

STRONGER CONVEX (STABILIZING) SELECTION ON HOMOLOGOUS SEXUAL DISPLAY TRAITS IN FEMALES THAN IN MALES: A MULTIPOPULATION COMPARISON IN *DROSOPHILA SERRATA*

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Received March 29, 2010

Accepted September 18, 2010

Mutual mate choice for homologous sexual display traits has been demonstrated in several recent studies yet little attention has been given to quantitative comparison of the strength and form of mate preferences between the sexes. Such comparisons may provide important insight into the evolution of mate choice for honest signals. In particular, because females generally provide the majority of resources for initial offspring development, female displays may trade-off with fecundity, causing preference evolution to differ between the sexes. Recent theory suggests that adaptive male preferences for honest displays in females are possible under certain conditions and may result in preferences that are convex (i.e., stabilizing) in form. We compared sexual selection on a suite of contact pheromones arising from mutual mate choice using nine separate geographic populations of *Drosophila serrata*. We show that the convex selection is stronger on females than on males overall in these populations, and that convex selection is the predominate form of nonlinear selection on females but not males.

KEY WORDS: Cuticular hydrocarbons, mutual mate choice, nonlinear selection, quadratic selection, sexual selection.

Since Darwin (1871) first recognized that mate preferences could be responsible for the evolution of the extravagant sexual display traits characteristic of many species, the evolutionary consequence of the resulting sexual selection has received much attention (Andersson 1994). Although the majority of work has focused on female preferences for male traits, male preferences have now been demonstrated in many species, including those with traditional sex roles (Bonduriansky 2001). Species exhibiting male and mutual mate choice may provide particularly insightful case studies for understanding the general conditions favoring the evo-

lution of mate choice (Bonduriansky 2001; Chenoweth et al. 2006; Kraaijeveld et al. 2007).

According to theory, the evolution of male mate choice in animals with conventional sex roles may be fundamentally different than its female counterpart. This is in part because males courting preferred females will tend to face stronger competition from other males, generating direct selection against the male preference (Servedio and Lande 2006). The evolution of male mate choice may therefore require that these costs be mitigated and recent models have explored a number of possibilities (Chenoweth

et al. 2006; Servedio and Lande 2006; Servedio 2007; Rowell and Servedio 2009). The often extreme variation in female fecundity that exists in many species suggests that adaptive male choice for females of high fecundity may be key (Bonduriansky 2001). Consistent with this, male mate choice appears to frequently target traits that directly indicate female fecundity such as body size (Itzkowitz et al. 1998; Bonduriansky 2001; Chenoweth et al. 2007; Long et al. 2009).

In some species, however, direct assessment of female fecundity may be not be possible. In this case, theory suggests that males seeking fecundity benefits should discriminate among females using traits that provide a reliable indication of their fecundity (Fitzpatrick et al. 1995), and studies of male mate choice appear to support this (Hunt et al. 1999; Amundsen and Forsgren 2001; Domb and Pagel 2001; Lebas et al. 2003; Cornwallis and Birkhead 2007). However, unlike males, the evolution of sexual displays in females may differ because female investment in offspring is necessarily substantial. Females that invest heavily in their sexual display may therefore suffer reduced fecundity and males should avoid mating with them when given a choice (Fitzpatrick et al. 1995). Female displays may therefore be constrained because their exaggeration is limited by this resource trade-off, raising the possibility that in species with mutual mate choice, the form of preference, and the resulting sexual selection they generate, may be fundamentally different.

Comparisons of the form and strength of sexual selection between the sexes are extremely rare. Here, using replicate two-stimulus choice trials conducted separately for each sex, we undertake a comprehensive comparison of sexual selection between males and females for nine separate geographic populations of *Drosophila serrata* recently collected from across much of the species range along the eastern seaboard of Australia. Mate preferences in this species are mutual and target a homologous suite of contact pheromones composed of cuticular hydrocarbons (CHCs; Chenoweth and Blows 2005). A number of lines of evidence suggest that CHCs are the direct target of sexual selection arising from female mate preferences as opposed to male–male (i.e., intrasexual) competition, and that two-stimulus choice trials are an appropriate technique for quantifying this selection (summarized in Delcourt et al. 2010; and see Higgie and Blows 2008). Nevertheless, the potential contribution of intrasexual selection in generating sexual selection on CHCs during two-stimulus choice trials has not been definitively ruled out and we return to this topic in the discussion. The results of these mate choice trials have been previously used to test for variation in linear and nonlinear sexual selection on CHCs among these nine geographic populations separately by sex and to associate this variation with large-scale biotic and abiotic variables (Rundle et al. 2008). We use these data here to compare sex-

ual selection on CHCs between males and females within each population.

