Evolutionary optimum for male sexual traits characterized using the multivariate Robertson–Price Identity

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Phenotypes tend to remain relatively constant in natural populations, suggesting a limit to trait evolution. Although stationary phenotypes suggest stabilizing selection, directional selection is more commonly reported. However, selection on phenotypes will have no evolutionary consequence if the traits do not genetically covary with fitness, a covariance known as the Robertson–Price Identity. The nature of this genetic covariance determines if phenotypes will evolve directionally or whether they reside at an evolutionary optimum. Here, we show how a set of traits can be shown to be under net stabilizing selection through an application of the multivariate Robertson–Price Identity. We characterize how a suite of male sexual displays genetically covaries with fitness in a population of *Drosophila serrata*. Despite strong directional sexual selection on these phenotypes directly and significant genetic variance in them, little genetic covariance was detected with overall fitness. Instead, genetic analysis of trait deviations showed substantial stabilizing selection on the genetic variance of these traits with respect to overall fitness, indicating that they reside at an evolutionary optimum. In the presence of widespread pleiotropy, stabilizing selection on focal traits will arise through the net effects of selection on other, often unmeasured, traits and will tend to be stronger on trait combinations than single traits. Such selection may be difficult to detect in phenotypic analyses if the environmental covariance between the traits and fitness obscures the underlying genetic associations. The genetic analysis of trait deviations provides a way of detecting the missing stabilizing selection inferred by recent metaanalyses.

cuticular hydrocarbons | evolutionary stasis

Directional selection on quantitative traits is both common in nature and frequently strong (1–4), but sustained evolutionary responses are rare (5, 6). Instead, phenotypes in natural populations tend to remain relatively stationary in value over various timescales (7), suggesting a limit to trait evolution. A simple lack of genetic variance in target traits is unlikely to provide a general explanation: genetic variance is found for almost all traits (8, 9), and although genetic covariances among traits may constrain their response to multivariate selection (10, 11), it is often insufficient to prevent it entirely (12–14). Although opposing natural selection that limits trait evolution during artificial selection is well-documented (14–18), the genetic basis of evolutionary limits in unmanipulated populations remains to be determined (19, 20).

Sexual selection provides a particularly striking example of how strong directional selection fails to change trait means in contemporary populations (6). An evolutionary response in male sexual displays is not generally observed, despite the presence of strong selection (3) acting on heritable traits (21). Opposing natural selection arising from pleiotropic effects on nonsexual fitness has long been suggested to limit display trait exaggeration (22), although direct evidence is rare (23, 24). A recent evolutionary manipulation in *Drosophila serrata* confirmed the potential of this mechanism to halt the evolution of a condition-dependent male contact pheromone display consisting of a suite of cuticular hydrocarbons (CHCs) (25). CHCs are a direct target of female mate preferences (*SI Text*), and artificial selection on them in the direction of these preferences conferred higher male mating success over control populations. However, the response to selection was stopped after seven generations by opposing natural selection, despite a substantial increase in additive genetic variance as the new evolutionary limit was approached (14). This experiment showed that alleles conferring higher male mating success were segregating at low frequencies in the base population, presumably because of their antagonistic effects on male sexual and nonsexual fitness.

Here, we provide evidence from an unmanipulated population for such antagonistic selection that ultimately causes these male sexually selected traits to reside at an evolutionary optimum. The identification of the pleiotropic costs of male sexual display phenotypes that result in opposing natural selection is difficult given that selection could potentially operate through a very large number of phenotypes. However, this problem can be overcome by recognizing that, in the presence of pleiotropy, stabilizing selection on the genetic variation in male traits can be detected using the framework of the Robertson–Price Identity (9, 26–28).

Results

Within a large breeding design, we measured the fitness of more than 2,000 sons and daughters (29) and subjected these traits to a genetic analysis together with the suite of CHCs measured on 1,114 other sons (25). As has been shown in previous studies (14, 30, 31), male CHC phenotypes were under strong directional sexual selection overall ($r^2 = 21.2\%$, $P < 0.0001$), with significant selection gradients on all eight traits (Table 1). The Robertson–Price Identity shows that selection on phenotypes will have no evolutionary consequence unless the traits genetically covary with fitness (Eq. **1**):

$$\Delta \mathbf{z} = \boldsymbol{\sigma}_A(w, \mathbf{z}), \qquad [1]$$

where in multivariate form, $\boldsymbol{\sigma}_A(w, \mathbf{z})$ is a vector of additive genetic covariances between fitness (w) and the vector of phenotypic traits ($\mathbf{z}$), and $\Delta \mathbf{z}$ is the response vector of trait means (32).

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Table 1. Directional sexual selection on the eight log-contrast CHCs (β), their genetic covariance with male [$\sigma_A(w_m, \mathbf{z})$] and female [$\sigma_A(w_f, \mathbf{z})$] fitness, and their first genetic principle component ($\mathbf{g}_{max}$)

Trait	β	$\sigma_A(w_m, \mathbf{z})$	$\sigma_A(w_f, \mathbf{z})$	$\mathbf{g}_{max}$
(Z,Z)-5,9-$C_{25:2}$	0.021*	−0.020	−0.055*	0.294
(Z)-9-$C_{25:1}$	−0.059†	−0.114*	−0.180†	0.028
(Z)-9-$C_{26:1}$	−0.037†	−0.028	0.015	0.347
2-Me-C_{26}	−0.066†	0.015	−0.043	0.288
(Z,Z)-5,9-$C_{27:2}$	−0.048†	−0.040	−0.009	0.414
2-Me-C_{28}	0.150†	0.016	−0.029	0.343
(Z,Z)-5,9-$C_{29:2}$	0.251†	−0.007	−0.015	0.483
2-Me-C_{30}	−0.131†	0.000	−0.015	0.433
CHC$\mathbf{g}_{max}$	0.078†	−0.004	−0.019	—
CHCβ	0.258†	−0.019	−0.048*	—

$\mathbf{g}_{max}$ was calculated as the first eigenvector of the sire-level covariance matrix and accounted for 93.9% of the total genetic variance in CHCs.
*$P < 0.05$.
†$P < 0.001$.

