not differ among populations, although it remains unknown how often this assumption may hold (Hine et al. 2009). Spatial heterogeneity in genetic-covariance structure may therefore influence evolutionary trajectories in addition to that expected from spatial variation in directional selection alone. To incorporate spatial variation in **G**, the approach represented by equation (3) can be extended by applying the multivariate breeders' equation, $\Delta\mathbf{z}_k = \mathbf{G}_k\boldsymbol{\beta}_k$, where $\boldsymbol{\beta}_k$ is the column vector of selection gradients on the n traits for population k and $\mathbf{G}_k$ is the population-specific genetic-covariance structure. This yields a vector of predicted response in mean ($\Delta\mathbf{z}_k$) for the n traits for each of the k populations. From this, the interpopulation variance-covariance matrix of predicted responses to selection ($\mathbf{R}_{\mathbf{G}}$) can be calculated as

$$\mathbf{R}_{\mathbf{G}} = \begin{pmatrix} \sigma^2(\Delta z_1) & \sigma(\Delta z_1, \Delta z_2) & \sigma(\Delta z_1, \Delta z_n) \\ & \ddots & \vdots \\ & & \sigma^2(\Delta z_n) \end{pmatrix}, \qquad (4)$$

where the elements of $\mathbf{R}_{\mathbf{G}}$ now represent divergence generated by the combined effects of among-population variation in selection and population-specific genetic-covariance structures of the traits under selection.

Comparing Observed and Predicted Phenotypic Divergence

The above framework characterizes both observed and predicted phenotypic divergence into a set of interpopulation covariance matrices of equal dimension. Comparison of predicted and observed divergence then allows the determination of both the extent to which divergent selection resembles observed divergence (**D** vs. **B**) and the extent to which evolutionary trajectories are altered because of genetic constraints (**D** vs. $\mathbf{R}_{\mathbf{G_w}}$) and among-

population differences in those constraints (**D** vs. $\mathbf{R_G}$). Although many techniques exist for the comparison of sets of covariance matrices (Steppan et al. 2002), we employ the Krzanowski (1979) method, a geometric approach to matrix comparison that provides a straightforward, bounded measure of the overall similarity in orientation of subspaces defined by each matrix. The utility of the Krzanowski method for the comparison of matrices has been outlined in detail elsewhere (Blows et al. 2004), and it has been employed to address several problems in evolutionary quantitative genetics (Hine et al. 2004; Petfield et al. 2005; Van Homrigh et al. 2007; Rundle et al. 2008). This approach was particularly useful here because the matrices we wished to compare were of equal dimension but did not represent the same kind of information (e.g., the two matrices were not both **G** matrices). Therefore, the two matrices could not be estimated within a single model to facilitate hypothesis testing, nor was it possible to decide on the appropriate analogous degrees of freedom for each matrix that is needed to implement such hypothesis-testing approaches as the Flury hierarchy (Phillips and Arnold 1999). Instead, the Krzanowski method allowed the subspaces that described the vast majority (>95%) of variance in both matrices to be compared geometrically, allowing us to determine to what extent predicted and observed divergence shared the same orientation in their common multivariate trait space.

Briefly, one selects a subset m of the n principal components of each of the two matrices being compared, where the choice of m is usually guided by an a priori decision based on the amount of variance to be captured by the principal components (note, however, that $m \leq n/2$). A projection is then performed that uses

$$\mathbf{S} = \mathbf{L}^{\mathrm{T}}\mathbf{M}\mathbf{M}^{\mathrm{T}}\mathbf{L}, \tag{5}$$

where **S** is the projection matrix and **L** and **M** each represent subspaces (dimensions $n \times m$) comprising the loadings for the chosen m principal components of the two matrices as columns. In our case, the two matrices were the observed (**D**) and predicted (**B**, $\mathbf{R_{G_w}}$, $\mathbf{R_G}$) divergence matrices. This projection finds the angles between the best-matching vectors within each of the two matrix subspaces being compared. The final unit of comparison is a sum of these angles (in the scale of squared cosines), calculated as the sum of the eigenvalues of the projection matrix **S**, and is on the scale 0–m, where 0 represents orthogonal subspaces and m indicates that the two subspaces share identical orientations.