We focus our analyses on nonlinear selection for two reasons (see Appendix S1 for the analysis of linear selection). First, it appears to be the predominate form of sexual selection on CHCs in these populations. Linear sexual selection on the entire suite of CHCs explained an average of $3.9\% \pm 2.5\%$ and $6.1\% \pm 2.7\%$ (mean $r_{\text{adj.}}^2 \pm \text{std. dev.}$) of the variance in mating success in females and males, respectively (Table 1). The addition of nonlinear sexual selection (quadratic and correlational) overall was highly significant in every population ($P < 0.008$ in all cases; Rundle et al. 2008) and explained an additional $12.4\% \pm 2.5\%$ and $13.8\% \pm 3.5\%$ of the variance in mating success in females and males, respectively (Table 1), two to three times that accounted for by linear selection.

Second, there is reason to think that nonlinear sexual selection may differ fundamentally between the sexes. Theory suggests that one possible route by which adaptive male mate preferences may evolve for female displays that trade-off with fecundity is if the preferences are stabilizing (Chenoweth et al. 2006). The reason is straightforward: stabilizing preferences allow males to avoid females that invest too few resources in fecundity, either because the females lack sufficient resources overall or because they allocate disproportionately to their sexual displays at a cost to their fecundity. Male preferences of this form should generate stabilizing selection on female displays. The evolutionary stability of such preferences depends critically on the presence and degree of stochastic variation in resource allocation in females to sexual displays and fecundity: error should be sufficient to make males avoid females with overly extravagant displays, but not so large that the display is not indicative of fecundity (Chenoweth et al. 2006).

Materials and Methods

Laboratory populations were founded from an average of 20 wild-caught females collected from each of nine geographic populations of *D. serrata* spanning approximately 15° latitude (1450 km) along the eastern Australian coastline (Chenoweth et al. 2008). Populations were maintained at an average census size of 200 individuals for 12 nonoverlapping generations (25°C , 12:12 light:dark cycle) prior to conducting mating trials. Sexual selection on CHCs was measured separately in males and females from each of the nine populations using replicate two-stimulus mate choice trials as outlined in Rundle et al. (2008). Briefly, a trial involved a single virgin individual (male or female) from one of the experimental populations choosing between two opposite-sex virgin individuals from the same population. Vials were observed and once mating occurred, the “choosing” fly was discarded and the chosen and rejected individuals were anaesthetized using CO_2 and had their

Table 1. Relative importance of linear and nonlinear sexual selection on the entire suite of CHCs in both males and females. Also shown are the results of tests for overall differences in nonlinear sexual selection between the sexes, including both correlational and quadratic selection.

Population	Females		Males		Sex differences in nonlinear selection	
	$r_{\text{adj.}}^2$ linear	$r_{\text{adj.}}^2$ nonlinear	$r_{\text{adj.}}^2$ linear	$r_{\text{adj.}}^2$ nonlinear	diff. in $-2LL$	P^*
1	0.045	0.109	0.088	0.100	36.679	0.437
2	0.023	0.110	0.061	0.121	59.986	0.007
3	0.040	0.128	0.088	0.057	53.103	0.033
4	0.031	0.170	0.043	0.165	63.544	0.003
5	0.046	0.090	0.106	0.145	36.727	0.435
6	0.096	0.140	0.029	0.180	84.407	<0.001
7	0.019	0.109	0.040	0.207	63.983	0.003
8	0.007	0.111	0.058	0.141	71.279	<0.001
9	0.047	0.151	0.037	0.130	50.700	0.053

Note: Linear and nonlinear sexual selection were estimated using a separate polynomial regressions in each sex and population, as described in Rundle et al. (2008). The proportion of total variance in mating success explained by CHCs is given by the coefficient of determination ($r_{\text{adj.}}^2$). For nonlinear selection, this values represent the amount by which $r_{\text{adj.}}^2$ increases with the addition of nonlinear selection.

