

Comparing Complex Fitness Surfaces: Among-Population Variation in Mutual Sexual Selection in *Drosophila serrata*

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ABSTRACT: Despite a dramatic increase in empirical estimates of phenotypic selection over the past two decades, we remain remarkably ignorant about variation in the multivariate fitness surfaces that shape the adaptive landscape. We develop a novel approach for quantifying patterns of spatial and/or temporal variation in multivariate selection that directly compares vectors of linear selection gradients (β) and matrices of nonlinear selection gradients (γ) that describe the multivariate fitness surface in each population. We apply this approach to estimates of sexual selection on a suite of cuticular hydrocarbons (CHCs) in males and females from nine geographic populations of *Drosophila serrata*. In males, variation in linear sexual selection was associated with the presence of the related species *Drosophila birchii*, suggesting that female mate preferences for male CHCs differ between sympatry and allopatry. This is consistent with previous experimental results suggesting that reproductive character displacement of male CHCs has resulted from selection caused by the presence of *D. birchii*. No significant associations were found for nonlinear sexual selection in males. In females, large-scale variation in both linear and nonlinear sexual selection was negatively associated with assumed-neutral population genetic structure, suggesting a key role for chance events in male mate preference divergence.

Keywords: cuticular hydrocarbons, fitness surfaces, mate preferences, linear selection, multivariate selection, nonlinear selection.

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Variation in phenotypic selection has important implications for fundamental evolutionary phenomena including, among others, the evolution of phenotypic diversity and speciation (Schluter 2000), the maintenance of genetic variation (Hedrick 2006), the existence of prolonged evolutionary stasis in many characters (Estes and Arnold 2007), and the stability of the genetic variance-covariance (\mathbf{G}) matrix (Arnold et al. 2001). That selection does vary is clear. Extensive research on local adaptation, for example, has revealed that trade-offs in mean performance are common enough that native forms outperform nonnative forms in their respective environments, thus strongly implicating divergent selection between environments (Schluter 2000). Such experiments, however, provide only a qualitative test of whether selection differs; a comprehensive understanding of how selection differs requires quantitative comparisons of its form and strength among populations from different areas or within a population at different times.

Quantitative descriptions of phenotypic selection, together with the relevant quantitative genetic parameters, form the basis of the microevolutionary equations for predicting the short-term response to selection (Lande 1979; Lande and Arnold 1983; Arnold and Wade 1984a, 1984b). The literature addressing variation in quantitative genetic parameters in natural populations is extensive (Mousseau and Roff 1987; Houle 1992; Blows and Hoffmann 2005). In contrast, we know much less about variation in the form and strength of selection as characterized by these formal quantitative descriptions, in spite of the dramatic increase in the use of these measures (Kingsolver et al. 2001) since Endler's (1986) seminal review of selection in the wild. In particular, we know virtually nothing about how multivariate selection on suites of traits varies spatially and temporally, despite a recognition that selection rarely acts on single traits in isolation (Lande and Arnold 1983; Schluter and Nychka 1994; Blows and Brooks 2003; Blows

et al. 2003). We therefore remain remarkably ignorant about variation in the multivariate fitness surfaces that shape the adaptive landscape, one of the most powerful heuristic images in evolutionary biology (Arnold et al. 2001).

Multivariate fitness surfaces can be quantified using first- and second-order polynomial regression, which provide the best-fit linear and quadratic approximations to the true surface (Lande and Arnold 1983; Phillips and Arnold 1989). The linear partial regression coefficients form a column vector (β) of linear selection gradients representing selection on each trait independent of the other traits in the model, depicted visually as the slope of the fitness surface with respect to that trait. For nonlinear selection, the quadratic partial regression coefficients form a symmetrical matrix (γ) representing selection affecting trait variances (γ_{ii}) and covariances (γ_{ij}), depicted visually as curvature of the fitness surface. Nonparametric techniques (e.g., cubic and thin-plate splines and projection-pursuit regression; Schluter 1988; Schluter and Nychka 1994; Blows et al. 2003) are often used for visual inspection of surfaces and are an important component of selection analyses because they may reveal local features of the fitness landscape that the best-fit linear and quadratic representations cannot. Unfortunately, current nonparametric approaches do not lend themselves readily to quantifying the strength and form of selection, and ultimately, when questions concern variation in selection, a quantitative framework for hypothesis testing is required.

Spatial and temporal variation in individual selection coefficients have been described in a number of studies (Schluter et al. 1991; reviewed in Schluter 2000). One approach to developing a quantitative framework for testing differences in these coefficients has been to extend the linear models used to estimate selection in one environment (or at one time, or in one sex) to multiple environments (or sampling times, or both sexes) by including site (or time, or sex) as a fixed factor in the model (e.g., Kalisz 1986; Galen et al. 1987; Conner 1989; Schemske and Horvitz 1989; Dudley 1996; Caruso 2000; Fornoni et al. 2004). The model becomes an ANCOVA, with the covariates being the individual traits that are the targets of selection. The interaction of each trait with the fixed effect then tests the assumption of homogeneity of slopes, in effect asking whether there is any evidence that the selection gradients differ between groups.

The ANCOVA approach can be extended to provide an overall test of whether selection varies on the entire suite of traits under consideration through the use of the partial *F*-test (Bowerman and O'Connell 1990). The partial *F*-test compares the fit of a full model, which includes all of the trait \times site/time/sex interactions, with a reduced one that excludes all of these interactions. This approach has been

used to test for differences in multivariate sexual selection between males and females (Chenoweth and Blows 2005) and among experimental populations adapted to different treatment environments (Rundle et al. 2005). However, although ANCOVA and partial *F*-tests can provide quantitative tests for differences in selection, these approaches become problematic as the number of traits increases because estimating the full model, including all of the interactions, can consume substantial degrees of freedom. This problem is particularly acute when nonlinear selection is considered because to fully account for all nonlinear selection on a set of n traits requires the inclusion of all $n(n-1)/2$ second-order terms that make up the γ matrix.

An alternative and potentially more informative approach is to directly compare the β vectors and γ matrices that describe the multivariate fitness surface in each population (Arnold et al. 2001). For linear selection, vector correlations can be calculated as the dot product of the standardized β vectors from two populations, providing a straightforward, bounded measure of similarity of linear selection on this entire suite of traits between these populations. The approach for nonlinear selection, however, is not so straightforward. Although Arnold et al. (2001) suggested the use of the Flury (1987) hierarchy to compare γ matrices, it is uncertain whether this method can be applied to matrices other than product-moment-based ones as originally intended.