A Case Study: Divergence Generated by Sexual Selection

Common in nature (Andersson 1994) and relatively strong (Kingsolver et al. 2001; Hereford et al. 2004), sexual selection has long been thought to be a key process generating phenotypic divergence among populations and consequently is the focus of a number of possible mechanisms of speciation (Panhuis et al. 2001; Schluter 2001; Gavrilets 2004). In particular, quantitative genetic models of the coevolution of female preferences with male traits (Fisher 1930; Lande 1981; reviewed by Mead and Arnold [2004]) predict unstable or multiple equilibria that have the potential to generate a diverse array of phenotypic outcomes (West-Eberhard 1983). Consistent with this, recently diverged species, including some of the most famous adaptive radiations, often exhibit spectacular diversity in sexually selected traits. The intensity of sexual selection also predicts diversity within some taxonomic groups (Barraclough et al. 1995; Arnqvist et al. 2000; Seddon et al. 2008).

It is surprising, therefore, that there are few examples of divergent sexual selection in natural populations (Schluter 2000; Panhuis et al. 2001), and evidence of contemporary evolution of traits under sexual selection is limited (Svensson and Gosden 2007). Although it has been possible to demonstrate the presence of divergent sexual selection in individual cases (Endler and Houde 1995; Seehausen et al. 1997; Boul et al. 2007) and although such selection is sometimes found in association with reinforcing natural selection (Saetre et al. 1997; Hoskin et al. 2005; Higgie and Blows 2007), the degree to which phenotypic divergence among populations is a consequence of divergent sexual selection is unknown. Isolating the contribution of divergent sexual selection is difficult, however, because sexually selected traits are likely to be condition dependent in their expression (Rowe and Houle 1996) and consequently may also be subject to natural selection. A means of isolating the contribution of sexual selection to an observed pattern of phenotypic divergence in sexually selected traits is therefore required to determine the importance of sexual selection in generating among-population divergence.

Population Divergence Driven by Sexual Selection in Drosophila

Drosophila serrata is an Australian native vinegar fly that has a long, narrow distribution along the coastal strip of eastern Australia from just south of Sydney, extending north into New Guinea (fig. 1). In this species, females exert sexual selection on a suite of male contact pheromones composed of well-characterized long-chain cutic-
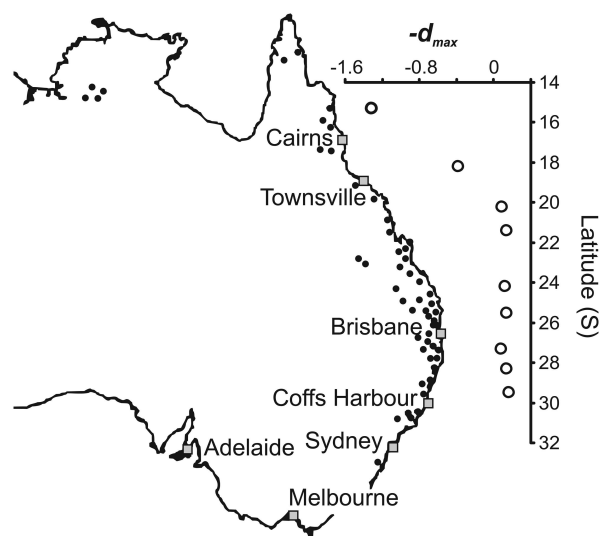
Figure 1: Phenotypic divergence in cuticular hydrocarbons (CHCs) among nine geographic populations of *Drosophila serrata*, shown relative to their latitudinal location along the Australian east coast. White circles represent mean values of all individuals within a population scored for their value of the trait combination represented by the first eigenvector ($\mathbf{d}_{max}$; table 1) of the interpopulation variance-covariance matrix for population mean CHCs, **D**. Standard errors do not extend beyond the individual points. Black points are indicative of the natural range of *D. serrata* and show capture locations from Jenkins and Hoffmann (2001).

ular hydrocarbons (CHCs; Blows et al. 2004; Hine et al. 2004). Sexual selection by females on these male traits varies among nine geographic populations sampled over a 1,450-km range (Chenoweth et al. 2008; Rundle et al. 2008). Male CHCs have diverged among these same geographic populations (Chenoweth et al. 2008), and neutral divergence due to genetic drift has been excluded as the cause of much of this divergence by both univariate and multivariate Q_{ST}-F_{ST} comparisons (Chenoweth and Blows 2008). Reinforcing natural selection, due to the presence of the closely related congener *Drosophila birchii*, has been shown to produce a pattern of reproductive character displacement between sympatric and allopatric populations (Higgie et al. 2000; Higgie and Blows 2007).