*Results of a likelihood ratio test with $df = 36$ in all cases. See text for details.

CHCs extracted. An average of 99 trials were performed for each sex and population.

CHC samples were analyzed by gas chromatography using established techniques as outlined in Rundle et al. (2008). CHC profiles for each individual were determined by integration of the area under nine peaks corresponding to those CHCs used in past studies and identified in order of their retention times as: (Z,Z)-5,9-C_{24:2}; (Z,Z)-5,9-C_{25:2}; (Z)-9-C_{25:1}; (Z)-9-C_{26:1}; 2-Me-C₂₆; (Z,Z)-5,9-C_{27:2}; 2-Me-C₂₈; (Z,Z)-5,9-C_{29:2}; and 2-Me-C₃₀. For each individual, relative amounts of each of the nine CHCs was determined by dividing the area under that peak by the total area under all nine peaks. Individual proportions were transformed by logcontrast to remove the unit-sum constraint characteristic of compositional data (Aitchison 1986), using the proportional area under (Z)-9-C_{26:1} as the divisor. This yielded eight logcontrast CHCs characterizing variation among individuals in relative CHC concentration. These values were then standardized to a mean of zero and a variance of one within each sex and population.

Overall variation between the sexes in nonlinear sexual selection, including both quadratic and correlational selection, was tested using a sequential model-building approach (Draper and John 1988; Chenoweth and Blows 2005). For each population, we determined whether estimating separate nonlinear selection gradients in males and females significantly improved the fit of the model compared to one that constrains nonlinear selection to be the same in each sex. Linear selection was allowed to vary between the sexes in both models, although results are essentially the same when linear selection is constrained to be equal in males and females (H. Rundle, unpubl. data). The full model was

$$w = \beta_0 + \alpha_0 \text{Sex} + \sum_{i=1}^8 \beta_i Z_i + \sum_{i=1}^8 \alpha_i Z_i \text{Sex} + \sum_{i=1}^8 \sum_{j=1}^8 \gamma_{ij} Z_i Z_j + \sum_{i=1}^8 \sum_{j=1}^8 \alpha_{ij} Z_i Z_j \text{Sex} + \varepsilon, \quad (1)$$

in which w is the binomial mating success score of an individual male or female (0 = rejected, 1 = chosen; converted to a relative measure by dividing each value by the population mean; Lande and Arnold 1983), β_0 is the y -intercept, Z_i and Z_j are the log-contrast concentrations for the i th and j th CHC in an individual, "Sex" is a dummy variable representing the sex of that individual (coded as 0 or 1), and ε is unexplained error. The β_i values represent linear (directional) sexual selection gradients for the eight CHCs across all individuals ignoring sex, and the eight $\alpha_i Z_i \text{Sex}$ terms represent the linear interaction of sex and the i th CHC, in effect estimating separate directional selection gradients for males and females. The γ_{ij} values form a symmetrical 8×8 matrix of second-order partial regression coefficients characterizing quadratic selection on this set of CHCs along the diagonal ($i = j$) and correlational selection on the off-diagonals ($i \neq j$). Positive and negative values of γ_{ij} indicate concave and convex selection, respectively. The 36 $\alpha_{ij} Z_i Z_j \text{Sex}$ terms represent the interaction of sex with the nonlinear selection gradients, in effect estimating separate quadratic and correlational selection gradients for males and females. Because mating success is binomial, a generalized version of the above model was fit using maximum likelihood and a logistic link function via the GENMOD procedure in SAS version 9.2 (SAS Institute, Cary, NC). A likelihood ratio test was

performed, separately for each population, to compare the fit of this full model with one lacking the $36 \alpha_{ij}Z_iZ_j\text{Sex}$ terms.