Despite significant additive genetic variance (V_A) in CHCs in this population (25), including the combination under directional sexual selection by female mate preferences (CHCβ; V_A = 0.130, likelihood ratio test: $\chi^2_1 = 33.9$, $P < 0.001$), male fitness genetically covaried with only a single CHC, (Z)-9-$C_{25:1}$ (Table 1). Furthermore, neither the phenotypic trait combination under directional selection (CHCβ) nor the trait representing the major axis of genetic variance in male CHCs (CHC$\mathbf{g}_{max}$; accounting for 93.9% of the additive genetic variance in these traits) was genetically correlated with male fitness, despite significant sexual selection gradients on both (Table 1). The latter was confirmed when estimated by a separate generalized linear mixed model, in which genetic males with zero fitness were included, and the fitness data were neither transformed nor standardized (mean CHC$\mathbf{g}_{max}$ – male fitness genetic correlation = −0.355, proportion of posterior correlations overlapping zero = 0.274) (*SI Text*). Male CHCs are, therefore, not predicted to evolve in a directional manner, despite substantial phenotypic sexual selection on them.

If male traits reside at an evolutionary optimum that arises as a consequence of opposing natural and sexual selection, little genetic covariance with net fitness will be detected by Eq. **1**. Instead, in the presence of an optimum, negative genetic covariance is expected between fitness and the squared deviations of the male trait phenotypes from their population means, representing stabilizing selection on the genetic variance (33, 34). To quantify change in CHC genetic variance because of nonlinear selection, the genetic covariance matrix ($\boldsymbol{\phi}$) between fitness and the products of the n traits when expressed as deviations from the population mean ($z_{d,n}$) can be estimated as (32) (Eq. **2**)

$$\boldsymbol{\phi} = \begin{bmatrix} \sigma_A\left(w, z_{d,1}z_{d,1}\right) & \cdots & \sigma_A\left(w, z_{d,1}z_{d,n}\right) \\ & \ddots & \vdots \\ & & \sigma_A\left(w, z_{d,n}z_{d,n}\right) \end{bmatrix}. \qquad [2]$$

Parameterization of Eq. **2** can be achieved by estimating each element as the genetic covariance between fitness and the appropriate trait deviations in a bivariate mixed model (Table S1). Diagonalization of $\boldsymbol{\phi}$ yields its eigenvectors and associated eigenvalues that characterize the form of nonlinear selection on CHC genetic variance arising from its association with male fitness. These findings were dominated in our case by negative eigenvalues, which are indicative of stabilizing selection (81.6% of the total selection present) (Table S2). Determining the significance of the overall pattern of the genetic associations between fitness and CHC genetic variance described by $\boldsymbol{\phi}$ cannot be accomplished easily, because separate mixed models were used to generate the elements of $\boldsymbol{\phi}$. We, therefore, focused on the CHC$\mathbf{g}_{max}$ phenotype as a simple univariate trait that captured almost all of the genetic variance in CHCs (93.9% of the total) (25). There was a strong and significant negative association between male fitness and the squared deviations of the CHC$\mathbf{g}_{max}$ phenotypic values (CHC$\mathbf{g}_{max}^2$), confirming the presence of stabilizing selection on the major axis of genetic variance in CHCs (Table 2). This association was also present when estimated by a separate generalized linear mixed model in which genetic males with zero fitness were included and the fitness data were neither transformed nor standardized (mean CHC$\mathbf{g}_{max}^2$ – male fitness genetic correlation = −0.530, proportion of posterior correlations overlapping zero = 0.052) (*SI Text*). This selection is not detected, however, in a phenotypic analysis of nonlinear sexual selection on CHC$\mathbf{g}_{max}$ ($\gamma = 0.0037$, $P = 0.487$), suggesting that opposing natural selection must be arising from pleiotropic effects of alleles underlying CHCs on other fitness components (34).

The change in genetic variance of the traits, when accounting for the effects of both linear and nonlinear selection, can then be estimated as (equation 9 in ref. 32) (Eq. **3**)

$$\Delta\mathbf{G} = \boldsymbol{\phi} - (\boldsymbol{\sigma}_A(w, \mathbf{z}))(\boldsymbol{\sigma}_A(w, \mathbf{z}))^{\mathrm{T}}. \qquad [3]$$

The predicted change was dominated by a reduction in CHC genetic variance (82.5% of the total response) (Table S2), showing the overall predominance of stabilizing selection arising through male fitness (Table S3). The trait combination for which genetic variance was reduced the most was closely associated (vector correlation of 0.847) with the trait combination for which genetic variance in male CHCs was greatest (i.e., $\mathbf{g}_{max}$, the first genetic principle component or leading eigenvector of CHCs).

Male sexually selected traits often have homologous traits in females, and selection can, therefore, be exerted on the genetic variance underlying male traits when expressed in females, mediated through the intersex genetic correlations (35). Female *D. serrata* express the same CHCs as males, and average intersex genetic correlations for these traits across nine populations vary between 0.61 and 0.89 (36). Female fitness exerted a similar pattern of selection on the male traits: directional selection on CHC genotypes was detectable for only two of eight traits (Table 1) and was absent on CHC$\mathbf{g}_{max}$, the trait representing the major axis of genetic variance in CHCs. In addition, most (70.3%) of the nonlinear selection on the genetic variance was stabilizing in nature (Table S3). The predicted change in genetic variance was again dominated by a reduction (72.5% of the response) (Table

Table 2. Sire-level covariance matrix of the squared deviations of CHC$\mathbf{g}_{max}$ phenotypes (CHC$\mathbf{g}_{max}^2$) and male and female fitness, with variances displayed along the diagonal, covariances displayed below, and genetic correlations displayed above (italics) along with the first two genetic principle components (i.e., eigenvectors $\mathbf{g}_1$ and $\mathbf{g}_2$) of this matrix

Trait	CHC$\mathbf{g}_{max}^2$	Female fitness	Male fitness	$\mathbf{g}_1$	$\mathbf{g}_2$
CHC$\mathbf{g}_{max}^2$	0.0240	*−0.162*	*−0.854**	−0.429	−0.563
Female fitness	−0.0052	0.0430	*−0.376*	−0.542	0.786
Male fitness	−0.0239	−0.0141	0.0328	0.722	0.256

Significance of the covariance of CHC$\mathbf{g}_{max}^2$ with male fitness was determined by 10,000 posterior estimates from a multiresponse generalized linear mixed model fit using a Markov chain Monte Carlo method (65). $\mathbf{g}_1$ and $\mathbf{g}_2$ account for 57.8% and 42.2% of the genetic variance, respectively.
*Mean = −0.824; 95% higher posterior density interval = −0.289 to −0.999.