In this case, we did not implement Hohenlohe and Arnold's (2008) complementary approach to the analysis of multivariate phenotypic divergence. Neutral-marker divergence among *D. serrata* populations is weak (average $F_{ST} = 0.026$) and resembles isolation by distance (Chenoweth and Blows 2008). Such weak population structure precludes the construction of a bifurcating population-level phylogeny, a requirement of the Hohenlohe and Arnold (2008) approach.

Analyses

Application of the geometric framework for multiple natural populations requires three substantial data sets for the estimation of divergence, selection, and genetic-covariance structures for the suite of traits under consideration. Although such a comprehensive set of microevolutionary parameters is rarely estimated within a single system, such data are now available for these nine geographic populations of *D. serrata*. Below we summarize the analyses conducted here and the sources of the different data sets.

In all analyses estimating divergence, selection, and genetic (co)variance, nine CHCs were quantified with standard gas chromatography methods: 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-$C_{29:2}$, and 2-Me-C_{30} (Blows and Allan 1998). The area under the nine chromatograph peaks was integrated and transformed into eight logcontrasts for subsequent statistical analyses (Blows and Allan 1998, following Atchison 1986), with Z-9-$C_{26:1}$ as the common divisor in all data sets. No further standardization of the traits was performed in any data set. The individual-trait phenotypic variances and narrow-sense heritabilities are supplied in tables B1 and B2 in the online edition of the *American Naturalist*.

The **D** matrix was estimated as the variance-covariance matrix among the nine population means for each of the eight logcontrast CHCs with data from a common-garden experiment, outlined in Rundle et al. (2008), that consisted of male CHC phenotypes from each of the nine populations. To estimate directional sexual selection, we reanalyzed data from Rundle et al. (2008), who tested for variation among the nine populations in linear and nonlinear selection gradients. Female choice for male CHCs in each population was characterized with standard two-stimulus mate choice tests involving a total of 1,809 lab-reared males (details in Rundle et al. 2008). We estimated the vector of linear sexual-selection gradients, $\boldsymbol{\beta}$, for each population arising from female mate choice, using multiple linear regression (Lande and Arnold 1983). Here, the only point of difference between our analyses and that of Rundle et al. (2008) was that we did not standardize the data in any way before analysis. The among-population variance-covariance matrix of directional selection gradients, **B**, was estimated from the nine $\boldsymbol{\beta}$ vectors in the same way that we estimated **D**.

Genetic (co)variance (**G**) matrices for male CHCs were estimated by means of paternal half-sib breeding designs conducted independently within each of the nine populations with the data of Chenoweth et al. (2008), with an average of 44 sires and 245 individuals per population. Briefly, for each population a hierarchical random-effects model was applied, with the random effects of sire, dam

Table 1: Major axes of interpopulation covariance matrices describing observed (**D**) and predicted (**B**, $\mathbf{R}_{G_w}$, $\mathbf{R}_G$) divergence due to sexual selection for cuticular hydrocarbons (CHCs) among nine natural populations of *Drosophila serrata*