To facilitate comparisons of the strength and form of nonlinear selection acting on this suite of logcontrast CHCs in males and females, we conducted a canonical rotation of the γ matrix of nonlinear selection gradients separately in each sex and population to identify the eigenvectors of γ that correspond to the major axes of the quadratic response surface (Blows and Brooks 2003). For this analysis, γ was calculated from equation 1, fit separately for each sex and population (data were standardized to mean of zero and standard deviation of one by sex and population), after removal of all the terms containing "Sex," constituting a standard second-order polynomial regression involving the eight logcontrast CHCs (Lande and Arnold 1983). The quadratic coefficients from this model were doubled to estimate the associated quadratic selection gradients appearing along the diagonal in γ (Stinchcombe et al. 2008), correcting previously published results that failed to do so (Table A2 of Rundle et al. 2008). Diagonalization of γ yielded the canonical axes, representing linear combinations of the original CHCs that display the strongest (largest absolute eigenvalues) and weakest (smallest absolute eigenvalues) nonlinear selection, with negative eigenvalues indicating convex selection and positive values indicating concave selection along the eigenvector in question (Tables A3 and A4). These canonical axes are orthogonal to one another and therefore integrate all nonlinear selection acting on the entire suite of traits, eliminating the distinction between correlational and quadratic selection. In the absence of a canonical rotation, focus is restricted to the original traits and the strength of stabilizing/disruptive selection may be substantially underestimated if it acts on combinations of traits (Blows and Brooks 2003).

To determine the predominate form of nonlinear selection in each sex, we calculated the sum of all eigenvalues (positive and negative) separately for males and females from each population. A one-sample t -test, again treating populations as replicates, was then used to determine whether this sum differed significantly from zero (i.e., the null hypothesis of equal overall strength of convex and concave selection) separately in males and females. To determine whether convex selection was stronger overall in females than in males, for each population we calculated the sum of all negative eigenvalues in each sex. A one-sample t -test, treating populations as replicates, was then used to determine whether the difference in this sum (females–males) differed from zero (i.e., the null hypothesis that the strength of convex selection does not differ between females and males in each population).

Stabilizing selection requires showing that a fitness maximum exists within the range of phenotypic expression of the fitness surface. We therefore performed a direct statistical test for stabilizing selection on all canonical axes subject to convex

selection (i.e., all eigenvectors with negative eigenvalues) as described in Mitchell-Olds and Shaw (1987). Details concerning the implementation of this approach are provided in Chenoweth et al. (2007). Using only those eigenvalues with statistical support for stabilizing selection, we then repeated the test above comparing the sum of all negative eigenvalues between males and females, thereby testing the null hypothesis of equal strength of stabilizing selection between males and females.

Results and Discussion

Differences in nonlinear sexual selection on the entire suite of CHCs (i.e., both quadratic and correlational gradients) between males and females were significant, or borderline so ($P = 0.053$), in seven of the nine populations (Table 1). Among populations in females, the predominate form of nonlinear sexual selection was convex ($t = -2.67$, $df = 8$, $P = 0.028$), whereas in males, there was no significant difference overall in the strength of convex versus concave selection, indicating that neither form tended to predominate (Fig. 1; $t = -0.616$, $df = 8$, $P = 0.555$). The total strength of convex selection was also significantly greater in females than in males among these nine populations ($t = -2.39$, $df = 8$, $P = 0.044$). This trend remains, although becomes marginally non-significant ($t = -1.80$, $df = 8$, $P = 0.109$), when the analysis is restricted to those trait combinations (i.e., eigenvectors of γ) for which an intermediate fitness optima was statistically supported

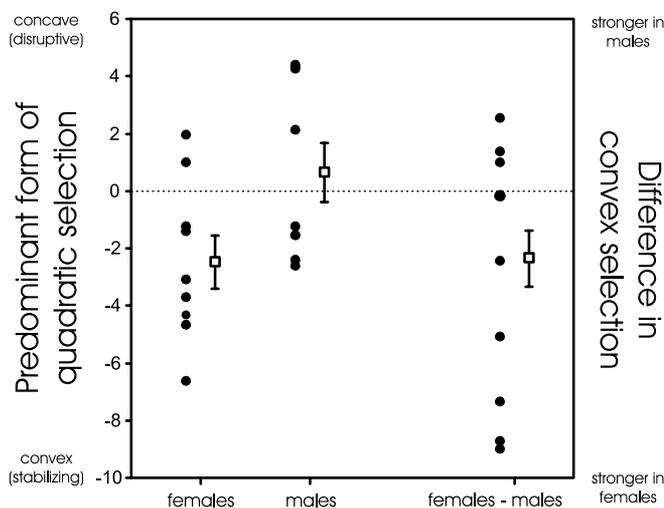


Figure 1. Predominant form of quadratic sexual selection on CHCs in females and males as determined by the sum of all the eigenvalues of γ . Positive values indicate concave and negative values convex selection, with zero implying equal strength of the two forms. Also shown is the difference in the overall strength of convex selection between females and males as determined by the difference in the sum of all negative eigenvalues. Points indicate each of the nine populations (filled circles) and the mean \pm SE across the nine populations (open boxes).

within the range of trait values, or in other words those traits on which selection was stabilizing (Tables A3 and A4).