EVOLUTION

S2). Consequently, genetic variation in male CHCs was subject to a similar overall pattern of stabilizing selection when expressed in either males or females. Unlike male fitness, however, the negative genetic correlation between CHC$\mathbf{g}_{max}^2$ and female fitness was small and nonsignificant (Table 2).

Diagonalization of the CHC$\mathbf{g}_{max}^2$ – fitness covariance matrix revealed two genetic principle components (i.e., eigenvectors $\mathbf{g}_1$ and $\mathbf{g}_2$) that accounted for all of the genetic variance in these three traits (eigenvalues: $\lambda_1 = 57.8\%$, $\lambda_2 = 42.2\%$). These eigenvectors differ primarily in the genetic association of male and female fitness, representing sexually antagonistic and sexually concordant fitness variation, respectively (i.e., segregating variants affecting the fitness of each sex in the opposite or same direction, respectively) (Table 2). These two axes of fitness variation, generated from a separate genetic analysis of male and female fitness alone, are displayed in Fig. 1. Because male and female fitness are necessarily measured on different individuals, we could not construct a single mixed model to directly test whether sexually concordant or sexually antagonistic variation was more closely associated with CHC$\mathbf{g}_{max}^2$. However, plots of the best linear unbiased predictors (BLUPs) of the three traits suggest stabilizing selection through sexually concordant but not sexually antagonistic fitness variation (Fig. 2). This finding suggests that individual males deviating in either direction from the CHC$\mathbf{g}_{max}$ mean tend to carry unconditionally deleterious alleles as opposed to those alleles harmful to males but beneficial to females (i.e., sexually antagonistic alleles).

Discussion

Characterizing evolutionary optima and hence, determining what limits directional evolutionary change have proven to be remarkably difficult empirical challenges (5, 11, 37). We have shown that a set of traits known to be under strong directional selection measured through one fitness component (male mating success) is, in fact, under net stabilizing selection when fitness is considered in a more complete fashion. In particular, male CHCs do not evolve in response to directional sexual selection, although alleles for higher male mating success are segregating in *D. serrata* populations (14). Instead, these sexually selected male displays are held at an evolutionary optimum by overall

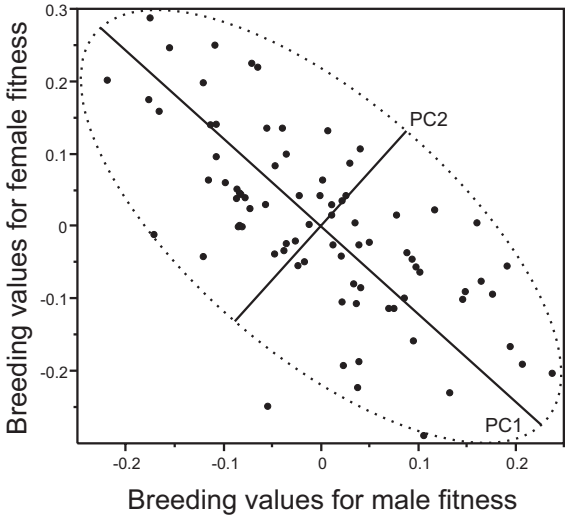

Fig. 1. Genetic association of male and female fitness. Breeding values for male and female fitness estimated as BLUPs from a genetic analysis of these traits. The first and second genetic principle components, depicted within a 95% confidence ellipse, represent genetic variance in fitness with either sexually antagonistic (PC1) or sexually concordant (PC2) effects.

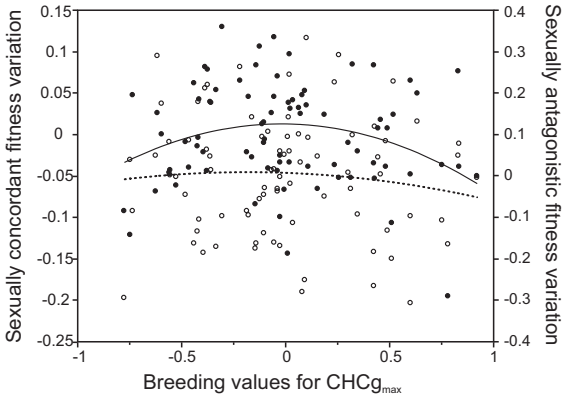

Fig. 2. Association between genetic variance in fitness and the major axis of genetic variance in CHCs (CHC$\mathbf{g}_{max}$). Genetic variance in fitness is expressed along two axes representing sexually antagonistic and sexually concordant variations (PC1 and PC2, respectively) (Fig. 1). Stabilizing selection seems to be more pronounced with respect to sexually concordant than sexually antagonistic fitness variation.

stabilizing selection on the genetic variation underlying the traits. The pleiotropic costs generating opposing natural selection are not known in *D. serrata*, although their existence is inferred from the rapid decay in the response of CHCs to artificial selection when this selection was stopped (14). Such costs may arise from the effects on other fitness components of CHCs themselves (e.g., desiccation resistance) (38, 39) or the pleiotropic effect of the underlying genes on other traits under selection (e.g., allocation tradeoffs arising through condition dependence) (25). Understanding the nature of these costs will be an important goal for future work.

Despite a predominance of stabilizing selection on CHC genetic variance, a negative genetic covariance was observed between log-contrast (Z)-9-$C_{25:1}$ and both male and female fitness (Table 1), indicating directional selection for a lower relative concentration of this trait through both sexes. Stabilizing selection was also strongest on this trait (Table S1), with the net effect of linear and nonlinear selection predicting a much larger reduction in genetic variance in this trait than in any of the other CHCs (Table S3). Consistent with this finding, genetic variance in (Z)-9-$C_{25:1}$ is more than an order of magnitude lower than for all other CHCs in this population (25). However, this CHC is not the smallest in relative concentration on the male cuticle (40), and why lower relative concentrations of this trait do not evolve in this population remains unexplained by our analysis. One possibility is that our fitness measures may have been incomplete in some way, giving a misleading picture of the selection acting on this trait (*SI Text*).