Trait	**D**		**B**		$\mathbf{R}_{G_w}$		$\mathbf{R}_G$	
	$\mathbf{d}_{max}$	$\mathbf{d}_2$	$\mathbf{b}_{max}$	$\mathbf{b}_2$	$\mathbf{r}_{G_w, max}$	$\mathbf{r}_{G_w, 2}$	$\mathbf{r}_{G, max}$	$\mathbf{r}_{G, 2}$
% variance explained	99.80	.14	95.90	2.58	81.66	15.41	98.41	1.15
Z,Z-5,9-$C_{24:2}$	−.209	.307	.021	−.241	.128	−.229	.124	.452
Z,Z-5,9-$C_{25:2}$	−.346	.530	.125	.889	.115	−.052	.135	.354
Z-9-$C_{25:1}$	−.281	.463	.016	−.020	.034	.019	.151	.343
2-Me-C_{26}	.061	.103	.249	.090	.513	−.598	.350	.438
Z,Z-5,9-$C_{27:2}$	.639	.473	.151	−.016	.290	.364	.119	.153
2-Me-C_{28}	.120	.225	−.828	−.047	.371	−.166	.380	.239
Z,Z-5,9-$C_{29:2}$	.547	−.037	−.111	−.023	.692	.369	.732	−.519
2-Me-C_{30}	.177	.352	.449	−.373	.077	.539	.357	−.106

Note: Shown are the loadings for the first two eigenvectors of the variance-covariance matrices of phenotypic divergence (**D**), among-population variation in sexual selection (**B**), and the predicted responses to selection based on a common genetic-covariance matrix for CHCs in males ($\mathbf{R}_{G_w}$) or unique covariance matrices in each of the nine populations ($\mathbf{R}_G$)

within sire, and individual within family, via the MIXED procedure in SAS, assuming an "unstructured" (type = UN) variance-covariance matrix for all random effects (SAS, ver. 9.2, SAS Institute, Cary, NC). The additive genetic variance-covariance matrix **G** was estimated as four times the sire (co)variance components (Lynch and Walsh 1998). Note that these estimates will differ from those presented in Hine et al. (2009) because in that work traits were converted to (population grand mean) standard normal deviates before analysis. The average within-population **G** matrix, $\mathbf{G}_w$, was estimated from a hierarchical multivariate random-effects model that included population, sire nested within population, dam within sire, and individuals within families, fitted with the software WOMBAT (Meyer 2007). The matrix $\mathbf{R}_{G_w}$ was subsequently calculated with equation (3). To estimate $\mathbf{R}_G$, the multivariate breeders' equation was used with the population-specific estimates of directional selection, $\boldsymbol{\beta}_k$ and $\mathbf{G}_k$. The nine resulting response vectors, $\Delta \mathbf{z}_k$, were then used to estimate the variance-covariance matrix $\mathbf{R}_G$ in the same way that we estimated **D** and **B**.

Finally, we compared the orientation of the matrix of observed divergence (**D**) with that of matrices of predicted divergence (**B**, $\mathbf{R}_{G_w}$, and $\mathbf{R}_G$), using the Krzanowski method. This required a principal-components analysis of each matrix to determine the number of principal components required to capture at least 95% of the variance in each matrix. In all cases, two principal components explained at least 95% of the variance, and these two-dimensional subspaces were then compared by means of equation (5). The Krzanowski method was implemented using SAS IML (SAS, ver. 9.2). The SAS code used to implement all analyses and tables of all parameter esti-

mates used in these analyses are available from the corresponding author upon request.

Results

Variation among populations in the direction of sexual selection alone was not closely associated with variation among populations in sexually selected traits. The pattern of population divergence in male traits represented in **D** (table B3 in the online edition of the *American Naturalist*) was dominated by a single trait combination, $\mathbf{d}_{max}$ (the first eigenvector, or principal component, of **D**), that accounted for almost 99.8% of the variation among population means. This trait combination contrasted the three shortest-chain CHCs with the others (table 1) and displays a steplike cline with latitude (fig. 1). Similarly, most (95.9%) of the divergent sexual selection among populations represented in **B** (table B4 in the online edition of the *American Naturalist*) was also accounted for by variation in selection on a single trait combination (the first eigenvector of **B**, $\mathbf{b}_{max}$; table 1). A comparison of the major subspaces of these two matrices indicated little similarity in orientation between them ($\sum \lambda_{S(B, D)} = 0.201$ out of a possible 2, or 10.1% of the maximum). Simply characterizing divergent sexual selection alone, assuming no genetic constraints, is therefore insufficient to explain much of the existing phenotypic divergence among these populations.

