

# ANALYSIS OF CHARACTER DIVERGENCE ALONG ENVIRONMENTAL GRADIENTS AND OTHER COVARIATES

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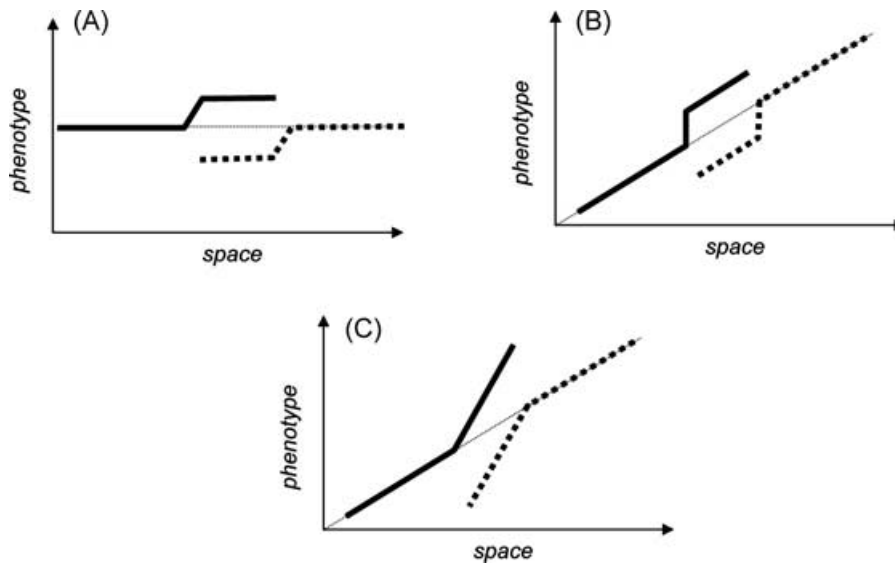
Character displacement is typically identified by comparing phenotypic differences in sympatry and allopatry. Recently, however, Goldberg and Lande (2006) pointed out that when phenotypic characters vary along an environmental gradient, the standard approach may fail to identify sympatric character divergence. Here we present a general analytical procedure for identifying sympatric character divergence while accounting for phenotypic changes that covary with environmental variables. Our approach uses residual randomization from a generalized linear model, and allows the statistical comparison of sympatric phenotypic divergence to allopatric phenotypic divergence while accounting for phenotypic variation along a gradient. Through simulation we demonstrate that our approach correctly identifies patterns of sympatric character divergence when they are present, and does not identify such patterns when they are not. Our analytical approach complements and extends the suggestions of Goldberg and Lande (2006), by allowing a full statistical assessment of the varied patterns of character displacement along environmental gradients, or while accounting for other covariates and sources of variation.

**KEY WORDS:** Character displacement, environmental gradient, generalized linear model, phenotypic change, residual randomization.

The competitive interactions of similar species have long been considered important for the evolution of phenotypic diversity. Competition may generate divergent selection, resulting in the evolution of phenotypic character shifts, or character displacement (Brown and Wilson 1956). Here, competition for limiting resources accentuates phenotypic differences between species in sympatry (Adams and Rohlf 2000; Losos 2000; Schluter 2000; Grant and Grant 2006). In the absence of alternative explanations, this pattern provides strong support of an evolutionary response to interspecific competition (Grant 1975; Schluter and McPhail 1992; Taper and Case 1992). Recent evidence suggests that character displacement may be widespread (Schluter 2000), and may be an important factor in determining community composition

(Dayan and Simberloff 2005) as well as a major cause of adaptive diversification (Schluter 2000; Pfennig and Murphy 2003; Pfennig and Pfennig 2005).

