

# Predictable and Divergent Change in the Multivariate P Matrix during Parallel Adaptation

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**ABSTRACT:** Adaptation to replicated environmental conditions can be remarkably predictable, suggesting that parallel evolution may be a common feature of adaptive radiation. An open question, however, is how phenotypic variation itself evolves during repeated adaptation. Here, we use a dataset of morphological measurements from 35 populations of threespine stickleback, consisting of 16 parapatric lake-stream pairs and three marine populations, to understand how phenotypic variation has evolved during transitions from marine to freshwater environments and during subsequent diversification across the lake-stream boundary. We find statistical support for divergent phenotypic covariance (**P**) across populations, with most diversification of **P** occurring among freshwater populations. Despite a close correspondence between within-population phenotypic variation and among-population divergence, we find that variation in **P** is unrelated to total variation in population means across the set of populations. For lake-stream pairs, we find that theoretical predictions for microevolutionary change can explain more than 30% of divergence in **P** matrices across the habitat boundary. Together, our results indicate that divergence in variance structure occurs primarily in dimensions of trait space with low phenotypic integration, correlated with disparate lake and stream environments. Our findings illustrate how conserved and divergent features of multivariate variation can underlie adaptive radiation.

**Keywords:** *Gasterosteus aculeatus*, genetic lines of least resistance, covariance tensor, quantitative genetics, parallel evolution.

## Introduction

Repeated evolution of similar trait values by independent populations in similar environments provides convincing evidence for evolution by natural selection (Reznick et al. 1996; Arendt and Reznick 2006). Such parallel evolution is, therefore, oft-invoked evidence for adaptation in nature (Blount et al. 2018). However, beyond the classic examples

of convergence from a handful of systems for a handful of traits (e.g., limb lengths in *Anolis* lizards [Losos 2009], armor loss in newly colonizing freshwater threespine stickleback [Colosimo et al. 2005]), multivariate, multipopulation convergence is more complex, and many systems show both shared and unique evolution across independent populations (Langerhans and DeWitt 2004): a continuum of so-called (non)parallelism (Bolnick et al. 2018). Variation among populations in chance events, phenotypic plasticity, heritability, demography, environments, and natural selection itself generates population-specific historical contingencies (Gould 1989; Beatty 2006, 2008; Losos 2017; Blount et al. 2018). Such contingencies result in nonparallel evolutionary trajectories, even for lineages adapting to ostensibly replicated environmental gradients where parallel evolution might be expected.

On the one hand, empirical evidence of (non)parallelism in trait means has become abundant (Bolnick et al. 2018; Jacobs et al. 2020; James et al. 2021; Weber et al. 2021). On the other hand, we know less about whether and why phenotypic variances and covariances might evolve predictably in these sorts of “natural experiments.” Yet variance-covariance structure (hereafter, “covariance” for simplicity) has an important role in evolutionary biology. Phenotypic variation underlies the evolutionary process at all timescales, providing the material on which natural selection acts; phenotypic variance must exist for natural selection to occur (Darwin 1859), and some of this variance must be heritable for evolutionary change (Fisher 1930; Lande 1976).

Variance is thus viewed as a key determinant of the rate of evolution and is often viewed as a source of constraint, biasing the direction of evolutionary change to directions of greater variability (Lande 1979; Schluter 1996, 2000; Hansen and Houle 2004). For example, under the multivariate breeders equation (Lande 1979), the

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evolutionary response is a product of the selection gradient  $\beta$  and the genetic variance-covariance matrix  $\mathbf{G}$  (a component of the phenotypic covariance matrix,  $\mathbf{P}$ ; see table 1). If the major axis of  $\mathbf{G}$  is misaligned with the direction of selection  $\beta$ , evolution should proceed away from the direction of selection and toward axes of greater covariance (Schluter 1996). Indeed, measures of within-population standing variance have been found to predict divergence among taxa or populations in a variety of organisms (Schluter 1996; Chenoweth et al. 2010; Costa e Silva et al. 2020; Hangartner et al. 2020; Opedal et al. 2023; Walter 2023), although counterexamples exist (Henry and Stinchcombe 2023a).

Although much of the above work is focused on genetic variance, it is important to realize that to the extent selection acts on variation, it will act through the phenotypic variance  $\mathbf{P}$ . Theory predicts that natural selection can change  $\mathbf{P}$  and  $\mathbf{G}$ , in particular with directional selection removing variance and nonlinear selection shaping patterns of covariance (Jones et al. 2003). The speed and frequency at which this happens determines whether and for how long multivariate variation acts as a constraint versus an adaptation in its own right. This is an open empirical question (Svensson et al. 2021). Thus, cases where variation is aligned with observed divergence in means may provide evidence of variational constraints or of natural selection's ability to shape variation (Schluter 1996).

Inferences of the shape of multivariate selection provide one step toward disentangling these interpretations (Hohenlohe and Arnold 2008; Punzalan and Rowe 2016; but see Henry and Stinchcombe 2023b), although estimates of contemporary selection carry their own limitations when interpreting historical adaptation (Grafen 1988). Alternatively, some studies have examined how estimates of within-population variation (either phenotypic or genetic) have themselves changed during adaptation (McGlothlin et al. 2018, 2022; Costa e Silva et al. 2020; Hangartner et al. 2020), in some cases finding support for conserved patterns of covariation consistent with hypotheses of constraint. Natural systems that show repeated adaptation to a common environmental gradient (Bolnick et al. 2018) provide an underexplored opportunity to understand the degree to which phenotypic variation itself evolves during adaptation and may provide insight into variational patterns as constraint versus variational patterns as an outcome of adaptation itself (McGlothlin et al. 2018, 2022).

Here, we use a replicated system of 16 lake-stream population pairs of threespine stickleback (*Gasterosteus aculeatus*) to quantify the predictability and repeatability of interpopulation variation in the phenotypic variance-covariance matrix,  $\mathbf{P}$ . Past work from this system (Stuart et al. 2017) has shown that evolutionary change across the lake-stream habitat boundary differs substantially across watersheds (i.e., the parapatric lake-stream pairs), although there is

**Table 1:** Glossary of terms

| Term                                     | Dimensions <sup>a</sup>        | Definition                                                                                                                                             |
|------------------------------------------|--------------------------------|--------------------------------------------------------------------------------------------------------------------------------------------------------|
| $\mathbf{P}$                             | $k \times k$                   | Phenotypic variance-covariance matrix, summarizing variation in trait expression among individuals                                                     |
| $\mathbf{G}$                             | $k \times k$                   | Genotypic variance-covariance matrix, summarizing variation in additive genetic effects among individuals                                              |
| $\mathbf{D}$                             | $k \times k$                   | Among-population divergence matrix, a variance-covariance matrix summarizing variation in trait means across populations or lineages                   |
| $\Delta\bar{z}$                          | $k$                            | Vector of evolutionary change in trait means between two populations, in this case, between lake and stream habitats within watersheds                 |
| $\Delta\mathbf{P}$                       | $k \times k$                   | Matrix describing change in phenotypic (co)variance between lake and stream habitats, taken as $\mathbf{P}_{\text{stream}} - \mathbf{P}_{\text{lake}}$ |
| $-\Delta\bar{z}\Delta\bar{z}^T$          | $k \times k$                   | Unit-rank matrix describing the expected change in $\mathbf{G}$ or $\mathbf{P}$ due to directional selection                                           |
| $\mathbf{d}_{\text{max}}$                | $k$                            | Leading eigenvector (PC1) of $\mathbf{D}$ , a vector describing the combination of traits that vary most in mean value among populations               |
| $\mathbf{p}_{\text{max}}$                | $k$                            | Leading eigenvector (PC1) of $\mathbf{P}$ , a vector describing the combination of traits that vary most among individuals                             |
| $\beta$                                  | $k$                            | Vector of directional selection gradients, describing selection on each trait                                                                          |
| $\Sigma_{\mathbf{P}}$                    | $k \times k \times k \times k$ | Fourth-order covariance tensor, describing variance and covariance in elements of $\mathbf{P}$ among populations                                       |
| $\Sigma_{\Delta\mathbf{P}}$              | $k \times k \times k \times k$ | Fourth-order covariance tensor, describing variance and covariance in elements of $\Delta\mathbf{P}$ among freshwater watersheds                       |
| $\Sigma_{-\Delta\bar{z}\Delta\bar{z}^T}$ | $k \times k \times k \times k$ | Fourth-order covariance tensor, describing variance and covariance in elements of $-\Delta\bar{z}\Delta\bar{z}^T$ among freshwater watersheds          |

Note: PC = principal component.