Here we utilize an alternative, geometric approach to asking whether nonlinear selection, as encapsulated by multiple γ matrices, varies among populations. Our approach employs the Krzanowski (1979) method for the comparison of the eigenstructure of two matrices. The Krzanowski method provides a straightforward, bounded measure of the overall similarity in orientation of subspaces defined by each matrix, and the method is not restricted in its application to any particular type of original matrices. The utility of the Krzanowski method for the comparison of matrices, including \mathbf{G} and γ , has recently been outlined in detail: it was employed in a study comparing the orientation of genetic variance in a suite of sexual display traits, as characterized by the \mathbf{G} matrix, with the multivariate nonlinear sexual selection on them, as characterized by the γ matrix (Blows et al. 2004; Hine et al. 2004). It has also been used to compare pairs of \mathbf{G} matrices in a study of indirect genetic effects (Petfield et al. 2005).

Using this geometric framework allows questions concerning large-scale patterns of variation in selection among multiple populations to be investigated. We test for patterns by assembling two similarity matrices, one for linear selection and one for nonlinear selection, that describe variation in multivariate phenotypic selection among pairs of populations. For linear selection, the individual ele-

ments of this matrix are vector correlation coefficients of linear selection on the suite of traits between pairs of populations. For nonlinear selection, the elements are Krzanowski values generated by the comparison of the γ matrices from the respective pairs of populations. We then analyze structure in these similarity matrices to determine whether any patterns exist in how multivariate selection varies among the suite of populations. We also associate these matrices with other similarity/distance matrices to determine what factors, if any, correlate between them. Although our interest here lies specifically in broad patterns of variation among populations in the strength and form of sexual selection on sexual display traits, our approach is equally applicable to questions concerning natural selection and temporal variation.

We studied variation in sexual selection among geographic populations of the fruit fly *Drosophila serrata*, a species that uses a suite of contact pheromones composed of cuticular hydrocarbons (CHCs) in both mate choice within populations (Blows and Allan 1998; Chenoweth and Blows 2003, 2005; Blows et al. 2004; Hine et al. 2004) and species recognition (Blows and Allan 1998). Mate choice in this species, which has been well characterized via a series of evolutionary and genetic experiments, is mutual: both males and females discriminate among potential mates based on CHCs (Chenoweth and Blows 2003, 2005). In the laboratory, both CHCs and mating preferences for them have been shown to respond when selection is manipulated (Blows 1998; Higgie et al. 2000; Rundle et al. 2005), demonstrating the evolutionary potential of these traits. In nature, CHCs vary among populations along the Australian east coast, and a pattern of reproductive character displacement has been generated by reinforcing selection arising from sympatry of the northern populations with the related species *Drosophila birchii* (Higgie et al. 2000).

Here we use individuals recently collected from nine populations spanning the species' range along the Australian east coast to examine large-scale among-population patterns of variation in sexual selection on CHCs separately in males and females. By conducting more than 1,800 independent mate choice trials involving more than 5,400 individual flies, we characterize the strength and form of linear and nonlinear sexual selection on CHCs separately in males and in females from each of these nine populations. This selection arises from mate choice and is therefore indicative of population-level mate preferences for CHCs in each sex (Rundle et al. 2005). We then compare this selection among populations, separately for each sex, determining whether any patterns exist in the variation and conducting quantitative tests to ask whether it correlates with among-population structure in assumed-

neutral genetic variance (F_{ST}), geographic distance, or the presence/absence (sympatry/allopatry) of *D. birchii*.

Comparisons with F_{ST} and geography were chosen to provide insight into the two main classes of models by which the evolutionary divergence of mate preferences can be initiated (Schluter 2000; Rundle and Nosil 2005), hence leading to differences in sexual selection arising from mate choice. In the first model class, divergence in mate preferences is initiated by chance events, such as genetic drift and/or unique mutations, with sexual selection amplifying this initial divergence to yield a wide array of possible outcomes. Examples of such models include sexual conflict (Chapman et al. 2003) and a Fisherian runaway process (Lande 1981). In the second model class, preferences diverge ultimately as a by-product of adaptation to different environments or niches. Examples of such models include spatial variation in natural selection on sexual display traits (Lande 1982) and sensory drive (Boughman 2002) in which sensory and/or communication systems adapt to their local habitats. In the former class of models, the divergence of mate preferences among populations, and hence the sexual selection they generate, is independent of environment, and correlations are therefore expected only with neutral population genetic structure. In the latter class of models, correlations should exist with the ecological factors generating divergent natural selection. Geographic distance was used in this study as a potential index of ecological differences among populations, and correlations above and beyond any influence of neutral population genetic structure would suggest a key role for divergent natural selection. Finally, comparisons of sexual selection between populations sympatric and allopatric with respect to *D. birchii* were made to investigate whether sexual selection varies with the natural pattern of reproductive character displacement in CHCs in males.

Material and Methods

Study Populations

We collected *Drosophila serrata* from nine natural populations spanning approximately 15° of latitude (1,450 km) along the eastern Australian coastline (table 1). Lab populations were founded from an average of 20 wild-caught females and were established as mass-bred populations at an average census size of 200 individuals for 12 generations before we conducted mating trials.

Mating Trials

Sexual selection on CHCs was measured separately in males and females from each of the nine populations, using replicate binomial mate choice trials. In each trial, a single

Table 1: Locations of populations and proportions of total variance in male and female mating success accounted for by linear and nonlinear sexual selection on all eight log-contrast cuticular hydrocarbons

Population	Latitude (S)	Longitude (E)	Males		Females	
			r_{adj}^2 (linear)	r_{adj}^2 (linear + nonlinear)	r_{adj}^2 (linear)	r_{adj}^2 (linear + nonlinear)
1	15°28.68'	145°15.47'	.088	.188	.045	.154
2	18°16'	146°1'	.061	.182	.023*	.133
3	20°17.16'	148°40.77'	.088	.145	.040	.168
4	21°35.16'	149°11.40'	.043	.208	.031	.201
5	24°24'	151°26'	.106	.251	.046	.136
6	25°49'	152°34'	.029*	.209	.096	.236
7	27°34'	152°59'	.040	.247	.019*	.128
8	28°32'	153°33'	.058	.199	.007 ^{NS}	.118
9	29°58.95'	153°13.77'	.037	.167	.047	.198

Note: Linear and nonlinear selection were estimated using separate regressions in each sex and population. Total variance is given by the adjusted coefficient of determination (r_{adj}^2). $P < .008$ in all cases for linear and nonlinear selection except where otherwise noted. NS = not significant ($P = .127$).

* $.01 < P < .05$.

male (or female) from one of the experimental populations (the “choosing” fly) was placed together with two females (or males) from the same population. Vials were observed, and once intromission had been achieved, all flies were anesthetized using CO_2 , and the choosing fly was discarded. The remaining individuals, one chosen and one rejected by the discarded choosing individual, then had their CHCs extracted for gas chromatography analysis using a standard protocol (Blows and Allan 1998). Individual flies were treated as independent replicates in subsequent analyses because past work indicates that this has no discernible effect on the magnitude or significance of the resulting selection gradients (Rundle et al. 2005).