Character displacement is most frequently identified by comparing sympatric differences to allopatric differences and determining whether phenotypic differences in sympatry are greater than differences among allopatric populations (see, e.g., Dayan and Simberloff 1994; Melville 2002; Adams 2004). However, Goldberg and Lande (2006) recently pointed out that this approach correctly identifies character divergence in the absence of significant associations of species' phenotypes with environmental gradients (Fig. 1A). If the optimum phenotype varies along an environmental gradient, then allopatric differences may be greater



**Figure 1.** Phenotypic differences between two competing species in sympatry and allopatry; (A) and (B) redrawn after Goldberg and Lande (2006). Three scenarios are presented: (A) the phenotype does not vary across space; (B) the phenotype spatially varies along an environmental gradient; and (C) the phenotype varies spatially along an environmental gradient, but the covariation pattern differs in allopatry and sympatry. In the latter two cases, the phenotypic differences in allopatry exceed those in sympatry, obscuring the pattern of character divergence.

than sympatric differences, even though character displacement is occurring (Fig. 1B, 1C). In such cases, character divergence is obscured by phenotypic variation associated with an environmental gradient, and the standard approach fails to accurately describe the actual amount of character divergence.

Goldberg and Lande (2006) recommended that observational studies of character displacement include careful spatial sampling, and when relatively smooth phenotypic clines are identified, the degree of change in the mean phenotype in allopatry and sympatry should be quantitatively compared. This approach is useful for comparing slopes of phenotypic change along environmental gradients between sympatry and allopatry (e.g., Fig. 1C). However, greater mean phenotypic differences in sympatry relative to allopatry can also be observed along an environmental gradient, even if slopes of phenotypic change along a gradient are similar (Fig. 1B). To identify such patterns, an analytical method is required that directly compares allopatric and sympatric phenotypic divergence while accounting for covariation of phenotype and environmental variables along an environmental gradient. In this note, we describe a general analytical procedure for identifying sympatric character divergence while accounting for phenotypic changes that covary with environmental variables. The approach is general, and may also be used for assessing patterns of character displacement when the phenotype covaries with other factors, such as body size and age. Finally, in the absence of a spatial–environmental gradient, our procedure reduces to the familiar form of comparing allopatric to sympatric phenotypic differences.

#### ASSESSING CHARACTER DIVERGENCE

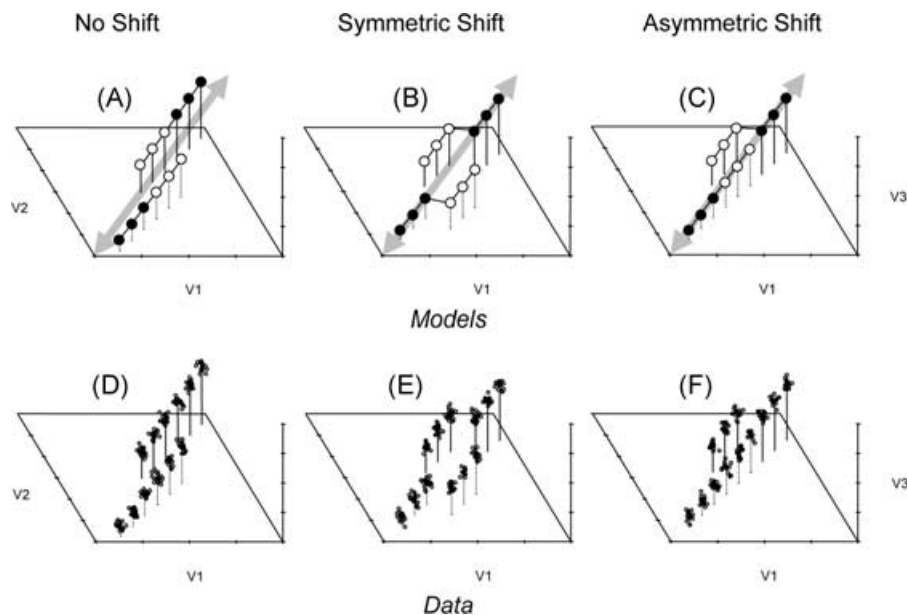
In many empirical studies of character displacement, phenotypic variation is first examined using the linear model  $\mathbf{Y} = \mathbf{X}\boldsymbol{\beta} + \boldsymbol{\epsilon}$ ; where  $\mathbf{Y}$  is the matrix of phenotypic data,  $\mathbf{X}$  is a design matrix describing the model effects of species identity, community types (allopatry and sympatry), and their interaction,  $\boldsymbol{\beta}$  is a matrix of partial regression coefficients, and  $\boldsymbol{\epsilon}$  is a matrix of residuals. Two-factor analysis of variance (ANOVA) or multivariate analysis of variance (MANOVA) statistically evaluates effects described by  $\boldsymbol{\beta}$  for inference of phenotypic variation in species differences, community type differences (allopatry and sympatry), and their interaction. A significant interaction identifies species-specific phenotypic changes from allopatry to sympatry, which may reflect a pattern of character displacement. To directly test this hypothesis, phenotypic differences are calculated between means of sympatric populations and between means of allopatric populations, and these values are statistically compared, frequently through a randomization procedure (e.g., Schluter and McPhail 1992; Adams and Rohlf 2000). For example, for multivariate phenotypic measures, the Euclidean distances between species–community phenotypic means are calculated in sympatry ( $D_{\text{symp}}$ ) and in allopatry ( $D_{\text{allo}}$ ), and the test statistic ( $D_{\text{symp}} - D_{\text{allo}}$ ) is evaluated to determine whether the observed sympatric phenotypic divergence is greater than expected from chance, frequently by randomly shuffling individuals across species–community type treatments and recalculating the test statistic (e.g., Adams 2004).