<sup>a</sup> Where  $k$  is the number of traits, in this case, seven.

some signature of shared directions of multivariate evolution (De Lisle and Bolnick 2020). In this article, we take a comparative approach to understand how multivariate phenotypic variance has changed during this radiation. Specifically, we were interested in three questions. First, what is the extent of variation in phenotypic covariance (**P**) among habitats and populations? Second, does within-population variation captured by **P** align repeatedly with among-population divergence? And third, can divergence in trait means among populations predict change in **P** itself?

## Methods

### *Stickleback Sampling*

The collection, preparation, and collation of these phenotypic data are reported in detail in Stuart et al. (2017). In brief, during the period May to July 2013, adult threespine stickleback were collected from 16 independent watersheds on Vancouver Island, British Columbia, Canada (Stuart et al. 2017). From each watershed, stickleback were sampled from one lake and its adjoining inlet or outlet stream. In addition to these 16 lake-stream pairs, marine fish were also collected from three sites spread around Vancouver Island, for a total of 35 populations (table S1; tables S1–S4 are available online). We hereafter use the term “population” to refer to this lowest level of sampling, “pair” to refer to any individual lake-stream sample, and “watershed” to refer to the watershed from which a pair was collected. Thirty-three linear and meristic measurements, including size, were measured from each fish (table S2). Digital landmarks were placed on left-side lateral photographs and on ventral photographs to measure traits. Left pectoral fins were cut from each fish and splayed for photography. Standard length and a few other traits were collected via caliper or dissection. The data we use for this article are a subset of those used in Stuart et al. (2017) in that we excluded geometric morphometric data for the present study a priori, to ease interpretation of subsequent results.

For this study, traits were scaled through natural log transformation followed by size correction for downstream analyses. Size correction was performed by saving residuals from a linear regression of the natural log of trait value against the natural log of standard length; a single regression model for each trait was fitted to maintain residual differences among populations. This trait standardization approach ensures that our interpretation of changes in variance and associations with evolution of traits means (see below) is conservative and not an inevitable outcome of mean-variance scaling. Nonetheless, similar qualitative conclusions were obtained in analyses without a log transformation.

### *Trait Selection*

With a sample of 39–55 individuals per population, we lacked data to confidently estimate phenotypic covariance matrices on a population-by-population level using all 33 traits originally measured by Stuart et al. (2017), noting that a single 33-dimensional covariance matrix contains 561 unique parameters. We thus focus on a subset of traits measured, targeting seven traits because a seven-dimensional covariance matrix contains 28 unique parameters. This dimensionality ensures the possibility of robust comparison of **P** matrices among populations because the most complex model allowing among-population variation in seven-dimensional **P** still contains far fewer parameters than the number of individuals per population. Because we were specifically interested in how variation evolves during repeatable lake-stream adaptation, we focus our analysis on the seven traits that show the most consistent change across lake-stream boundary. These seven traits were identified as the highest loading of the 32 size-corrected traits on principal component (PC) 1 of the among-pair correlation matrix of phenotypic change vectors (i.e., a matrix describing the correlations in lake-stream evolutionary change between different watersheds) describing divergence in means between lake and stream environments (table S3 from De Lisle and Bolnick 2020); these traits were body depth, width of the pelvic girdle, width of the ventral process of the pelvic girdle (the “diamond”; Stuart et al. 2017), gape width, gill raker density, caudal depth, and body width. Similar qualitative conclusions were obtained using an alternative selection of seven traits based on a priori natural history knowledge (body depth, pelvic girdle width, gape width, gill raker number, gill raker length, dorsal spine length, and pectoral fin width).

### *Statistical Analysis*

**Estimation of **P**.** We used a series of multiresponse mixed effects models to estimate **P** matrices and assess variation across the marine-freshwater boundary, across watersheds, and across the lake-stream boundary within pairs. In stickleback, several studies have shown that **P** and **G** align. For example, Schluter (1996) showed that the angle between the major axis of variation for a five-trait **G** matrix ( $\mathbf{g}_{\max}$ ) estimated from a limnetic freshwater population was only 16 degrees, on average, from the major axis of **P** matrices ( $\mathbf{p}_{\max}$ ) calculated from several other freshwater populations. Schluter (1996) also showed that  $\mathbf{p}_{\max}$  and  $\mathbf{g}_{\max}$  made similar predictions for the observed direction of evolutionary change. Similarly, Leinonen et al. (2011) found that  $\mathbf{p}_{\max}$  and  $\mathbf{g}_{\max}$  for body shape had a correlation of  $r = 0.88$  and an angle between them of 26 degrees, suggesting that the major axes of genetic and phenotypic variation are strongly

aligned. In general,  $\mathbf{P}$  and  $\mathbf{G}$  are typically similar to each other (Cheverud 1988; Roff 1995; Stepan et al. 2002; Hohenlohe and Arnold 2008). However, we note that neither our analysis nor our interpretation of it depend on the substitution of  $\mathbf{P}$  for  $\mathbf{G}$  in that, for example, we do not use  $\mathbf{P}$  to make predictions of evolutionary response, and we formulate our predictions for change in  $\mathbf{P}$  itself due to selection.

A major caveat in our estimate of  $\mathbf{P}$  is that our data came from wild-caught fish, so observed differences in  $\mathbf{P}$  could in part be due to environmental effects or genotype  $\times$  environment interaction. This caveat is one shared by most studies of phenotypic variation, and the alternative—estimates based on individuals reared in a common environment—carries its own set of limitations (namely, conflating the contribution of genotype  $\times$  environment interaction unless multiple common gardens are used; de Villemereuil et al. 2016). To ascertain the degree to which environmental effects may influence  $\mathbf{P}$ , we reanalyzed a previously published dataset (Oke et al. 2016) from a common garden experiment that reported traits measured from wild-caught fish as well as traits measured from individuals from the same population reared in a laboratory common garden. We found significant differences between  $\mathbf{P}$  matrices estimated from wild versus lab fish (see the supplemental PDF), so in our analysis of our own dataset we included potential explanatory variables related to environmental variation and population structure (elaborated below) and emphasize that interpreting variation in  $\mathbf{P}$  as evidence of differences in additive genetic variance would be premature.

Our mixed models to estimate  $\mathbf{P}$  were of the form

$$y_{k,j,i} = \mu_k + \alpha_{k,j} + \epsilon_{k,i}, \quad (1)$$

where  $y_{k,j,i}$  is the value of the  $k$ th trait from individual  $i$  in population  $j$ ,  $\mu_k$  is the grand mean for trait  $k$ ,  $\alpha_{k,j}$  is a random effect describing variation in the trait among populations, and  $\epsilon_{k,i}$  is the residual random effect describing variation among individuals. Fitting this model entails the estimation of two categories of random effect covariance matrices, the G-side covariance matrix of  $\alpha_{k,j}$ , which summarizes covariation in trait means across populations (i.e., the  $\mathbf{D}$  matrix; Lande 1979), and the residual covariance matrix of  $\epsilon_{k,i}$  describing among-individual variation and covariation in trait values, which is our estimate of  $\mathbf{P}$ . Note that because we have only one measurement per individual fish, this term will also contain measurement error, but since the fish were all measured the same way, we do not expect this to contribute in a biased way to any variation in  $\mathbf{P}$  we may uncover. Note also that all of our matrix comparisons accommodate uncertainty in our estimates of  $\mathbf{P}$  (see below).

We fit a series of five models of the general form of equation (1) but differing in their complexity: (1) a sim-

ple model with a single  $\mathbf{P}$  matrix estimated, forcing all populations and habitats to share a single within-population covariance structure; (2) a model with two separate  $\mathbf{P}$ s for freshwater versus marine environments, corresponding to a shared  $\mathbf{P}$  matrix structure across all populations within each environment; (3) a model with three separate  $\mathbf{P}$ s for lake, stream, and marine fish; (4) a model with a separate  $\mathbf{P}$  estimated for each watershed (19  $\mathbf{P}$ s; each marine population treated as its own watershed); and (5) a “saturated” model with a separate  $\mathbf{P}$  estimated for each population (habitat type  $\times$  watershed combination and the three marine populations; 35  $\mathbf{P}$ s). This model set was created by altering the random effects structure to estimate separate residual random effect covariance matrices across different factor levels corresponding to the models described above.

