

# Genetic Constraints on the Evolution of Mate Recognition under Natural Selection

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**ABSTRACT:** Field populations of *Drosophila serrata* display reproductive character displacement in cuticular hydrocarbons (CHCs) when sympatric with *Drosophila birchii*. We have previously shown that the naturally occurring pattern of reproductive character displacement can be experimentally replicated by exposing field allopatric populations of *D. serrata* to experimental sympatry with *D. birchii*. Here, we tested whether the repeated evolution of reproductive character displacement in natural and experimental populations was a consequence of genetic constraints on the evolution of CHCs. The genetic variance-covariance (**G**) matrices for CHCs were determined for populations of *D. serrata* that had evolved in either the presence or absence of *D. birchii* under field and experimental conditions. Natural selection on mate recognition under both field and experimental sympatric conditions increased the genetic variance in CHCs consistent with a response to selection based on rare alleles. A close association between **G** eigenstructure and the eigenstructure of the phenotypic divergence (**D**) matrix in natural and experimental populations suggested that **G** matrix eigenstructure may have determined the direction in which reproductive character displacement evolved during the reinforcement of mate recognition.

**Keywords:** natural selection, reinforcement, mate recognition, **G** matrix, experimental evolution.

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The reinforcement of mate recognition by natural selection may play an important role in the evolution of mate recognition in many animals, resulting in reproductive character displacement (Dobzhansky 1951). There are now a number of examples of reproductive character displacement in sympatric populations of closely related species across diverse taxonomic groups (Butlin 1989; Howard 1993; Noor 1995; Saetre et al. 1997). However, a dem-

onstration that a pattern of reproductive character displacement has been the result of natural selection on mate recognition has been much more elusive (Butlin 1987; Howard 1993; Noor 1999).

We have taken an experimental evolutionary approach to the question of how reproductive character displacement evolves (Higgie et al. 2000). *Drosophila serrata* and *Drosophila birchii* have overlapping distributions along the east coast of Australia (Ayala 1965) and are capable of producing viable and fertile hybrids (Ayala 1965; Blows 1998). Natural populations of *D. serrata* sympatric with *D. birchii* display the classic pattern of reproductive character displacement in cuticular hydrocarbons (CHCs), the mechanism of mate recognition between the two species (Blows and Allan 1998). Allopatric field populations placed in experimental sympatry for nine generations in the laboratory responded to natural selection by changing their CHCs to resemble those natural sympatric populations (Higgie et al. 2000). The CHCs of sympatric field populations exposed to experimental sympatry did not evolve, presumably because they had already responded to the presence of *D. birchii* under field conditions.

The repeated evolution of reproductive character displacement by independent geographic populations in response to sympatry in the field and to experimental sympatry in the laboratory suggested that reproductive character displacement may have been constrained to evolve in a particular direction. There are two hypotheses that are not mutually exclusive that may account for these repeatable evolutionary responses. First, the position of *D. birchii* in multivariate CHC space may have resulted in a single selective optimum toward which the CHCs of *D. serrata* evolved in order to optimize specific mate recognition in the presence of *D. birchii*. As long as genetic variation is present, the populations will eventually reach a single optimum irrespective of the pattern of genetic covariances between CHCs (Zeng 1988; Barton and Turelli 1989). Second, multiple peaks may exist, and the genetic covariances between CHCs may have constrained the direction in which the *D. serrata* populations evolved. The extent to which the direction of evolution is influenced

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by genetic constraint is a long-standing problem (Arnold 1992), and it is this explanation for the repeatable evolutionary responses that we consider in this article.

The genetic variance-covariance matrix ( $\mathbf{G}$ ) describes the genetic (co)variance of multiple traits and determines the multivariate response to selection, at least over the short term (Lande 1979). The eigenstructure of  $\mathbf{G}$ , and in particular the first principal component,  $g_{\max}$ , has been used to determine whether divergent natural populations have been constrained to evolve in the direction of greatest genetic variance (Schluter 1996; Arnold and Phillips 1999). However, unless  $\mathbf{G}$  eigenstructure remains constant during evolutionary change, its usefulness in predicting the direction of evolution, particularly over the longer term, may be limited (Turelli 1988). The extent to which selection will change the eigenstructure of  $\mathbf{G}$  depends on the genetic basis of the traits under consideration (Barton and Turelli 1987). If the pleiotropic genetic correlations between traits are the result of numerous alleles at each locus with a multivariate normal distribution of effects (Lande 1980),  $\mathbf{G}$  may remain relatively constant under selection (but see Turelli 1988 for a list of further restrictive assumptions). In contrast, if the distribution of allelic effects is leptokurtic and the response to selection is based on initially rare alleles, then genetic variances and covariances may change substantially (Turelli 1984; Barton and Turelli 1987). The effects of intermediate genetic architectures between these two extremes on genetic correlations do not suggest that either case may be more general than the other (Slatkin and Frank 1990).

Theoretical uncertainty concerning the stability of  $\mathbf{G}$  under selection has inspired a number of empirical comparisons of  $\mathbf{G}$  across various taxonomic levels that have shown that  $\mathbf{G}$  may remain constant in many instances (see reviews in Podolsky et al. 1997; Arnold and Phillips 1999; Roff 2000). Unfortunately, comparisons across populations or species must assume that the populations under study have a common ancestral population and that they are representative of the changes in  $\mathbf{G}$  through time (Podolsky et al. 1997). In addition, comparative approaches are limited by their inability to infer directly the evolutionary processes responsible for the patterns observed (Wilkinson et al. 1990; Arnold 1992). Direct experimental tests of the stability of  $\mathbf{G}$  under selection are less common, and they have been able to attribute changes directly to selection (Shaw et al. 1995). However, it is difficult to determine whether the intensity of selection employed to induce changes in such studies reflects field conditions and whether the traits under analysis in the laboratory experience natural selection in the field (Arnold 1992). Laboratory experiments that complement field-based studies are required to move beyond descriptions of genetic variance-covariances and selection (Barton and Turelli 1989).