All flies used in the above mating trials were collected as virgins from the stock populations, using light CO_2 anesthesia within 24 h of their emergence as adults. Individuals were subsequently held separately by sex and population for 4–5 days in glass vials containing 10 mL standard yeast media with live yeast sprinkled on top. Individuals for use as choosing flies were stored singly in vials, and the mating trials were subsequently conducted 4–5 days later within these same vials by introducing, by aspiration (i.e., without anesthesia), two individuals of the sex opposite to that of the choosing fly. Remaining flies were stored in groups of five flies per vial until their use in the mating trials.

An average of 99 (range 97–101) male choice and 99 (range 98–101) female choice mating trials were conducted for each of the nine populations over five consecutive days in February 2005. Both male and female choice trials were fully blocked such that approximately 20 trials were performed for each population on each day. Mating occurred in >98% of the trials overall.

Characterizing Sexual Selection within Populations

CHC samples from the mating trials were analyzed using gas chromatography and flame ionization detection on an Agilent (Wilmington, DE) 6890N gas chromatograph fitted with an HP5 column of 50 m \times 0.32 mm internal diameter and a pulsed splitless front inlet, running the temperature program outlined by Rundle et al. (2005). Individual CHC profiles were determined by integration of the area under nine peaks. These are the same peaks used in past studies (Hine et al. 2002; Chenoweth and Blows 2003, 2005; Blows et al. 2004; Petfield et al. 2005; Rundle et al. 2005); they are identified, in order of their retention times, as follows: (Z,Z)-5,9- $\text{C}_{24:2}$; (Z,Z)-5,9- $\text{C}_{25:2}$; (Z)-9- $\text{C}_{25:1}$; (Z)-9- $\text{C}_{26:1}$; 2-Me- C_{26} ; (Z,Z)-5,9- $\text{C}_{27:2}$; 2-Me- C_{28} ; (Z,Z)-5,9- $\text{C}_{29:2}$; and 2-Me- C_{30} (Howard et al. 2003).

Relative amounts of each of the nine CHCs were determined for each individual by integrating the area under that peak and then dividing by the total area under all nine peaks. Log-contrast values were then calculated, using the proportional area under (Z)-9- $\text{C}_{26:1}$ as the divisor, to break the unit-sum constraint imposed by the use of such proportions (Atchison 1986; Blows and Allan 1998). Six males and six females from the Cooktown population were excluded from the analyses because they lacked any detectable peak for (Z,Z)-5,9- $\text{C}_{24:2}$, and log-contrast values were therefore undefined. Because log-contrast peak values derive ultimately from proportional data, the selection analyses that follow address how differences in the relative abundances of these different CHCs within individuals predict their mating success. Statistical analyses were performed using SAS, version 9.1 (SAS Institute, Cary, NC).

Linear sexual selection on the eight log-contrast CHCs

was estimated separately by sex and population using the standard first-order polynomial regression model (Lande and Arnold 1983):

$$w = \alpha + \sum_{i=1}^8 \beta_i z_i, \quad (1)$$

where w is the mating success (0 = rejected, 1 = chosen) and z_i are the eight log-contrast CHC values for a given individual. These regressions yielded, for each sex and population, a column vector of linear selection gradients (β_i) characterizing directional selection on each of the eight log-contrast CHCs. It is these β vectors that were used subsequently to compare linear selection between pairs of populations. Because mating success is binomial, hypothesis testing for individual gradients within each population was performed using logistic multiple regression rather than standard least squares estimation techniques (Fairbairn and Preziosi 1996). This was done using a generalized linear model with a logistic link function by employing the GENMOD procedure in SAS.

To determine the overall importance of linear sexual selection in each sex and population, we used MANOVAs to calculate the canonical variate of choice—the linear combination of CHCs that best distinguishes between chosen and rejected individuals (Endler 1986; Hine et al. 2002; Blows 2007)—separately for each sex and population. This yielded a unique canonical variate for each sex and population, each of which was placed separately back into the standard first-order regression above in place of the original eight CHCs. The ability of CHCs to explain variance in mating success was then given by the adjusted coefficient of determination (r_{adj}^2) in each case, with significance given by the fit of the logistic model. Comparisons of selection among populations, however, must be performed on the same original set of traits (CHCs) and therefore cannot employ these canonical variates (each is a combination of the original eight CHCs that is unique to that sex and population).

Nonlinear sexual selection was estimated in a similar manner, separately by sex and population, using the standard second-order polynomial regression model (Lande and Arnold 1983; Brodie et al. 1995):

$$w = \alpha + \sum_{i=1}^8 \beta_i z_i + \frac{1}{2} \sum_{i=1}^8 \sum_{j=1}^8 \gamma_{ij} z_i z_j, \quad (2)$$

where w is the mating success (0 = rejected, 1 = chosen) and z_i and z_j are the log-contrast CHC values for a given individual. These regressions yielded, for each sex and population, an 8×8 symmetrical γ matrix characterizing quadratic selection on the eight log-contrast CHCs

along the diagonal (γ_{ii}) and on pairs of log-contrast CHCs on the off-diagonals (γ_{ij} ; also known as cross-product or correlational coefficients). Positive and negative values of γ_{ii} indicate concave and convex selection, respectively; the existence of disruptive and stabilizing selection requires the additional demonstration of stationary points within the sampled space (Mitchell-Olds and Shaw 1987). Correlational selection (γ_{ij}) represents selection on CHCs that is not parallel to the original trait axes. It is these γ matrices that were used subsequently to compare nonlinear selection among populations. Again, because mating success was binomial, significances for all individual nonlinear gradients were evaluated by fitting a generalized linear model with a logistic link function.

To determine the overall importance of nonlinear sexual selection in each sex and population, we conducted separate canonical rotations to condense all of the nonlinear selection onto the eight eigenvectors of the γ matrix, thus eliminating all of the cross-product (correlational) terms (Phillips and Arnold 1989; Blows and Brooks 2003; Blows 2007). Nonlinear selection on these eight eigenvectors was then analyzed using the standard second-order regression model above. The importance of nonlinear sexual selection on all eight log-contrast CHCs was indicated by the improvement in r_{adj}^2 over that of the respective linear model for that sex and population. In each sex and population, the overall significance of the addition of nonlinear selection was determined using a partial F -test that compared the fit of the models with and without the eight quadratic terms (Bowerman and O'Connell 1990; Chenoweth and Blows 2005; Rundle et al. 2005; analogous results were obtained when the best-fit models were selected using the Akaike Information Criterion). In addition, and again as with linear selection, comparisons of nonlinear selection among populations must utilize the same set of traits (i.e., γ matrices generated from the analysis of the original traits) and therefore cannot employ the eigenvectors of γ (because each is a combination of the original eight CHCs that is unique to that sex and population).