Although the procedure described above provides a straightforward test of sympatric character displacement, it does not allow the incorporation of environmental gradients or other covariates. Because such factors may obscure the signature of character displacement, the above procedure may fail to identify cases of sympatric character divergence. A useful alternative formulation, in which residual phenotypic values from a simplified (reduced) model are shuffled, rather than the original specimen values themselves, allows main effects (i.e., species and community type differences) to be held constant. For this “residual randomization” approach (ter Braak 1992; Gonzalez and Manly 1998), least squares means are estimated from a generalized linear model that contains the main effects of species and community type, but not the species  $\times$  community type interaction (spatial nonindependence among sampling locations can also be incorporated: see Appendix). Residuals from this model are then randomly assigned to least squares means calculated from the model to reconstruct “random” phenotypic values. These values are subsequently used to calculate random sympatric and allopatric differences, from which the significance of observed values can be inferred. With this approach, the observed sympatric character displacement can be statistically evaluated, but with the desirable property that nontargeted sources of variation, such as species differences and community type differences, are held constant throughout the procedure (Collyer and Adams 2007). Statistical details of the approach are found in Appendix.

Another important advantage of this approach is that spatial data and other variables which covary with phenotype can also be incorporated, analogous to analysis of covariance (see Appendix). Including the environmental variables allows phenotypic changes along a gradient to be accounted for while a direct test of sympatric character displacement is performed using residual randomization. With this analytical framework, phenotypic changes along the gradient no longer obscure the patterns between species in allopatry and sympatry, and sympatric and allopatric phenotypic divergences can be compared accounting for potential confounding factors.

### SIMULATION EXAMPLES

To illustrate the utility of our approach, we simulated three phenotypic variables for two species, plus one environmental variable along a spatial gradient for three different scenarios. For simplicity, we assume that locations along the spatial gradient are equally spaced and the spatial correlation of responses between sample locations is constant. The environmental variable was also assumed to have a linear increase along the spatial gradient. In the first simulation (Fig. 2A), phenotypic values for both species varied along the gradient, but there was no additional character shift in sympatry (i.e., no character displacement). In the second simulation (Fig. 2B), phenotypic values for both species varied along the gradient, and both species exhibited equally large additional



**Figure 2.** Simulated models and data for three phenotypic variables (V1–V3) across a gradient. Solid and open circles represent allopatric and sympatric population means, respectively. In all simulations, the mean phenotype changes monotonically along the gradient (shown as a grey arrow in the models). Differences in simulation models correspond to: (A) no character divergence; (B) symmetric character divergence; and (C) asymmetric character divergence. Panels (D)–(F) represent simulated specimens for each population (lines representing character shifts are included for illustrative purposes only).