Finding support for the saturated model and because we were specifically interested in potential replicated divergence in  $\mathbf{P}$  matrices between lake and stream habitats, we then fit separate linear mixed models for each freshwater pair of the form

$$y_{k,i} = \mu_k + \epsilon_{k,i},$$

where we compared a model with separate residual covariance matrices of  $\epsilon_{k,i}$  for lake and stream environments to a reduced model with a common within-pair  $\mathbf{P}$  matrix. This allowed us to assess statistical significance of lake-stream divergence in  $\mathbf{P}$  for each freshwater watershed. All models were fitted by Markov chain Monte Carlo (MCMC) using uninformative priors in MCMCglmm (Hadfield 2010), and deviance information criteria (DIC) were used to rank candidate models. Model convergence was confirmed by lack of trends in the Markov chain as well as low estimated autocorrelation in the posterior, for both the simple and the saturated models. All of the subsequent matrix comparisons (described below) were performed on the posterior distributions of  $\mathbf{P}$  to account for uncertainty in our estimates.

*Describing Variation in  $\mathbf{P}$ .* While the model comparison approach described above can indicate whether there is statistical support for variation in  $\mathbf{P}$ , other multivariate approaches are required to understand the nature of any variation that is found. We took two general approaches.

First, we performed pairwise comparisons between matrices of interest by calculating (i) the vector correlation of the leading eigenvector of each matrix and (ii) Krzanowski’s shared subspace (Krzanowski 1979), which identifies the degree to which the parts of multivariate trait space that contain most of the variation are shared between two matrices (Aguirre et al. 2014). This is calculated by summing the eigenvalues of  $\mathbf{S}$ , where

$$\mathbf{S} = \mathbf{A}^T \mathbf{B} \mathbf{B}^T \mathbf{A} \quad (2)$$

and where  $\mathbf{A}$  and  $\mathbf{B}$  are matrices that contain a subset of eigenvectors of the two  $\mathbf{P}$  matrices. A subset of three dimensions was chosen because this is the maximum number of dimensions that can be retained for a comparison of seven-dimensional covariance matrices ( $k$  less than or equal to  $n/2$ ; Aguirre et al. 2014). This approach allows a bounded sum for the eigenvalues of  $\mathbf{S}$  ranging from 0 to 3, representing no and complete shared subspace, respectively. We chose to retain as many dimensions in  $\mathbf{S}$  as possible given that we also compute the vector correlations between the leading eigenvectors of the matrices being compared. We used these pairwise approaches to compare the following matrices: (1) the  $\mathbf{P}$  matrix pooled across all 35 populations and the among-population divergence matrix  $\mathbf{D}$ , which is a test of the degree to which populations have diverged in the same set of traits that vary most across individuals within a population; (2) the pooled estimates of marine, lake, and stream  $\mathbf{P}$ ; and (3) lake and stream  $\mathbf{P}$  for each watershed, where statistical support for  $\Delta\mathbf{P}$  ( $\mathbf{P}_{\text{stream}} - \mathbf{P}_{\text{lake}}$ ) was found on the basis of model comparison of mixed models fitted separately for each watershed. Finally, because these approaches say little about the overall size of matrices, we also computed evolvability (Bolstad et al. 2014) and conditional evolvability (Hansen and Houle 2008) statistics using random skewers (Cheverud 1996; Cheverud and Marroig 2007). These statistics represent the expected evolutionary response in the direction of selection, where random selection gradients are sampled in multivariate traits space; the distribution of evolvability statistics from such an approach provides an indication of the degree of variational constraints imposed by  $\mathbf{P}$  and can be compared across  $\mathbf{P}$  matrices.

As a second approach to understanding variation in  $\mathbf{P}$ , we performed an analysis of the fourth-order covariance tensor  $\Sigma_{\mathbf{P}}$ , which summarizes variances and covariances of the elements of  $\mathbf{P}$  among populations (Melo et al. 2015). For seven-dimensional  $\mathbf{P}$  matrices,  $\Sigma_{\mathbf{P}}$  can be decomposed into 28 dimensions with corresponding eigentensors and their eigenvalues. These eigentensors can be interpreted like PC vectors, except that each eigentensor is a  $7 \times 7$  dimensional matrix (for seven traits). Each eigentensor can itself be decomposed into its eigenvectors and eigenvalues (which can be negative); the leading eigenvector from the first eigentensor is the linear combination of traits along which phenotypic variation has changed the most among populations. Hine et al. (2009) and Aguirre et al. (2014) provide an overview of this eigentensor approach. To summarize variation in  $\mathbf{P}$  captured by this covariance tensor, we plotted the first two eigentensors and also calculated the vector correlation between  $\Sigma_{\mathbf{P}_{\text{max}}}$ ,  $\mathbf{d}_{\text{max}}$ , and  $\mathbf{p}_{\text{max}}$  (calculated from the average of all population-level  $\mathbf{P}$  matrices; nearly quantitatively identical results are obtained using the pooled estimate of  $\mathbf{P}$  from the common model). These vector correlations indi-

cate the degree to which the major axis of variation in  $\mathbf{P}$  does or does not align with the major axis of variation among populations and individuals, respectively.

*Testing Predictions for Changes in  $\mathbf{P}$ .* Because lake-stream population pairs represent replicated cases of recent divergence into disparate environments, we can directly test microevolutionary predictions for change in  $\mathbf{P}$  under directional selection. Specifically, we can describe the within-generation change in phenotypic variance due to selection as

$$\Delta\mathbf{P}_s = \mathbf{P}\gamma\mathbf{P} - \mathbf{s}\mathbf{s}^T, \quad (3)$$

where  $\gamma$  is the matrix of nonlinear selection gradients and  $\mathbf{s}$  is the selection differential vector, which is equal to the change in mean values before and after selection (Lande and Arnold 1983). Focusing on the effects of directional selection only, equation (3) can be simplified to

$$\Delta\mathbf{P}_s = -\Delta\bar{z}\Delta\bar{z}^T, \quad (4)$$

where  $-\Delta\bar{z}\Delta\bar{z}^T$  is a matrix of unit rank (one nonnull dimension) describing loss of variance in the direction of evolution of trait means under directional selection. Equations (3) and (4) describe within-generation changes due to selection, yet these changes are expected to at least partially accrue to the next generation under realistic distributions of allelic effects (Barton 2022). Given the above, we expect the matrix  $\Delta\mathbf{P} = (\mathbf{P}_{\text{stream}} - \mathbf{P}_{\text{lake}})$  across lake-stream habitats to be aligned with  $-\Delta\bar{z}\Delta\bar{z}^T$  within a given freshwater watershed. Expanding out to consider the set of 16 replicate freshwater watersheds, we expect the covariance tensors  $\Sigma_{\Delta\mathbf{P}}$  and  $\Sigma_{-\Delta\bar{z}\Delta\bar{z}^T}$  to be aligned if adaptive divergence in trait means drives variation in phenotypic covariance (Hine et al. 2009). Thus, we compared leading eigentensors of these two covariance tensors  $\Sigma_{\Delta\mathbf{P}}$  and  $\Sigma_{-\Delta\bar{z}\Delta\bar{z}^T}$  and generated a null distribution of  $\Sigma_{-\Delta\bar{z}\Delta\bar{z}^T}$  for assessment of statistical significance by sampling random  $\Delta\bar{z}$  vectors.

*Environmental Predictors of Changes in  $\mathbf{P}$ .* Finally, we examined several potential environmental correlates that we expected could predict variation in  $\mathbf{P}$ . Specifically, given that much variation in  $\mathbf{P}$  occurred at the level of lake-stream pair, we explored how watershed-specific measures of lake-stream divergence may explain variation in change in  $\mathbf{P}$  captured by three possible metrics: the norm of the matrix  $\Delta\mathbf{P} = (\mathbf{P}_{\text{stream}} - \mathbf{P}_{\text{lake}})$ , the norm of  $\Delta\mathbf{P}$  standardized by the average size of  $\mathbf{P}$ , and Krzanowski's shared subspace (i.e., sum of eigenvalues of matrix  $\mathbf{S}$ ; eq. [2]). We explored how these measures of lake-stream change in  $\mathbf{P}$  correlate with measures of migration (average migration rate, average number of migrants, and average inferred population size, divergence time) and ecological differentiation between

lakes and streams captured by the length of the vector describing change in multivariate ecology between lakes and streams. These genetic and environmental measures were drawn from Stuart et al. (2017). We examined Pearson correlations between these variables but did not proceed with more sophisticated analyses, as there was little indication of environmental correlation with variation in  $\mathbf{P}$  when examining posterior modes of the above metrics.