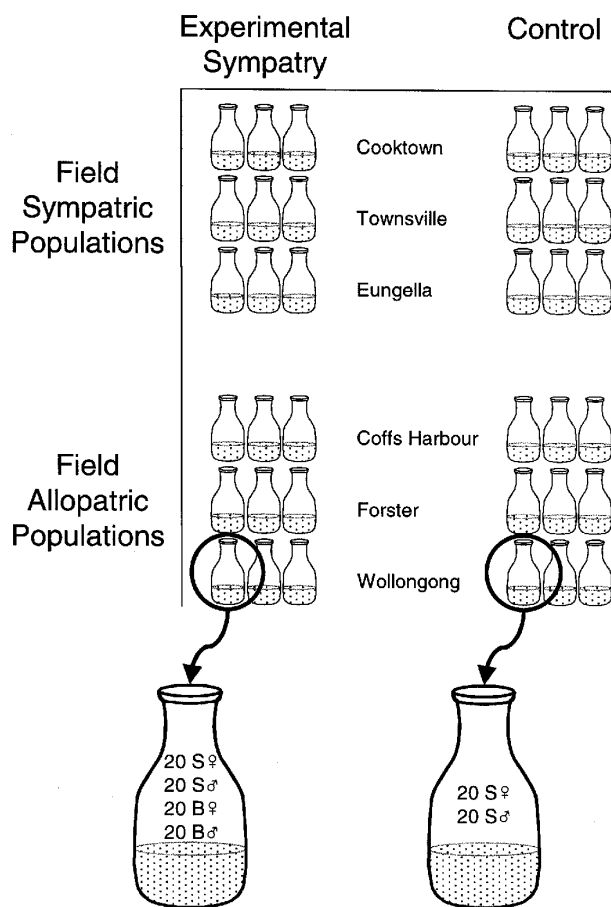
Here we combine the strengths of comparative and experimental approaches in a single experimental design to investigate the genetic basis of the repeated evolution of reproductive character displacement in *D. serrata* populations under field and experimental conditions. First, we use the comparative approach by comparing  $\mathbf{G}$  of the CHCs in field allopatric and sympatric populations to determine whether  $\mathbf{G}$  had been changed by natural selection on mate recognition in natural populations. We then determine whether the eigenstructure of  $\mathbf{G}$  was associated with the direction in which populations had evolved under field conditions. Second, we use an experimental approach to determine whether natural selection on mate recognition under experimental conditions changed  $\mathbf{G}$  and whether  $\mathbf{G}$  had constrained the experimental populations to evolve in the same direction as the field sympatric populations.

To compare  $\mathbf{G}$  matrices and to determine whether  $\mathbf{G}$  eigenstructure was associated with the direction of evolution, we employ common principal components (CPC) analysis (Flury 1988). Common principal components analysis has been recently adopted by evolutionary biologists (Phillips and Arnold 1999) as a way to determine whether eigenstructure is shared among  $\mathbf{G}$  matrices. In contrast, associating  $\mathbf{G}$  eigenstructure and the direction of evolution has relied on the angular comparison between  $g_{\max}$  and the major axis of phenotypic variation among divergent populations (Schluter 1996; Arnold and Phillips 1999). We argue that testing for associations between  $g_{\max}$  and the direction of evolution has limited statistical or biological validity, and we develop an approach based on CPC analysis to determine whether  $\mathbf{G}$  eigenstructure is associated with the direction of evolution.

## Methods

### *Natural Selection Experiment*

We exposed three field sympatric populations and three field allopatric populations of *Drosophila serrata* to an experimental application of sympatry (treatment) with *Drosophila birchii* for nine generations (Higgie et al. 2000). Control (allopatric) populations were maintained for each of the six geographic populations, which resulted in 12 experimental populations in total. The experimental design is summarized in figure 1, but full details of how we conducted the natural selection experiment can be found in Higgie et al. (2000). In the generation after selection had ceased, five *D. serrata* males (sires) were each mated to two virgin females (half-sib design) in each of the 12 populations, after which females were separated singly into vials to oviposit. When the offspring eclosed, two female and two male virgins from each half-sib family were held



**Figure 1:** Schematic representation of the experimental design for the natural selection experiment. Twelve experimental populations were established, a treatment and control population from each of the six localities of Cooktown, Townsville, Eungella, Coffs Harbour, Forster, and Wollongong. Three culture bottles were maintained per population. For experimental sympatry (treatment) populations, each bottle contained 20 *Drosophila serrata* females ( $S♀$ ) and 20 *D. serrata* males ( $S♂$ ) from the respective locality and 20 *Drosophila birchii* females ( $B♀$ ) and 20 *D. birchii* males ( $B♂$ ). Control populations were maintained in exactly the same way as the treatment populations except that they contained no *D. birchii*. All flies placed in bottles were 1-d-old virgins so that previous experience did not affect mate choice, and mate choice for *D. serrata* individuals in treatment populations began in the presence of *D. birchii*.

separately for 4 d, after which the CHCs of individual flies were analyzed using gas chromatography (Blows and Allan 1998).

A canonical discriminant analysis (CDA) on the population means of log contrasts was used to describe the multivariate variation in CHCs between sympatric and allopatric populations of *D. serrata* and how they responded to natural selection when exposed to experimental sympatry (Higgie et al. 2000). The use of log contrasts in the analysis of the multivariate CHCs of *D. serrata* has

been described in detail elsewhere (Blows and Allan 1998). Briefly, the area of each of 10 individual CHC peaks was divided by the total area of all 10 peaks from an individual's profile. Log contrasts were taken to remove the unit-sum constraint in this set of proportions (Aitchison 1986), reducing the data set to nine variables. The CDA and subsequent analysis of the selection response in Higgie et al. (2000) was conducted on the population means to avoid pseudoreplication because each geographic population, rather than individuals within a population, represented an independent application of allopatry or sympatry in the field for the purposes of hypothesis testing. The first four canonical variates were used in the analyses below and accounted for 99.4% of the variation in CHCs among the 12 experimental populations.

#### *Genetic Analysis of CHCs*

To conduct a genetic analysis of the canonical variates, it was necessary to calculate the canonical variate scores for each individual using the linear equations for each canonical variate from the CDA on population means. This was done by multiplying the unstandardized canonical discriminant function coefficients by the log contrasts and summing across the nine log contrasts (and constant) for each individual.

We present two genetic analyses. First, additive genetic components of variance were estimated using restricted maximum likelihood (REML) under the model

$$Y_{ijkl} = \mu + P_i + S_{j(i)} + D_{k(ij)} + e_{l(ijk)},$$

in which  $P$  was replicate geographic population,  $S$  was sire nested within population, and  $D$  was female nested within sire within population. Narrow-sense heritabilities were calculated using the among-sire variance components for the four treatment combinations: field allopatric control populations, field sympatric control populations, field allopatric treatment populations, and field sympatric treatment populations. This resulted in heritability estimates that were unbiased by replicate population-level differences in mean. By using sires from the three geographic populations to obtain estimates of genetic variances within each of the regions of allopatry and sympatry, we are assuming that the genetic basis of the pheromone system is constant across these randomly chosen replicate populations. Unfortunately, we do not have sufficient sample size within each population to make a convincing test of this assumption.

The sample size within each treatment combination was modest (15 sires) in comparison to genetic experiments conducted on single populations (we have 12 populations requiring genetic analysis). Our intention, however, was

not to determine the significance of individual heritability estimates using this analysis, as we directly demonstrate that genetic variance exists in these traits through their response to selection. Instead, these narrow-sense heritabilities were compared with approximations of genetic variances and covariances from a second genetic analysis conducted on sire means. Sire-mean estimates of genetic variance are potentially subject to bias from within-family sources of variance when traits are measured on the same individuals (Lynch and Walsh 1998). We show that the heritabilities based on REML estimates of additive genetic variance give qualitatively similar results to the sire-mean approximations with regard to the effect of natural selection on genetic variances.