Including the influence of a common genetic-covariance structure provided a substantial improvement in explaining the observed among-population divergence for these traits. The pattern of population divergence represented by $\mathbf{R}_{G_w}$ (table B5 in the online edition of the *American*

Naturalist) was also dominated by a single trait combination ($\mathbf{r}_{\mathbf{G}_\text{w,max}}$; table 1), which accounted for 81.7% of the predicted phenotypic divergence. There was greater similarity in the orientations of $\mathbf{R}_{\mathbf{G}_\text{w}}$ and $\mathbf{D}$ ($\sum \lambda_{\text{S}(\mathbf{R}_{\mathbf{G}_\text{w}}, \mathbf{D})} = 0.798$ of a possible 2, or 40.0% of maximum), indicating that accounting for genetic covariance via a single, common genetic variance-covariance structure ($\mathbf{G}_\text{w}$) improved the overall similarity in orientation over that due to sexual selection alone.

To explore the potential for among-population variation in $\mathbf{G}$ to contribute to phenotypic divergence, we used unique estimates of $\mathbf{G}$ from each population to calculate population-specific predicted responses to selection ($\Delta \mathbf{z}_k$) and, from these, the interpopulation covariance matrix, $\mathbf{R}_\mathbf{G}$ (table B6 in the online edition of the *American Naturalist*). The major axis of $\mathbf{R}_\mathbf{G}$, $\mathbf{r}_{\mathbf{G, max}}$ (table 1), accounted for 98.4% of the predicted phenotypic divergence due to sexual selection. The orientation of $\mathbf{R}_\mathbf{G}$ with respect to $\mathbf{D}$ remained high relative to the orientation of $\mathbf{B}$ with $\mathbf{D}$, and there was a minor improvement over that of a single common $\mathbf{G}$ ($\sum \lambda_{\text{S}(\mathbf{R}_\mathbf{G}, \mathbf{D})} = 1.071$, or 51.0% of maximum).

To determine whether the inclusion of genetic covariance significantly increased the degree of association between observed and predicted divergence, we estimated a confidence interval for $\sum \lambda_{\text{S}(\mathbf{R}_\mathbf{G}, \mathbf{D})}$ that considered error due to the estimation of $\mathbf{G}$ in each of the nine populations and determined whether it included the estimate of $\sum \lambda_{\text{S}(\mathbf{B}, \mathbf{D})}$, the measure of the orientation of divergence and directional sexual selection assuming no genetic constraints. To estimate the 95% confidence interval, we bootstrapped sire families from each of the nine populations, estimating $\mathbf{G}$ in each population for each of 100 bootstrap samples, and generated a null distribution of $\sum \lambda_{\text{S}(\mathbf{R}_\mathbf{G}, \mathbf{D})}$ values. The modest number of replicates was due to excessive computational demands associated with estimating 900 individual 8×8 $\mathbf{G}$ matrices via restricted maximum likelihood. We estimated confidence intervals for $\sum \lambda_{\text{S}(\mathbf{R}_\mathbf{G}, \mathbf{D})}$ rather than $\sum \lambda_{\text{S}(\mathbf{R}_{\mathbf{G}_\text{w}}, \mathbf{D})}$ because it was likely have a larger error due to the estimation of individual $\mathbf{G}$ matrices in each population. The null hypothesis of equal orientation of subspaces of $\mathbf{R}_\mathbf{G}$ with $\mathbf{D}$ and of $\mathbf{B}$ with $\mathbf{D}$ was rejected because the confidence interval for $\sum \lambda_{\text{S}(\mathbf{R}_\mathbf{G}, \mathbf{D})}$ (0.315–0.939) did not overlap the point estimate of 0.201. This indicated that the inclusion of genetic-covariance structure led to a significant improvement in the extent to which predicted divergence due to sexual selection is oriented in the direction of observed phenotypic divergence among populations.