Comparing Sexual Selection among Populations

Linear sexual selection was compared between pairs of populations within sexes using their vector correlation, calculated as the dot product of the two column vectors of eight linear selection gradients (β 's) standardized to unit length. These correlations, which represent a measure of the overall similarity between the pair of populations in the multivariate direction of linear sexual selection on these eight log-contrast CHCs, were calculated for each of the 36 unique combinations of the nine populations, and the resulting values were assembled into a symmetrical 9×9 similarity matrix representing among-population

variation in sexual selection. Individual coefficients range from +1 (indicating the same multivariate direction of selection) to -1 (indicating multivariate selection in precisely opposing directions), with 0 indicating orthogonal vectors of selection.

Nonlinear sexual selection was compared between pairs of populations within sexes using a technique for formal subspace comparisons originally developed by Krzanowski (1979):

$$\mathbf{S} = \mathbf{A}^T \mathbf{B} \mathbf{B}^T \mathbf{A}, \quad (3)$$

where the matrix \mathbf{A} is a subspace of γ_1 from population 1 that contains k of the n total eigenvectors of γ_1 as columns and \mathbf{B} is a subspace of γ_2 from population 2 of the same dimensions (k cannot exceed $n/2$ or the method will necessarily recover common dimensions between the subspaces; Blows et al. 2004). The similarity between the two subspaces can then be assessed using the single metric of the sum of the eigenvalues of \mathbf{S} , the upper bound of which equals k . The sum of the eigenvalues of \mathbf{S} has a straightforward interpretation and ranges—in our case, from four, indicating complete similarity in the orientation of the subspaces of \mathbf{A} and \mathbf{B} , to 0, indicating subspaces that are completely noncoincident (or, more precisely, the k principal vectors of each subspace are orthogonal; Blows et al. 2004; Petfield et al. 2005). Since each eigenvalue of \mathbf{S} is the squared cosine of the angle between two principal vectors in subspaces of \mathbf{A} and \mathbf{B} , intermediate values of the sum of these eigenvalues represent the extent to which the principal vectors in each subspace describe the same space. These between-population comparisons of nonlinear sexual selection were calculated for each of the 36 unique combinations of the nine populations and then assembled into a symmetrical 9×9 similarity matrix characterizing among-population variation in nonlinear sexual selection.

Large-scale patterns of variation in sexual selection were explored by testing for overall structure in the similarity matrices of linear and nonlinear selection. The eigenstructure of each of these two matrices was examined to determine their respective dominant axes of variation. Pearson correlation coefficients were also calculated separately between both the linear and nonlinear matrices and matrices of (1) pairwise geographic distances between populations (table A1 in the online edition of the *American Naturalist*), calculated from their GPS coordinates; (2) pairwise F_{ST} values between populations (table A1), calculated as outlined below; and (3) a model matrix (Manly 1985, p. 186; Rundle and Jackson 1996) specifying whether pairs of populations shared the same state for the presence/absence of *Drosophila birchii* (0 = *D. birchii* present in one population and absent in the other; 1 = *D. birchii*

present in both populations or absent in both populations). During the collection of our *D. serrata* populations, *D. birchii* was found in populations 1–4, corresponding with its published range (Higgie et al. 2000; Schiffer and McEvey 2006). Simple and partial Mantel tests (Mantel 1967) were used to test whether each of these correlations was significantly different from 0. These tests were conducted using the zt software package (Bonnet and Van de Peer 2002) and employed the complete enumeration of all possible permutations (362,880).

Values of F_{ST} were estimated by genotyping 24 females from each of the nine populations at seven previously developed microsatellite loci (*Dser10*, *Dser13*, *Dser15*, *Dser16*, *Dser18*, *Dros1*, and *Dros6*), using established screening techniques (Magiafoglou et al. 2002; Schiffer et al. 2004). Females rather than males were genotyped because two markers, *Dros1* and *Dros6*, are X-linked, and we thus needed to equalize the number of chromosomes sampled per locus. We calculated Rousset's (1997) linearized measure of population subdivision, $F_{ST}/(1 - F_{ST})$, among each pair of populations. Estimates of F_{ST} were calculated using Weir and Cockerham's (1984) unbiased estimator, θ , using the program GENEPOP 1.2 (Raymond and Rousset 1995).

Results

Sexual Selection on CHCs within Populations

Taken together, the eight log-contrast CHCs predicted mating success in both males and females from all nine populations, suggesting that these traits are key targets of mate choice in these populations. Linear sexual selection on the eight log-contrast CHCs was significant overall in males from all nine populations and in females from eight of the nine populations (table 1). The addition of nonlinear sexual selection was highly significant in both sexes in all nine populations, and linear and nonlinear selection combined explained $20.0\% \pm 3.5\%$ and $16.4\% \pm 4.0\%$ (mean $r_{adj}^2 \pm$ standard deviation) of the variance in male and female mating success, respectively (table 1). The 112 linear and 648 nonlinear sexual selection gradients that were calculated on the original log-contrast CHCs for both sexes from all nine populations and that were subsequently used to assemble the similarity matrices for among-population comparisons of β and γ are reported in table A2 in the online edition of the *American Naturalist*.

Variation in Sexual Selection among Populations

The correlations among populations in the multivariate direction of linear sexual selection on the suite of eight

log-contrast CHCs are given in table 2 for both males and females. Values ranged widely, from -0.737 to 0.951 in males and from -0.770 to 0.990 in females. The Krzanowski similarity matrix of between-population comparisons of nonlinear sexual selection on CHCs is given in table 3 for both males and females. Values ranged from 1.898 to 3.460 in males and from 2.107 to 3.165 in females.

In males, the dominant axis of among-population variation in linear sexual selection contrasted populations 1, 2, and 4 with the remaining ones (fig. 1A), indicating that the multivariate direction of linear sexual selection tended to be similar among populations within each of these groups but differed between groups. These groupings correspond with the distribution of *Drosophila birchii* (present in populations 1–4, absent in 5–9), with the exception of population 3 grouping with the allopatric populations. This association was reflected in the Mantel test; similarity in linear sexual selection on males was positively correlated with the presence/absence of *D. birchii* (table 4), although the correlation was marginally nonsignificant. There was no indication that variation in linear sexual selection was related to geographic distance or to the degree of among-population differentiation in neutral microsatellite loci (F_{ST} ; table 4).