Genetic variances and covariances from sire means for the four canonical variates for each sex were used as estimates of  $\mathbf{G}$  for each of the four treatment combinations. We used this method of approximation for two reasons. First, since the same canonical variates of CHCs were calculated for both males and females, the mean of each sex was taken for each full-sib family within sires so that the genetic covariance between males and females for each canonical variate could be estimated (Lynch and Walsh 1998) and subsequent analyses could be conducted on male and female components of the CHC profile separately. Second, the Flury (1988) method of matrix comparison, used to analyze the response of  $\mathbf{G}$  to natural selection, required product-moment-based variance-covariance matrices for parametric significance testing.

*Effect of Natural Selection on G Matrices*

We tested three specific hypotheses concerning the stability of  $\mathbf{G}$  under natural selection (fig. 2). Rather than comparing pair-wise estimates of heritability or genetic correlations across allopatric and sympatric treatments, the method of common principal components analysis (Flury 1988) was used to test for a hierarchy of similarity between  $\mathbf{G}$  matrices using the CPC program developed by Phillips (1998). There are two problems to be considered when applying this parametric methodology to genetic components of variance (Phillips and Arnold 1999). First, multivariate normality is required. The CHC data is particularly well behaved in this regard since ordination procedures tend to produce variables (i.e., canonical variates in our case) more likely to be normally distributed than original variables, and the analysis of means (sire means in our case) is recommended when extreme caution with multivariate normality is warranted (Pimentel 1979). Second, the appropriate degrees of freedom for the genetic components of variance are required to be known for the calculation of the log-likelihood ratios. It has not yet been established how the appropriate degrees of freedom should

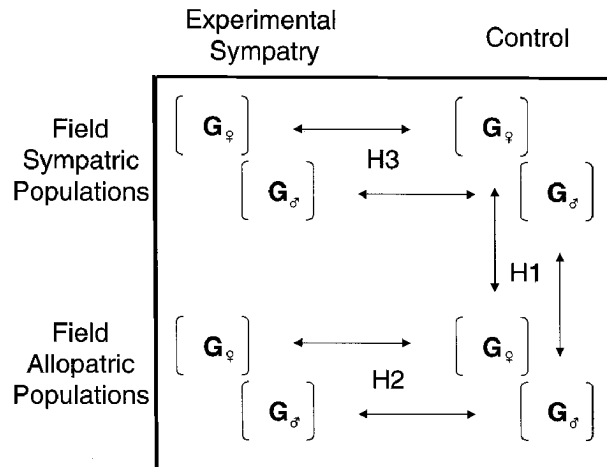


Figure 2: Comparisons between  $\mathbf{G}$  matrices made within the experimental design of the natural selection experiment. Each pair of male and female  $\mathbf{G}$  matrices are schematic representations of those submatrices in table 1 that are used in the analyses. Each matrix comparison made is indicated by an arrow. The comparisons are grouped to represent the three hypotheses under consideration: *H1*, Had the reinforcement of mate recognition by natural selection in the field changed  $\mathbf{G}$ ? *H2*, Did  $\mathbf{G}$  change in field allopatric populations that responded to natural selection on mate recognition when exposed to experimental sympatry? *H3*, Did  $\mathbf{G}$  change in field sympatric populations that did not respond to natural selection on mate recognition when exposed to experimental sympatry?

be determined for such analyses (Phillips and Arnold 1999). The number of sires for each  $\mathbf{G}$  matrix represented a lower limit to the number of degrees of freedom because it does not take into account the contribution of the number of observations within sires. We have therefore used 15 df (the number of sires in each analysis) for the matrix comparisons as a conservative approach to this problem. Using such an approach may increase the probability of concluding that the matrices being compared are less different than they actually are.

The Flury method was particularly useful here as it enabled the similarity between eigenvectors, the properties of the matrices of most interest when determining the influence of  $\mathbf{G}$  on the direction of evolution, to be directly assessed. The degree of similarity between any two matrices may be classified into one of five levels (Flury 1988): (1) both eigenvalues and eigenvectors are common (equality); (2) eigenvectors are common but eigenvalues differ by a set proportion (proportionality); (3) all eigenvectors are common but eigenvalues differ in a nonproportional manner (CPC); (4) some eigenvectors are common but others differ (partial principal components, designated CPC[2], for instance, if two eigenvectors are common; note that in the present case, since there are four canonical variates that comprise  $\mathbf{G}$ , only the CPC[1] and CPC[2] models can

be tested); (5) eigenvalues and eigenvectors differ (unrelated).

Model selection may be accomplished in three ways (Phillips and Arnold 1999). For each comparison, we present the log-likelihood ratio ( $\chi^2$ ) for each level of the hierarchy tested against the unrelated null hypothesis, the so-called jump-up approach. Significance testing using the jump-up approach is conducted by summing the partial  $\chi^2$ s (and their associated df) for each level in the hierarchy from the "bottom up" until a significant summed  $\chi^2$  is obtained, and then the level below is the accepted model. In addition to the jump-up approach, we used the step-up and model-building approaches to help determine the overall best-fitting model when decisions were made on borderline probability values involved in the jump-up approach. In the step-up approach, one proceeds up the hierarchy from unrelated structure until a model is rejected as indicated by a significant probability value associated with the relevant partial  $\chi^2$ , and then the level below that in the hierarchy is the accepted model. The model-building approach chooses the model with the minimum value of the Akaike Information Criterion to determine the best-fitting model in relation to the number of parameters used in the model. In most cases, these approaches were in close agreement as to the most appropriate model.

After the model representing the level of similarity between two matrices had been identified, it was desirable to relate the eigenvectors that were found to be common back to the original canonical variates that had responded to natural selection. Directional selection is likely to change genetic variances (Barton and Turelli 1987) and covariances (Bohren et al. 1966; Turelli 1988) as allele frequencies change. We therefore expected that those eigenvectors of  $\mathbf{G}$  most strongly associated with traits that responded to selection could be those that would change. So, after each comparison, we present the linear equations for each of the common principal components identified by the analysis and determine which canonical variates contributed significantly to each principal component. Significance was determined by the criterion suggested by Mardia et al. (1979); those canonical variates with coefficients  $> 0.7$  times the largest coefficient in an eigenvector were considered to have a significant association with that eigenvector. While this is essentially only a rule of thumb, it greatly simplified the discussion of each comparison and suited this particular data set well, as in most cases only a single canonical variate made a large contribution to any particular eigenvector. We have retained the notation of Schluter (1996) in referring to the eigenvector of greatest genetic variance as  $g_{\max}$ , and we refer to subsequent eigenvectors in descending order of their eigenvalues as  $g_2$ ,  $g_3$ , and  $g_4$ .