character shifts in sympatry (i.e., symmetric character displacement). In the third simulation (Fig. 2C), phenotypic values for both species varied along the gradient, but only one species exhibited an additional character shift in sympatry (i.e., asymmetric character displacement). Each simulation contained three allopatric and three sympatric populations for each species, whose population means were equally spaced along the gradient. Three measured traits represented the phenotype of each individual. Twenty individuals were generated from each population mean using isotropic error (Fig. 2D–F).

Two sets of analyses were performed on each simulated dataset. First, the “standard” analyses of character displacement were performed, which included a two-factor MANOVA and a direct randomization test of sympatric versus allopatric phenotypic divergence ( $D_{\text{symp}} - D_{\text{allo}}$ ), using the “residual randomization” method (reduced model lacked the interaction term). These analyses did not include information about the spatial–environmental gradient, and were thus “gradient naïve” analyses. Second, a “gradient informed” analysis was performed, where the two-factor MANOVA included gradient information as a covariate. In addition, the direct test of sympatric versus allopatric divergence ( $D_{\text{symp}} - D_{\text{allo}}$ ) was based on randomization of residuals from a model that included the gradient covariate. For all resampling tests, significance levels were assessed using 10,000 iterations (9999 random values plus the observed).

## SIMULATION RESULTS

Using the standard analysis, phenotypic differences between species were identified for all simulated scenarios, and differ-

ences between community types were only found for the asymmetric scenario (Table 1). Further, in all scenarios, a significant interaction between species and community type was revealed, implying that species-specific phenotypic changes were present (Table 1). However, for the first scenario, no phenotypic change was modeled, implying that the naïve analysis incorrectly identified an effect that was not present. This result illustrates that exclusion of the environmental gradient in the analysis can lead to erroneous conclusions about character displacement, by identifying significant effects that are really not present. This result supports Goldberg and Lande’s (2006) suggestion that when possible, gradient information should be included.

In contrast, when the environmental gradient was incorporated, both phenotypic differences between species and phenotypic change along the gradient were identified for all scenarios (Table 1). In addition, differences in community types were identified in only the asymmetric scenario, and a significant species  $\times$  community type interaction was revealed in both the symmetric and asymmetric divergence scenarios, but not in the gradient only scenario (Table 1). These outcomes are reasonable, as symmetric species’ phenotypic shifts in sympatry would not cause a change in the average sympatric phenotype (because interaction effects are in fact opposite) from the average allopatric phenotype. However, an asymmetric shift (i.e., phenotypic shift of only one species) results in a displacement of the average sympatric phenotype from the average allopatric phenotype. Therefore, when the environmental gradient was included as a covariate, all significant effects were correctly identified, and those effects that were not incorporated in the simulation model were correctly found to have no discernable effect on phenotypic variation.

**Table 1.** Wilks’  $\Lambda$  and associated  $P$ -values for multivariate analyses of variance (MANOVA) performed on simulated datasets. Additional direct tests of phenotypic divergence between sympatric and allopatric populations ( $D_{\text{symp}} - D_{\text{allo}}$ ) are also shown. Bold values indicate that significant effects that differ from what was simulated were statistically identified.

