E-NOTE

Experimental Hybridization Studies Suggest That Pleiotropic Alleles Commonly Underlie Adaptive Divergence between Natural Populations

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ABSTRACT: The alleles used for adaptation can pleiotropically affect traits under stabilizing selection. The fixation of alleles with deleterious pleiotropic side effects causes compensatory alleles to be favored by selection. Such compensatory alleles might segregate in interpopulation hybrids, resulting in segregation variance for traits where parents have indistinguishable phenotypes. If adaptation typically involves pleiotropy and compensation, then the segregation variance for traits under stabilizing selection is expected to increase with the magnitude of adaptive phenotypic divergence between parents. This prediction has not been tested empirically, and I gathered data from experimental hybridization studies to evaluate it. I found that pairs of parents that are more phenotypically divergent beget hybrids with more segregation variance in traits for which the parents are statistically indistinguishable. This result suggests that adaptive divergence between pairs of natural populations proceeds via pleiotropy and compensation and that deleterious transgressive segregation variance accumulates systematically as populations diverge.

Keywords: compensatory mutation, hybridization, pleiotropy, transgressive segregation.

Introduction

When populations adapt to their environment, they increase the frequency of (or fix) alleles that affect the phenotypes of traits under selection. The alleles that underlie adaptation can affect multiple traits at a time, a phenomenon known as pleiotropy (Stearns 2010). In recent years, evidence has accumulated, largely from evolutionary model systems, that suggests that pleiotropy is common (although it might only affect a small subset of an organism's traits; Wagner et al. 2008; Wang et al. 2010; Wagner and Zhang 2011; Hill and Zhang 2012). If the pleiotropic effects of alleles are deleterious, compensatory mutations that counteract this deleterious pleiotropy can be favored by natural selection (Phillips 1996; for empirical examples of compensatory mutation, see Adam et al. 1993; Poon and Chao 2005; Howe and Denver 2008; Merker et al. 2018). Although this model of adaptation via pleiotropy and compensation emerges in many theoretical models of adaptation (Orr 2000; Barton 2001), it is unclear whether such a process typically characterizes adaptation in natural populations.

Predictions from theoretical models of divergent adaptation and hybridization can be tested to infer whether adaptation in natural populations typically involves pleiotropy and compensation (Pavlicev and Wagner 2012). Barton (2001) conducted simulations of Fisher's (1930) geometric model of adaptation in a case where two populations with 10 traits experienced divergent selection on a single trait, while the other nine were subject to stabilizing selection. Following hybridization of the two populations, there was appreciable segregation variance in the nine traits under stabilizing selection. This segregation variance was caused by the recombinant hybrids inheriting alternative combinations of compensatory alleles. Importantly, the amount and/or average effect size of compensatory alleles should be positively correlated with the amount of phenotypic divergence between the parents. Thus, the theoretical prediction under adaptation via pleiotropy and compensation is as follows: as the phenotypic divergence between pairs of populations increases, so should the amount of segregation variance in nondivergent traits observed in their hybrids. See figure 1 for a visual overview of this prediction and figure S1 (figs. S1–S4 are available online) for the results of computer simulations illustrating the prediction more quantitatively. In this article, I test this theoretical