### *G Matrices and the Direction of Evolution*

Previous analyses searching for an association between  $\mathbf{G}$  and the direction of evolution have determined the angle between the dominant eigenvector of  $\mathbf{G}$  ( $g_{\max}$ ) and  $z$ , the major axis of phenotypic divergence between populations (Schluter 1996; Arnold and Phillips 1999). Although this approach has been an important first step and has been successful in uncovering associations between  $g_{\max}$  and divergence, it has two major limitations. First,  $g_{\max}$  represents the direction in which the greatest response to selection may occur, but it will not usually be the only axis for which genetic variation exists. A response to selection could easily be influenced by one of the other eigenvectors of  $\mathbf{G}$  if natural selection favored that direction over the direction of  $g_{\max}$ . Second, from a statistical point of view, the comparison of the dominant eigenvectors from two data sets tells one very little about the similarity in eigenstructure between them (Krzanowski 1979). For example, we might find that  $g_{\max}$  and  $z$  have a large angle between them, and yet the subspaces described by the eigenstructure of  $\mathbf{G}$  and the population means could still be identical;  $g_{\max}$  may simply be associated with the second principal axis of population divergence and not the first. Two sets of principal components must first be rotated to maximum similarity before such comparisons are very meaningful (Krzanowski 1979). Therefore, from both a biological and statistical perspective, testing for the overall similarity between  $\mathbf{G}$  and the direction of phenotypic divergence in the same multivariate space may result in a more comprehensive and robust test of the role of genetic constraint on the direction of evolution.

Here we have taken the same matrix comparison methodology used in the comparison of  $\mathbf{G}$  matrices to determine whether  $\mathbf{G}$  constrained the direction in which *D. serrata* populations evolved in response to sympatry in the field and in experimental sympatry. We introduce the  $\mathbf{D}$  matrix to describe phenotypic divergence of population means in multivariate space. The first principal component of  $\mathbf{D}$  is equivalent to  $z$ , the major axis of variation among population means employed by Schluter (1996). In our case, the  $\mathbf{D}$  matrix was the variance-covariance matrix calculated across six population means of the four canonical variates for any particular comparison. In this fashion, phenotypic divergence between field allopatric and field sympatric populations was represented by the variance-covariance matrix across the population means of the six control populations. Similarly, phenotypic divergence under experimental conditions was represented by the variance-covariance matrix across the six population means of the field allopatric control and experimental sympatry (treatment) populations. Since field sympatric populations did not respond to selection, we do not consider

the phenotypic divergence between field sympatric control and treatment populations. We refer to the eigenvectors of  $\mathbf{D}$  in descending order of their eigenvalues as  $d_{\max}$ ,  $d_2$ ,  $d_3$ , and  $d_4$ .

## Results

### Natural Selection in Natural Populations

*Effect of Natural Selection on G Matrices.* To determine the effect of natural selection in the field on  $\mathbf{G}$ , the  $\mathbf{G}$  matrix from the field allopatric control populations was compared to that from the field sympatric control populations (*H1*, fig. 2). Visual inspection of both matrices (table 1) revealed that all female genetic variances and three of the four male genetic variances were higher in the sympatric control populations, with male CV3 being the exception. The narrow-sense heritabilities calculated from REML estimates of additive genetic variances displayed the same pattern with the exception of female CV1, where in both sets of populations the REML analysis set estimates to zero. The increase in genetic variance resulted in the hypothesis of equality between matrices being rejected by the Flury matrix comparisons of  $\mathbf{G}$  between field allopatric and sympatric populations for both sexes (table 2). In females, the jump-up approach indicated that the matrices were proportional, but in males proportionality was rejected. Overall it appeared that the eigenvalues of  $\mathbf{G}$  had changed between sympatric and allopatric field populations, which was reflected in increased genetic variances in sympatric populations. There was no evidence supporting a change in  $\mathbf{G}$  eigenstructure between these populations (table 2).

*G Matrices and the Direction of Evolution.* The  $\mathbf{D}$  matrices representing the divergence of sympatric and allopatric populations in the field are presented in table 3. The comparison of  $\mathbf{G}$  and  $\mathbf{D}$  (table 4) indicated that for both sexes, all eigenvectors were common between  $\mathbf{G}$  and  $\mathbf{D}$ . Inspection of the principal component structure of the CPC model indicated that in females, the direction of greatest phenotypic divergence ( $d_{\max}$ ) was associated with  $g_2$  ( $-0.25CV1 + 0.95CV2 - 0.06CV3 + 0.20CV4$ ), which had a significant contribution from CV2. This was consistent with the major axis of divergence between field allopatric and sympatric populations that occurred along CV2 (fig. 3a). In males there were three eigenvectors of  $\mathbf{D}$  ( $d_{\max}$ ,  $d_2$ ,  $d_3$ ) with very similar eigenvalues explaining 34%, 33%, and 32.6% of the variance, respectively. One of these,  $d_3$ , was in the direction of  $g_4$  ( $-0.21CV1 + 0.82CV2 + 0.47CV3 + 0.26CV4$ ) and represented CV2, the axis of divergence between field allopatric and sympatric populations (fig. 3a).

### Natural Selection in Experimental Populations

*Response to Natural Selection.* The response to natural selection of field allopatric and sympatric populations of *Drosophila serrata* exposed to experimental sympatry with *Drosophila birchii* is displayed in figure 3. The response to selection on the first two canonical variates, CV1 and CV2 (fig. 3a), has been analyzed elsewhere using univariate split-plot ANOVAs (Higgie et al. 2000); field allopatric populations responded to natural selection on CV1 and CV2 in females and CV1 in males, but field sympatric populations did not evolve. Significant evolutionary change was also detected on CV3 in field allopatric females (fig. 3b) as indicated by the significant interaction between the field origin (allopatry or sympatry) and the treatment (control or experimental sympatry; split-plot ANOVA;  $F = 17.937$ ,  $df = 1, 4$ ,  $P = .013$ ). Similarly, an evolutionary response was suggested on CV4 in males (fig. 3c;  $F = 6.954$ ,  $df = 1, 4$ ,  $P = .058$ ).

*Effect of Natural Selection on G Matrices.* To determine the effect of natural selection in experimental sympatry on  $\mathbf{G}$ , the  $\mathbf{G}$  matrix from the field allopatric control populations was compared to that of the field allopatric treatment populations that had been exposed to experimental sympatry (*H2*, fig. 2). Visual inspection of the two matrices (table 1) revealed that all female genetic variances and three of the four male genetic variances were higher in the experimental sympatry populations, just as in the field sympatric populations. Once again, male CV3 was the only trait not to follow this pattern. Furthermore, REML-based, narrow-sense heritabilities displayed the same pattern, again with the exception of female CV1, for which the analysis set both estimates to zero. In females, equality of the two matrices was rejected, which supported a change in genetic variances under experimental conditions as in the field (table 5). In males, the matrices were found to be equal (table 5), which suggests that changes in male genetic variances under experimental conditions were not significant. However, in this case, the step-up and model-building approaches differed from the results of the jump-up approach and favored the CPC model, which supported a change in genetic variance under experimental sympatry conditions.