The classic expectation for the evolution of male sexual displays involves an initial phase of exaggeration driven either by sexual selection alone (41, 42) or a combination of natural and sexual selection (43–45) that is eventually halted by opposing natural selection. As shown by the Robertson–Price Identity, a directional change in the male trait mean requires a positive genetic covariance between it and fitness. After halted by natural selection, however, this covariance between the trait and total fitness can no longer be substantial. Given the likely rapid nature of this exaggeration phase (22), contemporary populations are more likely to be at an evolutionary limit for male display traits, experiencing no additional directional change, which has been observed in natural populations (6, 20, 46). If male traits reside at an evolutionary optimum, in the absence of direct benefits of mate choice, the maintenance of costly female preferences may depend on the indirect benefits that females gain from discriminating against males carrying a greater number of deleterious

mutations that shift their phenotypes farther from the optimum (34, 47, 48).

When the sexes were considered separately, our multivariate analyses suggest that stabilizing selection on male CHC genetic variance was generated through both male and female phenotypes. Nevertheless, male and female fitness covaried negatively (Fig. 1), consistent with other estimates of the intersex genetic correlation for fitness (49), and their joint analysis with CHC$\mathbf{g}_{max}$ suggested stabilizing selection primarily through male rather than female phenotypes (Table 2). Additional investigation suggested that stabilizing selection on CHC$\mathbf{g}_{max}$ genetic variance seemed to arise not from sexually antagonistic fitness variation but rather, from unconditionally deleterious fitness effects (Fig. 2). It has been recently suggested that a negative intersex correlation for fitness may result from a small number of loci at which sexually antagonistic alleles of potentially large effect are segregating at intermediate frequencies, whereas allelic effects at the majority of loci, including most new mutations, are likely to be sexually concordant (48). Our results suggest that, although they may contribute relatively little to standing genetic variance (25, 30, 50), alleles with sexually concordant deleterious effects may have a large impact on the evolution of male sexually selected traits and female preferences for them.

More generally, evolutionary biologists have had a preoccupation with directional selection that has resulted in much of the nonlinear selection estimated on traits being ignored (51). In particular, recent metaanalyses (3, 5, 7) have highlighted the current lack of evidence for stabilizing selection in nature. In the presence of pleiotropy, much of the stabilizing selection predicted to be present in natural populations is likely to be apparent in nature; selection measured on the phenotype of one trait is likely to be a consequence of selection on correlated traits (52). Pleiotropy also tends to restrict the distribution of the majority of genetic variance to a few trait combinations (11, 49). Under these conditions, stabilizing selection will be stronger on trait combinations than individual traits (34). Furthermore, detecting stabilizing selection directly on the genetic variance of traits will be more effective than phenotypic selection analyses if confounding effects of environmental covariances between traits and fitness obscure the fitness-trait genetic associations (32, 34, 53). Using the multivariate Robertson–Price Identity to quantify the genetic covariance between trait deviations and fitness may, therefore, provide a way of detecting this missing stabilizing selection.

Materials and Methods

Breeding Design. Using a previously described outbred and laboratory-adapted stock population of *D. serrata* (36, 54), we conducted a breeding design involving 91 sires mated to each of four dams implemented in three blocks consisting of 30, 30, and 31 sires, respectively, that spanned five generations of the laboratory population. As previously described, the fitness of 1,111 female and 1,371 male offspring was assayed in the standard laboratory environment (i.e., yeast medium) to which the population was long adapted (*SI Text*) (29). These assays were designed to capture the major components of fitness relevant to the normal maintenance of the stock population (*SI Text*). In brief, female fitness was measured as the total number of adults offspring produced over 48 h by a single female when held together with a random stock male. This measure includes her fecundity and the subsequent survival to emergence of her offspring. Male fitness was measured in an assay in which a single genetic male competed with two random stock males (each homozygous for a recessive orange eye mutation) for 72 h to fertilize a single random (homozygous orange eye) stock female. Under such conditions, the number of adult offspring sired by a male reflects his competitive mating success, the subsequent productivity of the female with which he mated, and the survival to emergence of his male and female offspring. In the analyses below, male fitness was calculated as the ratio of adult offspring sired by the genetic male relative to the two stock males after removing all genetic individuals that failed to produce any offspring (*SI Text*). Before analysis, these values were transformed as previously described (25, 29) and standardized [$N \sim (0, 1)$]; the resulting distributions were approximately normal (unimodal and symmetrical). To address concerns about the possible effects that such data culling and manipulation may have on the interpretation of the results (55), we repeated our main analyses by estimating the genetic association of both CHC$\mathbf{g}_{max}$ and CHC$\mathbf{g}_{max}^2$ with male fitness (see below) through a generalized linear mixed model that used all of the male fitness data (i.e., zeros included) on its original scale (*SI Text*, Tables S4 and S5, and Figs. S1–S3).

CHCs were assayed on 1,114 separate sons within the context of a sexual encounter by presenting the male with two random stock females (25). After mating occurred, CHCs were extracted using standard protocols and analyzed by gas chromatography (56), and the concentrations of each of nine previously identified CHCs (57) were determined by integration. Consistent with all previous work on these traits in this species, values were expressed as proportions of the total concentration for each individual to remove technical error associated with quantifying absolute abundances and then log contrast–transformed to break the unit sum constraint inherent in such compositional data (58) using (Z,Z)-5,9-$C_{24:2}$ as the common divisor. The resulting distributions of the log-contrast CHC were approximately normal. These traits were standardized [$N \sim (0, 1)$] before analysis.

As part of a separate analysis of the genetic basis of female mate preferences for male CHCs, other daughters from this breeding design were used in mate choice trials in which a single female was presented with five randomly chosen males from the same stock population (56). CHCs were extracted and quantified as above for the chosen male and one of the four rejected males (randomly chosen) from each trial, yielding 3,315 males in total.