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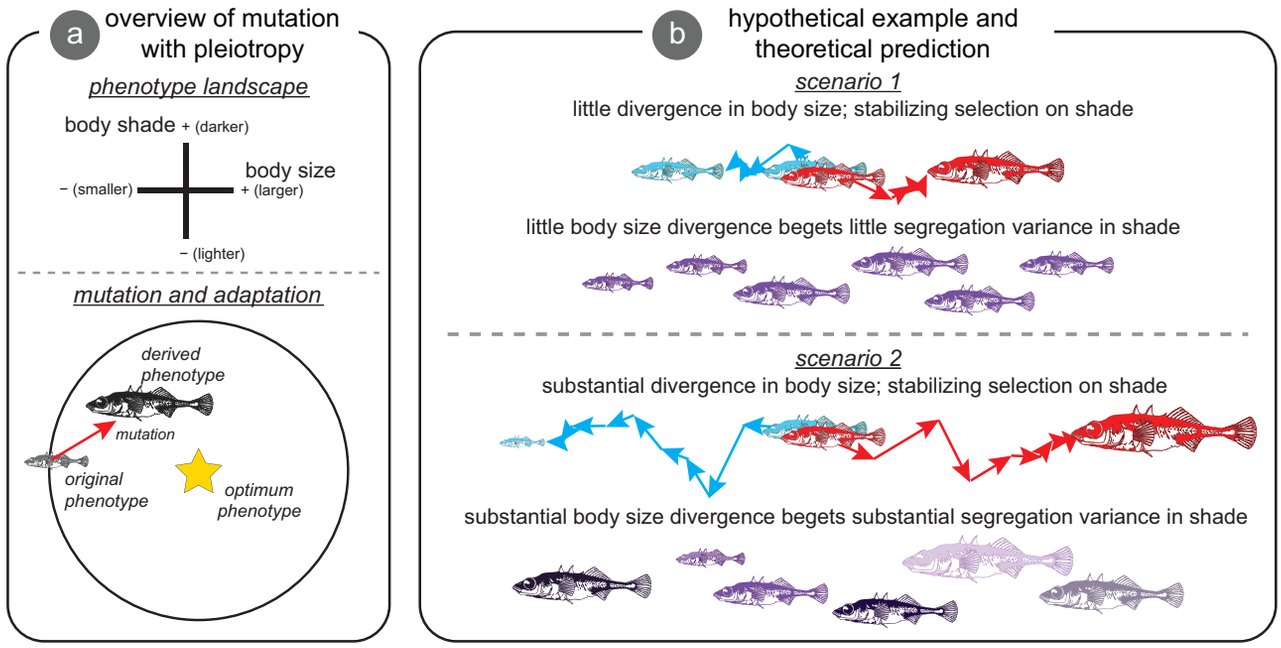

Figure 1: Overview of adaptation with pleiotropic alleles and corresponding theoretical prediction. *A* shows a general overview of Fisher's geometric model, which relies on pleiotropic mutation. The upper section shows the phenotype landscape under consideration, wherein the *X*-axis is body size and the *Y*-axis is body shade. The lower section illustrates the fixation of a pleiotropic allele during adaptation. The large circle defines the space wherein mutations are beneficial; mutations that point outside the circle are deleterious. The original phenotype is medium in size and shade, whereas the optimal phenotype is larger but is the same shade. A mutation arises that greatly increases size and has a deleterious pleiotropic effect to darken shade. Since the mutation is beneficial (points inside the circle), it has a high probability of fixation in spite of the deleterious side effect. *B* illustrates the theoretical prediction in two diverging populations—red and blue—with the same initial phenotype for size and shade—color here is just used to visually demarcate parent populations and hybrids (purple) and is not considered a trait. Arrows represent individual mutations as in *A*. In each of two scenarios, shade is under stabilizing selection in the two populations. Scenario 1 is a case where the two populations diverge little in body size, and scenario 2 represents a case with substantial divergence in body size. The lower section of each scenario illustrates the outcome of hybridization. The key insight is that the segregation variance in shade is greater in scenario 2 than in scenario 1. Body size segregates as well, but it would do so in a model without pleiotropy, whereas shade would not necessarily. Darker recombinant hybrid individuals inherited mostly compensatory alleles that darken shade (i.e., point up), and lighter individuals inherited mostly compensatory alleles that lighten shade (i.e., point down).

prediction using data collated from experimental hybridization studies. In doing so, I illustrate that alternative processes such as genetic drift are unlikely to underlie the observed patterns.