*Effect of Genetic Drift on G Matrices.* We did not detect any significant evolutionary change in CHCs between field sympatric populations subjected to experimental sympatry and those same field populations held in allopatry. Therefore, if natural selection on mate recognition was responsible for the increase in genetic variance in field allopatric populations exposed to experimental sympatry, field sympatric populations exposed to experimental sympatry

**Table 1: G matrices of field sympatric and allopatric populations of *Drosophila serrata* from control and experimental sympatry treatments in the natural selection experiment**

	$h^2$	Female				Male			
		CV1	CV2	CV3	CV4	CV1	CV2	CV3	CV4
Field allopatric control populations:									
Female:									
CV1	0	<b>32.945</b>	.458	-.358	-.076	.040	.049	-.020	-.133
CV2	.322	<b>7.548</b>	<b>8.235</b>	-.212	-.246	.041	.378	-.049	-.077
CV3	0	<b>-2.516</b>	<b>-.746</b>	<b>1.497</b>	.022	-.372	-.459	.270	.731
CV4	.107	<b>-.884</b>	<b>-1.421</b>	<b>.055</b>	<b>4.066</b>	-.453	-.422	-.310	.354
Male:									
CV1	0	1.123	.569	-2.204	-4.424	<b>23.490</b>	.385	.371	-.288
CV2	0	.609	2.331	-1.206	-1.827	<b>4.010</b>	<b>4.615</b>	-.554	-.711
CV3	.079	-.322	-.389	.912	-1.726	<b>4.971</b>	<b>-3.289</b>	<b>7.634</b>	.449
CV4	0	-1.413	-.410	1.653	1.319	<b>-2.578</b>	<b>-2.821</b>	<b>2.290</b>	<b>3.412</b>
Field sympatric control populations:									
Female:									
CV1	0	<b>41.982</b>	.252	.175	-.359	-.065	-.038	.022	.058
CV2	.427	<b>7.817</b>	<b>22.864</b>	-.052	-.190	-.104	-.235	-.257	.083
CV3	.198	<b>4.131</b>	<b>-.905</b>	<b>13.292</b>	-.406	.221	-.198	.519	.183
CV4	.155	<b>-8.082</b>	<b>-3.147</b>	<b>-5.142</b>	<b>12.039</b>	-.160	.084	-.115	.249
Male:									
CV1	.293	-2.596	-3.076	4.987	-3.451	<b>38.442</b>	.844	.421	-.618
CV2	.379	-.965	-4.365	-2.810	1.136	<b>20.360</b>	<b>15.148</b>	.068	-.497
CV3	0	.373	-3.190	4.905	-1.034	<b>6.774</b>	<b>.683</b>	<b>6.720</b>	-.483
CV4	.966	1.138	1.194	2.022	2.623	<b>-11.597</b>	<b>-5.860</b>	<b>-3.786</b>	<b>9.161</b>
Field allopatric treatment populations:									
Female:									
CV1	0	<b>41.695</b>	-.403	.235	-.041	.080	-.204	-.228	.623
CV2	.732	<b>-10.596</b>	<b>16.570</b>	-.438	-.362	-.288	.171	.355	.047
CV3	.220	<b>4.429</b>	<b>-5.206</b>	<b>8.533</b>	.481	-.047	-.449	.104	-.270
CV4	.439	<b>-.815</b>	<b>-4.583</b>	<b>4.369</b>	<b>9.680</b>	.070	-.581	.154	-.121
Male:									
CV1	.241	3.120	-7.112	-.834	1.322	<b>36.926</b>	.314	.461	.329
CV2	.361	-2.994	1.580	-2.977	-4.098	<b>4.325</b>	<b>5.141</b>	-.284	-.017
CV3	.011	-3.293	3.236	.677	1.075	<b>6.271</b>	<b>-1.439</b>	<b>5.003</b>	.163
CV4	.210	9.545	.452	-1.874	-.893	<b>4.743</b>	<b>-0.090</b>	<b>.864</b>	<b>5.628</b>
Field sympatric treatment populations:									
Female:									
CV1	0	<b>81.090</b>	.406	.592	-.722	.881	.552	-.488	-.248
CV2	.481	<b>13.460</b>	<b>13.580</b>	-.310	-.364	.418	.002	-.749	-.218
CV3	.833	<b>24.402</b>	<b>-5.238</b>	<b>20.956</b>	-.468	.494	.659	.012	-.192
CV4	.173	<b>-20.480</b>	<b>-4.230</b>	<b>-6.756</b>	<b>9.932</b>	-.755	-.685	.304	.362
Male:									
CV1	0	55.586	10.799	15.854	-16.689	<b>49.147</b>	.546	-.256	-.298
CV2	0	11.915	.015	7.237	-5.174	<b>9.173</b>	<b>5.751</b>	-.236	-.555
CV3	0	-9.029	-5.665	.112	1.966	<b>-3.684</b>	<b>-1.163</b>	<b>4.216</b>	.206
CV4	0	-6.052	-2.183	-2.763	3.098	<b>-5.662</b>	<b>-3.609</b>	<b>1.147</b>	<b>7.364</b>

Note: Narrow-sense heritabilities ( $h^2$ ) from restricted maximum likelihood estimates of variance components are given. Genetic variances from sire-mean analyses are along the diagonal of the  $8 \times 8$  matrices, genetic covariances are below the diagonal, and genetic correlations are above the diagonal. Male and female G used in the analyses are in bold.

**Table 2:** Comparison of **G** matrices between field allopatric and sympatric control populations of *Drosophila serrata* (H1, fig. 2)

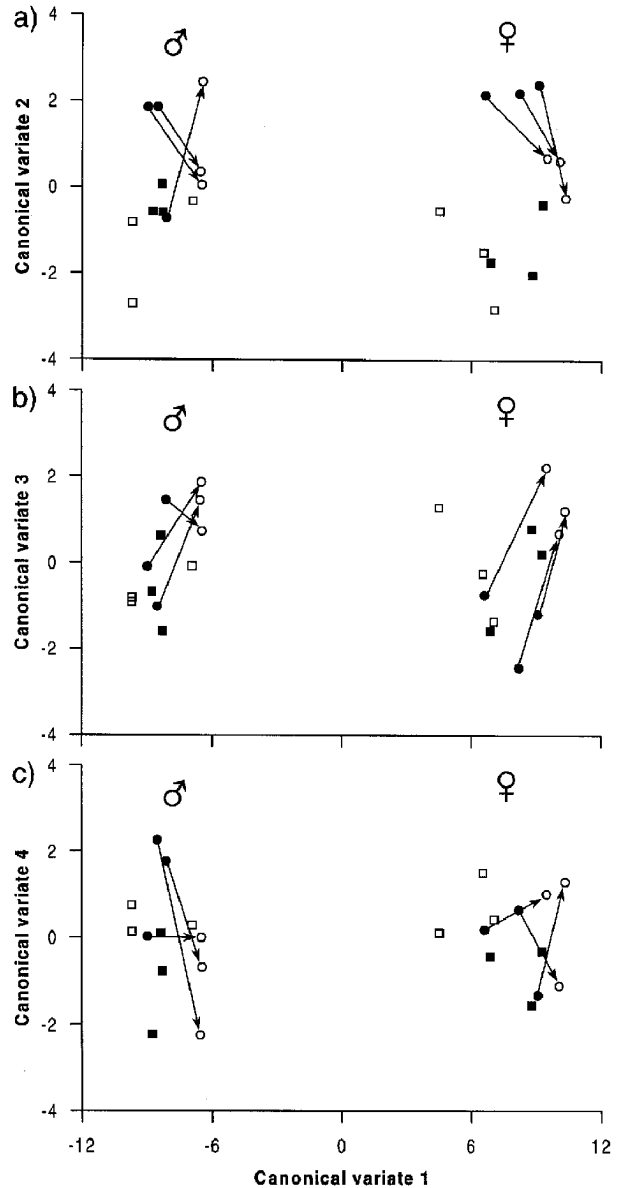
Hierarchy	df	Female		Male	
		$\chi^2$	<i>P</i>	$\chi^2$	<i>P</i>
Equality	10	24.54	.006	22.79	.012
Proportional	9	10.03	.348	19.76	.020
CPC	6	4.78	.572	11.44	.076
CPC(2)	5	1.86	.869	3.13	.679
CPC(1)	3	1.52	.677	2.48	.480
Unrelated					