In contrast to the linear results, there was little evidence of any structure in the Krzanowski matrix of among-population variation in nonlinear sexual selection in males (fig. 1B). Consistent with this, correlations between this matrix and the matrices of geographic distance, F_{ST} , and sympatry/allopatry with *D. birchii* were all small and nonsignificant (table 4).

In females, among-population variation in linear sexual selection was structured into two groups that contrasted populations 3 and 4 with the remaining ones (fig. 2A). This structure generated a small and positive, but nonsignificant, correlation with sympatry/allopatry with *D. birchii* and a stronger negative correlation with F_{ST} that approached sig-

nificance (table 4). This latter negative correlation indicates that as F_{ST} increases between pairs of populations, the overall similarity in their multivariate direction of linear sexual selection tends to decrease. Structure in the among-population variation in nonlinear sexual selection was stronger with geographically proximate populations tending to group together (e.g., populations 1 and 2; populations 5–7; populations 3 and 4; fig. 2B). This grouping produced negative correlations with both F_{ST} and geography, indicating that nonlinear sexual selection tended to be more similar between pairs of populations that were geographically near one another and less divergent at assumed-neutral microsatellite loci (table 4). However, geographic distance and F_{ST} between pairs of populations were themselves correlated (Mantel test: $r = 0.408$, $P = .017$), suggesting an overall pattern of isolation by distance. The correlation between differences in nonlinear sexual selection and geographic distance weakened and became nonsignificant when the effects of F_{ST} were taken into account (partial Mantel test: $r = -0.225$, $P = .109$), suggesting that the effect of geography reflected, at least in part, an association with neutral population genetic structure.

Discussion

CHCs predicted mating success in both males and females in each of the nine populations, consistent with results of past studies that have directly and indirectly identified CHCs as a primary target of sexual selection in *Drosophila serrata* (Hine et al. 2002, 2004; Chenoweth and Blows 2003, 2005; Blows et al. 2004; Petfield et al. 2005; Rundle et al. 2005; Skroblin and Blows 2006). In both sexes, selection had significant linear and nonlinear components, with nonlinear selection explaining an additional 12%–14% on average of the variance in mating success over linear selection alone (table 1). The presence of significant nonlinear sexual selection on males differs from early se-

Table 2: Overall similarity between pairs of populations in linear sexual selection on the suite of eight log-contrast cuticular hydrocarbons in males and females

Populations	1	2	3	4	5	6	7	8	9
1	1	.056	.188	.522	.258	.544	.003	.302	.261
2	.316	1	-.303	.141	.138	.471	-.316	.380	-.033
3	-.529	-.510	1	-.637	.472	.439	.951	.553	.931
4	-.194	.339	.565	1	-.236	-.127	-.737	-.163	-.610
5	.515	.395	-.304	.192	1	.240	.276	.431	.463
6	.797	.571	-.770	-.182	.697	1	.419	.745	.699
7	.452	.385	-.387	-.154	-.224	.210	1	.529	.921
8	.475	.713	-.159	.480	.649	.585	.289	1	.726
9	.779	.515	-.712	-.130	.731	.990	.136	.573	1

Note: Data for males are shown in the upper right portion of the table and data for females in the lower left. Values are correlation coefficients; a value of +1 indicates parallel (collinear) vectors of selection, -1 indicates directly opposing vectors, and 0 indicates orthogonal vectors.

Table 3: Comparisons of nonlinear sexual selection on a suite of eight cuticular hydrocarbons among pairs of populations in males and females

Populations	1	2	3	4	5	6	7	8	9
1	4	2.172	2.788	2.257	2.117	2.769	2.328	2.768	2.506
2	3.056	4	2.177	1.898	2.560	2.171	2.439	2.494	2.182
3	2.558	2.538	4	3.015	2.505	2.405	2.474	2.850	2.384
4	2.707	2.330	2.891	4	2.320	2.813	2.486	2.705	2.554
5	3.137	3.088	2.829	2.903	4	2.703	2.560	2.327	2.862
6	2.666	2.818	2.605	2.646	2.849	4	2.520	2.261	2.749
7	2.699	2.750	2.571	2.659	2.878	3.165	4	1.990	3.460
8	2.469	2.107	2.412	2.897	2.460	2.649	2.425	4	2.492
9	2.305	2.568	2.439	2.349	2.881	3.053	2.521	2.582	4

Note: Data for males are shown in the upper right portion of the table and data for females in the lower left. Values were derived using the method of Krzanowski (1979) and represent a measure of the overall similarity in orientation of the two eight-dimensional subspaces defined by the γ matrices of the two populations involved (see "Material and Methods" for details). Values can range from 4 (coincident subspaces) to 0 (orthogonal subspaces).

lection estimates using the previously described Forster laboratory population (Higgie et al. 2000; Hine et al. 2002), in which selection on males was found to be essentially linear, with no significant nonlinear component (Chenoweth and Blows 2005).

Given the presence of significant sexual selection within populations, our main goal was to use a quantitative framework to explore overall patterns in how this selection varied among populations in both sexes. There are two aspects of geographical variation in sexual selection that can be distinguished: (1) the similarity of sexual selection between pairs of populations, reflected by the magnitude of the elements of the similarity matrices of linear and nonlinear sexual selection (tables 2, 3, respectively); and (2) large-scale patterns in how sexual selection varies among populations, reflected in the structure of these two matrices (figs. 1, 2) and their overall associations with matrices of geographic distance, F_{ST} , and sympatry/allopatry with *Drosophila birchii* (table 4).

A biological interpretation of the magnitude of the individual similarity measures (aspect 1 above) requires estimates of the error associated with each measure. Empirical estimation of these errors would require multiple, independent measurements of sexual selection for both sexes in each population, which is logistically difficult in an experiment of this size. Unfortunately, however, a statistical estimate of these errors using resampling procedures (e.g., bootstrapping) is not straightforward because the similarity measures of linear and nonlinear selection are both bounded (linear: -1 to 1 , nonlinear: 0 to 4). Resampling cannot yield parameter estimates outside the bounds of these metrics, and all bootstrapped confidence intervals are therefore constrained to be significantly smaller and larger than their upper and lower bounds, respectively, making rejection of the null hypotheses of

complete similarity or complete difference guaranteed. This feature may also bias tests for differences between pairs of estimates.

As an alternative to the direct comparison of β vectors and γ matrices, it is conceivable that random-coefficient models (Longford 1993; Meyer and Kirkpatrick 2005) could be applied to these data to model the variation among populations in either directional selection (linear slopes) or nonlinear selection (second-order regression coefficients), in a restricted maximum likelihood framework. However, determining the significance of broader-scale patterns among populations, particularly in nonlinear selection, is likely to be a formidable task. Lacking estimates of the error association with the individual similarity measures, we therefore refrain from interpreting their magnitudes (aspect 1 above). In future studies, independent measurements of selection on the same population or at the same time will be crucial for interpreting comparisons of selection between populations or at different times.