Methods

I conducted a systematic literature search with the goal of identifying studies that measured phenotypic traits and variances in two parent taxa and their (intraspecific or interspecific) hybrids in a common environment. Most of the collected data are analyzed in a separate study investigating phenotypic dominance in F_1 hybrids (Thompson et al. 2019*b*). To be selected for inclusion in the larger data set, studies had to measure at least one nonfitness (i.e., "ordinary" [Orr 2001]) trait in two parent taxa (different species or divergent populations of the same species) and their F_1 hybrids. In addition, parent taxa had to be fewer than 10 generations removed from the wild (details of the literature search are given in the appendix, available online, and the reasons for excluding each study are included in the main literature search data frame [see the Dryad Digital Repository (https://doi.org/10.5061/dryad.qjq2bvqc3; Thompson 2019)]). In total, I (with help) screened more than 11,000 studies and collected data from 198. Of these 198 studies, all that met the following two additional criteria were included in the present analysis: (1) F_2 hybrids were measured and (2) the parents had significantly different phenotypes for at least one trait and were statistically indistinguishable for at least one other trait.

After identifying studies for possible inclusion, I filtered and binned the data to generate summary statistics for analysis. Filtering and binning decisions were—by necessity—somewhat subjective, and I present the test of the main hypothesis for summary data sets generated under

alternative filtering and binning criteria in table S1 (available online). Since the conclusions are generally robust (highest $P = .0523$) to alternative data-processing decisions, it seems unlikely that the study selection criteria bias my conclusions. In addition, further analysis with potentially low-power studies removed illustrates that the observed patterns are not caused by associations between sample size (number of individuals measured) and any variables (see also table S1). I also note that methods are only briefly detailed here in the main text, but full details with appropriate citations are given in the appendix. All analyses were conducted in R version 3.5.1 (R Core Team 2018), and all data underlying the article are deposited in the Dryad Digital Repository (https://doi.org/10.5061/dryad.qjq2bvqc3; Thompson 2019).

In the main text, I restrict my analysis to morphological traits—by far the most frequently measured trait type in the studies that met the criteria described above—to maximize the degree to which traits and units were comparable. In total, I retained data from 15 crosses (14 studies) for the present analysis (MacNair et al. 1989; Shore and Barrett 1990; Bradshaw et al. 1998; Bratteler et al. 2006; McPhail 2008; Raeymaekers et al. 2009; Koelling and Mauricio 2010; Jacquemyn et al. 2012; Pritchard et al. 2013; Selz et al. 2014; Hermann et al. 2015; Husemann et al. 2017; Mione and Anderson 2017; Vallejo-Marín et al. 2017). Of these 14 studies, nine crossed vascular plants, four crossed fish (one study contained two crosses), and one crossed copepods. Eight crosses were interspecific, and seven were intraspecific.

For each study, I divided traits into two groups: those that differed between the parents—which I assume was the result of divergent selection—and those that did not and were more likely (although not necessarily) subject to stabilizing selection. I classified traits as divergent if they were significantly different ($P < .05$) in a *t*-test. My conclusions are unchanged if parent divergence in phenotypic standard deviations is used (divergent if parents are >1 SD apart) as a binning criterion. For each trait, I calculated the degree of phenotypic divergence in units of parental phenotypic SDs using the smaller of the two parental values. For each study, I then calculated phenotypic divergence for both groups of traits as the mean of ln-transformed divergence values.

For traits that were statistically indistinguishable between parents, I determined the segregation variance of each as

$$\mathrm{var}(s) = \frac{4\mathrm{var}(\mathrm{F}_2)}{2\mathrm{var}(\mathrm{F}_1) + \mathrm{var}(\mathrm{P}_1) + \mathrm{var}(\mathrm{P}_2)} \quad (1)$$

Wright (1968). This quantity normalizes for the standing variation observed in each parent and F_1 hybrids and captures the variance due to the segregation of population-specific or species-specific alleles. For each cross, I took the mean of these values across all nondivergent traits after ln transformation as an estimate of segregation variance.