Factor	df	No shift		Symmetric shift		Asymmetric shift	
		$\Lambda$	$P$	$\Lambda$	$P$	$\Lambda$	$P$
Species	1	0.0207	<0.0001	0.0300	<0.0001	0.0800	<0.0001
Community	1	1.0000	0.3633	1.0000	0.9568	0.1300	<0.0001
Species $\times$ community	1	<b>0.2000</b>	<0.0001	0.0300	<0.0001	0.0900	<0.0001
Error	236						
$(D_{\text{symp}} - D_{\text{allo}})$		<b>-12.46</b>	0.0001 <sup>1</sup>	<b>-9.64</b>	0.0001	<b>-11.58</b>	0.0001
Gradient	1	0.0012	<0.0001	0.0016	<0.0001	0.0017	<0.0001
Species	1	0.0277	<0.0001	0.0161	<0.0001	0.0624	<0.0001
Community	1	1.0000	0.3580	1.0000	0.7929	0.1130	<0.0001
Species $\times$ community	1	1.0000	0.4764	0.1157	<0.0001	0.3573	<0.0001
Error	235						
$(D_{\text{symp}} - D_{\text{allo}})$		-0.064	0.4580	3.895	<0.0001	1.774	<0.0001

<sup>1</sup> $P$ -values of direct tests are empirically derived from a permutation procedure. These values represent two-tailed considerations.

Even more revealing were results from direct tests of sympatric versus allopatric divergence. In all cases, when the environmental gradient was ignored, highly significant ( $D_{\text{symp}} - D_{\text{allo}}$ ) statistics were attained, but in the wrong direction (Table 1). That is, in all cases, the phenotypic differences in allopatry were significantly greater than differences in sympatry. This result is consistent with those of Goldberg and Lande (2006). In contrast, when the gradient was included as a covariate, a significant ( $D_{\text{symp}} - D_{\text{allo}}$ ) statistic was found for the symmetric and asymmetric character divergence scenarios, but was not significant for the case where no additional character shift was included in the model (Table 1). Thus, for direct allopatry–sympatry comparisons, our approach incorporating the environmental gradient as a covariate correctly identified character displacement when it was present, and correctly identified no character displacement when it was not present.

## Discussion

Observational studies of character displacement provide important insights into the evolutionary responses of competing species. Recently, Goldberg and Lande (2006) pointed out that when phenotypic characters vary along an environmental gradient, the standard approach of comparing sympatric differences to allopatric differences may fail to identify sympatric character divergence. They recommended that such observational studies include spatial information on the locations of the samples, and when possible, comparisons of the slopes of change in the mean phenotype in allopatry and sympatry. In this study, we presented a general analytical procedure for identifying sympatric character divergence while accounting for phenotypic changes along an environmental gradient. Our approach uses residual randomization from a reduced generalized linear model, and allows the statistical comparison of sympatric phenotypic divergence to allopatric phenotypic divergence while accounting for phenotypic covariation along a gradient. Through simulation we demonstrate that our approach correctly identifies patterns of sympatric character divergence when they are present, and does not identify patterns when they are not.

One important aspect of our approach is that it is a simple extension of analytical methods commonly used in studies of character displacement. Therefore, there is no conceptual change in how one considers patterns of character displacement; rather, the linear model used is simply parameterized with or without environmental covariates. As such, when covariates are not included, our procedure reduces to the standard allopatry–sympatry comparison. On the other hand, if phenotypic values covary with an environmental variable, or multiple variables, they can be easily incorporated in the model. In addition, our approach is not restricted to accounting for environmental gradients, but can be

used to account for any nontargeted sources of variation, such as age, or the presence of a third potentially competing species at some localities. Therefore, the analytical procedure is truly general, as patterns of character displacement can be evaluated for simple ecological scenarios (with only two species in allopatry and sympatry), or considerably more complicated designs (e.g., where the phenotype covaries along a gradient, and where additional competitors are present at each location).

Finally, we note that our procedure can also be used to examine changes in the slopes of the gradient, as recommended by Goldberg and Lande (2006). For this, species  $\times$  community type  $\times$  gradient interactions are included in the original (full) model calculations, but are removed from the reduced model. Residuals from this reduced model can then be used to evaluate differences in the slopes for each species  $\times$  community type  $\times$  gradient combination. Therefore, our analytical approach complements and extends the suggestions of Goldberg and Lande (2006), by allowing a full statistical assessment of other patterns of character displacement along environmental gradients, or while accounting for other covariates and sources of variation.