Phenotypic Selection Analyses. Standardized directional sexual selection gradients on the eight log-contrast CHCs were calculated in a phenotypic analysis of these males using a standard first-order polynomial regression model (59), including experimental block as a fixed effect. Significance was determined using logistic multiple regression, because mating success is binomially distributed (60). Phenotypic sexual selection on CHC$\mathbf{g}_{max}$ was estimated by scoring these males for the first eigenvector of the sire-level covariance matrix for the eight log-contrast CHCs ($\mathbf{g}_{max}$) (Table 1), which is described in detail below. First- and second-order standard polynomial regressions of mating success on these scored phenotypes (59) were then used to estimate directional and quadratic selection (denoted β and γ, respectively) with significance determined by logistic regression.

Robertson–Price Identity for Multiple Traits. The sire-level additive genetic covariance matrix (**G**) for these eight traits was estimated by restricted maximum likelihood (REML) using a standard mixed model in which dam was nested within sire and including a fixed effect of experimental block (25). Genetic variance in CHC$\boldsymbol{\beta}$ was also estimated in a univariate version of this model after first scoring males for the vector of directional sexual selection gradients ($\boldsymbol{\beta}$) from the phenotypic analysis above using CHC$\boldsymbol{\beta} = \boldsymbol{\beta}^T\mathbf{Z}$, where **Z** is a row vector of the eight observed CHC values for an individual (34). The genetic covariances between fitness and the eight CHC traits were estimated in separate bivariate mixed models, and the covariances were then arranged as a column vector $\boldsymbol{\sigma}_A(w, \mathbf{z})$ separately for both male and female fitness (Table 1).

An approach for estimating $\boldsymbol{\phi}$ has not been previously determined (32). If we recognize that transforming each trait to a deviation from its mean allows a genetic analysis of squared trait deviations and hence, the measurement of selection on the genetic variance (33, 34, 61), $\boldsymbol{\phi}$ can be estimated at the sire level using the standard mixed model used above for estimating **G**. The diagonal elements of $\boldsymbol{\phi}$ are the genetic covariances between the squared deviations for each trait and fitness, whereas the off-diagonal elements are the genetic covariances between fitness and the cross-products of each bivariate combination of trait deviations. Unfortunately, estimating all of the required covariances would require a covariance matrix of very large dimensionality (37 in our case) and consequently, was not feasible within the context of a single mixed model. We, therefore, took the alternative approach of estimating each of the required covariances from separate bivariate mixed models, and we arranged the resulting genetic covariance estimates into the symmetrical matrix $\boldsymbol{\phi}$ (Table S1) separately for both male and female fitness. This approach precluded any hypothesis testing based on the entire $\boldsymbol{\phi}$ matrix within a single framework, a problem that we circumvented by confining our hypothesis testing to the major axis of genetic variance as explained below.

Given an estimate of $\boldsymbol{\phi}$ and **G**, the matrix of partial regression coefficients ($\boldsymbol{\Gamma}$) between genotypic values of fitness and the products of the trait deviations, analogous to $\boldsymbol{\gamma}$ in a phenotypic analysis, can be estimated as (equation 12 in ref. 32) (Eq. **4**)

$$\mathbf{\Gamma} = \mathbf{G}^{-1} \cdot \boldsymbol{\phi}\mathbf{G}^{-1}. \quad [4]$$

However, multicolinearity among our traits is high at the genetic level, and **G** is, therefore, ill-conditioned, making its inverse numerically unstable. This finding is likely to be a common problem, because it seems that genetic covariances among suites of traits typically concentrate genetic variance to a small number of independent trait combinations (34, 49). We therefore restrict our interpretation to $\boldsymbol{\phi}$ and $\Delta\mathbf{G}$.

Genetic Analyses Using CHC$\mathbf{g}_{max}$ as a Univariate Trait. Diagonalization of **G** yielded its eigenvectors and associated eigenvalues, the first of which represented the linear combination of the original traits with the greatest genetic variance ($\mathbf{g}_{max}$) (62, 63). As with any eigenvector, $\mathbf{g}_{max}$ of a set of metric traits is itself a metric trait, and phenotypic scores of individual sons from the breeding design (CHC$\mathbf{g}_{max}$) were calculated using CHC$\mathbf{g}_{max} = \mathbf{g}_{max}{}^{T}\mathbf{Z}$, where **Z** is a row vector of the eight observed log-contrast CHC values for an individual (34). These phenotypic scores for CHC$\mathbf{g}_{max}$ were then used in a genetic analysis along with male and female fitness to calculate the sire-level genetic covariance matrix (**G**) for these three traits by a three-dimensional factor-analytic model fit using REML fit (64). Significance of the individual (co)variances was determined by likelihood ratio tests. Directional selection on the major axis of genetic variance in CHCs (CHC$\mathbf{g}_{max}$) would be indicated by a significant additive genetic covariance between CHC$\mathbf{g}_{max}$ and male and/or female fitness (59).

Stabilizing selection on CHC$\mathbf{g}_{max}$ would not be detected as a covariance with fitness, because individuals deviating from the CHC$\mathbf{g}_{max}$ optimum in either direction would have similarly low fitness (34). These deviations were therefore calculated as the square of the individual CHC$\mathbf{g}_{max}$ scores when standardized to a mean of zero (CHC$\mathbf{g}_{max}{}^{2}$). These deviations were then used in place of the original CHC$\mathbf{g}_{max}$ scores in a genetic analysis along with male and female fitness. Because CHC$\mathbf{g}_{max}{}^{2}$ values were nonnormally distributed, significance of the genetic correlation between them and male fitness was determined by fitting a generalized linear mixed model by a Markov chain Monte Carlo algorithm as implemented in the MCMCglmm package in R (65); 10,000 posterior samples were generated using a weakly informative inverse-Wishart prior, and significance of the correlation was determined from whether the 95% bounds of the higher posterior density interval overlapped zero.

To characterize the covariance structure of male and female fitness, a genetic analysis was conducted on these two traits alone using the same multivariate mixed model that was fit by REML. Breeding values were estimated as BLUPs from this model. The two genetic principle components were extracted by diagonalization of the sire-level covariance matrix, representing sexually antagonistic and sexually concordant fitness variation. Fitness variation was reexpressed along these two axes by scoring the breeding values of male and female fitness separately for these two eigenvectors. Directional and stabilizing selection on CHC$\mathbf{g}_{max}$ arising from its association with these two new fitness axes was then characterized using first- and second-order polynomial regression on the CHC$\mathbf{g}_{max}$ breeding values calculated as BLUPs from a univariate model. We emphasize that these relationships are exploratory only, and we have not presented statistical analyses supporting them, because the BLUPS have not been estimated in a model that incorporated the genetic covariances of interest and hence are likely to be subject to considerable bias (66).