My prediction was that if adaptation commonly proceeds via pleiotropic and compensatory alleles, then there should be a positive relationship between parental divergence—for divergently selected traits—and segregation variance—for traits that do not differ between the parents. Visualization of linear models and statistical tests of heteroskedasticity clearly showed that the assumptions of parametric statistical analyses were violated (see fig. S2). I therefore tested all predictions using Spearman's rank-order correlations, which test whether more divergent pairs of populations beget hybrids with more (or less) segregation variance as compared with lesser divergent parental taxa.

A similar pattern to what is predicted above could be the result of genetic drift and have nothing to do with divergent natural selection. Specifically, if more phenotypically divergent pairs also diverged longer ago than less phenotypically divergent pairs, they might have fixed a greater number of compensatory mutations for all of their traits (if such mutations fix at a steady rate over time). If this was the case, one would detect the predicted pattern even if the alleles underlying divergence were not pleiotropic. It is therefore important to rule out this role for time by testing whether phenotypic divergence of parents is correlated with their divergence time in the studies analyzed herein. I did this using the three main approaches that follow: (1) by comparing phenotypic divergence of intraspecific cross parents to that of interspecific cross parents, (2) by evaluating the correlation between neutral gene sequence divergence and phenotypic divergence (units of base pairs), and (3) by evaluating the correlation between estimates of divergence time and phenotypic divergence (similar to [2] but in units of time based on fossil-calibrated phylogenies).

Results

I observed a positive correlation between the mean parental phenotypic divergence in statistically divergent traits and the mean segregation variance in statistically indistinguishable traits (Spearman's $\rho = 0.800$, $P = .000581$, $n = 15$; fig. 2). The magnitude of the phenotypic difference between parents for statistically indistinguishable traits was not significantly correlated with the segregation variance in those traits (Spearman's $\rho = 0.446$, $P = .0972$, $n = 15$; fig. S3). The patterns were generally robust to data-processing decisions (see table S1), only slightly surpassing the significance threshold when I included physiological and chemical traits ($P = .052$) in the analysis.

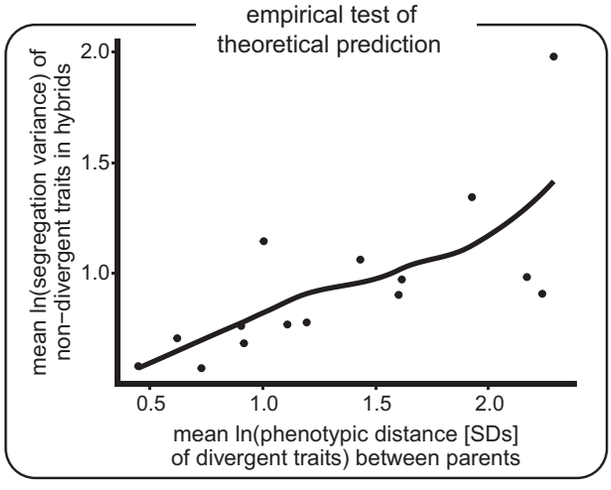

Figure 2: Scatterplot depicting the relationship between phenotypic divergence in parents (statistically divergent traits) and segregation variance in hybrids (statistically indistinguishable traits). Each point ($n = 15$) represents a unique cross between two populations or species. Points to the right on the *X*-axis represent crosses where the parent taxa exhibit a relatively large magnitude of phenotypic divergence for traits deemed "divergent" (Spearman's $\rho = 0.800$, $P = .000581$). The line is a loess fit.

Divergence time could correlate with phenotypic divergence between populations, which would render it difficult to disentangle the relative roles of time and phenotypic divergence in causing the pattern shown in figure 2. I found no evidence for a difference between intraspecific and interspecific crosses in parental phenotypic divergence ($F_{1,13} = 0.013$, $P = .912$; fig. S4*A*, S4*B*). Additional analyses found no support for associations between any variable and genetic divergence (fig. S4*C*), divergence time (fig. S4*D*), or phylogeny (phylogenetic signal test, all $P > .5$).