should not show the same pattern. To test this, we compared **G** between control and experimental sympatry treatment for field sympatric populations (H3, fig. 2). Visual inspection of the two matrices (table 1) revealed that there was no consistent change in genetic variances or heritabilities between the two sets of populations. In females, the matrices were equal (table 6). In males, the CPC model was rejected and only two eigenvectors were found in common,  $g_2$  ( $0.33CV1 - 0.28CV2 + 0.33CV3 + 0.84CV4$ ) and  $g_3$  ( $0.06CV1 - 0.51CV2 + 0.72CV3 - 0.47CV4$ ), which had significant contributions from CV4 and CV2 and from CV3 and CV4, respectively. Genetic drift has been shown to substantially alter **G** under laboratory conditions in populations of *Drosophila melanogaster* (Phillips et al. 2001). However, since our experimental design did not specifically manipulate genetic drift, we cannot rule out an effect of natural selection that was not discernible in trait means as the cause of these changes.

**G Matrices and the Direction of Evolution.** To determine whether the direction of evolution under experimental sympatry and **G** were associated, **G** in allopatric control populations (i.e., before selection) was compared to **D** generated from the means of field allopatric control and experimental sympatry populations (table 7). In females, the CPC model is almost rejected by the jump-up approach (table 8). The step-up and model-building approaches indicated that in females only two eigenvectors were common between **G** and **D**. The common eigenvectors under the CPC(2) model were  $d_2$  in the direction of  $g_2$  ( $-0.23CV1 + 0.65CV2 + 0.01CV3 - 0.72CV4$ ), which had a significant contribution from CV4, and  $d_{max}$  and  $g_4$  ( $0.08CV1 - 0.04CV2 + 0.99CV3 - 0.05CV4$ ), which represented CV3. In males, the CPC model was supported, which indicates that all eigenvectors were shared between **G** and **D**.

Overall, the association between **G** before selection and **D** was weaker in experimental populations than that displayed in field populations. There was little evidence that natural selection had significantly changed **G** eigenstruc-

ture in the nine generations. However, if natural selection had changed **G** eigenstructure but had remained undetected by the CPC analysis, it could explain the weaker association between **G** and **D** in experimental populations. If natural selection did change **G** eigenstructure, a more appropriate comparison between **G** and **D** would be be-



**Figure 3:** Response to natural selection in populations of *Drosophila serrata* exposed to experimental sympatry with *Drosophila birchii* for nine generations. Symbols represent control populations (filled circles, filled squares), experimental sympatry populations (open circles, open squares), field allopatric populations (circles), and field sympatric populations (squares). Evolutionary responses of field allopatric populations are indicated by the vectors. Field sympatric populations did not evolve.

**Table 3:** Divergence variance-covariance (**D**) matrices for field allopatric and sympatric control populations of *Drosophila serrata*

	Females				Male			
	CV1	CV2	CV3	CV4	CV1	CV2	CV3	CV4
CV1	1.309				.099			
CV2	-.110	4.279			-.227	1.496		
CV3	.529	-1.329	1.402		.108	-.266	1.254	
CV4	-.467	.776	-.611	.721	.208	.865	.663	2.718

tween the average **G** matrix and **D**. Average **G** was calculated by averaging the elements of the **G** matrices from field allopatric control (before selection) and experimental sympatry (after selection) populations (B. Walsh and M. Lynch, unpublished manuscript), and it assumes that selection remained constant over the nine generations. Using average **G** considerably improved the association between the genetic eigenvectors and the direction in which the experimental populations evolved (table 9). In both males and females, all eigenvectors were common between average **G** and **D**. This analysis suggests that **G** eigenstructure may have been changed by selection, but the CPC analysis may not have been sensitive enough to detect the change. However, we cannot discount the possibility that the larger sample size underlying average **G** may have dampened the effects of sampling error, which could have resulted in a similar improvement.

## Discussion

### *Effect of Natural Selection on G Matrices*

The genetic variance-covariance matrices of CHCs in allopatric and sympatric populations were found to be different under both field and experimental conditions. We found **G** matrices to be unequal between field allopatric and field sympatric populations as a consequence of genetic variance being substantially higher in field sympatric populations. After field allopatric populations were exposed to nine generations of experimental sympatry, we observed almost the same increase in genetic variance after this experimental manipulation as we had observed in field sympatric populations. This result indicated that natural selection on mate recognition was responsible for the increase in genetic variance under sympatric conditions.

There are at least two reasons why genetic variance may have increased in response to natural selection on mate recognition under field and laboratory conditions. First, the dynamics of genetic variance under selection critically depend on the genetic details of the selection response (Barton and Turelli 1987). Reeve (2000) has shown that genetic variance is expected to increase under allelic distributions assumed by both Gaussian (Lande 1980) and

house-of-cards (Turelli 1984) models but to a much lesser extent under the former set of assumptions. Reeve's simulations suggested that genetic variance may increase about 20% under the Gaussian model but by as much as sixfold in some traits under the house-of-cards model. Here, the increase in genetic variance ranged from about 30% to more than eightfold between field allopatric and sympatric populations, with five of the eight traits displaying an increase of more than twofold. The genetic variance of most traits therefore appeared to behave in a fashion consistent with a response to selection in the field and laboratory based on rare alleles, resulting in a substantial increase in genetic variance as these rare alleles increased in frequency (Barton and Turelli 1987). The increase in genetic variance was lower in experimental sympatry than in the field but followed the same pattern. Therefore, assuming that the level of genetic variance in field sympatric populations represented a new equilibrium level (Barton 1986) after natural selection on mate recognition, the field allopatric populations exposed to experimental sympatry do not seem to have reached equilibrium after nine generations of selection in the laboratory.