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Supporting Information

Delcourt et al. 10.1073/pnas.1116828109

SI Text

Sexual Selection on Cuticular Hydrocarbons in *Drosophila serrata*. Three lines of evidence have established cuticular hydrocarbons (CHCs) as a target of sexual selection in *Drosophila serrata*. First, sexual selection gradients on CHCs have been measured on several laboratory-adapted populations (1–3) and numerous natural populations within a few generations of their collection from the wild (4, 5), and in both cases, little genetic variance remains in the direction of this selection (2, 4, 6). Second, artificial selection on the multivariate combination of CHCs identified by these selection analyses as being preferred by females resulted in higher male mating success (7). Third, CHCs exhibit a pattern of reproductive character displacement along the Australian east coast that corresponds with the presence vs. absence of the related species, *D. birchii* (8, 9). Laboratory mate choice trials reveal genetically based differences in female mate preferences for male CHCs between sympatry and allopatry, and the resulting divergent sexual selection estimated in the laboratory corresponds with the pattern of character displacement in nature (9). Evolutionary manipulations have shown that the pattern of reproductive character displacement evolves as a consequence of the presence of *D. birchii* (8) and that sexual selection generated by female choice in the absence of *D. birchii* drives the evolution of the CHCs back to an allopatric blend (10).

Fitness Measures and Data Manipulation. How best to measure fitness is a controversial topic (11, 12) and often presents a substantial empirical challenge. The *D. serrata* stock population used in the current study was maintained on a schedule in which adults lifespan was only a few days, and our assays were designed to capture the key components of fitness under these conditions. Nevertheless, measuring fitness requires tracking individuals throughout their life, necessitating a change in environment (e.g., reduced density and hence, competition), and our measures must, therefore, be viewed as approximations.

Male fitness was calculated as the ratio of WT to orange-eye individuals in the adult offspring produced by the female (after adding a constant of one to each), and the resulting values were *ln*-transformed. Female fitness was calculated as the total number of adult offspring emerging in each vial, and values were square root-transformed. In both cases, the original distributions were bimodal because of a peak corresponding to genetic individuals (i.e., sons or daughters from the breeding design) that failed to produce any offspring. It is not known why these individuals failed to produce offspring, although experimental error was likely an important contributor (e.g., damage or death caused by handling). Removal of these individuals resulted in fitness distributions that were approximately Gaussian, and these data were used in subsequent analyses. However, we recognize growing concerns in the literature surrounding such data culling, transformation, and standardization (13). Therefore, as described below, we also confirm our central results concerning the genetic association of both $CHC\mathbf{g}_{max}$ and $CHC\mathbf{g}_{max}{}^2$ with male fitness by a generalized linear mixed modeling that used all of the male fitness data on its original scale.

Alternative Statistical Analyses Using Generalized Linear Mixed Models. We used a generalized linear mixed model, implemented in the MCMCglmm package (14) in R (15) and fit by a Markov chain Monte Carlo routine, to estimate the genetic correlation between $CHC\mathbf{g}_{max}$ and male fitness as well as the genetic correlation between $CHC\mathbf{g}_{max}{}^2$ and male fitness. Male fitness was measured as the absolute number of offspring sired by a genetic male (i.e., total number of WT offspring) and included those replicates in which the female produced only orange-eye offspring (i.e., the genetic male failed to sire any offspring). $CHC\mathbf{g}_{max}$ and $CHC\mathbf{g}_{max}{}^2$ were modeled in separate but otherwise identical analyses. Although the distribution of $CHC\mathbf{g}_{max}{}^2$ on the phenotypic scale seemed nonnormal, after conditioning on the random effects in the model (i.e., sire, dam nested with sire, and the residual), a Gaussian distribution fit the data well for both $CHC\mathbf{g}_{max}$ and $CHC\mathbf{g}_{max}{}^2$ (Figs. S1 *A* and *B* and S2 *A* and *B*). Fitness was modeled using a zero-inflated Poisson distribution, because preliminary analyses indicated that zero inflation was severe: the observed number of zeros exceeded that predicted under a standard Poisson model by ∼36 times. Hence, two components were estimated for fitness in each model, with the first estimating the variance in offspring production under the expectation of a Poisson model and the second estimating zero inflation under a binomial model. For the Poisson component of fitness, additive overdispersion, on the latent scale, was modeled as residual variance to estimate variation in the Poisson process not defined by the mean (λ). For the zero-inflation component of fitness, residual variation could not be estimated, and therefore, it was fixed at a value of one. Similarly, because at the individual level, zero and nonzero fitness cannot occur simultaneously, the residual covariance between the Poisson and the zero-inflated components of fitness could not be estimated. Likewise, because $CHC\mathbf{g}_{max}$ and fitness were measured on different individuals, the residual covariance matrix had no off-diagonal elements. Hence, for each variance component (i.e., sire, dam, and residual), the resultant (co)variance matrix was of order 3×3 (Tables S4 and S5).

The posterior distributions of the location effects and variance components were estimated from 1,300,000 MCMC iterations sampled at 100 iteration intervals after an initial burn-in period of 300,000 iterations. Weakly informative inverse Wishart priors were used, with shape and scale set to 0.001. Overall, autocorrelation between successive samples was low (<0.01), although the properties of the zero-inflation process should be interpreted with caution, because mixing was not as good for this component; the effective sample sizes of $CHC\mathbf{g}_{max}$ and the Poisson component of fitness at the sire level were approximated six times greater than the zero-inflation process for fitness. Nevertheless, the posterior predictive distributions indicate that our models fit the observed data well (Figs. S1 and S2). The component of greatest interest is the correlation between the CHC traits (i.e., $CHC\mathbf{g}_{max}$ and $CHC\mathbf{g}_{max}{}^2$) and the Poisson component of fitness, which we calculated by dividing the covariance between the two traits by the geometric mean of the variances [e.g., for the correlation between $CHC\mathbf{g}_{max}$ and fitness, $Corr_{CHC\mathbf{g}max,\,fitness} = Cov_{CHC\mathbf{g}max,\,fitness}/(Var_{CHC\mathbf{g}max} \cdot Var_{fitness})^{0.5}$] for each of the 10,000 samples of the posterior distribution (Fig. S3). We then assessed the significance of the correlations by examining the portion of higher posterior density (HPD) interval that did not overlap zero. The resulting sire-level (co) variance/correlation matrices are presented in Tables S4 and S5 for $CHC\mathbf{g}_{max}$ and $CHC\mathbf{g}_{max}{}^2$, respectively.