A repeated estimate of linear and nonlinear sexual selection is available in this study for the Forster population used in previous experiments. The vector correlation for two independent measurements of linear selection on the same suite of male CHCs in the Forster population, between that reported by Blows et al. (2004) and another measure taken in late 2006 (E. Hine, unpublished data), was 0.833, suggesting a high repeatability (only four of the 72 pairwise correlations among our nine populations exceed this value). In contrast, the Krzanowski value for the comparison of nonlinear selection on Forster male CHCs between these two sampling periods was 2.68, suggesting a lower repeatability. Approximately one-third of the 72 comparisons among populations exceed this value (table 3), implying the presence of substantial noise in these estimates. This lower repeatability is not unexpected, given the ratio of indepen-

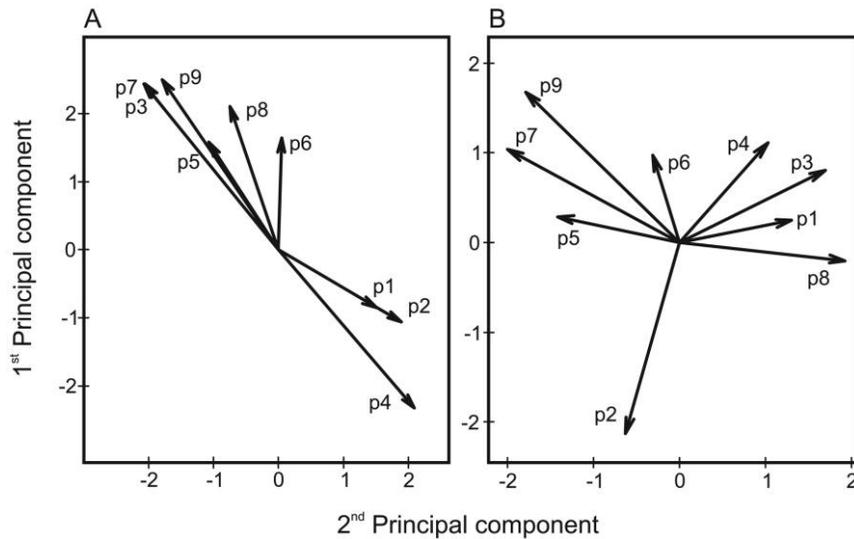


Figure 1: Two-dimensional biplots depicting the first and second eigenvectors resulting from an eigenanalysis of (A) the correlation matrix of among-population variation in linear sexual selection and (B) the Krzanowski matrix of among-population variation in nonlinear sexual selection on cuticular hydrocarbons in males.

dent observations (approximately 199) to the number of parameters in our models (44); estimating all nonlinear selection on a suite of eight traits is an empirical challenge even in *Drosophila*. Nevertheless, patterns across the entire set of estimates may still be detectable in the presence of sampling error of each individual estimate (aspect [2] above). Such tests treat similarity measures as individual observations, and measurement error itself should not produce large-scale structure in these similarity matrices, although its presence will reduce the ability to detect such patterns.

Linear Sexual Selection

In males, variation in linear sexual selection was associated with the presence versus absence of the related species *D. birchii*, although the effect was marginally nonsignificant (table 4). Because sexual selection arose in our study from female mate choice, such differences imply that female mate preferences vary between sympatry and allopatry. Similar differences in female preferences between four independent sympatric and allopatric populations of *D. serrata* were found by Higgin and Blows (2007) for males derived from a mixed population that comprised the full range of allopatric and sympatric phenotypes.

In females, among-population variation in linear sexual selection was correlated with neutral population genetic structure (F_{ST}), although, again, the effect was marginally nonsignificant ($P = .087$). Although a correlation with F_{ST} suggests that male mate preferences are diverging as es-

entially a neutral trait, caution is warranted for two reasons. First, recent analyses demonstrate clinal patterns in some CHCs among these same nine populations that are steeper than expected because of drift alone, thereby implicating selection (S. Chenoweth and M. Blows, unpublished manuscript). A similar adaptive cline in mate preferences would be difficult to detect if confounded with neutral population genetic structure. Such a problem is inherent to all correlational analyses in which inferences concerning mechanisms are weak. It remains possible, for example, that preferences may have diversified in part be-

Table 4: Large-scale patterns in among-population variation in linear and nonlinear sexual selection on a suite of eight cuticular hydrocarbons in male and female *Drosophila serrata*

Sex and selection type	Geography	F_{ST}	Sympatry
Males:			
Linear	-.051 (.33)	-.085 (.34)	.191 (.087)
Nonlinear	-.062 (.35)	-.078 (.33)	.037 (.40)
Females:			
Linear	.141 (.24)	-.302 (.087)	.125 (.15)
Nonlinear	-.329 (.038)	-.322 (.047)	.149 (.17)

Note: Values are correlation coefficients of the elements of a pair of matrices, one being a similarity matrix of linear or nonlinear selection among pairs of population (tables 2 and 3, respectively) and the other being a matrix of between-population values for geographic distance ("geography"), F_{ST} , or shared presence/absence of the related species *Drosophila birchii* ("sympatry"). P values are given in parentheses and derive from Mantel tests using the complete enumeration of all possible permutations (362,880).

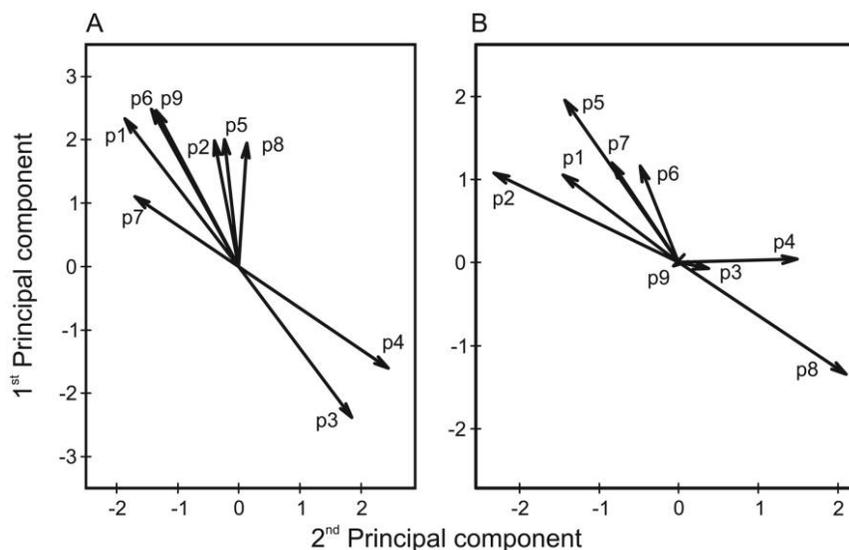


Figure 2: Two-dimensional biplots depicting the first and second eigenvectors resulting from an eigenanalysis of (A) the correlation matrix of among-population variation in linear sexual selection and (B) the Krzanowski matrix of among-population variation in nonlinear sexual selection on cuticular hydrocarbons in females.

cause of divergent natural selection and we have simply failed to identify the relevant environmental factor(s). Second, caution is also warranted because it is possible that mate preferences diverged via genetic drift during the 13 generations for which these populations were held in the lab before we estimated sexual selection. Ultimately, questions concerning mechanisms by which this pattern has arisen require manipulative experimental tests.