Discussion

I leveraged data from experimental hybridization studies to conduct a correlative test of the hypothesis that divergent adaptation is associated with transgressive phenotypic variation in recombinant hybrids. This prediction holds if the alleles underlying divergent adaptation are pleiotropic and does not hold if they are not pleiotropic (or if they are pleiotropic but have infinitely small individual effects [Barton et al. 2017]; see fig. S1). Given the lack of effect of divergence time (or its correlates) on phenotypic divergence in the data, the consistency between the results presented here and the theoretical prediction provides indirect and correlative evidence that the alleles used during adaptation are indeed pleiotropic and of appreciably large effect. The results might also hint at of the mode of adaptation for the taxa considered herein. For example, adaptation from standing variation causes greater transgressive segregation variance compared with adaptation from de novo mutation (Thompson et al. 2019*a*), and thus the observed patterns could be a consequence of adaptive divergence from standing variation being commonplace (Barrett and Schluter 2008). Even if large-effect pleiotropic mutations arise, models with slowly moving fitness optima predict that only alleles with very-small effects will be used during adaptation (Matuszewski et al. 2014). The analyses described above suggest that optima in nature move quickly enough for alleles of nontrivial effect sizes to be incorporated.

My findings might initially appear to contradict the results of previous studies of transgressive segregation. For example, Stelkens and Seehausen (2009) and Stelkens et al. (2009) found that genetic distance, but not phenotypic distance, predicts transgressive segregation. Although this seems to contradict the pattern shown in figure 2, the predictions are not directly comparable because I binned traits into categories of divergent and nondivergent and compared parental divergence in the former with hybrid variance in the latter. By contrast, Stelkens's studies investigated the degree to which individual hybrids are transgressive for traits considered on their own or across all traits. Thus, our analyses test separate hypotheses. Rieseberg et al. (1999) also predicted that genetic divergence and transgressive segregation will be positively correlated when parents experience stabilizing selection at a common optimum. This prediction arises purely from substitutions fixed by drift and subsequent compensatory mutations. In the present data set, genetic divergence is not correlated with transgressive segregation variance ($P = .801$; results not shown but analysis included in archived R script). It is likely that in wild and outbred taxa, any effect of drift on transgressive segregation is obscured by the segregation of large-effect pleiotropic alleles and compensatory mutations fixed during adaptive divergence in other traits.

Experiments can be conducted to directly test the prediction considered herein. In an experimental evolution system where individuals and traits are easily measured, parental lines could be selected for divergence to varying degrees and then hybridized with a common ancestor. The traits that responded to divergent selection should be identified and measured, as should the traits that did not diverge and were putatively subject to stabilizing selection. The expectation is that—if mutations are universally pleiotropic—the amount of segregation variance in nondivergent traits should increase with the phenotypic distance of divergent traits. Because alleles fixed from standing variation are expected to be more pleiotropic than those fixed from de novo mutation (Thompson

et al. 2019*a*), the transgressive segregation variance should be greater if the population is able to use standing variance for adaptation compared with if it must rely on de novo mutation. If desired, one could attempt to identify the causal alleles directly using quantitative trait locus mapping.