X-Linked Effects. The paternal half-sibling breeding design that we used includes autosomal but not X-linked effects in the estimated additive genetic (co)variances (because males are heterogametic and genetic sons, therefore, all carry a Y-chromosome from their father). X-linked additive genetic effects can be estimated by a mixed linear model that takes advantage of other relationships

within the breeding design but only if autosomal dominance is assumed negligible (16). The use of this method is not recommended for traits with low heritabilities (e.g., fitness), because the resulting estimates can be strongly biased in the presence of dominance (16). Inclusion of X-linked effects would, therefore, require a more complex breeding design.

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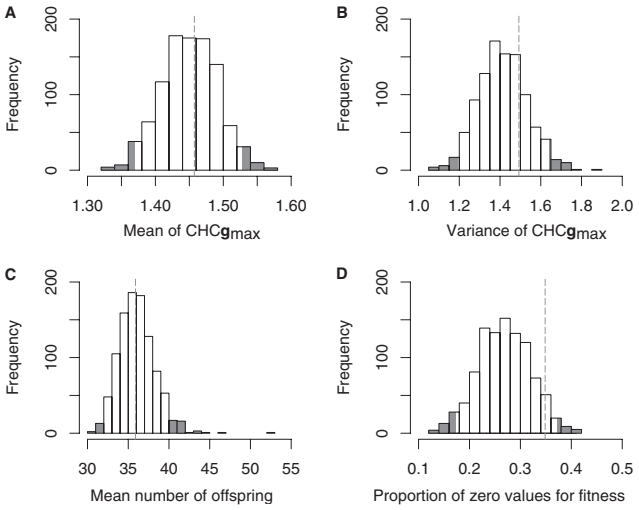

Fig. S1. Posterior predictive distributions for (*A*) the mean of CHC$\mathbf{g}_{max}$, (*B*) the variance of CHC$\mathbf{g}_{max}$, (*C*) male fitness from the Poisson process, and (*D*) the proportion of zero values for male fitness from the zero-inflation process. Dashed lines indicate the observed values. Shaded areas indicate 95% HPD intervals.

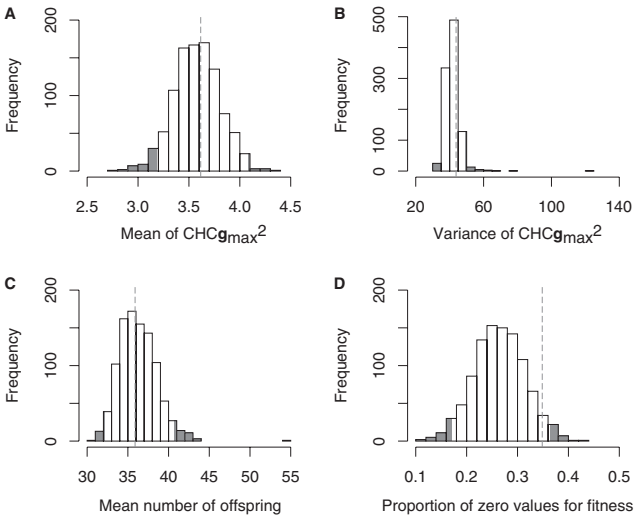

Fig. S2. Posterior predictive distributions for (*A*) the mean of CHC$\mathbf{g}_{max}^2$, (*B*) the variance of CHC$\mathbf{g}_{max}^2$, (*C*) male fitness from the Poisson process, and (*D*) the proportion of zero values for male fitness from the zero-inflation process. Dashed lines indicate the observed values. Shaded areas indicate 95% HPD intervals.

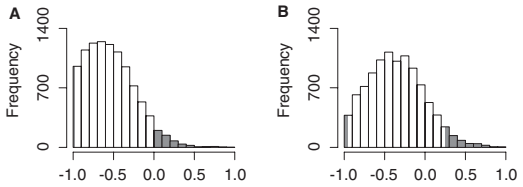

Fig. S3. Posterior distributions for the correlation between (*A*) CHC$\mathbf{g}_{max}$ and fitness and (*B*) CHC$\mathbf{g}_{max}^2$ and male fitness. Shaded areas indicate 95% HPD intervals.

Table S1. Genetic (i.e., sire-level) covariance matrices φ of male fitness (lower left) and female fitness (upper right) with the products of the eight log-contrast CHCs traits when expressed as deviations from the population mean with unit variance as described in Eq. 2

	(Z,Z)-5,9-$C_{25:2}$	(Z)-9-$C_{25:1}$	(Z)-9-$C_{26:1}$	2-Me-C_{26}	(Z,Z)-5,9-$C_{27:2}$	2-Me-C_{28}	(Z,Z)-5,9-$C_{29:2}$	2-Me-C_{30}	
	−0.0275	−0.0838	−0.0168	−0.0372	−0.0451	−0.0409	−0.0153	−0.0292	(Z,Z)-5,9-$C_{25:2}$
		−0.1958	−0.0803	−0.0628	−0.0907	−0.0678	−0.0847	−0.0663	(Z)-9-$C_{25:1}$
(Z,Z)-5,9-$C_{25:2}$	**−0.0173**		**−0.0056**	−0.0116	−0.0043	−0.0055	0.0219	0.0140	(Z)-9-$C_{26:1}$
(Z)-9-$C_{25:1}$	−0.0103	**−0.1058**		**−0.0114**	−0.0288	−0.0198	−0.0321	−0.0194	2-Me-C_{26}
(Z)-9-$C_{26:1}$	−0.0237	−0.0063	**−0.0324**		**−0.0197**	−0.0248	0.0096	−0.0071	(Z,Z)-5,9-$C_{27:2}$
2-Me-C_{26}	−0.0017	−0.0039	−0.0173	**0.0008**		**−0.0172**	−0.0074	−0.0047	2-Me-C_{28}
(Z,Z)-5,9-$C_{27:2}$	−0.0352	−0.0320	−0.0345	−0.0302	**−0.0335**		**0.0229**	0.0254	(Z,Z)-5,9-$C_{29:2}$
2-Me-C_{28}	−0.0137	0.0062	−0.0212	−0.0126	−0.0352	**−0.0220**		**0.0153**	2-Me-C_{30}
(Z,Z)-5,9-$C_{29:2}$	−0.0267	−0.0187	−0.0085	−0.0291	−0.0190	−0.0259	**−0.0014**		
2-Me-C_{30}	−0.0142	0.0290	−0.0240	−0.0170	−0.0320	−0.0247	−0.0164	**−0.0242**	