Nonlinear Sexual Selection

In males, there was little evidence for any large-scale structure in the among-population variation in nonlinear sexual selection (fig. 1), nor did we detect any significant associations between this matrix and the matrices of geographic distance, F_{ST} , and sympatry/allopatry with *D. birchii*. Although measurement error should not create any large-scale structure in our similarity matrices, its presence will reduce our ability to detect existing structure. As noted earlier, a repeated selection estimate on the Foster population suggests that error in similarity measures of nonlinear selection could be substantial, and it is therefore possible that measurement error was simply too great to permit patterns to be detected in this data set. Whether and how nonlinear selection varies among these populations will therefore require additional study.

In females, similar to results for linear sexual selection, variation in nonlinear sexual selection was correlated with neutral population genetic structure (F_{ST}). A significant

correlation with geography was also detected, but this correlation weakened and became nonsignificant when the effects of F_{ST} were controlled for ($r = -0.225$, $P = .109$). This result suggests that male mate preferences are diverging among populations as essentially a neutral trait and that geographic distance reflects, at least in part, neutral population genetic structure, probably through its effects on gene flow. Once again, however, caution is warranted because adaptive clines and neutral population genetic structure are likely to be confounded in this sampling design.

Conclusions

We have outlined a quantitative approach that allows investigation of broad-scale patterns of variation in selection among populations and of whether this variation is associated with possible causal factors that vary among populations. We applied the method to a large data set of linear and nonlinear sexual selection arising from mutual mate preferences in *D. serrata*, examining variation in the multivariate fitness surfaces for a suite of CHCs among nine natural populations in both males and females. Our comparisons suggested that variation in sexual selection on CHCs in males was associated with the presence versus absence of *D. birchii*, a biological pattern consistent with past observational and manipulative studies of reproductive character displacement in *D. serrata*. Selection was also found to be correlated with neutral population genetic

structure in females, suggesting that chance events may be playing a role in the diversification of male mate preferences. A first step in determining the evolutionary mechanisms responsible for the observed patterns will involve evolution experiments that permit variation among populations in mate preferences to be partitioned among treatments that manipulate the opportunities for these different mechanisms.

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Literature Cited

- Arnold, S. J., and M. J. Wade. 1984a. On the measurement of natural and sexual selection: applications. *Evolution* 38:720–734.
- . 1984b. On the measurement of natural and sexual selection: theory. *Evolution* 38:709–719.
- Arnold, S. J., M. E. Pfrender, and A. G. Jones. 2001. The adaptive landscape as a conceptual bridge between micro- and macroevolution. *Genetica* 112:9–32.
- Atchison, J. 1986. *The statistical analysis of compositional data*. Chapman & Hall, London.
- Blows, M. W. 1998. Evolution of a mate recognition system after hybridization between two *Drosophila* species. *American Naturalist* 151:538–544.
- . 2007. A tale of two matrices: multivariate approaches in evolutionary biology. *Journal of Evolutionary Biology* 20:1–8.
- Blows, M. W., and R. A. Allan. 1998. Levels of mate recognition within and between two *Drosophila* species and their hybrids. *American Naturalist* 152:826–837.
- Blows, M. W., and R. Brooks. 2003. Measuring nonlinear selection. *American Naturalist* 162:815–820.
- Blows, M. W., and A. A. Hoffmann. 2005. A reassessment of genetic limits to evolutionary change. *Ecology* 86:1371–1384.
- Blows, M. W., R. Brooks, and P. G. Kraft. 2003. Exploring complex fitness surfaces: multiple ornamentation and polymorphism in male guppies. *Evolution* 57:1622–1630.
- Blows, M. W., S. F. Chenoweth, and E. Hine. 2004. Orientation of the genetic variance-covariance matrix and the fitness surface for multiple male sexually selected traits. *American Naturalist* 163:329–340.
- Bonnet, E., and Y. Van de Peer. 2002. zt: a software tool for simple and partial Mantel tests. *Journal of Statistical Software* 7:1–12.
- Boughman, J. W. 2002. How sensory drive can promote speciation. *Trends in Ecology & Evolution* 17:571–577.
- Bowerman, B., and R. O'Connell. 1990. *Linear statistical models: an applied approach*. Duxbury, Belmont, CA.
- Brodie, E. D. I., A. J. Moore, and F. J. Janzen. 1995. Visualizing and quantifying natural selection. *Trends in Ecology & Evolution* 10:313–318.
- Caruso, C. M. 2000. Competition for pollination influences selection on floral traits of *Ipomopsis aggregata*. *Evolution* 54:1546–1557.
- Chapman, T., G. Arnqvist, J. Bangham, and L. Rowe. 2003. Sexual conflict. *Trends in Ecology & Evolution* 18:41–47.
- Chenoweth, S. F., and M. W. Blows. 2003. Signal trait sexual dimorphism and mutual sexual selection in *Drosophila serrata*. *Evolution* 57:2326–2334.
- . 2005. Contrasting mutual sexual selection on homologous signal traits in *Drosophila serrata*. *American Naturalist* 165:281–289.
- Conner, J. 1989. Density-dependent sexual selection in the fungus beetle, *Bolitotherus cornutus*. *Evolution* 43:1378–1386.
- Dudley, S. A. 1996. Differing selection on plant physiological traits in response to environmental water availability: a test of adaptive hypotheses. *Evolution* 50:92–102.
- Endler, J. A. 1986. *Natural selection in the wild*. Princeton University Press, Princeton, NJ.
- Estes, S., and S. J. Arnold. 2007. Resolving the paradox of stasis: models with stabilizing selection explain evolutionary divergence on all timescales. *American Naturalist* 169:227–244.
- Fairbairn, D. J., and R. F. Preziosi. 1996. Sexual selection and the evolution of sexual size dimorphism in the water strider, *Aquarius remigis*. *Evolution* 50:1549–1559.
- Flury, B. 1987. A hierarchy of relationships between covariance matrices. Pages 31–43 in A. K. Gupta, ed. *Advances in multivariate statistical analysis*. Reidel, Boston.
- Fornoni, J., P. L. Valverde, and J. Núñez-Farfán. 2004. Population variation in the cost and benefit of tolerance and resistance against herbivory in *Datura stramonium*. *Evolution* 58:1696–1704.
- Galen, C., K. A. Zimmer, and M. E. Newport. 1987. Pollination in floral scent morphs of *Polemonium viscosum*: a mechanism for disruptive selection on flower size. *Evolution* 41:599–606.
- Hedrick, P. W. 2006. Genetic polymorphism in heterogeneous environments: the age of genomics. *Annual Review of Ecology, Evolution, and Systematics* 37:67–93.
- Higgle, M., and M. W. Blows. 2007. Are traits that experience reinforcement also under sexual selection? *American Naturalist* 170:409–420.
- Higgle, M., S. Chenoweth, and M. W. Blows. 2000. Natural selection and the reinforcement of mate recognition. *Science* 290:519–521.
- Hine, E., S. Lachish, M. Higgle, and M. W. Blows. 2002. Positive genetic correlation between female preference and offspring fitness. *Proceedings of the Royal Society B: Biological Sciences* 269:2215–2219.
- Hine, E., S. F. Chenoweth, and M. W. Blows. 2004. Multivariate quantitative genetics and the lek paradox: genetic variance in male sexually selected traits of *Drosophila serrata* under field conditions. *Evolution* 58:2754–2762.
- Houle, D. 1992. Comparing evolvability and variability of quantitative traits. *Genetics* 130:195–204.
- Howard, R. W., L. L. Jackson, H. Banse, and M. W. Blows. 2003. Cuticular hydrocarbons of *Drosophila birchii* and *D. serrata*: identification and role in mate choice in *D. serrata*. *Journal of Chemical Ecology* 29:961–976.
- Kalish, S. 1986. Variable selection on the timing of germination in *Collinsia verna* (Scrophulariaceae). *Evolution* 40:479–491.
- Kingsolver, J. G., H. E. Hoekstra, J. M. Hoekstra, D. Berrigan, S. N.