Complete R script and data to reproduce all analyses and figures have been deposited in Zenodo (<https://doi.org/10.5281/zenodo.10512483>).

### Results

Model rankings indicate statistical support for variation in  $\mathbf{P}$  at all levels of analysis; the highest-ranked model includes variation in  $\mathbf{P}$  matrices among all 35 populations (table S3). The same overall conclusions were obtained in an analysis excluding the three marine populations. Thus, the model rankings indicate significant variation in phenotypic covariance structure between marine and freshwater, across freshwater watersheds, and at the lowest level of replication, the watershed  $\times$  habitat population. Pooled estimates of  $\mathbf{P}$  from each of lake, stream, and marine habitats are plotted in figure 1. Although we find statistical support for divergence in these pooled  $\mathbf{P}$  matrices,  $\mathbf{P}$  was generally similar among lake, stream, and marine environments, in terms of both Krzanowski subspace comparisons (fig. S1; figs. S1–S5 are available online) and evolvability metrics, although there was some evidence of greater evolvability in the pooled estimate of  $\mathbf{P}$  from marine populations (fig. S2). Consistent with this finding, we found evidence of increased size of marine  $\mathbf{P}$  in a linear model with the total size (trace) of population-specific  $\mathbf{P}$  as the response variable and habitat type (lake, stream, or marine) as a fixed effect ( $t = 2.999$ ,  $P = .00521$ ,  $df = 32$ ; we found no evidence of a difference between the average size of lake and stream  $\mathbf{P}$  in the same analysis:  $t = -.278$ ,  $P = .78$ ).

We found strong alignment between the  $\mathbf{D}$  matrix describing covariance in mean trait values among populations and the pooled estimate of within-population  $\mathbf{P}$  from our common model, both for association of leading eigenvectors (vector correlation = 0.91, 95% credible interval for  $|r| = 0.83$  to 0.97; beta distribution squared  $P$  for correlation = .0017; Watanabe 2023; fig. 2) and for shared Krzanowski subspace (sum of eigenvalues of  $\mathbf{S} = 2.58$ , 95% credible interval = 1.99 to 2.85; fig. 2). This indicates that the primary axes of phenotypic variance are nearly completely shared both among individuals within populations and among population means regardless of habitat. The lake and stream  $\mathbf{P}$  matrices tended to be more similar to each other than to the marine  $\mathbf{P}$  matrix in our estimates of  $\mathbf{P}$  from the habitat-specific (3  $\mathbf{P}$ ) model (figs. 1, S1). How-

ever, most of the variation in  $\mathbf{P}$  occurs across freshwater populations and watersheds, as illustrated by the first two eigentensors of  $\Sigma_{\mathbf{P}}$  (fig. 3), indicating that divergence across freshwater populations in both lake and stream environments is unique from variation in marine  $\mathbf{P}$ .

We found no evidence of a strong association between  $\Sigma_{\mathbf{P\_max}}$ , the major axis of variation in  $\mathbf{P}$  among populations, and  $\mathbf{d_{max}}$ , the leading eigenvectors of the  $\mathbf{D}$  matrix (vector correlation = 0.32, 95% credible interval =  $-0.57$  to  $0.73$ , beta distribution  $P$  for squared correlation = .44; Watanabe 2023), indicating that although phenotypic variation among individuals within populations tends to correspond to the pattern of divergence among populations (fig. 2), variation of phenotypic covariance structure among populations does not occur in these same combinations of traits. Moreover,  $\Sigma_{\mathbf{P\_max}}$  explained relatively little variation in population means summarized by the  $\mathbf{D}$  matrix (10.8%, 95% credible interval = 2.1% to 41.8%), and projecting  $\mathbf{D}$  onto  $\Sigma_{\mathbf{P}}$  indicates that the directions of divergence among populations does not explain much of the population-to-population variation in  $\mathbf{P}$  (only 0.012% of the variance in  $\mathbf{P}$ ). To appreciate these findings, we can consider the loadings for the principle components of phenotypic variation for the traits that load most and least strongly on  $\mathbf{p_{max}}$ . Figure 4A and 4C show the two traits that load most strongly on PC1 of the average  $\mathbf{P}$  matrix (taken across all freshwater and marine populations), body width and pelvic girdle diamond width. These two traits tend to be strongly correlated (see also fig. 1). Figure 4C shows not only alignment of average  $\mathbf{P}$  and the  $\mathbf{D}$  matrix but also relatively low variation in covariance across populations, as illustrated by the low loadings of these traits on  $\Sigma_{\mathbf{P\_max}}$ . In contrast, figure 4B and 4D show the two traits that load least strongly on this component, gill raker density and caudal depth. These two traits have low correlations with each other (figs. 1, 4B). Although PC1 of  $\mathbf{D}$  and average  $\mathbf{P}$  are aligned here despite low loadings, there is substantial population variation in the orientation and size of  $\mathbf{P}$ , reflected in the high loadings of these two traits on PC1 of the first eigentensor of  $\Sigma_{\mathbf{P}}$  (fig. 4D). Thus, these data indicate patterns of strongly conserved covariance structure within and among populations in some suites of traits (fig. 4A, 4C), along with divergence in covariance for ecologically important traits that are less strongly integrated (fig. 4B, 4D).

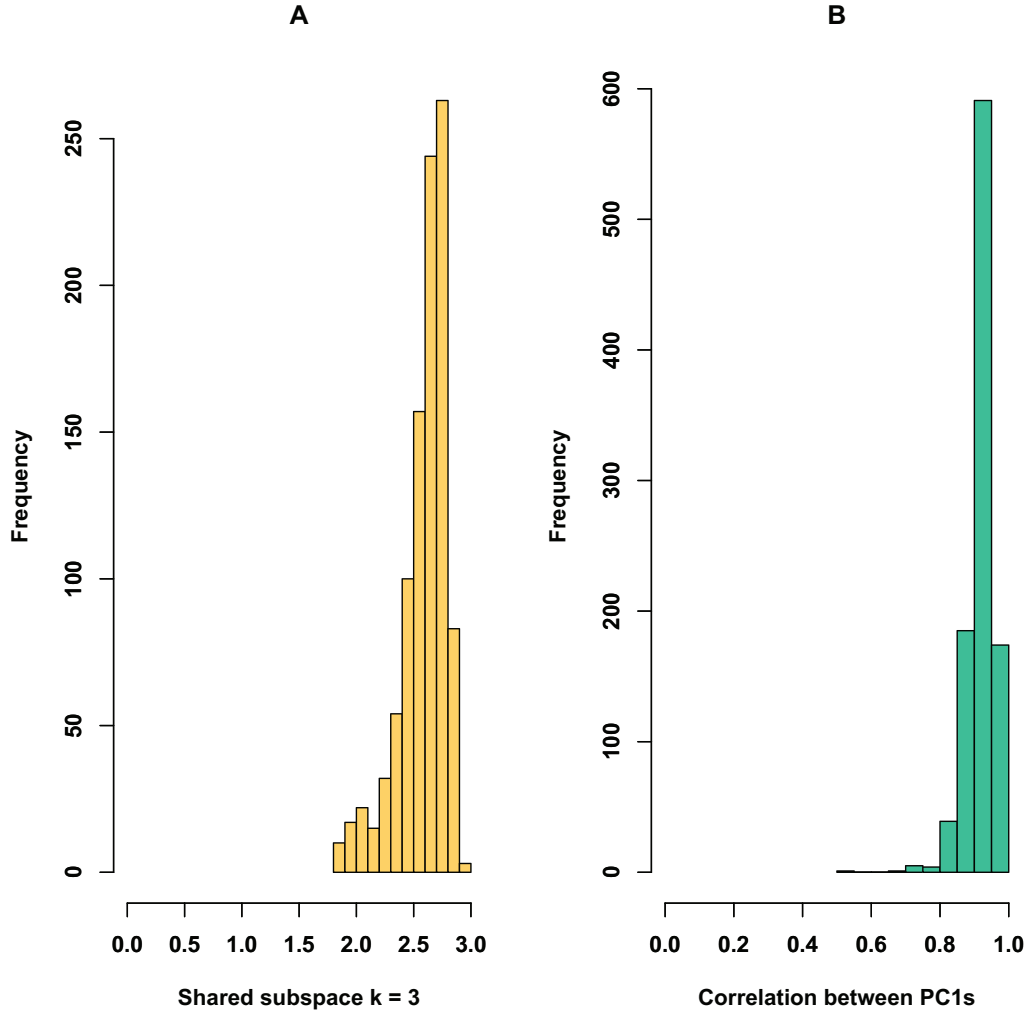
In our test of quantitative genetic predictions for change in  $\mathbf{P}$ , we found a striking relationship between variation in lake-stream  $-\Delta\bar{z}\Delta\bar{z}^T$ , the matrix describing expected change in variance due to directional selection, and lake-stream  $\Delta\mathbf{P}$ . That is, the correlation between the leading eigenvector of the first eigentensor of  $\Sigma_{\Delta\mathbf{P}}$  and  $\Sigma_{-\Delta\bar{z}\Delta\bar{z}^T}$  is near 1 and highly significant (vector correlation = 0.96, resampling  $P < .0001$ , beta distribution  $P$  for squared correlation = .00015; Watanabe 2023; fig. 5), indicating