Second, assortative mating in sympatry may also have contributed to the increase in genetic variance through the generation of linkage disequilibrium (Lynch and Walsh 1998; Kirkpatrick 2000). It is not known whether natural selection in sympatry has acted directly on both sexes or whether one sex had coevolved with the other through assortative mating. Blows (1999) has previously shown that the genetic covariance between male and female compo-

**Table 4:** Comparison between the field allopatric **G** matrix and **D** matrix between field allopatric and sympatric control populations of *Drosophila serrata*

Hierarchy	df	Female		Male	
		$\chi^2$	<i>P</i>	$\chi^2$	<i>P</i>
Equality	10	20.83	.022	43.83	.000
Proportional	9	14.92	.093	42.98	.000
CPC	6	5.44	.489	4.09	.665
CPC(2)	5	4.31	.505	4.07	.539
CPC(1)	3	3.57	.311	.948	.814
Unrelated					

**Table 5:** Comparison of **G** matrices between field allopatric control and experimental sympatry populations of *Drosophila serrata* (H2, fig. 2)

Hierarchy	df	Female		Male	
		$\chi^2$	<i>P</i>	$\chi^2$	<i>P</i>
Equality	10	20.49	.025	14.03	.172
Proportional	9	13.29	.150	11.17	.264
CPC	6	9.93	.130	4.75	.577
CPC(2)	5	6.70	.244	4.53	.475
CPC(1)	3	4.50	.212	3.43	.331
Unrelated					

nents of mate recognition in populations of hybrids between these two species can change substantially, which suggests that assortative mating may make a significant contribution to the evolution of mate recognition in this system. At present, we are unable to distinguish the contribution of assortative mating to the increase in genetic variance from changes in allele frequency. Nevertheless, although assortative mating may well be operating in these populations, it is unlikely to explain the larger increases in genetic variance observed in some traits without postulating a nearly perfect phenotypic correlation between mates (see fig. 7.7 in Lynch and Walsh 1998).

It is therefore likely that we have observed an increase in the genetic variance of male and female components of mate recognition as a consequence of the increase in rare alleles after a change in the direction of natural selection. Although such dramatic increases in genetic variance as a consequence of selection have been predicted by theory, they have rarely been observed (Barton and Turelli 1987), if at all (Keightley and Hill 1989). Large increases in genetic variance are more likely to occur if the traits in question have been under strong stabilizing selection before the advent of directional selection (Keightley and Hill 1989; Burger and Lande 1994). This is because the distribution of allele frequencies under strong stabilizing selection will be U-shaped, with few loci having alleles at intermediate frequencies (Keightley and Hill 1989). Mate recognition has been considered to be under strong stabilizing selection as a consequence of the requirement of coordination between male and female components to maintain effective communication (Butlin and Ritchie 1989; Ritchie 1996), particularly in relation to traits involved in species recognition (Butlin et al. 1985; Paterson 1985). Although the genetic consequences of stabilizing selection on mate preferences have received little attention (Bakker and Pomiankowski 1995), our experiment suggests that it may have a major effect on the standing genetic variation for both male and female components of mate recognition in natural populations.

### **G** Matrices and the Direction of Evolution

The eigenstructure of **G** in field allopatric populations of *Drosophila serrata* was closely associated with the direction in which the field sympatric populations had evolved as a consequence of the presence of *Drosophila birchii*. In contrast, there was less similarity between **G** in field allopatric control populations (i.e., before selection was applied) and the direction in which field allopatric populations exposed to experimental sympatry evolved. These results seem to conflict with the common assertion that **G** may be informative over the short term with respect to the direction of phenotypic evolution (Barton and Turelli 1989; Shaw et al. 1995) but not over the longer term (Turelli 1988). However, it is important to note that by using average **G** as the predictor of directional change, the association between genetic and divergence eigenvectors was restored in experimental populations. This suggested that **G** eigenstructure may have been changed by natural selection but remained undetected by our comparisons of **G** before and after selection. Statistical power in testing the Flury hierarchy of similarity is greater at higher levels in the hierarchy (e.g., equality) than at lower levels such as partial common principal components (Phillips and Arnold 1999). Perhaps with greater sample sizes we might have detected significant changes in the eigenvectors of **G** in addition to the changes in genetic variances that we observed.

If **G** was changed by selection in the short term (experimental sympatry), which diminished the association between **G** eigenstructure before selection and the direction that populations evolved, how can **G** accurately predict the direction of phenotypic evolution over the longer term (sympatry in the field)? It has been strongly suspected on theoretical grounds that **G** would not remain constant under directional selection primarily as a consequence of the sensitivity of genetic variances (Barton and Turelli 1987) and covariances (Bohren et al. 1966; Turelli 1988; Shaw et al. 1995) to changes in allele frequency. Nevertheless, it has been the case that most comparative studies

**Table 6:** Comparison of **G** matrices between field sympatric control and experimental sympatry populations of *Drosophila serrata* (H3, fig. 2)

Hierarchy	df	Female		Male	
		$\chi^2$	<i>P</i>	$\chi^2$	<i>P</i>
Equality	10	14.01	.173	20.81	.023
Proportional	9	12.65	.179	20.81	.014
CPC	6	3.54	.738	14.53	.024
CPC(2)	5	2.30	.806	2.96	.707
CPC(1)	3	.67	.879	1.77	.622
Unrelated					

of  $\mathbf{G}$  between populations and species have tended to find a high degree of conservation of  $\mathbf{G}$  as in the present case (Arnold and Phillips 1999; Roff 2000). While this discrepancy between theory and experiment may in part be a consequence of low statistical power in experimental studies (Shaw et al. 1995), our experiment suggested that a real difference may exist between the stability of  $\mathbf{G}$  in short-term experiments and comparative studies investigating longer-term changes. For example, under some conditions, selection will change the genetic variances and covariances over the short term, but the new equilibrium values reached in the long term may sometimes be very similar to those before selection (Keightley and Hill 1989; Reeve 2000; Agrawal et al. 2001). Alternatively, there may be the opportunity for mutation to restore variation over the long term (Lande 1980). This requires that the genetic variances and covariances associated with new mutations (the  $\mathbf{U}$  matrix; Lande 1979) must be aligned with those of the original  $\mathbf{G}$  matrix (Lande 1979; Turelli 1988). There is some evidence to suggest that mutation may change  $\mathbf{G}$  in a consistent way across populations (Camara and Pigliucci 1999), but currently there is no direct evidence to suggest that  $\mathbf{G}$  remains constant after mutation occurs.

Proportionality of  $\mathbf{G}$  and  $\mathbf{D}$  matrices was never strongly supported by any of the analyses. Since the eigenvalues of  $\mathbf{G}$  and  $\mathbf{D}$  therefore differed, the magnitude of the response to selection was not associated with the amount of genetic variation in a particular direction in either the field or laboratory environments. For example, the major axis of reproductive character displacement in field populations was not in the direction of greatest genetic variance but in the direction of  $g_2$ . This result argues against simply relying on an association between  $g_{\max}$  and the direction of greatest phenotypic divergence,  $d_{\max}$ , to test for the role of genetic constraints on the direction of evolution.

Under most circumstances, for  $\mathbf{G}$  to have a lasting influence on the direction of evolution, more than one selective optimum needs to exist (Barton and Turelli 1989). The reinforcement of mate recognition may be a good candidate for the existence of multiple peaks, as the position of *D. birchii* in multivariate CHC space may be the only selective agent acting during the reinforcement process, which results in the number of selective constraints being less than the number of genetic constraints (Arnold 1992). As long as a change in the CHCs of *D. serrata* results in effective avoidance of *D. birchii* individuals during mate choice, a change in any number of directions in multivariate CHC space may be sufficient for the reinforcement of mate recognition. It is important to note, however, that our experiment cannot exclude the possibility that a selective optimum lies in the direction of the eigenvectors of  $\mathbf{G}$  by chance. This possibility should not simply be discounted as unlikely, because under strong stabilizing

selection, the orientation of  $\mathbf{G}$  might well conform to the orientation of the fitness surface (Lande 1980; Cheverud 1984; Arnold et al. 2001). An important challenge to meet in future experiments of this type will be to explicitly distinguish between the position of a selective optimum and genetic constraint.