This sexual selection arises from mate choice and is therefore equivalent to the population-level mate preference within each sex (Wagner 1998; Chenoweth and Blows 2006). The predominance of convex selection on CHCs in females but not males is consistent with stronger stabilizing selection on the former than the latter sex, and differences in statistically detectable stabilizing selection support this, approaching significance despite the empirical challenges of demonstrating stabilizing selection on such a large suite of traits in replicate populations. The increased prevalence of stabilizing selection on females compared to males, in combination with the facts that linear selection is relatively weak and does not appear to differ in overall strength between the sexes, suggest that the evolution of exaggerated CHCs sexual displays may be less likely in females than in males, and that a fundamental difference exists in the information gained from CHCs in the opposite sex.

Adaptive male mate choice for fecundity benefits are expected when females vary substantially in their fecundity and males cannot inseminate all females they encounter (Parker 1983; Owens and Thompson 1994; Johnstone et al. 1996; Kokko and Monaghan 2001). Recent theory suggests that stabilizing preferences are one possible solution to males seeking fecundity benefits when choice is based on female displays that trade-off with fecundity (Chenoweth et al. 2006). The current pattern of stronger convex selection on CHCs in females than in males in multiple populations is consistent with this and with results from a previous study of male preferences for female CHCs in a single, laboratory-adapted population of *D. serrata* (Chenoweth and Blows 2005). Theory suggests that stabilizing selection on female displays should also translate into stabilizing selection on female fecundity due to the resource allocation trade-off between these traits. Such stabilizing sexual selection has been recently demonstrated on female mass in *D. serrata* (Chenoweth et al. 2007).

In contrast to stabilizing male preferences for costly female displays, theory also suggests an alternative situation in which male mating preferences may be maintained. If males can strategically allocate their courtship over the available distribution of females in a dynamic manner, male mate preferences may exist as a polymorphism in the absence of any costs of the female display or fecundity benefits to choosy males (Rowell and Servedio 2009). This is because males without the preference preferentially court females lacking the preferred display. Although this occurs for purely selfish reasons, it serves to alleviate the increased mate competition experienced by males with the preference, restoring a competitive balance across males. This model predicts directional as opposed to stabilizing male preferences, but relies on males freely adjusting their courtship in response to reliable information on the competitive landscape for female matings. As

suggested by Rowell and Servedio (2009), such conditions may be more likely in lekking species.

Direct evidence for an allocation trade-off between CHCs and fecundity in *D. serrata* has not been addressed but does exist in *D. melanogaster*. Gene-knockout studies have shown a shared genetic basis for CHCs and egg production (Wicker and Jallon 1995) that likely arises because the cuticle and ovaries appear to compete for a common pool of internal hydrocarbons in insects (Schal et al. 1994; Young et al. 1999), possibly because oenocytes (specialized cells responsible for CHC expression; Billeter et al. 2009) are also involved in lipid storage and metabolism (Gutierrez et al. 2007). Adaptation to severe desiccation stress in *D. melanogaster* has also been shown to cause significant changes in CHCs in females, along with a corresponding decrease in fecundity (Kwan et al. 2008; Kwan and Rundle 2009). Consistent with such a trade-off in *D. serrata*, female fecundity has been shown to be highly sensitive to resource availability in the adult diet (Chenoweth et al. 2007) and the same dietary manipulation also affects female CHCs (T. Gosden and S. Chenoweth, unpubl. data), suggesting the presence of a shared resource pool underlying these traits. A direct experimental test of this trade-off hypothesis is nevertheless an important goal for future work in this species.