**Figure 1:** Estimates of seven-dimensional  $\mathbf{P}$  matrices across marine, lake, and stream freshwater environments. Shown are the posterior modes from the habitat-specific (3  $\mathbf{P}$ ) model.

that variation in  $\Delta\mathbf{P}$  among freshwater watersheds was greatest in the direction of trait space where the average change in means also varied most. Moreover, 33% of the variation in  $\Delta\mathbf{P}$  can be explained by variation in  $-\Delta\bar{\mathbf{z}}\Delta\bar{\mathbf{z}}^T$  ( $P < .0001$ ; fig. 5). Thus, variation across watersheds in the change in  $\mathbf{P}$  between lake and stream environments is predicted by variation in evolution of mean trait values across these watersheds. This is consistent with multivariate

quantitative genetic expectations (Lande 1980, 1984; Phillips and Arnold 1989) and indicates that variation in change of  $\mathbf{P}$  between lake and stream environments matches variation in evolutionary change in means between these environments across watersheds. Thus, among-watershed differences in  $\Delta\mathbf{P}$  are predicted by among-watershed differences in  $\Delta\bar{\mathbf{z}}$ , although we note that this association cannot rule out the possibility that  $\Delta\bar{\mathbf{z}}$  was influenced by  $\Delta\mathbf{P}$  rather than vice versa.

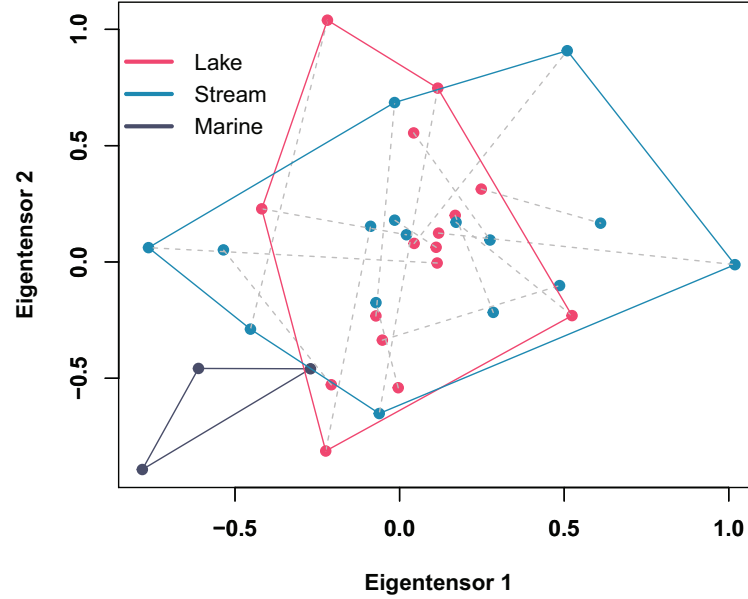


**Figure 2:** Within and between population variation align. A shows the Krzanowski shared subspace for three dimensions between  $\mathbf{D}$ , the matrix describing variation and covariation in trait means across populations, and  $\mathbf{P}$ , here the pooled estimate from the common model, sampled across the posterior distribution. This is essentially the proportion of the subspace captured by the three largest dimensions, with a value of 3 indicating very high similarity and a value of 0 indicating unrelated matrix structure. B shows the vector correlation between principal component (PC) 1 of  $\mathbf{D}$  ( $\mathbf{d}_{\max}$ ) and PC1 of  $\mathbf{P}$  ( $\mathbf{p}_{\max}$ ), sampled across the posterior.

We next explored within-watershed predictions, focusing only on watersheds where there was statistical support for change in  $\mathbf{P}$  between lake and stream environments (to avoid interpreting noise). We identified four watersheds where there was robust support for lake-stream divergence in  $\mathbf{P}$ , based on  $\Delta\text{DIC} > 7$  (table S4). For each of these four watersheds, we found significant change in phenotypic variance between lake and stream populations in the direction of evolution of trait means, as captured by the projection  $\mathbf{v}_{-\Delta\bar{z}\Delta\bar{z}^T}^T \Delta\mathbf{P} \mathbf{v}_{-\Delta\bar{z}\Delta\bar{z}^T}$ , where  $\mathbf{v}_{-\Delta\bar{z}\Delta\bar{z}^T}$  is the eigenvector associated with the most negative eigenvalue of the matrix  $-\Delta\bar{z}\Delta\bar{z}^T$  (fig. 6; Beaver, Bayesian  $P = .046$ ; Boot, Bayesian  $P = .034$ ; Moore, Bayesian  $P = .001$ ; Roberts, Bayesian  $P = .019$ ). These effects are visible by change in the 95% bi-

variate (co)variance ellipses for the two traits that load most strongly on  $\Delta\bar{z}$  for each watershed (fig. 6). Thus, we find significant change in variance in the combination of traits captured by  $\Delta\bar{z}$  in populations where there is strong statistical support for  $\Delta\mathbf{P}$ . We note that although theory predicts a reduction of variance, we cannot assess this because we do not know the polarity of lake-stream change. For reference, per theory, we found no consistent patterns of change in evolvability between lake and stream  $\mathbf{P}$  in these four watersheds (fig. S4) or change in variance along other eigenvectors of  $-\Delta\bar{z}\Delta\bar{z}^T$  (fig. S5; Phillips and Arnold 1989).

We found little evidence that multivariate lake-stream divergence in ecology explained observed variation in  $\mathbf{P}$  across the habitat boundary (in terms of the norm of



**Figure 3:** The first two eigentensors of the fourth-order covariance tensor  $\Sigma_P$ , which summarizes variation and covariation in elements of  $\mathbf{P}$  across populations. These two tensors capture approximately 50% of the variation in  $\mathbf{P}$ . Points show population-specific estimates from the population-specific model; colors and convex hulls show habitat types; and lake and stream populations from the same watershed are connected by dashed gray lines.