Segregation variance in nondivergent traits is expected to be deleterious and, accordingly, hybrid fitness should decline as the segregation variance increases. If segregation variance is observed for nondivergent traits, this directly implies that variance in the trait is deleterious—compensatory mutations would not be favored if not for their ability to counteract deleterious pleiotropy. The problem with relying entirely on phenotypic measurements for empirical tests is that segregation variance could manifest in unmeasured traits and thus easily be missed. It will therefore be useful, albeit difficult, to test predictions about fitness directly. If divergent experimental populations are hybridized, the fitness of F_1 and F_2 hybrids could be compared in a common environment. The clear prediction is that the loss in fitness of F_2 hybrids (due to segregating breakup of coadapted compensatory alleles) compared with F_1 hybrids will be greater in more divergently selected lines. A difficulty arises when attributing this loss in fitness to segregation variance of nondivergent traits, because segregation variance in the divergent trait(s) will affect fitness in an environment-dependent manner (see fig. 1 of Barton [2001]). For example, in an intermediate environment, the F_2 would have lower fitness than the F_1 even without pleiotropy due to deleterious segregation variance of the selected trait(s). However, if the F_2 has lower fitness than the F_1 in both the ancestral and derived environments, this implicates the segregation variance in nondivergent traits as the cause. Perhaps the best test would be to sequence the F_2s and look at selection on heterozygosity because the signature of selection against incompatible compensatory mutations in an F_2 is selection for heterozygosity (Simon et al. 2018). Thus, after measuring the fitness of F_2s in a particular environment, directional selection on the divergent trait(s) would manifest as selection favoring particular hybrid index, and selection against segregating phenotypic variance in nondivergent traits would manifest as selection for heterozygosity. Such an experiment would be valuable for establishing a general link between adaptive divergence and reproductive isolation.

Although I illustrated a correspondence between theory and data, I did so using a correlational approach and with a small sample size of 15 crosses. I offer no conclusive proof that pleiotropic alleles and compensatory mutations are the cause of the observed pattern. There are other plausible mechanisms besides pleiotropy that could underlie segregation variance in nondivergent traits. For example, parallel phenotypic evolution (if it has a nonparallel genetic basis; e.g., Ono et al. 2017) can cause segregation variance in traits that do not differ between the parent taxa (Chevin et al. 2014; Thompson et al. 2019*a*). For this mechanism to underlie the pattern shown in figure 2, there would have to be a correlation between parallel phenotypic evolution in some traits and divergent evolution in others—and this seems unlikely. Although results presented herein are consistent with theory, empirical tests using experimental evolution would be a stronger and more direct test of the underlying mechanistic hypothesis. The ability of such studies to make a direct link to hybrid fitness is also powerful. Such studies, paired with my indirect analysis across many taxa, would greatly strengthen our grasp on the generality of pleiotropy's role in adaptive evolution. At the very least, my analysis should serve to buttress the assessment that models fundamentally based on pleiotropy, such as Fisher's (1930) geometric model, are robust and useful abstractions of the evolutionary process.

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Data and Code Availability

All data and analysis code underlying the results reported in this article have been deposited in the Dryad Digital Repository (https://doi.org/10.5061/dryad.qjq2bvqc3; Thompson 2019).

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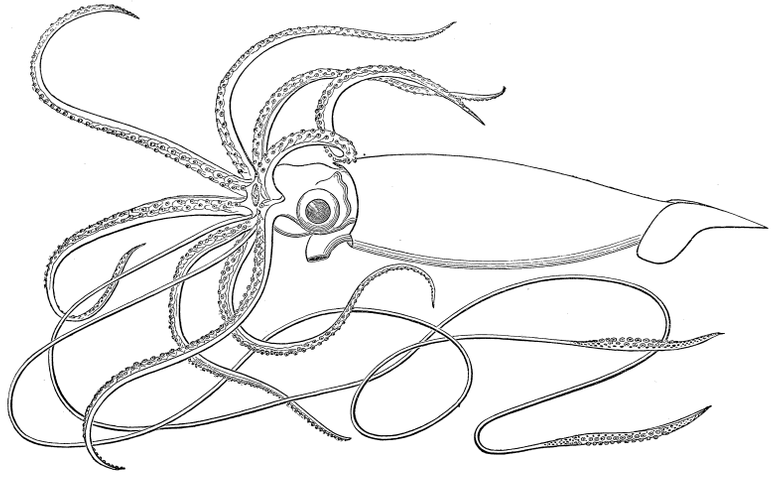

"The existence of several distinct species of these colossal ten-armed Cephalopods has been satisfactorily demonstrated in the various papers that have been written upon the subject both in Europe and America." From "The Colossal Cephalopods of the North Atlantic" by A. E. Verrill (*The American Naturalist*, 1875, 9:21–36).