To this point, our results demonstrate two key features of phenotypic divergence in this system. First, when considered in isolation, among-population differences in the direction of sexual selection are poor predictors of the direction of phenotypic divergence among these nine pop-

ulations. Second, when estimates of standing genetic (co)variation are included in predictions, the association becomes significantly stronger. We therefore examined in greater detail how the interaction between selection and genetic constraints have an influence on the direction of evolution in these populations. By use of a spectral decomposition of the multivariate breeders' equation, the contribution to the response to selection of each independent axis of additive genetic variance can be isolated from

$$\Delta \mathbf{z} = \mathbf{G}\boldsymbol{\beta}$$
$$= \lambda_1 \mathbf{g}_\text{max} \mathbf{g}_\text{max}^\text{T} \boldsymbol{\beta} + \lambda_2 \mathbf{g}_2 \mathbf{g}_2^\text{T} \boldsymbol{\beta} + \ldots + \lambda_n \mathbf{g}_n \mathbf{g}_n^\text{T} \boldsymbol{\beta}, \quad (6)$$

where λ_n is the nth eigenvalue of $\mathbf{G}$, $\mathbf{g}_n$ is a column vector containing the nth eigenvector of $\mathbf{G}$, and $\boldsymbol{\beta}$ is the vector of directional selection gradients (Walsh and Blows 2009). The first term on the right of equation (6) describes the vector of change in trait means due to a combination of selection and $\mathbf{g}_\text{max}$, the linear combination of traits exhibiting the greatest additive genetic variance. When applied to each of the nine populations using population-specific estimates of $\mathbf{G}$, the response to sexual selection was largely dominated by $\mathbf{g}_\text{w, max}$ (table 2); on average, 59% of the within-population response to sexual selection was oriented in this direction. This occurred despite the fact that, in almost all cases, the direction of sexual selection was close to 90° from $\mathbf{g}_\text{w, max}$, which represents the maximum possible difference in orientation between these two vectors (table 2). The end result is a predicted response to selection that is substantially biased away from the direc-

Table 2: Orientation (angle) between the vector of sexual selection gradients ($\boldsymbol{\beta}$) and the major axis of genetic variance ($\mathbf{g}_\text{w, max}$) within each of the nine natural populations of *Drosophila serrata*

Population	Angle (°)	Contribution from $\mathbf{g}_\text{w, max}$ (%)	$\mathbf{r}_{(\boldsymbol{\beta}, \Delta \mathbf{z})}$
Cooktown	77.5	92.7	.428
Cardwell	88.5	19.8	.459
Airlie Beach	98.3	97.1	.152
Sarina	99.5	26.0	.444
Colosseum Creek	111.9	94.4	.461
Bauple	95.6	81.1	.058
Brisbane	89.6	1.5	−.246
Brunswick Heads	81.7	70.5	.281
Red Rock	85.6	46.3	.195

Note: Populations are listed in north-to-south order, corresponding to figure 1. Also shown is the fraction of the total phenotypic response to sexual selection influenced directly by the major axis of genetic variance, $\mathbf{g}_\text{w, max}$, calculated via the spectral decomposition of the breeders' equation (6) as $\|\lambda_1 \mathbf{g}_1 \mathbf{g}_1^\text{T} \boldsymbol{\beta}\| / \|\Delta \mathbf{z}\|$ and expressed as a percentage. For all calculations, the genetic-covariance matrix for each population was used. Vector correlations between $\boldsymbol{\beta}$ and $\Delta \mathbf{z}$, $\mathbf{r}_{(\boldsymbol{\beta}, \Delta \mathbf{z})}$, are also provided.

tion of selection, as indicated by the vector correlation between Δ**z** and *β* given in table 2 (Blows and Walsh 2007; Hansen and Houle 2008; Marroig et al. 2009).

Discussion

Sexual selection has long been thought to be a powerful process driving the evolution of reproductive isolation between populations (Panhuis et al. 2001; Schluter 2001). This is in part because traits under the influence of sexual selection have been hypothesized to evolve in arbitrary ways, producing an infinite variety of outcomes (West-Eberhard 1983). Our study challenges this classic view of the ability of sexual selection to generate divergence in arbitrary directions for two reasons. First, the matrix **B** characterizing spatial variation in phenotypic sexual selection alone was dominated by a single trait combination. If sexual selection caused populations to evolve in arbitrary directions, the **B** matrix would contain many independent vectors of similar length. Instead, it is likely that much of the spatial divergence seen in sexual selection arising from female mate choice in *Drosophila serrata* reflects preference differences generated in response to reinforcing natural selection generated by the presence/absence of a related species, *Drosophila birchii* (Higgie et al. 2000; Higgie and Blows 2007; Rundle et al. 2008).