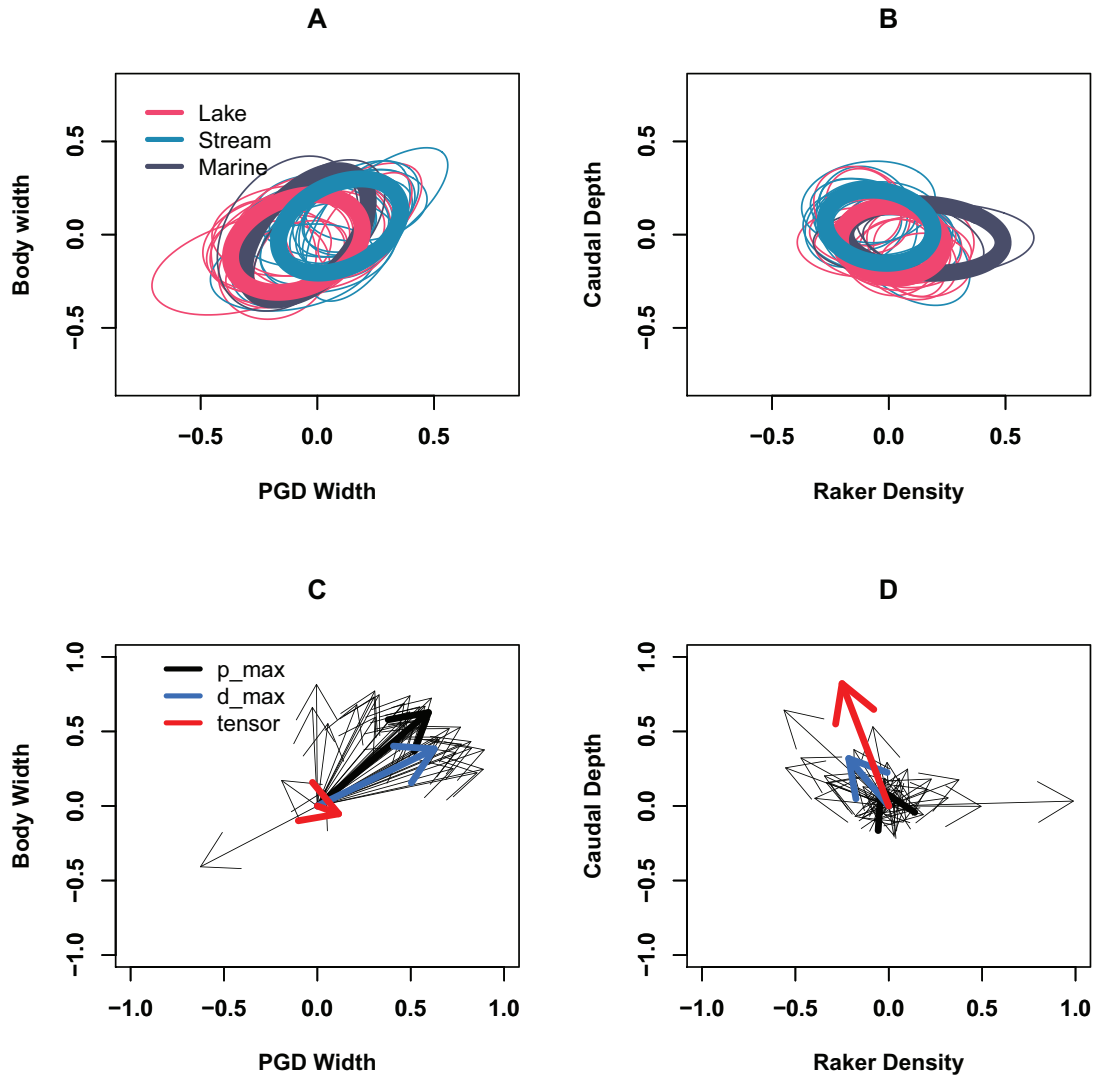
lake-stream  $\Delta\mathbf{P}$  [ $r = -0.24$ ,  $P = .36$ ], the norm of  $\Delta\mathbf{P}$  standardized by the average size of  $\mathbf{P}$  [ $r = 0.01$ ,  $P = .96$ ], or Krzanowski's shared subspace [ $r = 0.035$ ,  $P = .89$ ]). We also found little evidence that measures of genetic population structure explained variation in  $\mathbf{P}$ , for (i) the norm of lake-stream  $\Delta\mathbf{P}$  (lake-stream divergence time:  $r = -0.38$ ,  $P = .14$ ; number of migrants:  $r = 0.12$ ,  $P = .66$ ; migration rate:  $r = 0.0005$ ,  $P = .99$ ; population size:  $r = -0.03$ ,  $P = .89$ ), (ii) the norm of  $\Delta\mathbf{P}$  standardized by the average size of  $\mathbf{P}$  (lake-stream divergence time:  $r = -0.43$ ,  $P = .09$ ; number of migrants:  $r = 0.25$ ,  $P = .35$ ; migration rate:  $r = 0.13$ ,  $P = .63$ ; population size:  $r = 0.026$ ,  $P = .92$ ), and (iii) Krzanowski's shared subspace (lake-stream divergence time:  $r = 0.15$ ,  $P = .56$ ; number of migrants:  $r = -0.037$ ,  $P = .89$ ; migration rate:  $r = 0.0017$ ,  $P = .99$ ; population size:  $r = -0.06$ ,  $P = .82$ ).

### Discussion

We analyzed multivariate phenotypic variation across 35 populations of threespine stickleback (*Gasterosteus aculeatus*) to understand how phenotypic covariance has changed during replicated adaptation to freshwater environments (Stuart et al. 2017). We found evidence of significant divergence in  $\mathbf{P}$  matrices across populations against a background of what is, largely, shared covariance structure. The major axis of phenotypic variance is strongly

aligned across populations and is further aligned with divergence in mean trait values across populations, with traits such as body width and pelvic girdle width showing stable patterns of covariance. Change in phenotypic variance, in contrast, has occurred in suites of traits that tend to exhibit low correlations with other traits. Simple microevolutionary predictions for evolution of phenotypic variance are surprisingly successful in predicting change in  $\mathbf{P}$  matrices across lake and stream environments within watersheds, indicating that watershed-specific changes in the covariance of ecologically important traits, such as gill rakers and caudal depth, have occurred during adaptation to disparate freshwater environments.

By far, most variation in phenotypic (co)variance occurred between freshwater and marine habitats and among freshwater watersheds; we found less evidence of consistent differences between lake and stream  $\mathbf{P}$  matrices. This suggests that variation in  $\mathbf{P}$  was largely idiosyncratic and nonparallel across the replicated instances of adaption to lake and stream environments, consistent with non-parallelism in means observed in the same set of populations (Stuart et al. 2017; De Lisle and Bolnick 2020). This is illustrated in our analysis of the covariance tensor describing among-population variation in  $\mathbf{P}$ , where dispersion along the first two eigentensors illustrates the substantial variation in  $\mathbf{P}$  and lack of consistent differences between lakes and streams. This result is also demonstrated in our comparison of candidate models where the most

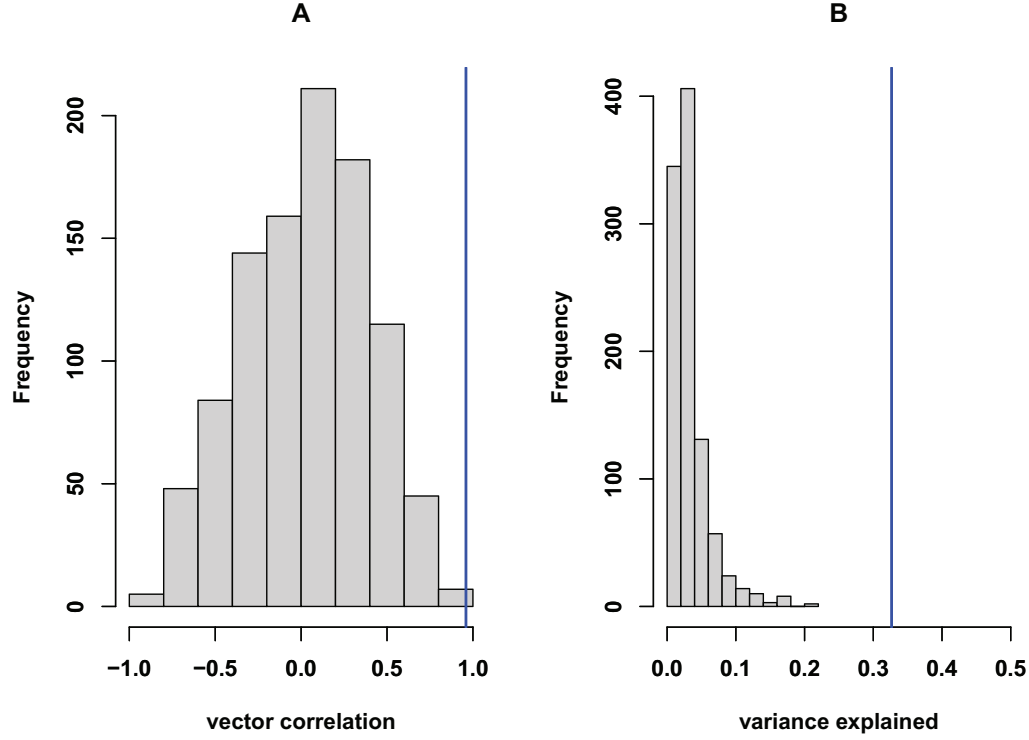


**Figure 4:** Variation in  $\mathbf{P}$  matrices among populations. A and B show estimates (posterior modes) of  $\mathbf{P}$  (plotted for two different traits in each panel) for each of the 35 populations sampled, as well as the pooled estimates for lake, stream, and marine environments, shown by thick ellipses centered on the corresponding means. C and D show variation in the orientation of major axes of variation across populations for the same sets of traits. C shows the loadings for the two traits that load most strongly on principal component (PC) 1 from the average  $\mathbf{P}$  matrix, or  $\mathbf{p}_{\max}$ ; the average is shown by the thick black arrow, and thin black arrows show population-specific estimates. The blue arrow shows PC1 from the  $\mathbf{D}$  matrix, or  $\mathbf{d}_{\max}$ ; this is the direction of maximum variation among population means. The red arrow shows PC1 from the first eigentensor of  $\Sigma_{\mathbf{p}}$ , which describes the combination of traits that vary most in their degree of (co)variance among populations. D shows the same but for raker density and caudal depth, two traits that do not load strongly on average on  $\mathbf{p}_{\max}$ . As C shows, these two traits, body width and pelvic girdle diamond (PGD) width, tend to be tightly correlated within and across populations, with relatively little variation in the degree of this correlation. Alternatively, caudal depth and raker density show substantial variation in their (co)variance among populations, shown in D.

dramatic drops in information criteria were observed when including separate  $\mathbf{P}$  matrices for freshwater versus marine and, subsequently, for separate watersheds. Marine populations exhibited less variation in  $\mathbf{P}$  and exhibited tighter covariance in traits related to body shape and substantially more variance in gill raker density relative to freshwater populations. However, it is notable that despite these

disparities in  $\mathbf{P}$ , our pooled estimates of marine, lake, and stream  $\mathbf{P}$  matrices were similar in structure.