- Vignieri, C. E., Hill, A., Hoang, P., Gibert, and P. Beerli. 2001. The strength of phenotypic selection in natural populations. *American Naturalist* 157:245–261.
- Krzanowski, W. J. 1979. Between-groups comparison of principal components. *Journal of the American Statistical Association* 74:703–707.
- Lande, R. 1979. Quantitative genetic analysis of multivariate evolution, applied to brain : body size allometry. *Evolution* 33:402–416.
- . 1981. Models of speciation by sexual selection on polygenic traits. *Proceedings of the National Academy of Sciences of the USA* 78:3721–3725.
- . 1982. Rapid origin of sexual isolation and character divergence in a cline. *Evolution* 36:213–223.
- Lande, R., and S. J. Arnold. 1983. The measurement of selection on correlated characters. *Evolution* 37:1210–1226.
- Longford, N. T. 1993. *Random coefficient models*. Oxford University Press, Oxford.
- Magiafoglou, A., M. E. Carew, and A. A. Hoffmann. 2002. Shifting clinal patterns and microsatellite variation in *Drosophila serrata* populations: a comparison of populations near the southern border of the species range. *Journal of Evolutionary Biology* 15:763–774.
- Manly, B. F. J. 1985. *The statistics of natural selection on animal populations*. Chapman & Hall, London.
- Mantel, N. 1967. The detection of disease clustering and a generalised regression approach. *Cancer Research* 27:209–220.
- Meyer, K., and M. Kirkpatrick. 2005. Up hill, down dale: quantitative genetics of curvaceous traits. *Philosophical Transactions of the Royal Society B: Biological Sciences* 360:1443–1455.
- Mitchell-Olds, T., and R. G. Shaw. 1987. Regression analysis of natural selection: statistical inference and biological interpretation. *Evolution* 41:1149–1161.
- Mousseau, T. A., and D. A. Roff. 1987. Natural selection and the heritability of fitness components. *Heredity* 59:181–197.
- Petfield, D., S. F. Chenoweth, H. D. Rundle, and M. W. Blows. 2005. Genetic variance in female condition predicts indirect genetic variance in male sexual display traits. *Proceedings of the National Academy of Sciences of the USA* 102:6045–6050.
- Phillips, P. C., and S. J. Arnold. 1989. Visualizing multivariate selection. *Evolution* 43:1209–1222.
- Raymond, M., and F. Rousset. 1995. GENEPOP (version 1.2): population genetics software for exact tests and ecumenicism. *Journal of Heredity* 86:248–249.
- Rousset, F. 1997. Genetic differentiation and estimation of gene flow from *F*-statistics under isolation by distance. *Genetics* 145:1219–1228.
- Rundle, H. D., and D. A. Jackson. 1996. Spatial and temporal variation in littoral-zone fish communities: a new statistical approach. *Canadian Journal of Fisheries and Aquatic Sciences* 53:2167–2176.
- Rundle, H. D., and P. Nosil. 2005. Ecological speciation. *Ecology Letters* 8:336–352.
- Rundle, H. D., S. F. Chenoweth, P. Doughty, and M. W. Blows. 2005. Divergent selection and the evolution of signal traits and mating preferences. *PLoS Biology* 3:1988–1995.
- Schemske, D. W., and C. C. Horvitz. 1989. Temporal variation in selection on a floral character. *Evolution* 43:461–465.
- Schiffer, M., and S. F. McEvey. 2006. *Drosophila bunnanda*: a new species from northern Australia with notes on other Australian members of the *montium* subgroup (Diptera: Drosophilidae). *Zootaxa* 1333:1–23.
- Schiffer, M., M. E. Carew, and A. A. Hoffmann. 2004. Molecular, morphological and behavioural data reveal the presence of a cryptic species in the widely studied *Drosophila serrata* species complex. *Journal of Evolutionary Biology* 17:430–442.
- Schluter, D. 1988. Estimating the form of natural selection on a quantitative trait. *Evolution* 42:849–861.
- . 2000. *Ecology of adaptive radiation*. Oxford University Press, Oxford.
- Schluter, D., and D. Nychka. 1994. Exploring fitness surfaces. *American Naturalist* 143:597–616.
- Schluter, D., T. D. Price, and L. Rowe. 1991. Conflicting selection pressures and life-history trade-offs. *Proceedings of the Royal Society B: Biological Sciences* 246:11–17.
- Skroblin, A., and M. W. Blows. 2006. Measuring natural and sexual selection on breeding values of male display traits in *Drosophila serrata*. *Journal of Evolutionary Biology* 19:35–41.
- Weir, B. S., and C. C. Cockerham. 1984. Estimating *F*-statistics for the analysis of population structure. *Evolution* 38:1358–1370.

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