Sexual selection on CHCs in *D. serrata* is interpreted as arising from mate preferences and indirect evidence supports this (Higgie and Blows 2008; Delcourt et al. 2010). Nevertheless, two-stimulus choice trials on their own cannot experimentally separate the effects of intrasexual competition from mate preferences. Although female–female competition during male choice trials is unlikely (females do not interact with one another; Hoikkala and Crossley 2000), males will fight (Hoikkala et al. 2000). Ultimately, however, females can prevent males from mounting and achieving intromission (Hoikkala et al. 2000), suggesting a central role for female mate choice. Detailed behavioral studies that separate the effects of intra- and intersexual selection on CHCs are nevertheless another important component of future research.

Our comparisons of nonlinear selection were replicated across a spatial scale rarely seen in such analyses, consisting of more than 1700 independent mate choice trials involving nine separate populations recently collected from across much of the range of *D. serrata*. Comprehensive studies of the form and strength of sexual selection on females, and comparisons with that acting on males, are rare and restricted to single populations. Bussière et al. (2008) compared sexual selection on males and females for a set of morphological traits in a wild population of dance flies (*Rhamphomyia longicauda*), finding contrasting directional selection on wing and tibia length in the two sexes but no significant nonlinear selection in either sex. Quadratic selection was detected by LeBas et al. (2003) on pinnate scale area in females of another species of dance fly (*R. longicauda*), but was found to be

escalating (concave) as opposed to stabilizing. A directional male preference was recently inferred for larger female yellow breast patches in the rock sparrow (*Petronia petronia*), although selection gradients were not estimated in this case (Griggio et al. 2009). Replication across populations may be important in such studies because spatial variation in sexual selection may be common, as recently demonstrated for CHCs among these nine populations of *D. serrata* using the current data. In particular, differences among populations in both linear and nonlinear sexual selection on females were associated with neutral population genetic structure; in males, differences in linear sexual selection were associated with the presence versus absence of a related species, *D. birchii* (no significant large-scale associations were found in males for variation in nonlinear selection; Rundle et al. 2008). With respect to the current article, however, there is no evidence that the difference between the sexes in nonlinear sexual selection among these nine populations is associated with either geographic distance or the presence versus absence of *D. birchii* (H. Rundle, unpubl. data). Nevertheless, quantitative comparisons of nonlinear selection are statistically challenging because selection is quantified on different traits in every sex and population; this is an area in need of further methodological development.

Male mate choice may be underestimated, in part because common methods such fixed-stimulus mate choice assays are unable to detect nonlinear preferences (Wagner 1998). More generally, quadratic and especially correlational gradients are often not published even when they can be obtained (Kingsolver et al. 2001), and the strength of quadratic selection may often be underestimated due to an incomplete application of appropriate analytical techniques (Blows and Brooks 2003; Stinchcombe et al. 2008). Our results demonstrate that male and female mate preferences can differ with important consequences for the sexual selection they generate. Our results are also consistent with theory suggesting that males may gain direct fecundity benefits from their choice of mates, although direct experimental tests remain needed.

ACKNOWLEDGMENTS

We thank B. Foley, M. Higgie, E. Hine, G. Joseph, D. Petfield, and A. Skroblin for laboratory assistance. The article was improved by comments from M. Webster and two anonymous reviewers. This research was funded by grants from the Australian Research Council and the University of Queensland to SFC and HDR, and was performed in the laboratory of M. Blows at the University of Queensland.

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Associate Editor: M. Webster

Supporting Information

The following supporting information is available for this article:

Appendix S1. Comparing linear sexual selection between the sexes.

Table S1. Results of likelihood ratio tests ($df = 8$ in all cases) for overall differences in linear (directional) sexual selection between the sexes separately for each population.

Table S2. Partial regression coefficients indicating the standardized strength of nonlinear (quadratic selection: γ_{ii} ; correlation selection: γ_{ij}) on male (lower left) and female (upper right) logcontrast-transformed CHCs from nine populations of *D. serrata*. Values, but not significance, differ from those presented in Rundle et al. (2008) due to a failure to double the quadratic regression coefficients in the previous manuscript.

Table S3. Eigenvectors and their associated eigenvalues corresponding to the major axes of the quadratic response surface for females from all nine populations as obtained from a canonical rotation of the γ matrix of nonlinear selection gradients separately by population.

Table S4. Eigenvectors and their associated eigenvalues corresponding to the major axes of the quadratic response surface for males from all nine populations as obtained from a canonical rotation of the γ matrix of nonlinear selection gradients separately by population.

Supporting Information may be found in the online version of this article.

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