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## Appendix

### STATISTICAL DETAILS

Phenotypic variation,  $\mathbf{Y}$ , can be expressed by a generalized linear model,  $\mathbf{Y} = \mathbf{X}\boldsymbol{\beta} + \boldsymbol{\epsilon}$ ; where  $\mathbf{X}$  is an  $n \times k$  design matrix describing the  $k$  model effects for  $n$  objects,  $\boldsymbol{\beta}$  is a  $k \times p$  matrix of partial regression coefficients for  $p$  response variables, and  $\boldsymbol{\epsilon}$  is the  $n \times p$  matrix of residuals. For a typical character displacement study,  $\mathbf{X}$  takes the form of a two-factor MANOVA, with species, community type (allopatry and sympatry) as main effects, and includes their interaction (we represent this as the design matrix for the full model,  $\mathbf{X}_f$ ). Parameter estimates are solved as  $\hat{\boldsymbol{\beta}}_f = (\mathbf{X}_f^t \mathbf{X}_f)^{-1} \mathbf{X}_f^t \mathbf{Y}$ , where  $t$  and  $-1$  correspond to matrix transpose and inverse, respectively; and the least-squares

means are estimated ( $\bar{\mathbf{Y}} = E[\mathbf{Y}|\mathbf{X}_f, \hat{\boldsymbol{\beta}}_f]$ ). It is likely that incorporating information about an environmental gradient in the analysis means that responses will be spatially correlated (samples will be more similar in proximate locations than distal ones). It is therefore useful to use a generalized least squares (GLS) estimator of  $\hat{\boldsymbol{\beta}}_f$ :  $\hat{\boldsymbol{\beta}}_{f(\text{GLS})} = (\mathbf{X}_f^t \boldsymbol{\Sigma}^{-1} \mathbf{X}_f)^{-1} \mathbf{X}_f^t \boldsymbol{\Sigma}^{-1} \mathbf{Y}$ , where  $\boldsymbol{\Sigma}$  is an  $n \times n$  covariance matrix that describes the spatial covariance among observations (Gotway and Stroup 1997; Dalthorp 2004).

The observed phenotypic divergence in sympatry and allopatry are then calculated as the Euclidean distances between least squares means for the proper species-community type treatments ( $D_i = (\Delta \bar{\mathbf{Y}}_i \Delta \bar{\mathbf{Y}}_i^t)^{1/2}$ ), where  $\Delta \bar{\mathbf{Y}}_i = \bar{\mathbf{Y}}_{ij} - \bar{\mathbf{Y}}_{ik}$ , for species  $j$  and  $k$  in the  $i$ th community type. The difference between sympatric and allopatric phenotypic divergences is determined as ( $D_{\text{symp}} - D_{\text{allo}}$ ).

To evaluate the significance of this value, a residual randomization is performed. First, the interaction term is removed, resulting in the reduced model,  $\mathbf{X}_r$ . The reduced model is solved, and predicted values ( $\hat{\mathbf{Y}}_r = \mathbf{X}_r \hat{\boldsymbol{\beta}}_r$ ) and residuals ( $\boldsymbol{\epsilon}_r$ ) are determined. Residuals ( $\boldsymbol{\epsilon}_r$ ) are randomized and are added to predicted values to produce random values ( $\mathbf{Y}^* = \hat{\mathbf{Y}}_r + \boldsymbol{\epsilon}_r^*$ ) such that nontargeted effects are held constant. The value ( $D_{\text{symp}} - D_{\text{allo}}$ ) is determined and the process is repeated many times to compare the observed value to a distribution of random values.

To incorporate an environmental gradient (i.e., environmental variables that vary along a spatial gradient), the above procedure is identical, except that additional covariates are included in both the full ( $\mathbf{X}_f$ ) and reduced ( $\mathbf{X}_r$ ) models. Thus, these models examine patterns of sympatric and allopatric phenotypic divergence, while accounting for variation of phenotype along an environmental gradient. By including other variables (e.g., size or age covariate) in the design matrices, more complicated designs can also be examined.