Although we found little evidence of consistent parallel change in  $\mathbf{P}$  across the lake-stream boundary, within-watershed differences in lake and stream  $\mathbf{P}$  matrices were in some cases substantial. Moreover, these differences in lake and stream  $\mathbf{P}$  within watersheds were predictable based on simple



**Figure 5:** Variation in  $\Delta\mathbf{P}$  across freshwater watersheds is explained by variation in  $\Delta\bar{z}$ . *A* shows the vector correlation between the leading eigenvector of the first eigentensor of  $\Sigma_{\Delta\mathbf{P}}$  and  $\Sigma_{-\Delta\bar{z}\Delta\bar{z}^T}$  (blue = observed; gray = null); that is, the single linear combination of traits that explains the most variation in each of these two matrices is nearly identical. *B* shows the variance in  $\Delta\mathbf{P}$  explained by the first eigentensor of  $\Sigma_{-\Delta\bar{z}\Delta\bar{z}^T}$  (blue = observed; gray = null).

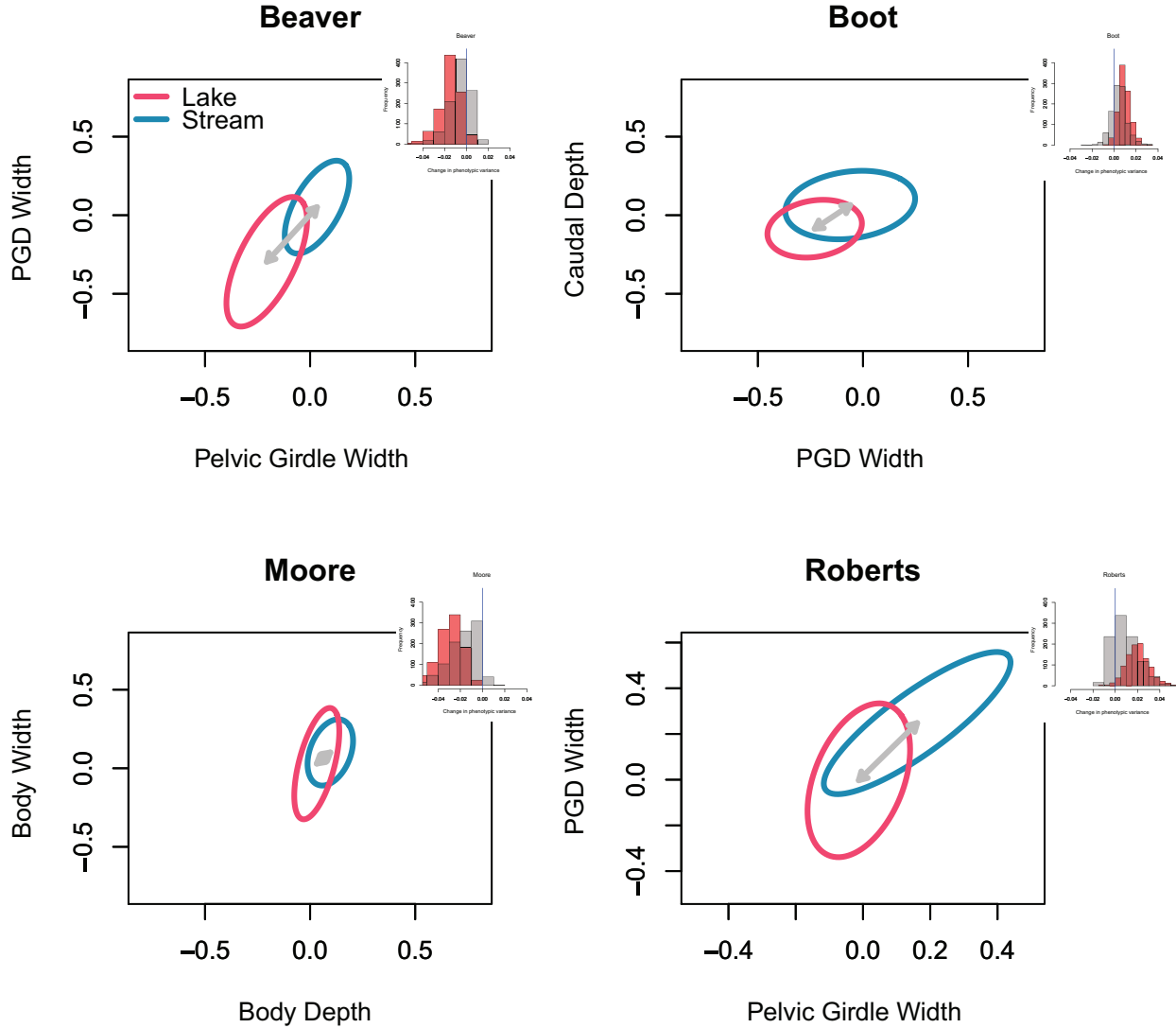
quantitative genetic predictions for within-generation change in phenotypic variance due to selection. More precisely, variation in lake-stream  $\Delta\mathbf{P}$  across watersheds was predictable based on variation in lake-stream  $\Delta\bar{z}$ , indicating that seemingly idiosyncratic variation in  $\mathbf{P}$  is in fact explainable based on nonparallel evolution of population means across watersheds.

This variation in  $\Delta\mathbf{P}$  indicates that changes in phenotypic covariance structure occur in the same suites of traits that show greatest divergence in mean value across lake and stream habitats within specific watersheds. Such within-watershed predictability of change in  $\mathbf{P}$  does not scale up to generate consistent differences in average lake and stream  $\mathbf{P}$  matrices across watersheds, however, likely because of the substantial variation in multivariate lake-stream differences in environment. That is, in analyses of trait means, Stuart et al. (2017) found little evidence of repeatable lake-stream divergence. Applying another analytical approach to the same data, De Lisle and Bolnick (2020) supported the conclusion that divergence across lake-stream habitat is more complex than can be described by a single dimension of parallel evolution (i.e., the lake-stream axis). Our study suggests that this among-watershed variation in the

direction of multivariate evolution has driven subsequent change in phenotypic covariance structure across this suite of populations—a continuum of (non)parallelism in  $\mathbf{P}$  matrices.

Noteworthy in this regard is that our analysis is based on a subset of traits that have contributed most to parallel evolution of multivariate mean phenotypes in this suite of populations (De Lisle and Bolnick 2020); even with this focus on the traits contributing most to parallel evolution, we found substantial variation across watersheds in how the  $\mathbf{P}$  matrix changed between lake and stream environments.