Second, applying the geometric framework to sexually selected trait divergence in *D. serrata* revealed that the predicted response to divergent sexual selection was closely aligned with the observed pattern of among-population divergence. However, the association was strong only when the influence of genetic covariance was included; sexual selection alone was only weakly associated with divergence. Genetic-covariance structure appears, therefore, to have biased the evolutionary trajectories of these populations. Thus, the direction in which sexual selection generates divergence may depend critically on the pattern of genetic constraint in individual populations, restricting how sexually selected traits may ultimately diversify.

Although we have been able to explain up to 50% of the phenotypic divergence among populations as a consequence of the action of sexual selection, the possible effects of divergent natural selection operating on these traits have been ignored. Insect cuticular hydrocarbons not only act as contact pheromones but are also involved in ecologically important functions such as maintaining water balance and stress resistance (Gibbs 2002; Howard and Blomquist 2005). Clinal variation in abiotic factors such as temperature and humidity may therefore also contribute in some way to among-population divergence in CHCs (Chenoweth and Blows 2008). Unfortunately, it is difficult to quantify the forces of natural selection in species in which it is not possible to follow individuals in the field.

It is important to recognize some further limitations of our approach, which arise primarily as a consequence of trying to reconstruct a series of evolutionary events that are likely to have been the outcome of a relatively long process. Population genetic analyses have indicated a pattern of isolation by distance among these populations (Chenoweth and Blows 2008), and it is therefore likely they have been separated for a substantial period of time, as will be the case for many populations that are under investigation in evolutionary studies. In contrast, the multivariate breeders' equation predicts only the single-generation response to selection; this prediction will change if the direction of selection and/or **G** changes through time.

Environments fluctuate through time, causing temporal changes in the direction of selection. The use of our approach for systems in which natural selection gradients have been estimated may therefore be problematic if this selection has fluctuated over time or has permanently changed direction. In the case of sexual selection in *D. serrata*, the spatial similarity of sexual-selection gradients among populations within a geographic region that either contains or does not contain the species *D. birchii* (Higgie et al. 2000; Higgie and Blows 2007; Rundle et al. 2008) suggests that the direction of sexual selection may remain relatively constant over the time frame of divergence represented by these populations. Similarly, although our approach accommodates both direct estimates of the direction of selection and heterogeneity in genetic-covariance structure, it shares with previous retrospective selection analyses the assumption that extant patterns of genetic covariance are representative of the orientation of the **G** matrices throughout the period of divergence (Turelli 1988). This issue of the constancy of **G** within this context is a complex one that we address in greater detail below.

How Does the **G** Matrix Influence Phenotypic Divergence?

The way in which the genetic-covariance structure is likely to bias the direction of evolution is best illustrated by the spectral decomposition of the predicted response within each population. Because these **G** matrices are relatively ill conditioned (i.e., some trait combinations have far more genetic variance than others), the predicted response is dominated by the trait combination with the most genetic variance. This result is important because it means that even when selection is almost orthogonal to the direction of the major axis of genetic variance (i.e., selection is in a direction close to 90° away from the direction of $\mathbf{g}_{max}$), $\mathbf{g}_{max}$ still dominates the predicted responses within each population (Schluter 1996). In other words, in the presence of ill-conditioned **G** matrices, evolution may sometimes proceed in directions very different from that actually favored by selection.

Our analyses suggest that the extent to which populations will tend to diverge along $\mathbf{g}_{max}$ as a consequence of selection, as suggested by Schluter (1996), will be determined by two attributes of the **G** matrix. First, the extent of the ill-conditioned nature of **G**, which is reflected by the relative sizes of eigenvalues of **G** (i.e., their evenness; Kirkpatrick 2009), affects the opportunity for a given response to selection to be heavily biased by $\mathbf{g}_{max}$. If the **G** matrix is essentially spherical (i.e., all eigenvalues are of roughly equal size), then $\mathbf{g}_{max}$ is unlikely to play a dominant role biasing the response. Second, selection must be oriented in a direction that exhibits little genetic variance. If substantial levels of genetic variance exist in the direction of selection, the relative contribution of $\mathbf{g}_{max}$ to the response will be substantially reduced.