Genetic variances are given in bold, and covariances are not bold.

Table S2. Distribution of eigenvalues from the covariance matrices quantifying the change in CHC genetic variance caused by nonlinear selection alone (φ) or the combined effects of linear and nonlinear selection (ΔG) arising separately through male and female fitness

	Eigenvalues (males)		Eigenvalues (females)	
Eigenvector	φ	**ΔG**	φ	**ΔG**
1	0.0472	0.0471	0.1264	0.1222
2	0.0150	0.0147	0.0294	0.0293
3	0.0050	0.0045	0.0108	0.0108
4	0.0015	0.0015	0.0082	0.0076
5	−0.0075	−0.0075	−0.0027	−0.0030
6	−0.0158	−0.0167	−0.0222	−0.0231
7	−0.1175	−0.1261	−0.0303	−0.0304
8	−0.1634	−0.1695	−0.3588	−0.3915
Sum (absolute)	0.3729	0.3877	0.5887	0.6178
Prop (negative)	0.816	0.825	0.703	0.725

The proportion of negative eigenvalues was calculated as Prop(negative) = Σ|(negative eigenvalues)|/ Σ|(all eigenvalues)|.

Table S3. Within-generation change in G (i.e., ΔG) for eight log-contrast male CHCs caused by the combined effects of linear and nonlinear selection through male fitness (lower left) and female fitness (upper right) calculated using Eq. 3

	(Z,Z)-5,9-$C_{25:2}$	(Z)-9-$C_{25:1}$	(Z)-9-$C_{26:1}$	2-Me-C_{26}	(Z,Z)-5,9-$C_{27:2}$	2-Me-C_{28}	(Z,Z)-5,9-$C_{29:2}$	2-Me-C_{30}	
	−0.0306	−0.0937	−0.0159	−0.0396	−0.0456	−0.0425	−0.0162	−0.0300	(Z,Z)-5,9-$C_{25:2}$
		−0.2282	−0.0775	−0.0706	−0.0923	−0.0729	−0.0874	−0.0690	(Z)-9-$C_{25:1}$
(Z,Z)-5,9-$C_{25:2}$	**−0.0177**		**−0.0059**	−0.0110	−0.0041	−0.0051	0.0221	0.0142	(Z)-9-$C_{26:1}$
(Z)-9-$C_{25:1}$	−0.0126	**−0.1188**		**−0.0133**	−0.0291	−0.0210	−0.0328	−0.0200	2-Me-C_{26}
(Z)-9-$C_{26:1}$	−0.0242	−0.0095	**−0.0332**		**−0.0198**	−0.0250	0.0094	−0.0072	(Z,Z)-5,9-$C_{27:2}$
2-Me-C_{26}	−0.0014	−0.0022	−0.0169	**0.0006**		**−0.0181**	−0.0078	−0.0051	2-Me-C_{28}
(Z,Z)-5,9-$C_{27:2}$	−0.0360	−0.0366	−0.0356	−0.0296	**−0.0351**		**0.0227**	0.0252	(Z,Z)-5,9-$C_{29:2}$
2-Me-C_{28}	−0.0134	0.0080	−0.0208	−0.0129	−0.0345	**−0.0222**		**0.0151**	2-Me-C_{30}
(Z,Z)-5,9-$C_{29:2}$	−0.0269	−0.0195	−0.0086	−0.0290	−0.0193	−0.0258	**−0.0015**		
2-Me-C_{30}	−0.0142	0.0290	−0.0240	−0.0170	−0.0320	−0.0247	−0.0164	**−0.0242**	

Changes in genetic variance are given in bold, and covariances are not bold.

Table S4. Sire-level covariance matrix of $CHCg_{max}$ and male fitness

Trait	$CHCg_{max}$	Fitness	Zero-inflated fitness
$CHCg_{max}$	**0.21 (0.12, 0.30)**	*−0.36 (−0.96, 0.25)*	*0.29 (−0.67, 1.00)*
Fitness	−0.02 (−0.06, 0.02)	**0.02 (<0.00, 0.05)**	*−0.48 (−1.00, 0.63)*
Zero-inflated fitness	0.03 (−0.05, 0.11)	−0.02 (−0.06, 0.01)	**0.04 (<0.00, 0.14)**

Variances are in bold on the diagonal; genetic covariances are below the diagonal, and genetic correlations are above the diagonal (italics). Values in parentheses denote 95% HPD intervals.

Table S5. Sire-level covariance matrix of the squared deviations of $CHCg_{max}$ phenotypes ($CHCg_{max}^2$) and male fitness

Trait	$CHCg_{max}^2$	Fitness	Zero-inflated fitness
$CHCg_{max}^2$	**5.86 (3.45, 8.25)**	*−0.53 (−0.98, 0.01)*	*0.47 (−0.44, 1.00)*
Fitness	−0.17 (−0.37, 0.05)	**0.02 (<0.00, 0.05)**	*−0.53 (−1.00, 0.48)*
Zero-inflated fitness	0.21 (−0.20, 0.65)	−0.02 (−0.06, 0.01)	**0.04 (<0.00, 0.14)**

Variances are in bold along the diagonal; genetic covariances are below the diagonal, and genetic correlations are above the diagonal (italics). Values in parentheses denote 95% HPD intervals.