#### *A Comment on Matrix Comparison Methodology in Evolutionary Studies*

The method of CPC has become a popular tool for the comparison of variance-covariance matrices in evolutionary studies (Phillips and Arnold 1999; Houle et al. 2002). The utility of CPC for testing evolutionary hypotheses, however, has recently been questioned by Houle et al. (2002), who make two broad points, one biological and one statistical, that we consider here. First, CPC analysis does not appear to be very effective in identifying similarity in underlying biological causal factors. The heuristic example that Houle et al. (2002) give is a situation in which two causal factors, genetic variation and developmental temperature, affect both size and shape in a set of morphological measurements. Now imagine two populations that have been measured for this set of traits, and the two populations differ in just one causal factor, say developmental temperature. If the phenotypic variance-covariance matrices of the two populations are compared using CPC, all eigenvectors, not just one, are likely to be different. This is because a principal components analysis, on which CPC is based, is likely to allocate the variation in morphology to a "size" component (PC1) and then subsequent "shape" components, which spreads the effect of the two causal components across all eigenvectors. In this case, and probably in many others, as Houle et al. (2002) argue, principal components do not necessarily reflect underlying biological causal factors, and therefore any inference concerning causal factors is likely to be misleading. Houle et al. (2002) effectively demonstrate that CPC analysis is only relevant for hypothesis testing if principal components are meaningful in the context of the hypothesis.

However, many of the hypotheses of interest to evolutionary biologists using CPC as a method of matrix comparison are not directly concerned with causal components. In the comparison of  $\mathbf{G}$  matrices, for example, one is interested in whether  $\mathbf{G}$  changes under selection. In the context of our experiment, we do not know how many genes or independent developmental pathways may be involved in the selection response in sympatric environments, which underlies the increase in genetic variance. This is an important question but one that could only be addressed by combining quantitative trait loci (QTL) analyses with  $\mathbf{G}$  matrix comparisons (Agrawal et al. 2001). A CPC analysis is an effective tool for determining whether

**Table 7:** Divergence variance-covariance (**D**) matrices for field allopatric control and experimental sympatry populations of *Drosophila serrata*

	Females				Males			
	CV1	CV2	CV3	CV4	CV1	CV2	CV3	CV4
CV1	1.906				1.321			
CV2	-1.515	1.184			-.222	1.566		
CV3	1.414	-1.562	2.957		.871	-.996	1.209	
CV4	.000	.514	.590	1.211	-1.255	-.121	-.860	2.711

two **G** matrices differ, but no inferences can be drawn concerning the number or developmental relationships of loci that may underlie the difference.

Flury (1988, p. 158) makes the point concerning the meaningful nature of principal components in another context in which the traits measured may be on different scales, as in life-history studies, for example. Comparison of **G** matrices comprised of life-history traits using CPC should be approached with caution, as principal components extracted from the covariance matrix may simply reflect scale to a large extent. Extension of the CPC model to the comparison of correlation matrices, which would nullify the effects of scale, has been considered by Schott (1998).

The second set of (statistical) points that Houle et al. (2002) make concerns the performance of significance testing in CPC analysis. A major problem highlighted by Houle et al. (2002), and one we continually encountered in this study, concerns how Phillip’s CPC program considers the similarity of the eigenvector with the largest eigenvalue (PC1) in the hypothesis test for a single partial common principal component (CPC[1]) by default. Without reordering of the principal components as they are considered in the hierarchy of partial common principal component hypotheses, matrices may be found to be completely unrelated (if PC1 is different) even when there is considerable similarity between the matrices in eigenvectors other than PC1. Reordering is therefore critical in determining the level of similarity between matrices, and the comparisons we present in this article are the final result of numerous analyses to explore the behavior of CPC as the order of entry of the principal components into the analysis was changed. Such analyses would be a daunting task if the number of traits involved was large.

Finally, and perhaps most problematically for the CPC method, Houle et al.’s (2002) simulations suggest that a lack of statistical power in testing the lower levels of the hierarchy sometimes resulted in the (statistically) wrong model being favored. For example, when the expected statistical result in cases 2.2 and 4.1 of Houle et al. (2002) was no similarity, all eigenvectors were found to be common (the CPC model) at an appreciable frequency when the effect size was modest. We suspect that such a lack of

power may have resulted in our analyses indicating that **G** eigenstructure was not changed by natural selection in our experiment, as average **G** was a better predictor of the direction of evolution. Since it is the eigenvectors of **G** that are of most interest when testing hypotheses concerning genetic constraints on the direction of evolution, perhaps alternative methods to test eigenstructure similarity should be employed in addition to CPC analysis. Direct angular comparison of principal components is one alternative, but simply comparing  $g_{\max}$  between two groups or  $g_{\max}$  with  $d_{\max}$  is statistically meaningless, as the eigenstructure of the two matrices may be identical even if PC1 differs. Krzanowski’s (1979) method of subspace comparison is based on the simultaneous determination of critical angles between principal components from different groups and results in a quantitative measure of the similarity of two or more subspaces that could perhaps be used in conjunction with randomization protocols for hypothesis testing.

In summary, this experiment has demonstrated that genetic variances can change substantially under natural selection. The experimental manipulation of sympatry replicated the effect of natural selection on mate recognition in the field not only in phenotypic means but also by reproducing the field pattern in genetic variances. The association between the genetic covariance structure and the direction of evolution under field and laboratory conditions suggested that experimental sympatry populations evolved in the direction of field sympatry populations as

**Table 8:** Comparison between the allopatric **G** matrix and the **D** matrix between field allopatric control and experimental sympatry populations of *Drosophila serrata*

Hierarchy	df	Females		Males	
		$\chi^2$	<i>P</i>	$\chi^2$	<i>P</i>
Equality	10	29.83	.001	24.97	.005
Proportional	9	25.20	.003	20.767	.014
CPC	6	12.55	.051	11.04	.087
CPC(2)	5	7.39	.193	5.04	.411
CPC(1)	3	2.85	.415	4.57	.201
Unrelated					

**Table 9:** Comparison between the average **G** matrix (before and after selection) and the **D** matrix between field allopatric control and experimental sympatry populations of *Drosophila serrata*

Hierarchy	df	Females		Males	
		$\chi^2$	<i>P</i>	$\chi^2$	<i>P</i>
Equality	10	31.95	.000	26.31	.003
Proportional	9	15.23	.085	15.85	.070
CPC	6	9.38	.153	7.16	.306
CPC(2)	5	9.23	.100	6.93	.226
CPC(1)	3	4.90	.179	2.93	.403
Unrelated					

a consequence of genetic constraints. By combining experimental and comparative approaches to the investigation of the evolution of traits known to be under natural selection in the field, we may be able to discern how the quantitative genetic basis of traits change under natural selection and how evolution is constrained by the available patterns of genetic (co)variation.

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