Two general questions therefore arise: first, are **G** matrices typically ill conditioned, and second, is genetic variance low in the direction of selection? Although the data are limited at present, it does appear that the genetic variance in multiple traits resides in fewer dimensions than the number of traits that have been measured (Kirkpatrick 2009). In addition, ill-conditioned **G** matrices may be expected under mutation-drift equilibrium, even with a spherical distribution of mutation effects (i.e., mutations are equally likely in all directions of phenotypic space; Griswold et al. 2007). Therefore, ill-conditioned **G** matrices may turn out to be a common feature of the distribution of multivariate genetic variance (Blows 2007).

The answer to the second question depends on how genetic variances change under selection, a long-standing problem in evolutionary genetics (Lande 1980; Barton and Turelli 1987; Johnson and Barton 2005). Although under the assumptions of the infinitesimal model genetic variance changes very little during the response to selection (Lande 1979), there is little doubt that these assumptions are likely to be violated for many traits (Barton and Turelli 1987; Reeve 2000), particularly where genes of major effect respond to selection (Agrawal et al. 2001). More specifically, there is now considerable evidence that genetic variance in the sexually selected traits considered here has been depleted by persistent sexual selection (Blows et al. 2004; Hine et al. 2004, 2009). This highlights the fact that selection itself may contribute to the generation of ill-conditioned **G** matrices by reducing genetic variance for the trait combinations that are under strong selection (Walsh and Blows 2009). Consequently, the response to selection during divergence may first be a result of genetic variance lying in the direction of selection that would soon be depleted, and then further and longer-term responses might be dominated by major axes of genetic variance that are not closely associated with the direction of selection.

Divergence in Genetic Variance

The fact that the predicted response to selection is dominated by $\mathbf{g}_{max}$ helps to explain why the orientation of observed divergence and that predicted by sexual selection were similar when either the average within-population **G** or independent estimates of **G** for each population were employed. Despite large differences in trait means that have arisen as a consequence of divergent selection (Chenoweth and Blows 2008), the orientation of **G** does not itself differ greatly among these populations. Hine et al. (2009) established two important attributes of **G** matrix divergence among these populations. First, it was shown that the combination of traits constituting $\mathbf{g}_{max}$ within populations was very similar among the nine populations. Second, more than 70% of all divergence in genetic variance that has occurred among these nine populations is itself oriented along $\mathbf{g}_{max}$. It is therefore not surprising that individual **G** matrices and the average within-population **G** matrix had similar effects on predicted divergence, given that $\mathbf{g}_{max}$ was shown to heavily bias the predicted direction of evolution.

While trait means have diverged as a consequence of selection in a direction that has been biased by $\mathbf{g}_{max}$, Hine et al. (2009) interpreted the divergence in genetic variance that has occurred primarily in $\mathbf{g}_{max}$ as being consistent with divergence as a consequence of genetic drift. Because the selection response along $\mathbf{g}_{max}$ would be expected to be very slow, given the almost orthogonal nature of $\mathbf{g}_{max}$ and β in these populations, it may be that drift has a much greater influence on the level of genetic variance in $\mathbf{g}_{max}$ than selection does in this situation. This is in contrast with what appears to be a very strong effect of selection on the genetic variance in the direction of selection itself. Therefore, not only may major aspects of divergence in trait means and genetic variance be driven to some extent by different processes but divergence in the genetic variance of different trait combinations may also be differentially affected by these same processes.

Conclusion

We have presented a framework that allows microevolutionary estimates of selection and genetic variance in multiple traits to be used to predict how phenotypic divergence would occur as a consequence of selection. This predicted divergence can then be associated with that observed among natural populations to determine the extent to which phenotypic divergence may have been biased by genetic constraints. In the case of sexually selected male traits of *D. serrata*, the direction of divergence among natural populations was significantly biased by the multivariate patterns of genetic constraint. These results sug-

gest that evolution may at times proceed in directions that are determined more by the pattern of genetic constraint than by the direction favored by selection.

Acknowledgments

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