We have focused on understanding variation in patterns of phenotypic ( $\mathbf{P}$ ), rather than genotypic ( $\mathbf{G}$ ), variance. A justification for this is that selection acts on phenotypic variance (and thus  $\mathbf{P}$  is of interest in its own right) and that change in  $\mathbf{P}$  must be shaped by the same phenomena that affect evolution of  $\mathbf{G}$  (Cheverud 1988). Nonetheless, a major caveat is that we cannot account for changes in  $\mathbf{P}$  induced by variation in environmental (co)variance across habitats and populations, and moreover  $\mathbf{P}$  matrices may often exhibit more dimensions of meaningful variation than  $\mathbf{G}$ . Indeed, reanalysis of a previous study of some of the same populations (Oke et al. 2016) indicates that  $\mathbf{P}$  matrix



**Figure 6:** Change of phenotypic variance along predicted direction of  $\Delta \bar{z}$ , from watersheds with robust statistical support for  $\Delta \mathbf{P}$ . Each panel illustrates change in  $\mathbf{P}$  for the two traits that contribute most to  $\Delta \bar{z}$  in the watershed in question; gray arrows show  $\Delta \bar{z}$ . Inset red histograms show  $v_{-\Delta \bar{z} \Delta \bar{z}^T}^T \Delta \mathbf{P} v_{-\Delta \bar{z} \Delta \bar{z}^T}$ , where  $v_{-\Delta \bar{z} \Delta \bar{z}^T}$  is the eigenvector associated with the most negative eigenvalue of the matrix  $-\Delta \bar{z} \Delta \bar{z}^T$ , sampled from the posterior distribution with bootstrapped  $\Delta \bar{z}$ . Light gray shows samples generated using random samples from the entire dataset rather than population lake or stream habitats. These inset panels thus show the observed change in variance in the direction of  $\Delta \bar{z}$ . PGD = pelvic girdle diamond.

estimates can differ between wild-caught fish and those reared in the laboratory, suggesting an important contribution of environmental effects on  $\mathbf{P}$  in stickleback (although they share similar structure; see the supplemental PDF). We found no evidence that environmental differences or differences in population structure between lake and stream habitats explained lake-stream divergence in  $\mathbf{P}$ , suggesting that environment may not affect  $\mathbf{P}$ . Furthermore, a study in Baltic sea stickleback reported  $\mathbf{G}$  of lower rank (fewer dimensions of meaningful variation) than  $\mathbf{P}$ , suggesting that small eigenvalues of  $\mathbf{P}$  may be associated with dimensions of

null genetic variance (Leinonen et al. 2011). Future studies examining the contribution of genotype  $\times$  environment interaction to patterns of phenotypic variation in this radiation would be informative.

Bias in response to selection produced by multivariate genetic constraints can generate an association between major axes of genetic variance and mean population divergence (Schluter 1996; Blows and Higgie 2003; McGuigan et al. 2005; Chenoweth et al. 2010; Punzalan and Rowe 2016; McGlothlin et al. 2018). More generally, there is a long-standing empirical finding that across traits within a study

system, measures of within-population variation coincide with among-lineage divergence (Kluge and Kerfoot 1973; Bolstad et al. 2014; Houle et al. 2017; Opedal et al. 2023). An underappreciated (although noted by Schluter and others; e.g., Punzalan and Rowe 2016) alternative interpretation of such patterns is that selection shapes evolution of both trait means and genetic variance on timescales relevant to produce a correspondence between the two. Our finding of an association between variation in  $\Delta\bar{z}$  and  $\Delta\mathbf{P}$  supports this alternative interpretation of patterns of evolution along genetic lines of least resistance: selection may be shaping both standing phenotypic variance and divergence in trait means across lake and stream environments in our study. Although we cannot exclude a likely role for genetic drift in contributing to divergence in **P** matrices across this set of populations, we note that we found no evidence for an association between change in **P** and population size of lake-stream pairs.

Our findings that multivariate variation largely aligns with divergence concomitantly with subtle changes in (co)variance is partly consistent with patterns found in the *Anolis* adaptive radiation. McGlothlin et al. (2018, 2022) found that evolution of **G** in *Anolis* occurred primarily in the same combinations of traits that varied most among individuals and among populations. This differs from our finding, where we observe that most of the divergence in **P** matrices occurs largely orthogonally to  $\mathbf{p}_{\max}$  or  $\mathbf{d}_{\max}$ , indicating that most change in variance occurs in different suites of traits than those involved in divergence within and among stickleback populations. However, consistent with our finding, McGlothlin et al. (2022) found that predictable features of **G** matrix evolution in *Anolis* ecomorphs have occurred primarily in suites of traits that show less integration with other traits. This is consistent with our findings, where changes in stickleback **P** occurred in suites of traits that were not tightly integrated.

We found that  $\Delta\bar{z}$  across lake-stream pairs predicts variation in change in **P** between lake and stream habitats. This is consistent with microevolutionary predictions for change in **P** due to directional selection, although it is unclear what environmental factors may have contributed to change in **P**. Moreover, we emphasize that we cannot exclude environmentally induced plasticity that contributed to observed variation in **P**, as well as that environmental variation could contribute to observed correspondence between  $\Delta\bar{z}$  and  $\Delta\mathbf{P}$ . That is, environmental effects could drive variation in both  $\Delta\bar{z}$  and  $\Delta\mathbf{P}$ , contributing to the observed relationship between the two. However, we found no evidence that  $\Delta\mathbf{P}$  was related to environmental distance between lake and stream habitats.

Our work paints a nuanced picture of divergence in phenotypic variation in stickleback. On the one hand, patterns of variation of tightly covarying traits, such as pelvic traits

and body width, remain deeply conserved within and across populations. On the other hand, some combinations of traits show change in (co)variance between habitats that is predictable from simple quantitative genetic theory. Thus, our study adds to a growing body of work (Hohenlohe and Arnold 2008; Punzalan and Rowe 2016; McGlothlin et al. 2018, 2022) that indicates a potential role for selection in contributing to apparent correspondence between within-population variation and among-population divergence. Our work highlights how both conservation and divergence of multivariate variation can each contribute to adaptive radiation.

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### Statement of Authorship

Y.E.S. and D.I.B. designed the field sampling and collected the data. S.P.D. and Y.E.S. analyzed the data. S.P.D. and Y.E.S. drafted the manuscript. All authors contributed to writing and revising.

### Data and Code Availability

Complete R script and data to reproduce all analyses and figures have been deposited in Zenodo (<https://doi.org/10.5281/zenodo.10512483>; De Lisle et al. 2024).

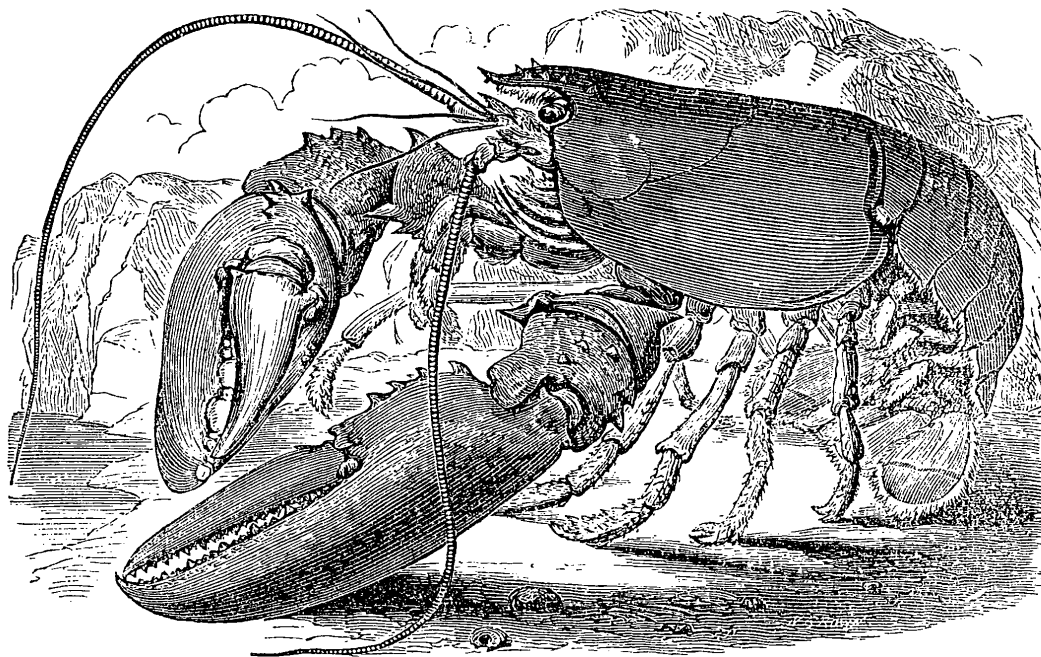
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“What a wonderful chemistry these animals must possess to enable them to convert refuse animal matter into the delicious white muscle which most of us relish so much! And here it may be remarked that crustaceans are among the few scavengers whose flesh is prized for food by man. Most scavengers are of benefit to man only by aiding in keeping the air and the waters pure.” Figured: “American Lobster (*Homarus americanus*).” From “A Few Words About Scavengers” by Sanborn Tenney (*The American Naturalist*, 1877, 11:129–135).